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RESEARCH ARTICLE

Physiological responses of wild zebra finches (*Taeniopygia guttata*) to heatwaves

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ABSTRACT

Desert birds inhabit hot, dry environments that are becoming hotter and drier as a consequence of climate change. Extreme weather such as heatwaves can cause mass-mortality events that may significantly impact populations and species. There are currently insufficient data concerning physiological plasticity to inform models of species' response to extreme events and develop mitigation strategies. Consequently, we examine here the physiological plasticity of a small desert bird in response to hot (mean maximum ambient temperature=42.7°C) and cooler (mean maximum ambient temperature=31.4°C) periods during a single Austral summer. We measured body mass, metabolic rate, evaporative water loss and body temperature, along with blood parameters (corticosterone, glucose and uric acid) of wild zebra finches (*Taeniopygia guttata*) to assess their physiological state and determine the mechanisms by which they respond to heatwaves. Hot days were not significant stressors; they did not result in modification of baseline blood parameters or an inability to maintain body mass, provided drinking water was available. During heatwaves, finches shifted their thermoneutral zone to higher temperatures. They reduced metabolic heat production, evaporative water loss and wet thermal conductance, and increased hyperthermia, especially when exposed to high ambient temperature. A consideration of the significant physiological plasticity that we have demonstrated to achieve more favourable heat and water balance is essential for effectively modelling and planning for the impacts of climate change on biodiversity.

KEY WORDS: Blood parameters, Climate change, Evaporative water loss, Metabolic rate, Physiological stress, Temperature

INTRODUCTION

Anthropogenically driven climate change is increasing the frequency, severity and duration of extreme weather events, such as storms, floods, fires and heatwaves (Meehl and Tebaldi, 2004; Tebaldi et al., 2006; Rahmstorf and Coumou, 2011; Diffenbaugh and Field, 2013; IPCC, 2014). Twenty-five percent of wild animal mass mortality events are related to extreme climate events, so extreme events can significantly impact populations, and even entire species (McKechnie et al., 2012; Fey et al., 2015; Ruthrof et al., 2018), particularly when they are superimposed on a general pattern of increasing heat and aridity (Harris et al., 2018). Therefore, potential management of biological diversity in the face of anthropogenic climate change requires a clear understanding of how species, populations and communities respond not only to a change in long-term climatic factors such as mean ambient temperature and annual rainfall, but also to more acute extreme weather events (Harris et al., 2018).

Birds, and especially desert birds which inhabit already hot, dry environments that are becoming hotter and drier, are particularly susceptible to extreme events such as heatwaves (McKechnie and Wolf, 2010). Desert birds may already be close to the upper limit of their thermal tolerance. Once ambient temperature (T_a) exceeds body temperature (T_b) the only avenue for heat loss is evaporation, so there is little scope to avoid lethal hyperthermia without the risk of dehydration if temperatures become even more extreme (Wingfield et al., 2017). This trade-off is exacerbated when drinking is associated with activity or exposure to solar radiation (Wolf, 2000). A detailed mechanistic understanding of physiological function under variable environmental conditions is required to better understand the potential impacts of global warming, including heatwaves (Huey et al., 2012; McKechnie et al., 2012). However, there are currently insufficient physiological data to inform models that can be applied to develop meaningful mitigation strategies for the majority of these extreme events (Martin et al., 2014; Denny and Helmuth, 2009; Ratnayake et al., 2019).

One issue with predicting species responses to extreme conditions is that the majority of predictive species distribution models fail to incorporate the potential for physiological flexibility or plasticity (Chown et al., 2010; Fuller et al., 2010). This is despite the fact that physiological plasticity is well appreciated (Piersma and Drent, 2003; Martin et al., 2014), including for birds (e.g. Chaffee and Roberts, 1971; Tieleman et al., 2003; Klaassen et al., 2004; Cavieres and Sabat, 2008; Maldonado et al., 2009). Wild birds acclimate to seasonal changes in weather and food availability, and in captivity acclimatise to differing thermal regimes, although patterns of acclimation and acclimatisation are not necessarily consistent, suggesting that temperature may not be the only driver of seasonal physiological plasticity (McKechnie and Swanson, 2010). If this is the case, and the documented physiological plasticity for wild birds is driven more by indirect environmental cues such as photoperiod rather than temperature (McKechnie and Swanson, 2010), then there is potential for a mismatch in physiological responses to environmental conditions such as heatwaves. These mismatches are likely to become more frequent and pronounced with climate change (McCormick and Romero, 2017). However, these general seasonal responses may be moderated by recent ambient conditions, at least during cold winter periods (McKechnie, 2008). The potential for physiological plasticity by wild birds in response to extreme, short-term, high temperature events is, however, not known despite being an essential aspect of the understanding of physiological function necessary to effectively plan for the biodiversity impacts of climate change (Martin et al., 2014; Denny and Helmuth, 2009).

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To address the current paucity of data for how wild birds respond physiologically to acute changes in temperature, especially at high T_a (Noakes et al., 2016), we examine here how physiological responses of wild zebra finches (*Taeniopygia guttata* Gould 1837) vary during hot (mean maximum T_a=42.7°C) compared with cooler periods (mean maximum T_a=31.4°C) during a single Austral summer. Zebra finches are small estrildid finches endemic to arid regions of Australia, and have become an iconic model for studying the physiology, ecology, and reproduction of desert birds (Zann, 1996). Wild zebra finches can maintain energy and water balance during periods of extreme high temperature, facilitated by a high body water content that buffers them from fatal dehydration, and pre-emptive foraging during the mornings to reduce the need to eat and drink during the heat of the day (Cooper et al., 2019). However, the acute mechanistic adjustments of metabolic, hygric, thermal and endocrine physiology during hot and cool periods that facilitate survival during periods of extreme temperature are still unclear. Here we measure the plasticity of T_b, metabolic rate (MR) and evaporative water loss (EWL) at thermoneutrality (T_a=30°C; Calder, 1964; Cade et al., 1965) and in response to a physiologically challenging higher T_a (40°C). We also examine how blood parameters immediately after capture and in response to acute non-invasive stress vary during hot and cooler periods, by measuring corticosterone (CORT) as an index of the major stress response, glucose (GLU) as the main metabolic substrate, and uric acid (UA) as the main product of muscle degradation and the major circulating antioxidant in birds (Deviche et al., 2016).

