Avian thermoregulation in the heat: phylogenetic variation among avian orders in evaporative cooling capacity and heat tolerance

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ABSTRACT

Little is known about phylogenetic variation in avian evaporative cooling efficiency and heat tolerance of birds in hot environments. We quantified thermoregulatory responses to high air temperature (T_a) in ~100-g representatives of three orders: African cuckoo (*Cuculus gularis*, Cuculiformes), lilac-breasted roller (*Coracias caudatus*, Coraciiformes), and Burchell's starling (*Lamprotornis australis*, Passeriformes). All three species initiated respiratory mechanisms to elevate evaporative heat dissipation when body temperature (T_b) approached 41.5°C in response to increasing T_a, with gular flutter observed in cuckoos and panting in rollers and starlings. Resting metabolic rate (RMR) and evaporative water loss (EWL) increased by quantitatively similar magnitudes in all three species, although maximum EWL was proportionately lower in starlings. Evaporative cooling efficiency—defined as the ratio of evaporative heat loss (EHL) to metabolic heat production (MHP) —generally remained below 2.0 in cuckoos and starlings, but reached a maximum of ~3.5 in rollers. The high value for rollers reveals a very efficient evaporative cooling mechanism, and is similar to EHL/MHP maxima for similarly sized columbids which rapidly dissipate heat via cutaneous evaporation. These findings shed new light on the ecology, physiology and evolution of birds that routinely have to maintain T_b below environmental temperature.

Keywords: evaporative heat loss, heat dissipation, upper critical limits of thermoneutrality, panting, gular flutter

Summary statement

Avian evaporative cooling efficiency and heat tolerance show substantial taxonomic variation. We show that a 100-g coraciiform show highly efficient evaporative cooling capacity and tolerate higher air temperatures, compared with a similar sized cuculiform and passerine.

These findings highlight that we currently have a poor understanding of the mechanisms underpinning taxonomic variation in avian thermoregulation under hot conditions.

Introduction

Evaporative heat loss (EHL) is the only mechanism whereby birds can maintain body temperature (T_b) below lethal limits in hot environments where environmental temperature exceeds Tb, and rapid increases in evaporative water loss (EWL) appear to be a ubiquitous avian response to such temperatures (Bartholomew and Cade, 1963; Dawson and Bartholomew, 1968; Smith et al., 2015; Whitfield et al., 2015). Water requirements for thermoregulation thus have shaped the ecology and evolution of birds living in hot environments, and lead to important trade-offs between dehydration and hyperthermia avoidance in arid-zone species (Smit et al., 2013; Tieleman and Williams, 2000; Tieleman and Williams, 2002; Tieleman et al., 2003).

Avian heat tolerance and evaporative cooling efficiency (quantified as the maximum ratio of heat dissipated evaporatively, EHL, to that generated metabolically, MHP) appears to vary substantially among orders (Lasiewski and Seymour, 1972; Smith et al., 2015), within orders (McKechnie et al., 2016a; McKechnie et al., 2017), and even within species (Noakes et al., 2016). For example, recent studies have shown that the efficiency of evaporative cooling is generally very high in members from the order Columbiformes and Caprimulgiformes (McKechnie et al., 2016a; O'Connor et al., 2017; Smith et al., 2015; Talbot et al., 2017), and less so in Passeriformes, Pteroclidiformes, Galliformes and Strigiformes (Bartholomew et al., 1968; McKechnie et al., 2016b; McKechnie et al., 2017; Smith et al., 2015; Whitfield et al.,

2015). Many avian taxa also allow their T_b to increase (described as facultative hyperthermia) and thereby are at risk of reaching lethal limits during heat exposure. Limited phylogenetic sampling means we still have a limited understanding of the diversity of avian EHL mechanisms and their functional significance in tolerance of heat and evaporative cooling efficiency.

