

RESEARCH ARTICLE

Avian thermoregulation in the heat: evaporative cooling in five Australian passerines reveals within-order biogeographic variation in heat tolerance

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ABSTRACT

Evaporative heat loss pathways vary among avian orders, but the extent to which evaporative cooling capacity and heat tolerance vary within orders remains unclear. We quantified the upper limits to thermoregulation under extremely hot conditions in five Australian passerines: yellow-plumed honeyeater (*Lichenostomus ornatus*; ~17 g), spiny-cheeked honeyeater (*Acanthagenys rufogularis*; ~42 g), chestnut-crowned babbler (*Pomatostomus ruficeps*; ~52 g), grey butcherbird (*Cracticus torquatus*; ~86 g) and apostlebird (*Struthidea cinerea*; ~118 g). At air temperatures (T_a) exceeding body temperature (T_b), all five species showed increases in T_b to maximum values around 44–45°C, accompanied by rapid increases in resting metabolic rate above clearly defined upper critical limits of thermoneutrality and increases in evaporative water loss (EWL) to levels equivalent to 670–860% of baseline rates at thermoneutral T_a . Maximum cooling capacity, quantified as the fraction of metabolic heat production dissipated evaporatively, ranged from 1.20 to 2.17, consistent with the known range for passerines, and well below the corresponding ranges for columbids and caprimulgids. Heat tolerance limit (HTL, the maximum T_a tolerated) scaled positively with body mass, varying from 46°C in yellow-plumed honeyeaters to 52°C in a single apostlebird, but was lower than that of three southern African ploceid passerines investigated previously. We argue this difference is functionally linked to a smaller scope for increases in EWL above baseline levels. Our data reiterate the reliance of passerines in general on respiratory evaporative heat loss via panting, but also reveal substantial within-order variation in heat tolerance and evaporative cooling capacity.

KEY WORDS: Body temperature, Evaporative water loss, Hyperthermia, Passeriformes, Respiratory evaporative water loss, Resting metabolic rate

INTRODUCTION

Habitats where environmental temperatures regularly exceed normothermic body temperature (T_b) pose substantial physiological challenges to birds. In many deserts at subtropical

latitudes, maximum mid-summer air temperatures (T_a) in the 45–50°C range are combined with solar heat loads in excess of ~1000 W m⁻², creating conditions under which birds may need to defend a T_b setpoint 15°C or more below operative temperature (Williams and Tieleman, 2005; Wolf and Walsberg, 1996b; Wolf et al., 2000). Even at milder T_a in the 30–40°C range, thermoregulatory demands may be manifested as consequential trade-offs between several fitness components, including foraging efficiency, opportunity and body condition (du Plessis et al., 2012), nestling provisioning and growth rates (Cunningham et al., 2013), and nest predation risk (Tieleman et al., 2008).

Understanding phylogenetic variation in the thermal physiology of arid-zone birds, particularly thermoregulatory limits during extremely hot conditions, is vital for elucidating the ways in which hot, desiccating environments have driven the evolution of avian physiology and life history. Moreover, a clearer picture of how heat tolerance and evaporative cooling capacity vary among and within avian taxa is vital for developing mechanistic, physiologically informed models predicting how arid-zone avifaunas will respond to the higher maximum T_a and more frequent heat waves associated with anthropogenic climate change (IPCC, 2007, 2011; Urban et al., 2016).

Around half of all extant bird species belong to a single order, the Passeriformes. The passerines originated in Australia, with their diversification beginning in the Oligocene and accelerating in the early Miocene (20–25 mya), at which time the formation of islands in the Wallacea region between Australia and southeast Asia provided a dispersal route for the passerines to reach Asia and then other continents (Moyle et al., 2016). The contemporary avifauna of Australia includes conspicuous representation of several basal passerine clades, most notably the honeyeaters and allies (Meliphagidae) and the corvids.

Australia's predominantly hot, arid climate, combined with the long evolutionary history of the Passeriformes on this continent, means that the Australian passerine avifauna is particularly important for understanding phylogenetic variation in avian heat tolerance and evaporative cooling capacity. Historical accounts of catastrophic avian mortality events during extreme heat events in Australia include mention of several passerine species (Finlayson, 1932; Serventy, 1971). In addition, recent analyses have revealed body size shifts in Australian passerines correlated with climate change over recent decades (Gardner et al., 2014, 2016, 2011). Collectively, these findings reiterate the need for a better understanding of relationships between body mass and physiological variables related to tolerance of high environmental temperatures.

Heat tolerance and evaporative cooling capacity varies substantially among avian orders, with columbids and

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List of symbols and abbreviations

AB	apostlebird (<i>Struthidea cinerea</i>)
CB	chestnut-crowned babbler (<i>Pomatostomus ruficeps</i>)
EHL	evaporative heat loss
EWL	evaporative water loss
GB	grey butcherbird (<i>Cracticus torquatus</i>)
M_b	body mass
MHP	metabolic heat production
RER	respiratory exchange ratio
REWL	respiratory evaporative water loss
RMR	Resting metabolic rate
SH	spiny-cheeked honeyeater (<i>Acanthagenys rufogularis</i>)
T_a	air temperature
T_b	body temperature
T_{uc}	upper critical limit of thermoneutrality
TNZ	thermoneutral zone
\dot{V}_{CO_2}	carbon dioxide production
YH	yellow-plumed honeyeater (<i>Lichenostomus ornatus</i>)

caprimulgids possessing much more efficient evaporative cooling pathways compared with passerines (McKechnie et al., 2016b; O'Connor et al., 2017; Wolf and Walsberg, 1996a; Gerson et al., 2014). However, comparisons between columbids and passerines, in particular, are confounded by a lack of overlap in body mass (M_b). For instance, the heat tolerance limits and maximum evaporative cooling capacities of three ploceid passerines were lower than those of four columbids (McKechnie et al., 2016b; Whitfield et al., 2015), but there was almost no overlap in the M_b ranges in these two studies (passerines: 10–40 g; columbids: 37–186 g). In order to meaningfully compare these variables across taxa and examine variation in their scaling, we need data from larger-bodied passerines, for which many opportunities exist among Australian birds.

Previous studies of thermoregulation in Australian passerines have generally not involved measurements of T_b , evaporative water loss (EWL) or resting metabolic rate (RMR) at T_a values above 45°C (Ambrose et al., 1996; Burton and Weathers, 2003; Collins et al., 1980; Williams and Main, 1976). Moreover, investigations into passerine evaporative cooling elsewhere have typically focused on species smaller than 60–70 g (Dmi'el and Tel-Tzur, 1985; Tieleman and Williams, 2002; Ward and Pinshow, 1995; Whitfield et al., 2015; Williams, 1999; Wolf and Walsberg, 1996a; but see also Marder, 1973). Consequently, it remains unclear (a) how heat tolerance and evaporative cooling capacity scale with M_b in larger passerines, and (b) whether these traits vary among passerine families and/or among the passerine avifaunas of different continents.