MATERIALS AND METHODS

Animals

Experiments were conducted at Fowlers Gap Arid Zone Research Station (31°05′ S, 142°42′ E; 178 m above sea level), approximately 112 km north of Broken Hill, New South Wales, Australia, during the 2018–2019 Austral summer. At this time, the population of zebra finches at the site had been exposed to an extended period of drought and above-average temperature for 2 years, with no significant (>10 mm) rainfall events since December 2016 (Australian Bureau of Meteorology, 2018; http://www.bom.gov.au/climate/data-services/). Zebra finches were captured using mist nets near the empty Gap Hills dam, where two small drinking troughs had been maintained at the site for approximately 2 months before the study (Cooper et al., 2019). Finches were readily netted between the surrounding vegetation and the water troughs. All birds involved in the study were banded with an individually numbered metal leg band (Australian Bird and Bat Banding Scheme, size 02, Parkes, ACT, Australia) to prevent re-measurement and pseudoreplication. Physiological parameters were measured for birds after at least three consecutive days of maximum T_a≥39°C (hot days; maximum T_a mean=42.7°C, range 39–45.2°C) or maximum T_a≤36.3°C (cool days; maximum T_a mean=31.4°C, range 27–36.3°C). Experiments followed the Australian Code of Practice for the care and use of animals for scientific purposes, approved by the Macquarie University and Curtin University animal ethics committees (ARA 2017/024 and ARE2017-16), and were conducted under licence from the New South Wales National Parks and Wildlife Service.

Respirometry

For measurement of metabolic rate (oxygen consumption, V_{O_2}, and carbon dioxide production, V_{CO_2}) and evaporative water loss (EWL), three zebra finches were captured per day, between 10:00 and 11:00 h, and were kept in a small cage in a shaded outdoor aviary with water but no food until approximately 19:30 h (around sunset). They were then placed individually into a metabolic chamber (500 ml glass jar) and open-flow respirometry was used to measure their V_{O_2}, V_{CO_2} and EWL. Finches remained in the chambers until variables were minimal and constant (generally until 02:00 to 03:00 h); birds were then removed from the chamber and their T_b measured immediately with a plastic-tipped thermocouple connected to a RadioSpares (Smithfield, NSW, Australia) digital thermocouple meter. Finches were weighed to the nearest 0.1 g with an electronic balance before and after each measurement, and the mean was used for calculations; they were banded the morning following experiments, before release. Baselines of background O_2, CO_2 and relative humidity (RH) were established for each system for at least 30 min before and after each experiment. Finches were measured during 13 hot and 8 cool periods (reflecting the prevalence of hot conditions at the site during the study period), with 20 finches (9 male, 11 female) measured at T_a=30°C and 18 (10 male, 8 female) at T_a=40°C during hot conditions, and 12 finches (7 male, 5 female) at T_a=30°C and at T_a=40°C (4 male, 8 female; Table S1) during cool conditions.

Three separate respirometry systems were used, one for each chamber. Each system consisted of a Sable Systems FoxBox O_2 and CO_2 analyser (Las Vegas, NV, USA), which also received the digital input of a Vaisala RH/T_a probe (MNP45A, Helsinki, Finland). The serial output from the Foxbox was recorded every 30 s by a PC running custom-written (Visual Basic V6; Microsoft, Redmond, WA, USA) data acquisition software (Philip Withers, University of Western Australia). Ambient temperature was maintained by placing the chambers within a refrigerator set to 4°C, with a custom-built thermostat and heater maintaining T_a at the desired set point (30 or 40°C). Outside ambient air was drawn through columns of drierite (W. A. Hammond Co., Xenia, OH, USA) using the Foxboxes' built-in pump and flow controller, and then pushed through the chambers and gas analysers at 300 ml min^{-1}. A small column of drierite was located between the RH/T_a probe and the gas analysers. The gas analysers were calibrated using compressed nitrogen (0% O_2 and CO_2) and two precision gas mixes of 0.53 and 0.153% CO_2 (CO_2; BOC gases, Perth, Western Australia) or dry ambient air (20.95% O_2). The T_a probe and thermocouple meter were calibrated against a precision mercury thermometer traceable to a national standard (T_a). The RH probe was calibrated at five RHs, from 2% (dry, using drierite) to 85%, generated with a Sable Systems DG4 humidity controller. Flow rates were calibrated with a Sensodyne Gillian Gilibrator (Clearwater, FL, USA) bubble flow meter.

A custom-written VB (V6) data analysis program (Philip Withers, University of Western Australia) was used to calculate V_{O_2}, V_{CO_2} and EWL after Withers (2001). These variables were averaged over a period of approximately 20 min, when they were minimal and stable. The respiratory exchange ratio (RER; V_{CO_2}/V_{O_2}) was used to determine the appropriate oxy-calorific and hygric conversion to calculate metabolic heat (MHP) and water production (MWP; after Withers et al., 2016) from V_{O_2}. As V_{CO_2} mirrored V_{O_2} (there were no temperature or hot/cool period effects on RER, $F_{3,58}$=2.16, P=0.102), V_{CO_2} is not presented separately here. Evaporative heat loss (EHL) was calculated from EWL as 2.4 J mg H_2O^{-1} (Withers et al., 2016) and wet (C_{wet}) and dry (C_{dry}) thermal conductance as MHP/(T_b−T_a) and (MHP−EHL)/(T_b−T_a) respectively. Relative water economy (RWE) was calculated as MWP/EWL. Temperature coefficient (Q_{10}) calculations were after Withers et al. (2016).