Body mass represents one of the most prominent sources of variation in heat stress and the efficiency of EHL (McKechnie and Wolf, 2010), but even species of similar size can still show substantial variation. For example, Lasiewski and Seymore (1972) showed that four similar sized species from different avian orders show substantial variation (in terms of magnitude of elevations in T_b, metabolism, EWL, and subsequently EHL/MHP) in their responses to heat. Recent studies have suggested that some of the underlying mechanisms of this variation may include differential reliance on respiratory versus cutaneous pathways of evaporative cooling (McKechnie et al., 2016a), and also morphological variation (e.g. bill size) (Danner et al., 2016; Tattersall et al., 2009; van de Ven et al., 2016). Variation in the rate and efficiency of evaporative cooling in avian taxa may represent physiological costs, such as risk of dehydration and hyperthermia (McKechnie and Wolf, 2010), but may also include behavioural [e.g. reduced foraging efficiency correlated with heat dissipation efforts (du Plessis et al., 2012)] and ecological costs [e.g. reliance of surface water sources (Smit et al., 2016)]. Thus far, evaporative cooling efficiency and heat tolerance are most studied in the orders Passeriformes and Columbiformes, and data from more unrepresented avian orders are needed to better quantify evolutionary drivers and mechanisms linked to variation in these traits during hot weather.

Here we present data on thermoregulation under hot conditions in three 100-g avian species representing three orders: Cuculiformes, Coraciiformes and Passeriformes. The

Cuculiformes and Coraciiformes remain largely unstudied in terms of thermoregulation at high temperatures; both represent diverse taxa that include many species occurring in hot, arid regions. To the best of our knowledge, the only thermoregulatory data from a cuculiform under hot conditions was for the roadrunner (Calder and Schmidt-Nielsen, 1967). In the latter study high humidity levels may have confounded the efficiency of EHL at experimental temperatures (T_a) below T_b. Recent advances in carbon dioxide and water analysers has allowed us to maintain low humidity levels during testing by utilizing high flow rates, thus avoiding complications associated with high humidity that plagues early studies (Gerson et al., 2014; Lasiewski et al., 1966; Smith et al., 2015; Whitfield et al., 2015). In addition, our inclusion of a large passerine (>100g) will allow more rigorous analyses of the scaling of traits related to heat tolerance and evaporative cooling in this speciose taxon (McKechnie et al., 2017).

Methods

Study species and study sites

We measured resting metabolic rate (RMR), evaporative water loss (EWL), and T_b in species representing three avian orders in the southern Kalahari Desert in the Northern Cape province of South Africa, an arid area with a mean annual rainfall of ~200 mm and summer daily maximum T_a ranging from ~20-43°C (Whitfield et al., 2015). We followed the methodology of Whitfield et al. (2015) to quantify thermoregulatory responses to high T_a in a field laboratory from January to April 2012 at Wildsgenot Game Ranch (27°04´S, 21°23´E), and from January to March 2013 at Leeupan Game Ranch (26°58´S, 21°50´E).

We trapped six African cuckoos (*Cuculus gularis*, order Cuculiformes, hereafter cuckoos) with a mean ± SD body mass (M_b) of 109.6 ± 5.6 g at Leeupan Game Ranch during early 2013 (austral summer) using mistnets. We trapped 10 lilac-breasted rollers (*Coracias caudatus*; Order Coraciiformes, hereafter rollers) with a M_b of 95.4 ± 8.5g; two individuals were trapped in February 2012 at Wildsgenot Game Ranch, and the remaining individuals were trapped at Leeupan Game Ranch between January 2013 and March 2013. We trapped seven Burchell's starlings (*Lamprotornis australis*; order Passeriformes, hereafter starlings) with a M_b of 109.1 ± 9.3g using mistnets and/or flap traps baited with tenebrionid beetle larvae on Wildsgenot Game Ranch in February 2012, and Leeupan Game Ranch from December 2013 to February 2013. Measurements were carried out on the same day of capture. If birds were not run within the first three hours subsequent to being trapped, they were housed in cages constructed of shade-cloth, and were provided with tenebrionid beetle larvae and water ad libitum, until physiological measurements commenced.