We quantified heat tolerance and evaporative cooling capacity in five Australian passerines spanning a ~7-fold range in body mass from ~15 to ~120 g, using the same methods as those employed in a number of recent studies (McKechnie et al., 2016b; Smith et al., 2015; Whitfield et al., 2015). We predicted that these species would show positive scaling of heat tolerance limits, as has been reported for passerines elsewhere (Whitfield et al., 2015). We also expected that thermoregulation at high T_a would be characterized by the same clearly defined upper critical limits of thermoneutrality (T_{uc}) and rapid increases in RMR at $T_a > T_{uc}$ evident among passerines in general, contrary to the situation in taxa such as columbids and caprimulgids (McKechnie et al., 2016b; O'Connor et al., 2017; Whitfield et al., 2015).

MATERIALS AND METHODS**Study species and site**

We measured heat tolerance and evaporative cooling capacity in five species: yellow-plumed honeyeater [YH; *Lichenostomus ornatus* (Gould 1838); Meliphagidae, mean±s.d. M_b =16.72±1.65 g, n =20], spiny-cheeked honeyeater (SH; *Acanthagenys rufogularis* Gould 1838; Meliphagidae, M_b =41.68±4.71 g, n =20), chestnut-crowned babbler [CB; *Pomatostomus ruficeps* (Hartlaub 1852); Pomatostomidae, M_b =51.96±3.90 g, n =17], grey butcherbird [GB; *Cracticus torquatus* (Latham 1801); Artamidae, M_b =86.01±6.62 g, n =16] and apostlebird (AB; *Struthidea cinerea* Gould 1837; Corcoracidae, M_b =117.55±10.20 g, n =7).

The study took place in Gluepot Reserve, South Australia (33°46'S, 140°07'E), between 30 January and 16 March 2014. Birds were captured using Japanese mist nets, and initially held in cloth bags before being transported to a field laboratory. None of the study species are sexually dimorphic, and individuals used in the study were not sexed. All birds used in the study were adults and appeared to be in good condition. No individuals showing evidence of a brood patch were used. Measurements took place on the same day as each individual was captured. Birds were held in cages constructed of shade cloth with food and water available *ad libitum*. Birds were always offered water before experimental measurements, and a feeding tube attached to a syringe was used to introduce 5–10 ml of water directly into the crop if they were unwilling to drink, which was the case for most individuals. Measurements typically lasted 2–3 h, resulting in M_b loss (expressed as a percentage of initial M_b at the start of measurements) of 3.9±2.0% in YH, 3.3±1.4% in SH, 3.1±1.2% in CB, 6.6±2.2% in GB and 5.3±1.5% in AB. Time in captivity did not exceed 24 h, after which birds were released at the site of capture.

Habitats at Gluepot Reserve consist of summer, red and yorrell mallee scrub and black oak woodland. The climate is hot and arid, with a mean annual precipitation of 276±96 mm. Maximum T_a during January (the hottest month) averages 33.8±5.7°C, and maximum T_a exceeds 40°C on 12.9±5.2 days year⁻¹ (weather records for 2000–2016 obtained from Bureau of Meteorology www.bom.gov.au).

All experimental procedures were approved by the Animal Ethics Committees of the University of Adelaide (S-2013-151A) and the University of Pretoria (protocol EC054-16), and the Institutional Animal Care and Use Committee of the University of New Mexico (12-1005370-MCC). Birds were captured under a permit issued by the Department of Environment, Water and Natural Resources South Australia (E26141-2).

Gas exchange and temperature measurements

The same methods and equipment described by Whitfield et al. (2015) were used to measure T_a and T_b . A temperature-sensitive passive integrated transponder (PIT) tag (Biomark, Boise, ID, USA) was injected intraperitoneally into the abdominal cavity of each bird, with a reader and transceiver system (model FS2001, Biomark) subsequently used to measure T_b . We measured carbon dioxide production (\dot{V}_{CO_2}) and EWL over the T_a range 30–52°C, also using the same experimental setup as described by Whitfield et al. (2015) for their measurements during the 2013 season. Birds were placed individually in 4 l plastic chambers. Within the chambers, birds stood on a platform of plastic mesh 10 cm above a 1 cm layer of mineral oil to trap excreta. Flow rates of 2.5–40 l min⁻¹ were used depending on the experimental T_a and chamber volume, in order to keep chamber humidity below ~5 ppt so that humidity within the chamber remained low. As was the case in several previous studies

(McKechnie et al., 2016a,b; Whitfield et al., 2015), we found that reducing chamber humidity by means of increasing flow rate during the course of measurements usually kept birds calm while serving the dual purpose of maintaining water vapour gradients similar to natural desert conditions.

Experimental protocol

Experiments took place during the day using the protocol described by Whitfield et al. (2015), with birds exposed to progressively higher T_a using a stepped profile of $T_a=30, 35$ and 40°C , and thereafter 2°C increments at $T_a>40^\circ\text{C}$. Each individual was exposed to a maximum of three T_a values $>40^\circ\text{C}$, with the exception of one CB and one AB that were each exposed to four T_a values. Birds spent a minimum of 10 min, and on average ~ 30 min, at each T_a value, and were continually monitored during measurements using a video camera and an infrared light source (Whitfield et al., 2015). Our approach to identifying the heat tolerance limits was the same as that described by Whitfield et al. (2015) and followed by McKechnie et al. (2016a,b) and Smith et al. (2015), with measurements terminated when a bird was either (a) obviously stressed (sustained escape behaviour such as agitated jumping, pecking and/or wing flapping), or (b) showing signs of extreme heat stress such as loss of coordination or balance, or a sudden decrease in EWL, RMR and/or an uncontrolled increase in T_b . In the last-mentioned situation, the bird was considered to have reached its upper limit of heat tolerance, and the T_a associated with the onset of these signs of heat stress and/or T_b approaching 45°C was considered the thermal endpoint for that individual. During measurements, the behaviour

of birds while in the chamber was scored on a scale from 0 to 5, where 0 corresponded to a completely calm individual sitting immobile and 5 corresponded to a highly agitated bird exhibiting sustained escape behaviour. Data analyses were restricted to data corresponding to an activity score of 0–3. Any bird that reached its thermal endpoint was removed from the chamber and held in front of an air-conditioner producing chilled air, often with a cotton pad soaked in ethanol rubbed on the bird's body in order to augment heat loss (Whitfield et al., 2015).