Blood sampling

Blood samples for CORT, GLU and UA analysis were obtained from the brachial vein of 39 finches (21 male and 18 female) into a

heparinised capillary tube. Different individual finches were captured for blood sampling, but under the same environmental conditions as for metabolic studies, i.e. during hot (21 birds) or cooler (18 birds) periods. Birds used for metabolic measurements were not used for blood sampling and vice versa. Birds were caught between 08:30 and 11:50 h. They were removed from the net and an initial blood sample (<75 µl) was taken within ∼2.5 min of capture (Romero and Reed, 2005) to provide basal (pre-stress) CORT and plasma metabolite levels. An acute stress response was then induced by keeping birds in individual cloth bags in the shade for 30 min to stimulate the hypothalamic–pituitary–adrenal (HPA) axis (Wingfield et al., 2017; Romero et al., 2000; Deviche et al., 2016). A second sample (<75 µl) was then taken before finches were banded and released. Blood was kept on ice in the field, returned to the field laboratory, and then centrifuged to obtain the plasma, which was stored frozen until analysis. Approximately 20 µl plasma was obtained from each blood sample, and then analysed for all three compounds where possible.

Plasma sample analysis

We assayed plasma samples for CORT, GLU and UA in duplicate and following validated methods (Deviche et al., 2014). Briefly, we quantified plasma CORT using commercial enzyme-linked immunoassay kits (Enzo Life Sciences, Farmingdale, NY; Deviche et al., 2010, 2014). Plasma was firstly diluted 20× in an assay buffer containing displacement reagent (see manufacturer's specifications). For CORT, the average inter- and intra-assay coefficients of variation were 4.20% and 14.34%, respectively, and the average assay sensitivity was 0.08 ng ml^{-1}. We quantified plasma GLU and UA using colorimetric assay kits (GLU: Cayman Chemical Co., Ann Arbor, MI; UA: BioAssay Systems, Hayward, CA; Deviche et al., 2014, 2016). Plasma used for the UA assay was first diluted 4× in the assay buffer. The average inter- and intra-assay coefficients of variation for the GLU assay were 2.21% and 1.57%, respectively, and the assay sensitivity was 5 mg dl^{-1}. For UA, the average inter- and intra-assay coefficients of variation were 1.37% and 4.73%, respectively, and the assay sensitivity was 2.5 mg dl^{-1}.

Data analysis

A small number of samples (2 for CORT, 7 for UA and 1 for GLU) could not be assayed for technical reasons, but we were able to estimate these missing values using multiple imputation and the NORM program (http://sites.stat.psu.edu/~jls/misoftwa.html; Schafer, 1999). Multiple imputation is more appropriate than other approaches, such as case deletion or replacement of missing values by group means, to deal with missing values because it relies on more plausible assumptions, properly accounts for uncertainty about missing values (leading to appropriate standard errors), and retains adequate sample sizes (Little, 1988). Grubb's test identified some statistical outliers (2 for CORT and 1 for UA) that were removed before analyses. For CORT, we analysed data for 20 finches (12 male, 8 female) during hot conditions and 17 (9 male, 8 female) during cool conditions. During hot periods UA data were analysed for 20 finches (11 male, 9 female) and for 18 (9 male, 9 female) during cool periods, while for GLU we analysed data for 19 finches during hot periods (10 male, 9 female) and 18 during cool periods (9 male, 9 female; Table S2).

Data are presented as means±s.e.m., with N=number of individuals. For metabolic, thermal and hygric variables, the effects of weather (hot versus cool) and T_a (30 or 40°C) were determined by full-factorial two-way ANOVA. Analyses were for ranked rather than absolute values (i.e. analyses were non-parametric) because of the heterogeneity of variance and non-normality of these variables. To explore the consequences of interaction between weather and T_a, two-sample t-tests (accounting for heterogeneity of variance) were used to hypothesise about varying responses to hot and cool conditions at T_a=30 and 40°C individually. For plasma CORT, UA and GLU, a multivariate repeated measures ANOVA (Withers and Cooper, 2011) was used to assess the effect of weather and sex (factors) on the two measurements (repeat) for each variable for each bird. In the case of a significant interaction between the repeat (sample one or two) and weather (hot and cold), a two-sample t-test (with a test, and if necessary correction for, heterogeneity of variance) was used to hypothesise about the effect of hot and cool conditions on the first and second sample separately. The effect of weather (hot versus cool period) on the magnitude of change between the first and second sample was assessed by two-sample t-test, and for GLU a linear regression was used to examine the impact of maximum and minimum T_a of the sampling day. Potential correlations between the changes in CORT, UA or GLU for individual birds were examined with simple correlations. The potential for the time between capture and the initial blood sample to impact on the first measure for each bird, or the difference between the first and second measure, was assessed by linear regression for all blood variables. All statistical analyses were accomplished with statisti*XL* (www.statistiXL.com, Perth, Western Australia).

RESULTS

The mean body mass of all finches over all respirometry experiments was 10.9 g (N=62), and did not differ significantly for measurements at T_a=30 or 40°C, or during hot or cold periods ($F_{3,58}$=1.12, P=0.353). There were highly significant effects of measurement T_a for all metabolic, hygric and thermal variables ($F_{1,58}$≥8.61, P≤0.005), with all variables higher at T_a=40°C compared with 30°C except RWE, which was lower at T_a=40°C (Fig. 1; Table S1). Hyperthermia of 3–3.5°C occurred at T_a= 40°C compared with T_b at T_a=30°C, accompanied by increases in MR of 3% (hot) and 57% (cool), representing a Q_{10} of 1.1 and 3.5, respectively. The increase in EWL at T_a=40°C compared with that at T_a=30°C was 167 and 201% of that at T_a=30°C. The proportion of total heat loss attributed to EHL increased from ∼0.15 at T_a=30°C to as high as 0.33 at T_a=40°C. RWE was <1 (i.e. finches were losing more water by evaporation than they were gaining from MWP) for all measurement conditions, with mean RWE≤0.40±0.023.