Birds were placed in respirometry chambers for 2-3 hr, a period that typically limited M_b loss to <5% of initial M_b (mean M_b loss during measurements was 4.0 ± 1.8 % of initial values) and time in captivity did not exceed 24 hr, after which birds were released at the site of capture following, Whitfield et al. (2015). All experimental procedures were approved by the Animal Ethics Committee of the University of Pretoria (protocol EC071-11) and the Institutional Animal Care and Use Committee of the University of New Mexico (12-1005370-MCC). A permit to trap the birds was issued by the Northern Cape Department of Environmental Affairs (ODB 008/2013).

Gas exchange and temperature measurements

We used the same experimental set-up as described by Whitfield et al. (2015) to obtain gas exchange, T_a and T_b measurements. We measured T_b using calibrated temperature-sensitive passive integrated transponder (PIT) tags (Biomark, Boise, ID, USA) which were injected intraperitoneally into the abdominal cavity of each bird shortly after capture. We monitored T_b throughout gas exchange measurements, using a reader and transceiver system (model FS2001, Biomark, Boise, ID, USA). We obtained carbon dioxide production ($\dot{V}_{CO_2}$) and EWL measurements over the T_a range of 25-56°C (depending on the species), also using the same experimental setup as described by Whitfield et al. (2015). All three species were placed individually in 9-L plastic chambers, within which they stood on a platform of plastic mesh 10 cm above a 1-cm layer of mineral oil to trap excreta. We used flow rates between 9 and 30L min^{-1} for cuckoos; 9 and 70 L min^{-1} for rollers, and 10 and 55 L min^{-1} for starlings, depending on the experimental T_a, in order to keep chamber humidity below 5 ppt. As was the case in previous studies using the same methodology (Smith et al. 2015, Whitfield et al. 2015, McKechnie et al. 2016a, McKechnie et al. 2016b), birds remained calmer at very high T_a when we increased flow rate (and hence decreased humidity).

Experimental protocol

Following the protocol described by Whitfield et al. (2015), we exposed birds to progressively higher T_a, with increments of approximately 5°C between 25 and 40°C, and 2°C increments between 40°C and the maximum T_a (ranging from 48 to 56°C depending on the species). Tests were conducted during the day, as this was the active phase of all three species. Birds spent between 10 and 30 min at each T_a value. We continually monitored birds during measurements using a live video feed and an infrared light source (Whitfield et al., 2015). We

recorded behavioural responses of birds within the respirometry chambers every two minutes. Behaviours noted included scoring the activity state of the individual as calm, turning around, and jumping. We also noted respiratory EHL mechanisms, including panting (defined as gaping) and gular flutter (defined as an obvious pulsation of hyoid bone in the gular area while gaping). For each individual, we recorded the air temperature and body temperature where heat dissipation behaviour was initiated.

We followed Whitfield et al. (2015) in terminating test runs when a bird a) exhibited escape behaviour such as agitated jumping, pecking and/or wing flapping), b) showed signs of a loss of coordination or balance accompanied by T_b > 44°C, or 3) exhibited a decrease in EWL and RMR accompanied by an uncontrolled increase in T_b. In the last instance, a 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 was considered the thermal endpoint for that individual. When a bird reached any the conditions described above, we removed the individual from the chamber by hand, gently rubbed a cotton pad soaked in ethanol onto its body, and held it in front of an air-conditioner producing chilled air in order to facilitate rapid heat loss (Whitfield et al., 2015).

Data analyses

Data were analysed following Whitfield et al.(2015). We calculated physiological estimates by extracting $\dot{V}_{CO_2}$, EWL and T_b as the lowest 5 min mean at each T_a using Expedata (Sable Systems, Las Vegas NV, USA). We present whole-animal values, although we also calculated the slope of mass-specific EWL vs. T_a to compare our values with the allometric equation presented by McKechnie and Wolf (2010). We converted rates of EWL to evaporative heat

loss (W) assuming a latent heat of vaporisation of water of 2.406 J mg^{-1} at 40°C (Tracy et al. 2010). Since none of our study species display crops we assumed all birds were post-absorptive at the time of measurements, but we were unable to confirm this. Measurements typically took place more than 1 hr after capture, and food (mealworm larvae) was only offered to individuals not measured within 3 hrs of capture. We therefore assumed a respiratory exchange ratio of 0.85, representative of a mix of carbohydrate and lipid metabolism in post-absorptive birds (Walsberg and Wolf 1995), and converted rates of $\dot{V}_{CO_2}$ to metabolic rate (Watt, W) using 24.4 J mL^{-1} CO_2 (Withers 1992).