Data analyses

In order to ensure comparability among studies, we analysed our data in the same way as Whitfield et al. (2015), McKechnie et al. (2016a,b) and Smith et al. (2015). We present whole-animal values, although in the case of EWL we calculated the slope of mass-specific EWL versus T_a to facilitate comparisons with the allometric equation presented by McKechnie and Wolf (2010). Rates of EWL were converted to rates of evaporative heat loss (EHL, W) assuming a latent heat of vaporisation of water of 2.406 J mg^{-1} at 40°C (Tracy et al., 2010). We suspect that birds were post-absorptive at the time of measurements, but were unable to confirm this. The high flow rates we used to keep chamber humidity values low precluded the measurement of oxygen consumption and hence respiratory exchange ratio (RER). We hence assumed $\text{RER}=0.71$, indicative of lipid metabolism (Walsberg and Wolf, 1995), and converted rates of \dot{V}_{CO_2} to metabolic rate (W) using $27.8 \text{ J ml}^{-1} \text{ CO}_2$ (Withers, 1992).

We used broken-stick linear regression models fitted in the R package *segmented* (Muggeo, 2008) to identify inflection points,

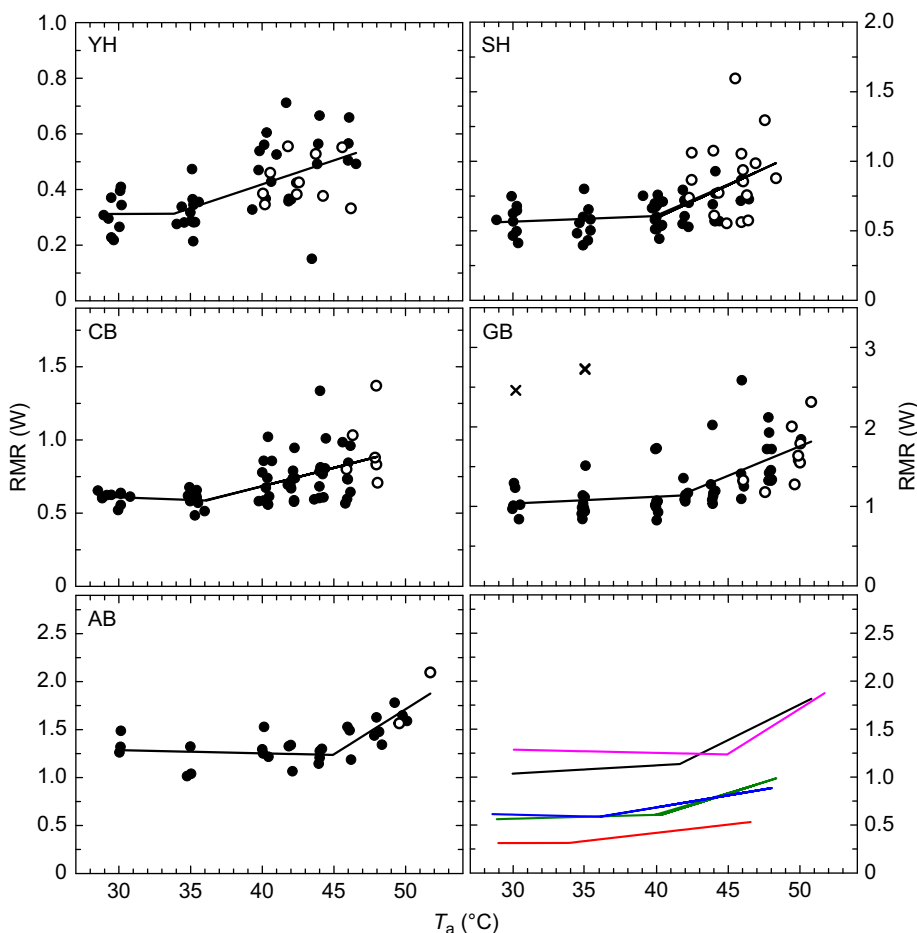


Fig. 1. Relationship between resting metabolic rate (RMR) and air temperature (T_a) in five Australian passerines. Data are for yellow-plumed honeyeater (*Lichenostomus ornatus*, YH), spiny-cheeked honeyeater (*Acanthagenys rufogularis*, SH), chestnut-crowned babbler (*Pomatostomus ruficeps*, CB), grey butcherbird (*Cracticus torquatus*, GB) and apostlebird (*Struthidea cinerea*, AB). Open circles indicate individuals that reached thermal endpoints; crosses indicate two anomalous RMR points in the GB data that we treated as outliers. For each species, the solid line is the segmented linear regression model that provided the best fit. Note that the scaling of the y-axes varies among panels. The bottom right panel shows the regression models for all five species plotted together for comparative purposes (red, YH; green, SH; blue, CB; black, GB; pink, AB).

and then fitted linear mixed effects models that included individual identity as a random factor in the R package *nlme* (version 3.57; <https://CRAN.R-project.org/package=nlme>). In the case of GB, three very high RMR data at $T_a=30$ and 35°C were excluded from analyses. Despite these individuals being inactive (behaviour score=0), these data were not associated with elevations in T_b or EWL, and we consider them to represent outliers (Fig. 1). All values are presented as means \pm s.d.

RESULTS

T_b and thermal endpoints

Normothermic T_b at $T_a=30^\circ\text{C}$, which appeared to fall within the thermoneutral zone (TNZ, i.e. the range of T_a values below the inflection point for RMR) of all five species (Fig. 1), varied over $\sim 0.6^\circ\text{C}$ from $40.1\pm 0.9^\circ\text{C}$ in YH to $40.7\pm 0.3^\circ\text{C}$ in CB (Table 1). All five species showed significant increases in T_b when exposed to high T_a (Fig. 2). The inflection point above which T_b increased was at $T_a\approx 35^\circ\text{C}$ in all species except AB, in which the inflection in T_b occurred only at $T_a\approx 46^\circ\text{C}$ (Table 1, Fig. 2). Excluding the single AB datum at $T_a\approx 52^\circ\text{C}$ causes the inflection point to shift downwards to $T_a=42.2^\circ\text{C}$, which does not change the conclusion that the inflection point for T_b is substantially higher in this species than in the other four. In all five species, at T_a above the inflection point, T_b increased approximately linearly to maximum values that varied from $44.2\pm 0.2^\circ\text{C}$ ($n=4$) in CB to 45.3°C ($n=1$) in AB (Table 1). The slope of T_b above the inflection point was not related to M_b in any consistent way, but varied from 0.23°C per 1°C change in T_a in CB to 0.58°C in AB. The maximum T_a reached varied from 46°C in YH to 52°C in a single AB, and was positively related to M_b (Table 1).