EWL was lower ($F_{1,58}$=4.07, P=0.048) and T_b higher ($F_{1,58}$=4.45, P=0.039) for birds during hot periods compared with during cooler weather. At T_a=40°C, birds lost 7.3±0.63 mg g^{-1} H_2O h^{-1} or 0.97% of their body water h^{-1} [assuming 75.4% body water content, measured by Cooper et al. (2019) for this population during the same period] and this increased to 9.2±1.20 mg g^{-1} H_2O h^{-1} or 1.2% of their body water h^{-1} during cool periods. For MR and C_{wet}, there was a significant interaction between weather and measurement T_a ($F_{1,58}$≥4.4, P≤0.039). MR was statistically indistinguishable at T_a=30 and 40°C during hot periods (2.70±0.176 and 2.78±0.179 ml O_2 g^{-1} h^{-1}, respectively; t_{36}=0.513, P=0.611), but MR measured at T_a=40°C during cool periods, increased (t_{22}=3.73, P=0.001) to 3.25± 0.313 ml O_2 g^{-1} h^{-1} from 2.07±0.184 ml O_2 g^{-1} h^{-1} at T_a=30°C. This generated an additional 109 J h^{-1} of MHP, but provided an additional MWP of 79 mg H_2O h^{-1}, which if all evaporated, would dissipate 174% of the extra heat generated. At T_a=40°C, C_{wet} was lower (t_{28}=2.31, P=0.027) during hot (30.6±3.92 J g^{-1} h^{-1} $°C^{-1}$) than cool (59.3±14.34 J g^{-1} h^{-1} $°C^{-1}$) conditions, although at T_a=30°C the weather effect was insignificant (t_{30}=0.764, P=0.451).

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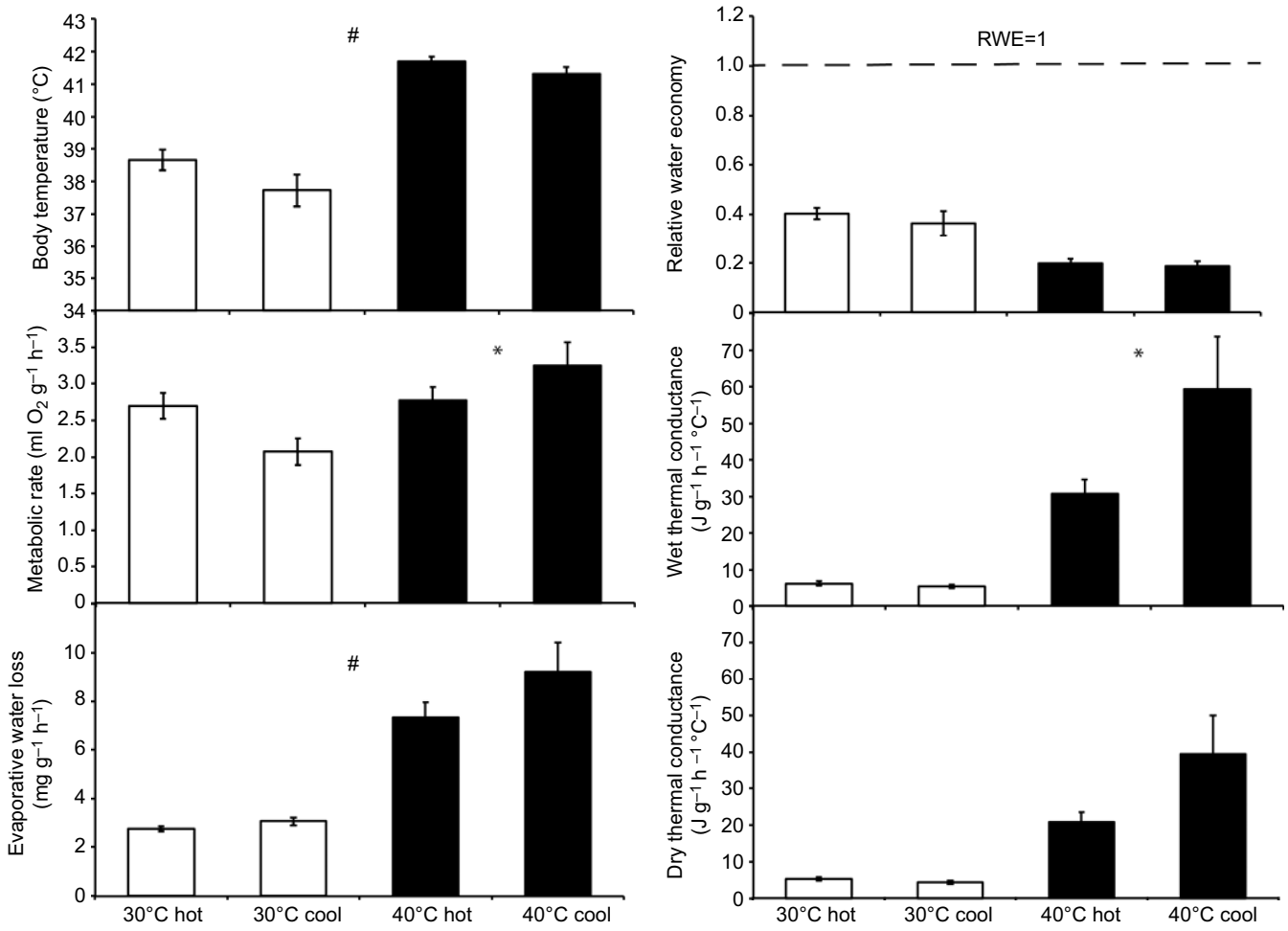

Fig. 1. Thermal, metabolic and hygric physiological variables for zebra finches (*Taeniopygia guttata*) measured at an ambient temperature (T_a) of 30°C or 40°C after at least 3 days of hot weather (mean maximum T_a=42.7°C) or cooler weather (mean maximum T_a=31.4°C). There was a significant effect of measurement temperature for all variables. #Overall difference between hot and cool weather; *significant interaction between measurement T_a and weather, with higher values during periods of cool weather than during hot weather, at a measurement T_a=40°C. The dashed line represents a relative water economy (RWE) of one. Values are means±s.e.m., N=20 measured at T_a=30°C and 18 at T_a=40°C during hot conditions, and N=12 at both T_a=30°C and 40°C during cool conditions.