We used segmented linear regression models fitted in the *R* package *segmented* (Muggeo, 2009) to estimate inflection T_a in the physiological data. Although our use of these models violates assumptions of independent samples, we used segmented models purely to aid us in identifying inflection T_a values in physiological variables. We subsequently used linear mixed effects models that included individual identity as a random factor in the *R* package *nlme* (Pinheiro et al., 2009), to obtain estimates of physiological variables as a function of T_a, using subsets of the data above respective inflection T_a. We contrasted the slopes of physiological parameters obtained from these mixed effects models among species, by including data previously collected from laughing doves (*Stigmatopelia senegalensis*, 89.4 ± 13.0, hereafter doves) studied at the same time and site as our species (McKechnie et al., 2016a), and freckled nightjars (*Caprimulgus tristigma*, 64.7 ± 6.3, hereafter nightjars) studied from a desert site in Namaqualand, South Africa (O'Connor et al., 2017). Thermoregulatory data in the doves and nightjars were collected in the same manner as described here, although we recalculated physiological estimates of VCO2, EWL and T_b in the dove, as the lowest 5 min mean at each T_a, to facilitate comparison with our study species and the nightjar. Our small sample size of species studied here precluded us from conducting more rigorous

statistical analyses that test of phylogenetic inertia and, if necessary, account for phylogenetic relatedness. However, the five avian orders representing these taxa all diverged from a common ancestor between 71.5 and 79.6 million years ago (Jetz et al., 2012), and given the current uncertainty in the fine-scale relatedness of these avian orders, we argue that it will be difficult to obtain an accurate phylogenetic signal at this level. We therefore calculated the upper and lower 95% confidence limits (CL) of each coefficient, and considered species to differ quantitatively in the parameters when there was no overlap in the 95% CL.

Results

Body temperature

Normothermic T_b measured at T_a < 35°C, or the respective T_{uc} of each species, varied by 0.7°C in the three species, from a mean of 40.0 ± 0.6°C in cuckoos to 40.7 ± 0.8°C in rollers (Table 1). The inflection T_a above which T_b increased was below 37°C in all three species (Table 1), and in all three species T_b increased linearly and significantly above the inflection T_a (cuckoo, $t_{1,26}$=14.275, p<0.001; rollers, $t_{1,56}$= 12.10, p<0.001; starlings, $t_{1,28}$=7.71, p<0.001) (Fig. 1). The maximum T_a reached was 50°C for both cuckoos and starlings, and 56°C for rollers. Any further increases in T_a resulted in these individuals becoming severely agitated. Average maximum T_b ranged between 42.8° and 44.0°C in the three species at the highest T_as tested. In two calm cuckoos, T_b exceeded 44°C at T_a = 46° and 48°C, respectively, whereupon they lost their ability to right themselves and the experiment was terminated immediately. With the exception of T_b exceeding 44°C in one roller individual (with no ill-effects), T_b remained <44°C in calm rollers and starlings at the maximum T_as. At the highest T_a (46°C) shared among the three

species, rollers regulated a lower T_b than cuckoos and starlings (Table 1). The mean T_a where birds initiated respiratory EHL mechanisms varied from 35.9°C in starlings to ~41°C in cuckoos and rollers, typically as T_b approximated 41.5°C in all three species (Table 1). Our observations of the cuckoos revealed that gaping was always accompanied by gular flutter, which was visibly evident from the pulsating throat and moving hyoid apparatus. Rollers and starlings panted while gaping (the gape is wide in rollers) and we did not observe gular flutter (hyoid movement) in either species.