RMR

Minimum RMR values within the TNZ varied from 0.32 W in YH to 1.13 W in GB (Table 1). All five species showed a clear T_{uc} above which RMR increased (Fig. 1), with the T_{uc} generally higher in larger species, ranging from $T_a=33.9^\circ\text{C}$ in YH to $T_a=44.9^\circ\text{C}$ in AB. Above the T_{uc} , RMR increased to maximum values equivalent to 154–196% of minimal thermoneutral values in YH, SH, CB and GB. In AB, maximum RMR in four individuals at $T_a=50^\circ\text{C}$ was equivalent to 146% of minimum thermoneutral values, but the equivalent value for a single bird that reached its thermal limit at $T_a=52^\circ\text{C}$ was 186% (Fig. 1). In all five species, panting commenced at T_a between 40.5 and 41.7°C (Table 1).

EWL

Minimum EWL at T_a within the TNZ varied from 0.12 g h^{-1} in YH to 0.59 g h^{-1} in GB (Table 1, Fig. 3). All five species showed clear inflection points above which EWL increased rapidly; these varied from $T_a=37.6^\circ\text{C}$ in YH to $T_a=42.2^\circ\text{C}$ in CB and GB (Table 1, Fig. 3). The slope of the relationship between EWL and T_a above the inflection point scaled positively with M_b , whereas the slope for mass-specific EWL scaled negatively (Table 2). Maximum rates of EWL ranged from 0.83 g h^{-1} in YH to 4.07 g h^{-1} in AB, with the fractional increase in EWL (i.e. the ratio of maximum values to those in the TNZ) ranging from ~ 6.7 (GB) to ~ 8.6 (SH) (Table 1). Maximum EHL/metabolic heat production (MHP) generally increased with M_b (Fig. 4) from 1.20 in YH to 1.67 in AB, exceeding 2 only in GB (Table 1). It should be noted, however, that these values are based on the assumption that RER=0.71, i.e. the metabolic substrate during gas exchange measurements consisted entirely of lipids. If this assumption is incorrect, the actual

Table 1. Variables related to thermoregulation at high air temperatures (T_a) and evaporative cooling in five species of Australian passerines

Variable	Yellow-plumed honeyeater	Spiny-cheeked honeyeater	Chestnut-crowned babbler	Grey butcherbird	Apostlebird
M_b (g)	16.72 \pm 1.65 (20)	41.68 \pm 4.71 (20)	51.96 \pm 3.90 (17)	86.01 \pm 6.62 (16)	117.55 \pm 10.20 (7)
T_b					
Min. T_b ($^\circ\text{C}$)	40.09 \pm 0.88 (9)	40.28 \pm 0.65 (9)	40.70 \pm 0.33 (9)	40.03 \pm 0.31 (7)	40.67 \pm 1.28 (3)
Inflection T_a ($^\circ\text{C}$)	34.97	34.59	35.53	34.78	45.70
T_b versus T_a slope	0.35	0.30	0.23	0.28	0.58
Max. T_b ($^\circ\text{C}$)	45.01 \pm 0.55 (6)	44.37 (2)	44.15 \pm 0.21 (4)	44.52 \pm 0.42 (8)	43.91 \pm 0.90 (4) 45.35 (1)
Max. T_a ($^\circ\text{C}$)	46	48	48	50	50 (4) 52 (1)
T_b at onset of panting ($^\circ\text{C}$)	43.04 \pm 1.13 (17)	42.31 \pm 0.62 (20)	42.31 \pm 0.91 (17)	41.25 \pm 0.69 (14)	41.30 \pm 1.22 (6)
T_a at onset of panting ($^\circ\text{C}$)	40.77 \pm 1.07 (17)	40.89 \pm 1.08 (20)	40.71 \pm 2.68 (17)	40.44 \pm 2.54 (14)	41.71 \pm 3.64 (5)
RMR					
Min. RMR (W)	0.32 \pm 0.07 (9)	0.56 \pm 0.12 (9)	0.59 \pm 0.06 (9)	1.13 \pm 0.30 (11)	1.12 \pm 0.17 (3)
T_{uc} ($^\circ\text{C}$)	33.87	40.34	36.11	41.62	44.93
RMR slope (mW $^\circ\text{C}^{-1}$)	17.2	47.4	25.1	74.1	94.0
Max. RMR (W)	0.52 \pm 0.18 (9)	1.09 \pm 0.30 (2)	0.95 \pm 0.29 (4)	1.75 \pm 0.32 (8)	1.64 \pm 0.10 (4) 2.09 (1)
Max. RMR/min. RMR	1.64	1.96	1.60	1.54	1.46 1.86 (1)
EWL					
Min. EWL (g h^{-1})	0.12 \pm 0.02 (12)	0.23 \pm 0.10 (9)	0.30 \pm 0.11 (9)	0.59 \pm 0.18 (11)	0.58 \pm 0.04 (3)
Inflection T_a ($^\circ\text{C}$)	37.55	38.06	42.23	42.20	41.39
EWL slope ($\text{g h}^{-1}\text{ }^\circ\text{C}^{-1}$)	0.09	0.18	0.23	0.32	0.30
Max. EWL (g h^{-1})	0.82 \pm 0.18 (6)	1.94 \pm 0.26 (2)	2.15 \pm 0.40 (4)	3.91 \pm 0.59 (8)	4.07 \pm 0.38 (4) 4.51 (1)
Max. EWL/min. EWL	6.88	8.59	7.21	6.67	7.08 7.84 (1)
Min. EHL/MHP	0.25 \pm 0.03 (9)	0.27 \pm 0.09 (9)	0.33 \pm 0.11 (9)	0.47 \pm 0.19 (11)	0.28 \pm 0.02 (3)
Max. EHL/MHP	1.20 \pm 0.85 (9)	1.32 \pm 0.16 (12)	1.57 \pm 0.32 (4)	2.17 \pm 0.37 (10)	1.67 \pm 0.24 (4)

Study species: yellow-plumed honeyeater, *Lichenostomus ornatus*; spiny-cheeked honeyeater, *Acanthagenys rufogularis*; chestnut-crowned babbler, *Pomatostomus ruficeps*; grey butcherbird, *Cracticus torquatus*; apostlebird, *Struthidea cinerea*.