None of the measurements of blood compounds (Table S2) were affected by the time taken to obtain the first blood samples after capture ($F_{1,35–36}$≤2.01, P≥0.165). Mean initial plasma CORT values were 4.2±0.42 ng ml^{-1}, UA 37.1±2.08 mg dl^{-1} and GLU 299±6.1 mg dl^{-1} There was a significant effect of acute stress on all three plasma compounds (Fig. 2; $F_{1,33–34}$≥35, P<0.001), with plasma CORT and GLU increasing by 547±63% and 49±4.9%, respectively, and UA decreasing by 22.6±3.2% of the initial value, after birds were held in a bag for 30 min. Neither sex ($F_{1,33–34}$≤1.09, P≥0.304) nor weather (hot versus cool; $F_{1,33–34}$≤1.10, P≥0.304) influenced any of the plasma compounds, but there was a significant interaction between stress and weather for GLU ($F_{1,33}$=4.89, P=0.034). In response to stress, plasma GLU increased more during hot than cool weather (168 versus 111 mg dl^{-1} increase, respectively; $t_{26.8}$=2.29, P=0.030). There were significant positive relationships between baseline GLU and daily maximum and minimum T_a (R^2=0.197, $F_{1,36}$=8.6, P=0.006 and R^2=0.112, $F_{1,36}$=4.4, P=0.043). There were no significant correlations between changes in CORT, UA or GLU for individual finches (t≤1.8, P≥0.086).

DISCUSSION

Zebra finches did not lose body mass after several days of hot compared with cooler weather. Therefore this study, over 13 periods of hot weather lasting 3 or more days for 38 individual finches, confirms previous findings from only a single 3 day period of hot weather and 10 finches (Cooper et al., 2019) that wild, free-living zebra finches can maintain body mass, hence energy and water balance, over several days of maximum T_a of up to 45.2°C. This is in contrast to some South African birds that lose body mass during consecutive days of hot weather, even at much lower maximum T_a of <38°C (du Plessis et al., 2012; Van de Ven et al., 2019). It is possible that the highly digestible granivorous diet of zebra finches facilitates maintenance of body mass during hot weather, provided that drinking water is available (Cooper et al., 2019). However, daily body mass maintenance of another Australian species, the insectivorous Australian magpie (*Gymnorhina tibicen*), was also not impacted by high T_a of up to 43°C, despite a reduction in foraging efficiency (Edwards et al., 2015). It may be that Australian birds are particularly resilient to extreme T_a, considering their mostly non-migratory life-history and the continent's generally hot and arid climate (McKechnie et al., 2012). Although mass mortality events are well documented for Australian birds (McKechnie et al., 2012), they maintain this resilience to close to the limits of their thermal tolerance, and it is when extreme conditions are unpredictable or extended that their tolerances are exceeded (Cooper et al., 2019). We explore here the physiological

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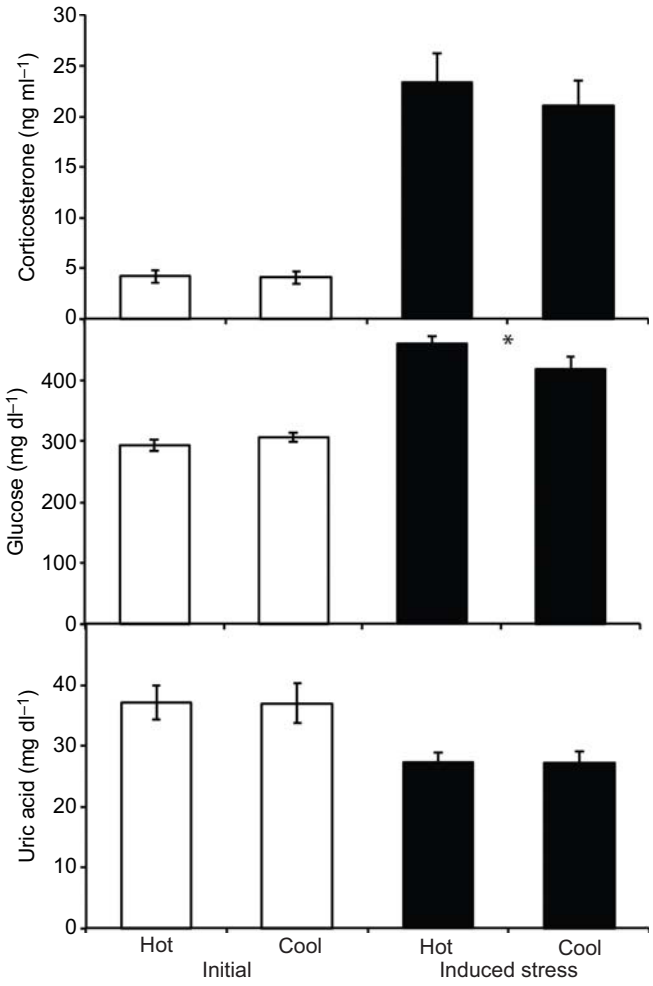

Fig. 2. Blood chemistry for wild-caught zebra finches after at least 3 days of hot weather (mean maximum T_a=42.7°C) or cool weather (mean maximum T_a=31.4°C). Finches were sampled within ~2.5 min of capture to obtain background levels of each parameter (Initial) and again after being held in a cloth bag for 30 min (Induced stress). Data are means±s.e.m., total N=39. There were significant differences between initial and induced stress values for all three variables. *Significant interaction between weather and blood glucose, with higher values during hot than cool periods for birds after 30 min.

mechanisms employed by finches at high T_a, and the potential for physiological plasticity to facilitate maintenance of energy and water balance during periods of hot weather.