Resting metabolic rate

One cuckoo showed abnormally high RMR values (ranging from 1.03W at T_a = 30°C, to 5.20W at T_a = 50°C, i.e. > 6 x SD greater than mean for the remaining cuckoos), and we excluded data from this individual from our RMR analyses and descriptive statistics. Minimum RMR values measured T_a < 35°C, or the respective T_{uc} of each species, varied from 0.64 ± 0.20W and 0.69 ± 0.13W in cuckoos and rollers, respectively, to 1.19 ± 0.31W in starlings (Table 1). Minimum mass-specific RMR ranged from 5.85 ± 1.79 mW g^{-1} in cuckoos to 10.76 ± 1.98 mW g^{-1} in starlings (Table 2). In rollers and starlings T_{uc} (T_a inflection) ranged from 31°C in starlings to 47.5°C in rollers. No clear inflection point could be identified for cuckoos, and RMR increased across the entire range of T_a tested (Fig. 2). In all three species, RMR increased linearly and significantly at $T_a > T_{uc}$ (cuckoo, $t_{1,23}$=2.67, $p<0.05$; rollers, $t_{1,25}$ = 2.5866, $p<0.05$; starlings, $t_{1,29}$=4.13, $p<0.001$) (Fig. 2). Maximum RMR observed at maximum T_a was higher in the starlings, at an average of 1.91W, and lowest in the rollers at ~ 1.10W. Maximum RMR was 0.96W and 2.77W in two cuckoo individuals, respectively (Table 1). The average magnitudes of RMR elevations above estimated thermoneutral levels (max. RMR / min. RMR) were generally below 1.6, with the exception of a single cuckoo that showed a 3-fold increase RMR

3-fold (Table 1). At the highest test T_a shared among the three species, RMR was quantitatively similar in cuckoos and rollers, but ~2-fold higher in the starlings (Table 1).

Evaporative water loss

Minimum EWL values measured at T_a < 35°C, or the respective T_{uc} of each species ranged from 0.48 ± 0.14 g h^{-1} in cuckoos to 1.00 ± 0.67 g h^{-1} in starlings (Table 1). Similarly, mass-specific EWL was lowest in cuckoos and highest in starlings (Table 2). The inflection T_a varied by more than 10°C among the three species; inflection T_a was 33.3°C in starlings and greater than 42°C in cuckoos and rollers (Table 1). Whereas cuckoos and starlings showed stable EWL rates below their respective inflection T_as, rollers showed a shallow but significant increase in EWL to 1.65 ± 0.35 g h^{-1} at T_a = 44.7 °C ($t_{1,22}$=3.22, p<0.01; Fig. 3). Above the inflection T_a EWL increased linearly and significantly in all three species (cuckoo, $t_{1,20}$=13.22, p<0.001; rollers $t_{1,33}$=11.64, p<0.001; starlings, $t_{1,28}$=11.133, p<0.001; Fig. 3). Mass-specific slopes varied from around 2.1 mg g^{-1} h^{-1} °C^{-1} in cuckoos and starlings to >4 mg g^{-1} h^{-1} °C^{-1} in rollers (Table 2). Maximum elevations in EWL was around 5-fold (>5.5-fold in cuckoos and rollers) above thermoneutral values in the three species (Fig. 3, Table 1). Whereas rollers showed maximum EHL/MHP ratios exceeding 3 at the maximum T_as tested, cuckoos and starlings generally showed values below 2 (Fig. 3). At the highest shared T_a cuckoos and rollers showed similar EWL rates and evaporative efficiencies (Table 1). In contrast, starlings showed EWL ~ 3.40 g h^{-1}, and a slightly lower efficiency compared to the former species (Table 1).