M_b , body mass; T_b , body temperature; T_a , air temperature; RMR, resting metabolic rate; T_{uc} , upper critical limit of thermoneutrality; EHL, evaporative heat loss; MHP, metabolic heat production.

Additional values for the apostlebird with $n=1$ refer to a single individual that reached $T_a=52^\circ\text{C}$, whereas for most individuals the maximum T_a reached was 50°C .

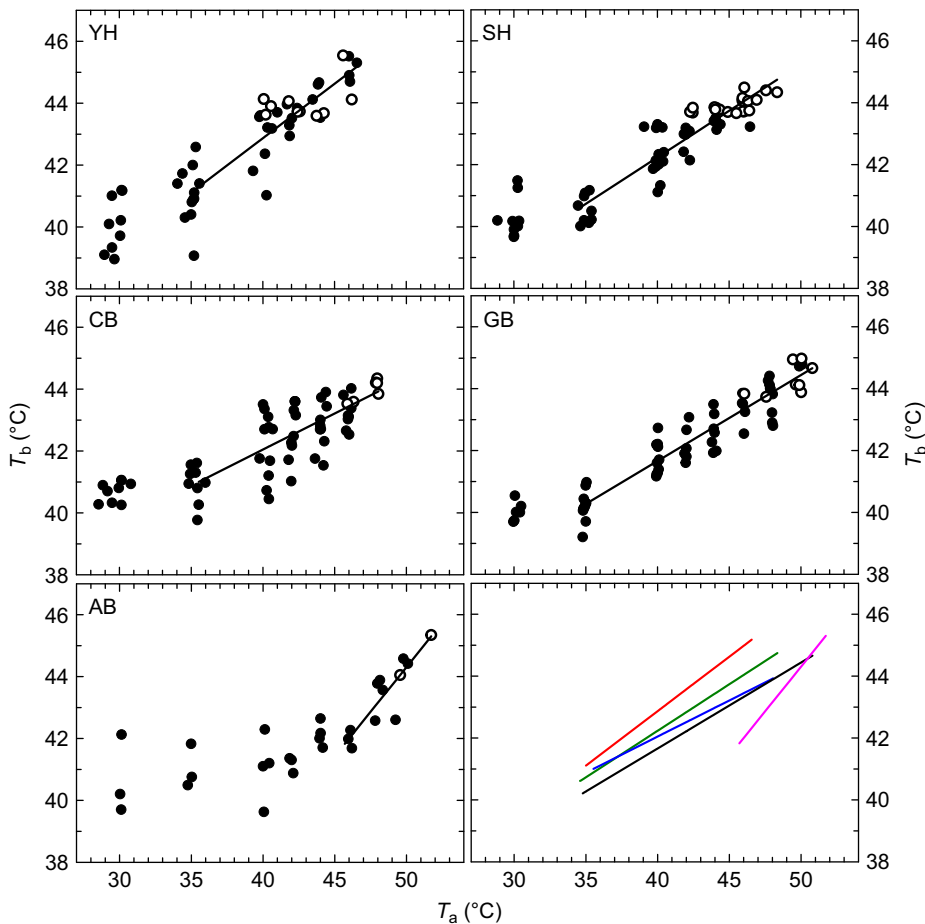


Fig. 2. Relationship between body temperature (T_b) and T_a in five Australian passerines. Species as in Fig. 1. Open circles indicate individuals that reached thermal endpoints. For each species, the solid line is the upper portion of the segmented linear regression model that provided the best fit. The bottom right panel shows the regression models for all five species plotted together for comparative purposes (red, YH; green, SH; blue, CB; black, GB; pink, AB).

EHL/MHP values could be higher, ranging from 1.56 in YH to 2.84 in GB if the metabolic substrate consists solely of carbohydrates (i.e. RER=1.00).

DISCUSSION

All five species showed patterns of thermoregulation at high T_a broadly similar to those of other passerines, with T_b increasing from normothermic values of $\sim 40^\circ\text{C}$ to maxima around $44\text{--}45^\circ\text{C}$. The regulation of T_b below T_a was achieved via large increases in rates of evaporative heat dissipation, with EWL increasing to rates equivalent to 670–860% of resting values at thermoneutral T_a . These increases in EWL were brought about primarily via panting, with concomitant increases in MHP; all five species showed clearly defined upper critical limits of thermoneutrality.

T_b and heat tolerance limits

The normothermic T_b of the five species investigated here were all lower than the mean \pm s.d. resting active-phase T_b of $41.6\pm 1.13^\circ\text{C}$ reported for 298 passerines by Prinzing et al. (1991). The inflection T_a above which T_b increased was remarkably consistent among four of the species, varying by less than 1°C and averaging $\sim 35^\circ\text{C}$. In contrast, AB showed an inflection T_a that was $\sim 10^\circ\text{C}$ higher than that of the other four species. It is also noteworthy that the inflection point for T_b of YH, the smallest species, was less clearly defined than in any of the larger species (Fig. 2).

Maximum T_b values associated with acute heat stress for all five species were within the same range as those recently reported for southern African passerines (Whitfield et al., 2015), columbids from southern Africa and North America (McKechnie et al., 2016b; Smith et al., 2015) and a southern African sandgrouse (McKechnie

Table 2. Mass-specific RMR and EWL rate at high T_a in five species of Australian passerines

Variable	Yellow-plumed honeyeater	Spiny-cheeked honeyeater	Chestnut-crowned babbler	Grey butcherbird	Apostlebird
RMR					
Min. RMR (mW g^{-1})	19.6 ± 3.4 (9)	13.5 ± 2.2 (9)	11.6 ± 1.5 (9)	13.8 ± 4.0 (11)	9.8 ± 1.5 (3)
Max. RMR (mW g^{-1})	32.3 ± 4.9 (6)	24.1 ± 6.0 (2)	19.9 ± 6.0 (4)	20.9 ± 4.3 (8)	14.7 ± 1.3 (4) 19.6 (1)
EWL					
EWL in TNZ ($\text{mg g}^{-1} \text{h}^{-1}$)	8.6 ± 2.1 (12)	5.4 ± 2.1 (9)	5.8 ± 2.0 (9)	7.1 ± 2.0 (11)	4.9 ± 0.8 (3)
EWL slope ($\text{mg g}^{-1} \text{h}^{-1} \text{ }^\circ\text{C}^{-1}$)	5.58	4.36	3.77	2.95	2.95
Max. EWL ($\text{mg g}^{-1} \text{h}^{-1}$)	51.7 ± 2.1 (6)	43.2 ± 6.9 (2)	45.3 ± 8.7 (4)	46.5 ± 5.9 (8)	36.5 ± 4.5 (4) 42.2 (1)

Study species as in Table 1. TNZ, thermoneutral zone.