Both MR and EWL measured here at T_a=30°C for wild zebra finches were lower than those of early studies of captive finches (e.g. 2.08–2.70 ml O_2 g^{-1} h^{-1} compared with 3.28–4.9 ml O_2 g^{-1} h^{-1} for MR and 2.7–3.1 compared with 8.1–9.7 mg H_2O g^{-1} h^{-1} for EWL; Calder and King, 1963; Calder, 1964; Cade et al., 1965; Bennett and Harvey, 1987). Recent measures for captive MR of 2.70–2.76 ml O_2 g^{-1} h^{-1} by Rønning et al. (2005) and Cooper et al. (2020) more closely approximate our values at T_a=30°C. However, during cool periods MR at T_a=30°C was significantly lower (one-sample t-test t_{11}=3.39, P=0.006), and EWL was significantly higher during both hot and cool periods (t_{31}=6.88, P<0.001) than even these more recent estimates. This presumably reflects physiological differences between wild and captive finches (e.g. Skadhauge and Bradshaw, 1974; Weathers et al., 1983; Warkentin and West, 1990; McKechnie et al., 2006). Our calculations of RWE confirm earlier reports (Cade et al., 1965) that despite being iconic desert birds well-known for their ability to survive on dry seed alone (Bartholomew and Cade, 1963), MWP is not sufficient to achieve water balance in zebra finches at T_a at or above thermoneutrality. At least during summer, wild zebra finches must drink to maintain water balance (Calder, 1964; Cooper et al., 2019; Cooper et al., 2020). At T_a=40°C, our values for the various physiological variables for wild finches were at the lower end of the range recorded previously for zebra finches in captivity (e.g. Calder, 1964; Cade et al., 1965; Cooper et al., 2020).

A T_a of 40°C clearly presented a heat challenge, but was not a physiological stressor, for wild zebra finches. During short periods of hot weather, birds modified their physiological response to accommodate even better this high T_a. When T_a exceeds T_b, birds must rely on EHL to dissipate metabolic and environmental heat loads. Therefore, they must trade off the risk of lethal hyperthermia with the risk of dehydration (Albright et al., 2017). Substantial hyperthermia at T_a=40°C compared with T_a=30°C maintained a positive body to environment temperature gradient (a positive C_{dry}) to dissipate heat, with changes in posture, feather positioning and possibly blood flow presumably facilitating the observed increase in C_{dry}. Hyperthermia was more pronounced during hot periods and this, together with reduced MHP during hot periods, contributed to the observed water savings.

EWL was never sufficient to dissipate MHP; in fact only 24± 1.7% of MHP was dissipated by EHL over all conditions, suggesting that finches prioritised hydration over maintaining homeothermia, at least while T_b remained several degrees below a lethal T_a of 45–46°C (Wingfield et al., 2017). However, a considerable increase in EWL was still required to maintain T_b within tolerable limits at T_a=40°C, and EHL became more important for heat balance as T_a increased. Despite this, finches did maintain EWL below 1% of body water h^{-1} during both hot and cool conditions. If we assume that 11% loss of body water (0.9 ml H_2O for a 10.9 g finch) is lethal (Wolf, 2000), then inactive finches recently acclimatised to hot periods could survive an additional 3 h without drinking compared with those acclimatised to cool periods (10.7 h compared with 13.8 h to fatal dehydration, accounting for MWP).

Constancy of MR at T_a=30 and 40°C during hot periods, together with a much lower Q_{10} than expected for a biophysical effect on MR of increased T_b, and a reduced C_{wet}, suggests an upward shift in the TNZ for finches during hot periods. In contrast, during cool periods, MR increased considerably at T_a=40°C compared with that at 30°C. A Q_{10} of 3.5 is higher than the 2.5–3 expected based on the T_b increase observed at T_a=40°C (Guppy and Withers, 1999), and suggests that at T_a=40°C during cool periods the increase in MR is not just a consequence of a higher T_b, but also the metabolic cost of heat dissipation. This shift in the TNZ with short-term changes in T_a is advantageous in both minimising energy expenditure during cool periods when lower T_a are more likely to require MHP, and reducing the EHL required for thermoregulation during hot periods.

The absence of a relationship between the concentration of blood compounds, or the magnitude of their increase after capture, and time taken to sample individual birds indicates that our initial samples were obtained before the concentrations of circulating compounds had changed relative to baseline because of the effects of capture stress (Romero and Reed, 2005). We therefore interpret our initial samples as representing baseline levels of these compounds. Constancy of baseline levels of blood compounds after hot and cool periods, together with absolute values and responses to imposed stress that conform to species predictions, further indicate that zebra finches were not heat-stressed after several days with high maximum T_a.

Our baseline and stress-induced values of CORT for wild zebra finches are within the range measured for captive zebra finches in other studies (e.g. Wada et al., 2008; Spencer et al., 2009; Xie et al., 2017), and variation between individual birds (baseline 7–8 times, stress-induced 6–7 times) falls at the lower end of the range described by others (Wada et al., 2008). This is somewhat surprising considering studies on captive finches may be expected to produce lower CORT levels and less inter-individual variation as a result of individuals likely experiencing more favourable, constant and similar environmental conditions than wild birds. Perhaps the daily stresses of captivity, such as proximity to humans and necessary husbandry routines are more stressful, at least for some individuals, than the challenges faced by wild, free-living birds (Crino et al., 2017).

Environmental events such as unfavourable weather may result in a hormonal stress response that activates the HPA axis, stimulating the production and secretion of glucocorticoids, which in birds is predominately CORT (Siegel, 1980; Romero et al., 2000; Cockrem, 2007; Krause et al., 2016). Exposure to high T_a can increase CORT (Siegel, 1980; Xie et al., 2017), but CORT declines as the T_a exposure continues, presumably due to exhaustion of high-level CORT synthesis (Siegel, 1980). However, acute (2 h) exposure of captive zebra finches to T_a of 45°C did not increase CORT (4.87±2.70 ng ml^{-1}) compared with a T_a of 35°C (3.78±2.63 ng ml^{-1}; Xie et al., 2017). These findings are consistent with our data (4.11±0.62 ng ml^{-1}, cool and 4.22±0.60 ng ml^{-1}, hot); there is no hormonal evidence that daily maximum T_a up to 45°C is a stressor, even for wild birds that must modify their access to food and water during the heat of the day (Funghi et al., 2019; Cooper et al., 2019). Xie et al. (2017) suggest that a lack of a CORT response by zebra finches (as well as budgerigars, *Melopsittacus undulatus*) may explain their susceptibility to high T_a and propensity for mass die-offs during heatwaves (e.g. Finlayson, 1932; Birdlife Australia, 2014), owing to the potential role of CORT in influencing activity and foraging. Jimeno et al. (2017) described a positive relationship between MR and CORT for captive zebra finches at T_a below thermoneutrality (T_a=22 and 12°C), reflecting the role of CORT in mobilising glucose that we also observed (see below). However, our respirometry data demonstrate that wild zebra finches do have the physiological plasticity to reduce MR at high T_a during hot periods without changes in CORT.