Variation in thermoregulation at high air temperatures

Our data, combined with those for doves and nightjars, provide an opportunity to compare heat tolerance and evaporative cooling capacity among five avian orders. The T_b of cuckoos and doves increased more rapidly compared to those of the remaining species, although the 95% CL overlapped largely in most species (Fig. 5, Table 3). The slope of RMR at T_a above thermoneutrality in doves was similar to those of our three present study species, with overlapping 95% confidence intervals. In contrast, nightjars showed almost no increase in RMR above 40°C and no overlap in 95% CL with any of the other species (Fig. 5, Table 3). Similarly, the slope of EWL above the inflection T_a was also lower in nightjars compared to the other four species (no overlap in 95% CL). Rollers showed a higher slope in EWL above the inflection T_a compared to cuckoos, starlings and the dove (Fig. 5, Table 3). Nightjars achieved more rapid increases in EHL/MHP at T_a above the inflection, compared to our three study species and doves (Fig. 5; Table 3); whereas nightjars showed a higher EHL/ MHP values compared to rollers and cuckoos, the 95% CL overlapped marginally (Table 3). Unexpectedly, the slopes of EHL/MHP were similar in doves and starlings, both lower than in cuckoos and rollers showing no overlap in 95% CL (Table 3).

Discussion

All three species studied here showed elevations in T_b, RMR and EWL at T_a above 40°C qualitatively consistent with typical avian patterns. Despite their similarity in M_b, however, they showed substantial variation in patterns of T_b, RMR and EWL at high T_a. The comparatively shallow elevations in RMR in rollers were associated with the highest evaporative efficiency, and this species tolerated the highest T_{air} among the three species

Additionally, our five-order comparison reveals that all species, with the exception of caprimulgids (O'Connor et al., 2017), clearly showed elevated metabolic costs of heat dissipation (as defined by the slope of RMR above the inflection point). Yet, the T_{air}s where these responses were initiated, patterns of T_b regulation, and EWL elevations, were strikingly different between these taxa. Rollers showed very small elevations in T_b, associated with high evaporative efficiency. In contrast, at lower T_{air}s both starlings and cuckoos showed sharp elevations in T_b, rapid elevations in RMR, and lower evaporative efficiencies. These findings illustrate how little we know about the phylogenetic variation in avian evaporative cooling pathways, and specifically the functional significance of these pathways for heat tolerance.

Body temperature and heat tolerance limits

The mean T_b of cuckoos at T_a below 35°C (39.8 ± 0.30°C) was 1.5 °C lower than the mean active phase T_b values for Cuculiformes (41.3°C), but mean T_b of starlings and rollers were within 0.5°C of the mean values reported for Passeriformes and Coraciiformes, respectively (Prinzinger et al., 1991). In all three species, the inflection T_a for T_b was below 40°C. This is lower than the inflection T_a recorded previously in columbids, quail, and smaller passerines (generally T_a ~ 40°C) (McKechnie et al., 2016a; Smith et al., 2015; Whitfield et al., 2015). Two species of Caprimulgiform also show a T_b inflection at T_a below 40°C (O'Connor et al., 2017).

In calm birds, maximum T_b was >44.5°C in a cuckoo at T_a = 49.6°C, 44.1°C in a starling at T_a = 49.2°C and 44.6°C in a roller at T_a = 53°C. The two cuckoos lost coordination at T_b > 44°C, which suggests this taxon may have lower critical thermal limits than Passeriformes and Columbiforms, where several species have been shown to tolerate T_b>45°C (Dawson, 1954; McKechnie et al., 2016a; Whitfield et al., 2015). Several smaller passerines also reached thermal limits as T_a approached 50°C (McKechnie et al., 2017; Whitfield et al., 2015).

However, T_a = 50°C is probably an underestimation of the heat tolerance limits of cuckoos and starlings, as we did not record instances where calm individuals reached a plateau EWL accompanied by an increasing T_b (Whitfield et al., 2015). In contrast to cuckoos and starlings, rollers defended their T_b over a much larger T_a-T_b gradient, tolerating T_a ~ 56°C, with a mean T_b of ~ 43°C. The T_b patterns of the coraciiform in our study at T_a approaching 60°C was therefore quantitatively similar to the thermoregulatory patterns observed in Columbiformes (McKechnie et al., 2016a; Smith et al., 2015).