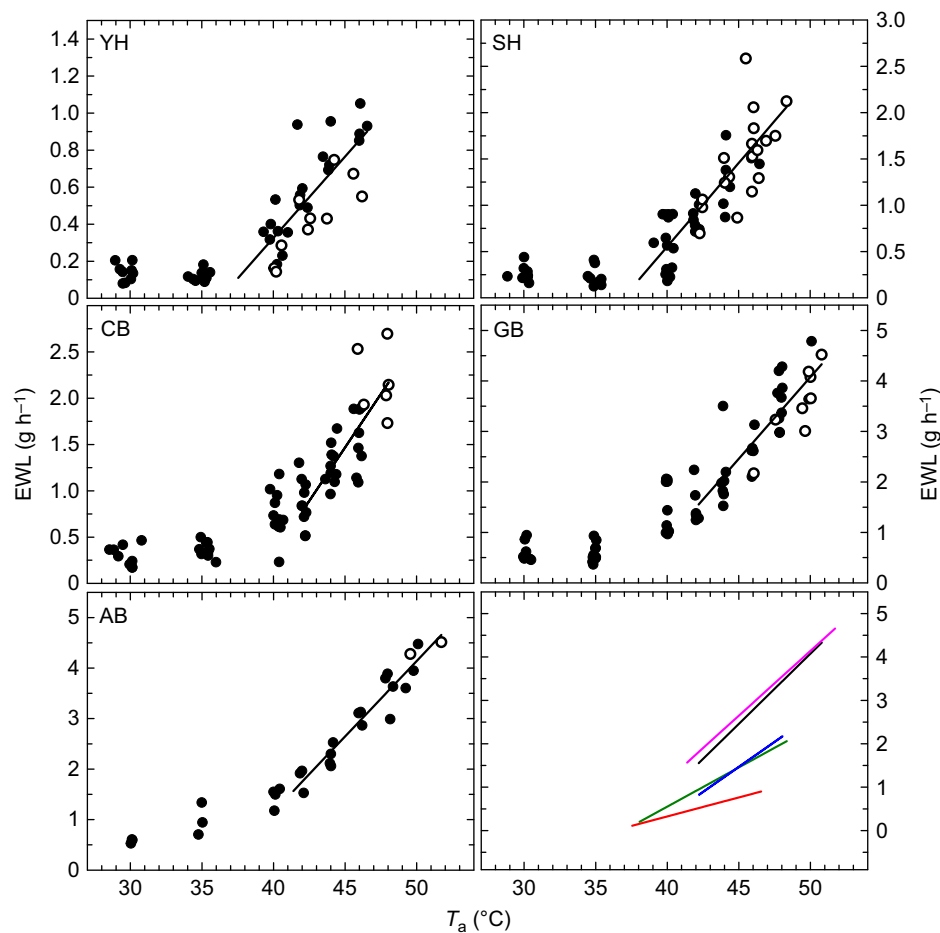


Fig. 3. Relationship between evaporative water loss (EWL) and T_a in five Australian passerines. Species as in Fig. 1. Open circles indicate individuals that reached thermal endpoints. For each species, the solid line is the upper portion of the segmented linear regression model that provided the best fit. Note that the scaling of the y-axes varies among panels. The bottom right panel shows the regression models for all five species plotted together for comparative purposes (red, YH; green, SH; blue, CB; black, GB; pink, AB).

et al., 2016a), with all these studies involving the same methods as we used here. Similar T_b maxima have been reported for other Australian passerines, including Spinifexbirds (*Eremiornis carteri*; 44.0°C; Ambrose et al., 1996) and Gouldian finches (*Erythrura gouldiae*; ~44.5°C; Burton and Weathers, 2003). Maximum T_b values for Australian passerines are also similar to those reported in four Nearctic passerines ranging in M_b from 11 to 46 g by Weathers (1981) in his seminal analysis of the scaling of avian thermoregulation at high T_a . Although some authors have reported birds being able to tolerate $T_b \approx 46^\circ\text{C}$ for brief periods (Brush, 1965; Dmi'el and Tel-Tzur, 1985) and avian lethal T_b as high as ~48°C (Randall, 1943), the picture that emerges from recent studies is that, in general, $T_b \approx 45^\circ\text{C}$ is the approximate upper limit to physiological function for most birds. That these values are slightly more conservative than some reported in the earlier literature may reflect the fact that we actively avoided exposing birds to lethal T_b .

The heat tolerance limits of the five species investigated here scaled positively with M_b (Fig. 5A), with the maximum T_a tolerated being lowest in YH, the smallest species investigated, and highest in AB, the largest. The heat tolerance limits of these species were conspicuously lower than those of the three southern African ploceids investigated by Whitfield et al. (2015). For instance, SH and CB tolerated maximum $T_a = 48^\circ\text{C}$, whereas the similarly sized white-browed sparrow-weaver (*Plocepasser mahali*) tolerated $T_a = 54^\circ\text{C}$ (Whitfield et al., 2015). The limited sample sizes preclude drawing general conclusions, but the currently available data suggest that there may be considerable phylogenetic and/or biogeographic variation in the scaling of passerine heat tolerance limits (Fig. 5A). We suspect these differences may be related to the

generally greater fractional increases in EWL among southern African ploceids compared with the Australian species investigated in the present study, which we discuss further below.

RMR

All five species showed a clear T_{uc} above which RMR increased linearly. However, the T_{uc} varied among species by ~11°C, and was not related to M_b in any obvious way. The large variation in T_{uc} among species was not reflected by variation in the T_a associated with the onset of panting; the latter varied by less than 1.5°C among the five species. This absence of a clear link between the onset of panting and increases in RMR above thermoneutral values contrasts with recent data for Burchell's sandgrouse (*Pterocles burchelli*) (McKechnie et al., 2016a). The T_{uc} for AB (~45°C) is one of the highest yet reported in a passerine (Tieleman et al., 2002; Weathers, 1981; Whitfield et al., 2015). It is also noteworthy that the estimated T_{uc} for this species is nearly 4°C higher than the mean T_a associated with the onset of panting (Table 1). Overall, the lack of a clear link between the onset of panting and the T_{uc} of the five species investigated here is difficult to explain.