Initial GLU (16.6 mmol l^{-1} l^{-1}) of our zebra finches very closely approximated the predicted baseline value for a 10.9 g bird (16.2 mmol l^{-1} l^{-1}; Braun and Sweazea, 2009). Acute stress resulted in significantly higher blood GLU, presumably as a consequence of the raised CORT levels and in preparation for the increased energetic demand of a flight-or-fight response (Deviche et al., 2016). During periods of high T_a, this stress-induced elevated GLU was higher than during cool periods, and was positively correlated with T_a, as observed previously for both rufous-winged sparrows (*Peucaea carpalis*) and blue tits (*Cyanistes caeruleus*; Kaliński et al., 2014; Deviche et al., 2016). This suggests that zebra finches were more physiologically challenged during cool periods and this is consistent with the findings of Cooper et al. (2019) who concluded that cooler periods are more challenging because of increased requirements for MHP for thermoregulation, particularly during an extended drought when food availability is presumably limited. For rufous-winged sparrows, GLU only increased above baseline as a consequence of induced stress during pre-breeding periods, and remained constant or decreased during breeding and post-breeding moult periods (Deviche et al., 2014, 2016), when birds were experiencing increased energetic demand (Cyr et al., 2008; Bicudo et al., 2010).

Uric acid is an important avian antioxidant, and the blood concentration of UA often decreases in response to stress, which is presumably due in part to movement from the blood to the tissues in response to increased CORT (Cohen et al., 2008; Davies et al., 2013; Gormally et al., 2019; Haskins et al., 2017). Higher levels of baseline stress typically relate to low UA levels and may result in a limited reduction, or even an increase, in UA with imposed stress (Cohen et al., 2007). Baseline UA of our zebra finches (37.1±2.08 mg dl^{-1}) was well within the range of 0.93–110.4 mg dl^{-1} measured for 92 species of wild-caught American birds (Cohen et al., 2007), and the ratio of baseline:stress UA for our zebra finches (0.737) also conformed closely to the negative linear relationship described for these species.

Overall, our assessment of the physiology of wild zebra finches during summer heatwaves compared with cooler periods during the same season indicate that physiological plasticity can be moderated by recent periods of high ambient temperatures. These small desert birds can modify their metabolic, hygric and thermal response to environmental conditions during heatwaves to achieve more favourable heat and water balance, which consequently facilitate survival during periods of high T_a. Days with maximum temperatures of up to 45°C are not significant stressors; they do not result in modification of blood parameters or an inability to maintain body mass. Understanding this significant physiological plasticity of wild birds in response to extreme, short-term, high temperature events is essential for effectively modelling and planning for the biodiversity impacts of climate change. We provide evidence that the ability of birds to react to short-term changes in environmental conditions with plastic physiological responses must be considered in future predictive species distribution and climate resilience models.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: C.E.C., P.D., S.C.G.; Methodology: C.E.C., L.L.H., P.D., S.C.G.; Validation: C.E.C., L.L.H., P.D.; Formal analysis: C.E.C.; Investigation: C.E.C., L.L.H., P.D.; Resources: C.E.C., P.D., S.C.G.; Data curation: C.E.C., L.L.H.; Writing - original draft: C.E.C.; Writing - review & editing: L.L.H., P.D., S.C.G.; Visualization: C.E.C.; Project administration: C.E.C., S.C.G.; Funding acquisition: C.E.C., P.D., S.C.G.

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Table S1.

Physiological variables (body temperature, T_b; oxygen consumption, VO_2; carbon dioxide production, VCO_2; evaporative water loss, EWL) measured at an ambient temperature of 30 or 40°C, for wild zebra finches (*Taeniopygia guttata;* Gould 1837) at Fowlers Gap, NSW Australia during summer. Measurements occurred after at least three consecutive days of hot (maximum T_a mean = 42.7°C) or cool (maximum T_a mean = 31.4°C) weather.

Bird	Sex	T_a	Weather	Mass	T_b	VO_2	VCO_2	EWL
		°C		g	°C	mL g^{-1} h^{-1}	mL g^{-1} h^{-1}	mg g^{-1} h^{-1}
1	M	30	Hot	10.7	38.3	3.56	2.17	3.06
2	M	30	Hot	11.4	39.9	2.28	2.32	2.33
3	M	30	Hot	11.5	39.3	3.69	1.81	2.18
4	M	30	Cool	11	38.6	1.05	1.54	2.57
5	M	30	Cool	10.8	35.3	2.09	1.47	2.39
6	F	30	Cool	10	36.3	2.87	2.96	2.26
7	F	30	Cool	11.7	38.4	1.48	1.33	3.45
8	F	30	Cool	10.8	38.4	2.28	1.82	3.55
9	M	30	Cool	10.0	36.2	1.67	1.25	3.41
10	M	40	Cool	11.3	39.9	2.55	1.41	3.18
11	F	40	Cool	10.9	41	1.79	2.43	10.91
12	F	40	Cool	10.4	41.1	3.26	2.29	13.47
13	F	30	Hot	10.7	37.4	2.07	1.40	3.04
14	F	30	Hot	10.4	39.4	3.35	1.61	2.75
15	F	30	Hot	11.1	39.6	4.40	1.58	4.01
16	F	40	Hot	9.3	41.8	2.60	2.35	10.40
17	F	40	Hot	10.9	41.5	3.10	2.15	3.75
18	F	40	Hot	10.6	41	2.93	2.21	8.09
19	M	30	Cool	11.5	38.6	3.07	1.60	3.83
20	F	30	Cool	11.3	40.1	2.51	1.67	3.12
21	M	30	Cool	11	40.2	2.81	1.71	2.70
22	M	40	Hot	11.9	41.5	2.60	1.71	4.71
23	M	40	Hot	10.7	40.8	2.58	2.29	5.69
24	F	40	Hot	10.8	41.3	3.65	1.91	8.30
25	M	30	Hot	10.2	37.8	2.34	1.50	2.45
26	F	30	Hot	11.1	40.5	2.02	1.52	2.24
27	F	30	Hot	11.7	40.7	3.76	1.41	2.80
28	M	40	Hot	11.1	41.7	4.10	2.05	8.49
29	F	40	Hot	10.7	41.1	3.15	2.19	8.95
30	M	40	Hot	12.1	42.3	3.59	1.93	8.26
31	M	30	Hot	11.2	38.7	2.65	1.65	2.93
32	M	30	Hot	11	39.7	2.53	1.86	3.12
33	F	30	Hot	10.7	38.5	2.30	1.46	2.67
34	M	40	Hot	10.8	40.6	1.50	1.69	6.40
35	F	40	Hot	10	41.6	1.62	1.85	5.65