Resting metabolic rate

The upper inflection T_a in RMR versus T_a, i.e., the T_{uc} indicating increased energetic costs of respiratory EHL, generally occurs at T_{air} < 40°C (Calder and Schmidt-Nielsen, 1967; Dawson and Bennett, 1973; McNab and Bonaccorso, 1995; Tieleman and Williams, 2002; Whitfield et al., 2015). In all three species studied here, T_{uc} was not clearly related to increased efforts of respiratory EHL, unlike the case in Burchell's sandgrouse (McKechnie et al. 2016a). Our data suggest that the initiation of gular flutter or panting was related to a threshold T_b of ~41.5°C, rather than a threshold T_a. The cuckoos in our study did not show a clear upper inflection T_a, contrasting with the marked T_{uc} associated with the onset of gular flutter in Burchell's sandgrouse (*Pterocles burchelli*) (McKechnie et al., 2016b). At the T_a value at which we observed cuckoos to initiate gular flutter (~42°C), RMR had already increased significantly by more than 50% above minimum resting levels. Similarly, in the starlings, the inflection T_a for RMR was well below the T_a where this species initiated gaping behaviour, and lower than recorded in smaller passerines (Whitfield et al. 2015). The patterns in these two species are perhaps explained by a Q_{10} effect associated with T_b elevations, but our small sample sizes precluded us from making any firm conclusions; it is noteworthy that Weathers (Weathers,

1981) has shown that elevations in body temperature do not effect RMR in a number of passerines, but this idea has not been rigorously tested. In contrast to the starlings and cuckoos, rollers maintained a low and stable RMR up to T_a = 47°C, much higher than the T_a associated with gaping and suggesting that rollers kept metabolic heat loads low during the initial stages of panting. Our estimate of the upper T_{uc} in rollers is thus substantially higher than typically observed in passerines, which also make use of panting (McKechnie et al., 2017; Weathers, 1981; Whitfield et al., 2015).

Our data reveal considerable overlap in the slope of RMR increases above the T_{uc} in all three species studied here when compared to a similar-sized dove (*S. senegalensis*). Our estimate of the slope of RMR *vs.* T_a above the inflection T_a was higher than reported for this species of dove (same data) by McKechnie et al. (McKechnie et al., 2016a), as we calculated RMR over a 5-min period, instead of the 1-minute period used by the latter authors. This discrepancy does not influence our conclusions; doves showed quantitatively similar metabolic elevations at high T_as compared to cuckoos, rollers and starlings. At least one previous study noted that the metabolic costs in species that pant (e.g. village weaver, *Ploceus cucullatus*) are similar to those in species which supplement panting with gular fluttering (e.g. Chinese painted quail, *Excalfactoria chinensis*), and additionally cutaneous EHL (e.g. Inca dove, *Scardafella inca*) (Lasiewski and Seymour 1972) (Lasiewski and Seymour, 1972). However, one striking difference among the species we compare here is that doves and rollers reached maximum RMR at higher T_as and maintained lower T_bs compared with cuckoos and starlings.

The fractional elevations in RMR at T_a up to 50°C were lower in one cuckoo individual (~24% above thermoneutral values) compared to starlings (~60%); in the latter species values were quantitatively similar to those recorded in passerines (30-90%; (McKechnie et al., 2017;

Whitfield et al., 2015). However, fractional RMR elevations at T_a = 50°C in cuckoos and starlings were higher than those observed in the roller (~10% higher; this study), and Columbiformes (approximately 7% higher than in seven species; (McKechnie et al., 2016a), over a similar T_a range (30°C to 50°C). The lack of metabolic elevations at T_as above those where gular flutter was initiated in the nightjars (T_a ~ 40°C) contrasts strongly with all the above-mentioned species. O'Connor et al. (2017) argued that the low RMR of nightjars contributes to low metabolic heat load at high T_a s. However, the negligible metabolic elevations at high T_a previously also observed in the common poorwill [linked to the unique mode of gular flutter (Bartholomew et al. 1968, Lasiewski and Seymour 1972)], strongly suggest that this is characteristic of caprimulgids in general (O'Connor et al., 2017; Talbot et al., 2017).