The fractional increases in RMR at $T_a > T_{uc}$ (increases of 50–100% above thermoneutral values) are quantitatively similar to those of three southern African ploceid passerines (30–60%; Whitfield et al., 2015), and smaller than those reported for a sandgrouse (~150%; McKechnie et al., 2016a).

EWL

In all five species, the relationship between EWL and T_a followed the typical avian pattern of low, relatively constant EWL at moderate

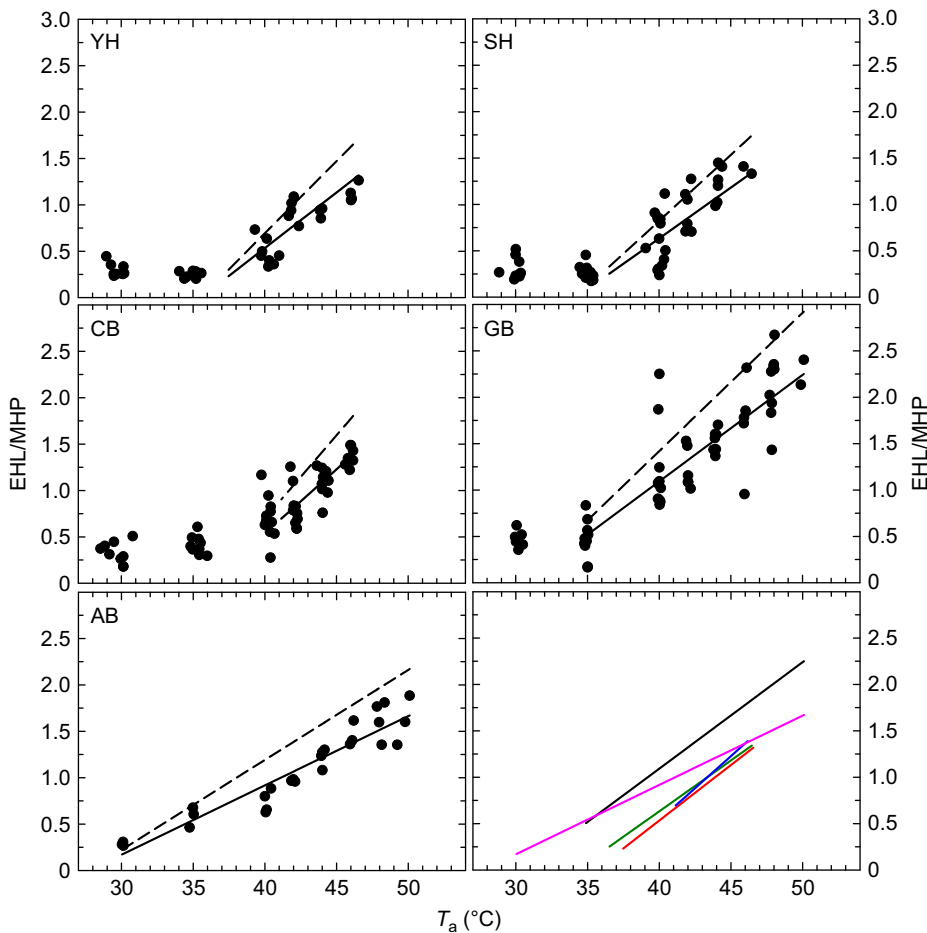


Fig. 4. Relationship between evaporative heat loss/metabolic heat production (EHL/MHP) and T_a in five Australian passerines. Species as in Fig. 1. For each species except AB, the solid line is the upper portion of the segmented linear regression model that provided the best fit. In the case of AB, no inflection point was evident, and the solid line shows a linear regression. The dashed lines show the relationship if EHL/MHP is recalculated assuming carbohydrate metabolism, i.e. respiratory exchange ratio=1.0. The bottom right panel shows the regression models for all five species plotted together for comparative purposes (red, YH; green, SH; blue, CB; black, GB; pink, AB).

T_a , and rapid, approximately linear increases in EWL at T_a approaching or exceeding normothermic T_b (Fig. 3). Minimum rates of EWL at thermoneutral T_a were higher than those predicted by the equation for arid-zone passerines presented by Williams (1996), being equivalent to 154–214% of predicted values. However, that equation was based on EWL typically measured at $T_a=25^\circ\text{C}$, below the T_a range involved in the present study. The inflection T_a above which EWL began to increase was close to normothermic T_b in all five species investigated here, ranging from 37.6°C in YH to 42.2°C in CB and GB.

The slopes of mass-specific EWL versus T_a above the inflection point for all five species were substantially above those expected, ranging from 158% (YH) to 219% (AB) of predicted values (Fig. 5D). Maximum rates of EWL were equivalent to 6.5–8.5 times baseline levels at $T_a=30$ – 35°C , with no obvious relationship between M_b and the ratio of maximum to baseline EWL. Maximum cooling efficiencies (i.e. maximum EHL/MHP values) were similar to the range reported for southern African ploceids (1.41–2.22; Whitfield et al., 2015), with only GB showing EHL/MHP > 2.0. Passerines in general hence appear to have maximum cooling efficiencies equivalent to EHL/MHP < 2.0; the notion that this applies to much larger passerines too is supported by the EHL/MHP of 1.67 at $T_a=50^\circ\text{C}$ in the brown-necked raven (*Corvus corax ruficollis*) reported by Marder (1973).

One consistent difference between the five Australian passerines investigated here and the three southern African species examined by Whitfield et al. (2015) concerns the ratios of maximum EWL to EWL at moderate temperatures (i.e. at T_a below the inflection point). In the five Australian species, this ratio averaged 7.3 ± 0.8 ,

approximately half the corresponding mean for the three southern African species (15.1 ± 3.9 ; Whitfield et al., 2015). Whereas data exist for too few species to permit a rigorous statistical examination, a cursory assessment of the scaling of baseline and maximum EWL in the eight species involved suggests that the larger fractional increases in the southern African species arise from a combination of lower baseline and greater maximum rates of EWL (Fig. 5C). We argue that these differences in the scope for increases in EWL above baseline levels are probably the mechanism underlying the apparent variation in the scaling heat tolerance limits (Fig. 5A), whereby three southern African ploceids have substantially higher heat tolerance limits than the five Australian species. One environmental variable that may be related to maximum rates of EWL, and hence upper limits to heat tolerance, is water availability. Although a greater scope for increasing rates of EWL above baseline levels confers higher heat tolerance, higher maximal rates of water loss also reduce time to dehydration and require the availability of surface water and/or may constrain diets to those with high preformed water availability. Therefore, future evaluations of this trait should also consider potential differences in behaviour and ecology to fully appreciate the drivers and constraints experienced by birds inhabiting these different biogeographic regions.