36	M	40	Hot	11.3	42.7	2.19	1.76	4.91
37	F	30	Hot	10.2	35.3	3.45	1.56	3.24
38	F	30	Hot	11.6	39.7	3.07	1.89	2.78
39	M	30	Hot	11.7	39.4	2.20	1.64	2.34
40	M	40	Hot	12	41.3	1.45	1.69	6.76
41	F	40	Hot	11.6	42.4	2.74	2.01	4.03
42	M	40	Hot	10.2	41.9	3.20	2.14	14.54
43	F	30	Hot	11.1	37	1.96	1.26	2.30
44	M	30	Hot	11.4	39.7	1.75	1.54	2.06
45	F	30	Hot	12.4	39.1	1.62	1.38	3.01
46	F	40	Hot	11.7	42.6	2.39	1.79	8.62
47	M	40	Hot	11.4	42.2	3.01	2.06	5.27
48	M	40	Hot	11.1	42.2	3.67	2.06	9.29
49	F	30	Hot	11.4	36.9	3.02	1.89	2.89
50	M	30	Hot	10.2	36.3	1.90	1.01	2.75
51	M	30	Cool	9.15	36.8	1.62	1.07	2.82
52	F	30	Cool	10.6	35.4	1.95	1.32	3.02
53	M	30	Cool	11.2	38.4	1.52	1.43	3.60
54	F	40	Cool	10.3	41.3	2.80	2.31	7.27
55	F	40	Cool	10.5	42.2	2.69	2.24	8.73
56	M	40	Cool	9.9	42.7	5.98	3.11	18.55
57	F	40	Cool	9.5	41.5	3.56	2.10	8.21
58	M	40	Cool	11.3	40.6	2.91	2.06	6.06
59	F	40	Cool	10.3	41.4	2.36	2.34	8.26
60	F	40	Cool	12.0	40.9	3.50	2.55	10.06
61	F	40	Cool	10.7	41.4	3.23	2.23	4.43
62	M	40	Cool	11.3	41.7	4.41	2.33	11.46

Table S2.

Corticosterone (CORT), uric acid (UA) and glucose (GLU) concentrations in the blood of wild zebra finches (*Taeniopygia guttata;* Gould 1837) captured at Fowlers Gap, NSW Australia during summer, after at least three consecutive days of hot (maximum T_a mean = 42.7°C) or cool (maximum T_a mean = 31.4°C) weather. Blood samples were taken after ~2.5 min of capture (1) and after 30 min in cloth bag (2).

Bird Band No.	Sex	Temperature	CORT1 ng mL^{-1}	CORT2 ng mL^{-1}	UA1 mg dL^{-1}	UA2 ng dL^{-1}	GLU1 mg dL^{-1}	GLU2 mg dL^{-1}
10000	M	Hot	2.46	17.36	29.1	32.5	269	444
18945	M	Hot	10.76	33.48	28.5	10.2	362	454
23420	F	Cool	4.96	26.97	39.2	34.5	303	432
23590	F	Hot	1.75	25.91	41.8	27.1	298	416
23619	F	Cool	6.46	20.5	25	23.5	314	410
23625	M	Hot	6.58	20.69	41.7	37.1		
23815	M	Cool	3.24	13.47	47.9	33.4	311	463
23850	F	Hot	4.81	18.07	40.8	32.5	255	397
23851	F	Hot	6.68	17.63	36.1	30.3	305	445
23857	M	Cool	2.23	7.58	36.1	34.3	372	386
23858	F	Cool	4.52	31.41	43.3	31	315	328
23862	M	Cool	8.62	39.18	31.8	26.7	345	380
23863	F	Cool	2.33	9.55	33.7	27.7	258	392
23864	F	Cool	1.8	25.74	42.8	29.5	331	363
23865	F	Cool	5	27.32	79.5	40.5	306	422
23866	F	Cool			26.6	20.2	302	407
23867	M	Cool	2.24	34.21	36.8	14.8	342	435
23869	M	Cool	4.8	6.66	36.3	22.9	321	481
23870	M	Cool	4.72	17.75	27.3	19.3	324	700
23882	F	Hot			28.4	24.2	313	446
23883	M	Hot	3.74	33.05	21.6	21.1	330	461
23887	F	Hot	3.36	14.9	25.6	26.6	350	424
23888	M	Hot	1.96	6.84	34.3	24.6	279	495
23889	F	Hot	2.2	7.48	29.6	17.4	290	455
23890	M	Hot	1.62	16.41	31.2	29.6	286	461
23891	M	Hot	3.19	30.94	24.3	22.9	287	527
23895	M	Hot	8.57	39.06	47.8	39.6	208	438
23896	M	Hot	2.27	9.33	42.3	30.1	291	399
23919	F	Hot	2	25.64	23.9	21.3	310	559
24142	M	Hot	4.83	48.43	57.9	25.6		
24505	F	Cool	1.36	14.14	48.9	39.2	244	458
24509	M	Cool	2.8	11.24	28.9	19.3	256	387
24520	M	Hot	4.79	50.04	67.3	35.7	322	561
24521	F	Hot	2.51	11.25	33.4	27.4	318	508
24522	M	Hot	1.96	12.44			201	414
24523	F	Hot	8.32	28.49	56.8	30.7	296	458
24570	M	Cool	2.04	26.44	32.9	24.1	271	296
25430	F	Cool	2.21	13.79	35.4	31.5	303	485
90583	M	Cool	10.59	32.99	15	19.4	300	294