Conclusions

Our data on thermoregulation at environmental temperatures above T_b in five Australian passerines varying ~ 7 -fold in M_b support the notion that evaporative cooling in the Passeriformes is generally less efficient than is the case in orders such as Columbiformes and Caprimulgiformes. Compared with these last two orders, passerines

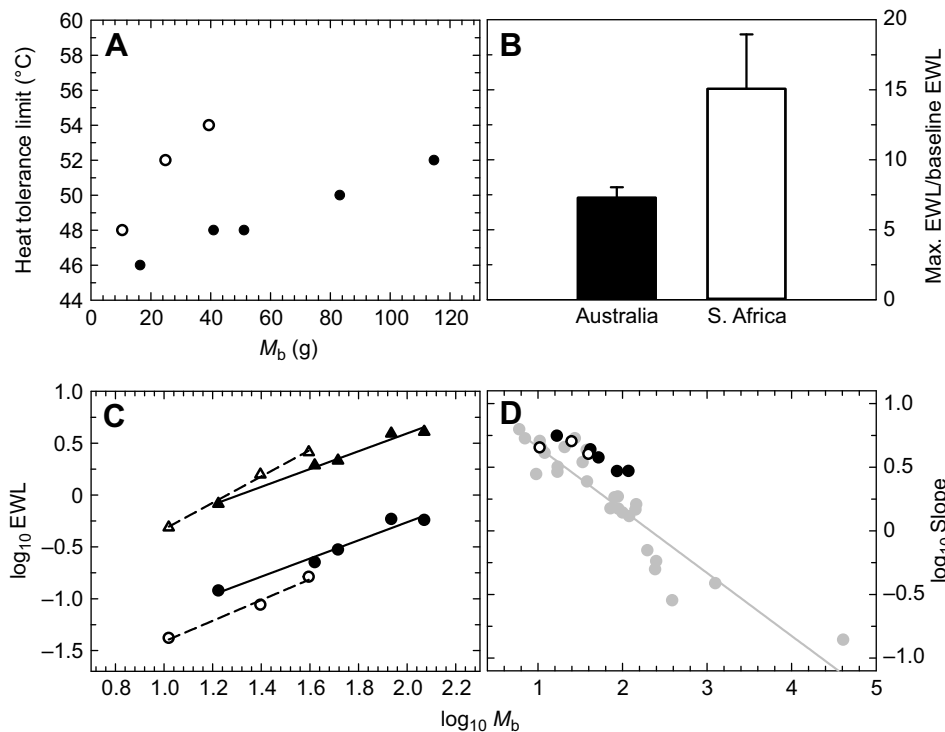


Fig. 5. Scaling of heat tolerance and evaporative cooling parameters in five Australian and three southern African passerines. Australian species as in Fig. 1; data for the southern African species are from Whitfield et al. (2015). (A) Heat tolerance limits were consistently higher for the three southern African ploceids (open symbols) than for the five Australian species (filled symbols). (B) The ratio of maximum EWL to baseline EWL in the thermoneutral zone was also substantially higher in southern African ploceids than in the Australian species investigated in the present study. (C) This difference in fractional increases in EWL appears to arise from a combination of lower baseline EWL (circles) and higher maximum EWL (triangles) in the southern African ploceids (open symbols) compared with the Australian species (filled symbols). EWL was measured in g h^{-1} and M_b in g. (D) The slopes of mass-specific EWL versus T_a (in $\text{mg g}^{-1} \text{h}^{-1} \text{°C}^{-1}$) in the five Australian species investigated here (filled symbols) are all relatively high compared with allometrically expected values. M_b was measured in g. Data for the three southern African ploceids are shown by open symbols; the grey symbols and line show the avian data included and regression model fitted by McKechnie and Wolf (2010).

show more rapid increases in EWL, RMR and T_b with increasing T_a , and lower maximum heat dissipation capacity measured as maximum EHL/MHP [passerines: 1.2–2.2 (Whitfield et al., 2015; present study); columbids: 2.3–4.7 (McKechnie et al., 2016b; Smith et al., 2015); caprimulgids: 3.0–5.2 (Dawson and Fisher, 1969; O'Connor et al., 2017)]. This marked among-order variation in evaporative cooling efficiency appears to arise from the primary avenues of heat dissipation involved: panting in passerines, cutaneous evaporation in columbids, and gular flutter using a comparatively massive buccal surface in caprimulgids.

Although our results highlight some quantitative traits common to all passerines investigated to date, they also reveal marked variation within this order. The five Australian species examined here had markedly lower heat tolerance than three southern African ploceids investigated previously, tolerating T_a maxima 2–6°C lower. The mechanism responsible for this more modest heat tolerance in the Australian species appears to involve a reduced scope for increasing EWL above baseline levels; the fractional increases in EWL were approximately half those in the southern African ploceids.

It remains unclear whether these differences between Australian and southern African passerines arise from phylogenetic differences or adaptive variation, and our sample sizes are too small to rigorously identify the sources of variation involved. The present study involved members of four families, whereas the study by Whitfield et al. (2015) involved just one. The two study sites are climatically similar, although the two sites in the Kalahari Desert where Whitfield et al. (2015) collected their data are hotter, with a mean mid-summer maximum T_a of $37.7 \pm 3.7^\circ\text{C}$ (data for Twee Rivieren, Noakes et al., 2016) versus $33.8 \pm 5.7^\circ\text{C}$ for Gluepot Reserve. Testing the hypothesis that broad differences among passerine communities in terms of their heat tolerance and evaporative cooling capacity are related to climate will require far more data than are currently

available. Nevertheless, it is striking that 7 g verdins (*Auriparus flaviceps*) from North America's intensely hot Sonoran Desert were able to tolerate $T_a = 50^\circ\text{C}$ (Wolf and Walsberg, 1996a,b), a T_a higher than those tolerated by scaly-feathered weaver and YH, two comparatively sized (10–16 g) southern African and Australian species (Whitfield et al., 2015; present study). This observation is consistent with the idea that biogeographic variation in passerine heat tolerance may be functionally linked to broad-scale climatic differences, and moreover suggests that testing hypotheses concerning adaptive correlations between climate and passerine evaporative cooling capacity may prove fruitful.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.E.M., B.O.W.; Formal analysis: A.E.M., A.R.G.; Investigation: A.R.G., T.J.M., E.S., W.T.; Resources: B.O.W.; Writing - original draft: A.E.M.; Writing - review & editing: A.E.M., A.R.G., T.J.M., E.S., W.T., B.O.W.

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