Evaporative heat loss

Patterns of EWL followed the typical trend observed in birds, where EWL was minimal at T_as around 25-35°C and increased approximately linearly at T_as approaching or exceeding 40°C. Average minimum EWL rates recorded in our three study species were higher than those predicted by (Williams 1996) for all birds (eqn. 2) at T_a = 25°C, at 124%, 153%, and 169% of expected values for the cuckoos, rollers, and starlings, respectively, when compared to the expected values for arid-zone species (Williams 1996); these discrepancies may reflect the fact that most of our measurements of calm individuals were obtained at T_a > 30°C, as opposed to T_a = 25°C in the latter study. The inflection T_as above which we observed rapid elevations in EWL in the three species studied here varied by > 10°C. McKechnie and Wolf (2010) showed that inflection T_as vary with body mass, and show large inter-specific variation of a quantitatively similar magnitude to what we observed here. McKechnie and Wolf (2010)

found that the slope of mass-specific EWL *versus* T_a scales negatively with body mass, explaining ~90% of variation. However, we observed substantial variation in the slopes of EWL above the respective inflection T_as, among these species we studied. This variation in the sensitivity of EWL to T_a is difficult to explain. Comparisons of slopes in EWL with other species are also not feasible as the data from the literature analysed by McKechnie and Wolf (McKechnie and Wolf, 2010) were obtained under different experimental conditions, most notably varying chamber humidity levels, which has been shown to influence evaporative cooling efficiency (Gerson et al., 2014).

The capacity for evaporative cooling, expressed as maximum EHL/MHP, was qualitatively related to the maximum T_a reached our three study species. On average, cuckoos and starlings evaporated less than 200% of metabolic heat production at the maximum T_a reached, patterns similar to those observed in passerines (McKechnie et al., 2017; Whitfield et al., 2015). The comparatively high RMR of starlings likely constrained their evaporative cooling efficiency as they had to evaporate water almost twice as rapidly (compared to the other species) to dissipate 100% of their metabolic heat. This may have broad-scale implications for heat loss in the order Passeriformes as a recent study conclusively shown this order to have higher energy demands than other orders (Londoño et al., 2015). Cuckoos showed a slope of EHL/MHP versus T_a only marginally lower than rollers, but despite their low RMR at T_a < 35°C, they did not reach a very high efficiency; even though a very calm cuckoo dissipated 200% of its metabolic heat load at T_a = 50°C, the T_b of this individual was already ~ 43°C.

Rollers showed a mean maximum EHL/MHP of 3.64 (> 4 in one individual), quantitatively similar to values reported in Columbiformes (ranging from 2.27 to 4.66), a taxon in which we expect significant reliance on cutaneous EHL (McKechnie et al., 2016a;

Smith et al., 2015). In fact, rollers evaporated a greater fraction of their metabolic heat by a substantial margin compared to medium-sized columbids (the 140-g *S. capicola*, and 90-g *S. senegalensis*) occupying the same habitat in the Kalahari Desert (McKechnie et al. 2016b). We do not know if rollers rely on cutaneous evaporation at high T_a, although we propose that evaporative cooling may be enhanced by the large gape of rollers increasing the surface area of EWL over the respiratory region (personal observation); the large gape and high evaporative efficiency is also seen Caprimulgids. Our understanding of the capacity for different avian taxa to partition EWL into cutaneous and respiratory pathways remains very poorly studied, and besides for a few studies focussing on Columbiformes and Passeriformes, we have limited empirical data on how increased reliance on CEWL at high T_a would reduce the energetic demands for respiratory heat dissipation.

Phylogenetic variation in thermoregulation under hot conditions: ecological and evolutionary consequences

The metabolic cost of heat dissipation seems to vary widely amongst avian orders, and explain most variation in evaporative cooling efficiency. With the exception of the patterns of RMR observed in nightjars, the other four species compared here show elevations in RMR, apparently correlated with phylogenetic variation in the use of gular flutter and/or panting. Similar variation was observed by (Lasiewski and Seymour 1972) (Lasiewski and Seymour, 1972), using four taxa from different orders with varying modes of respiratory EHL, from panting only in a passerine, synchronous panting and gular flutter in a galliform and columbid, and gular flutter in a caprimulgid; all species, expect the caprimulgid, showed elevations in RMR at high T_a.

Although our study was not designed to directly quantify the relative importances of gular flutter versus panting in our study species, we suspect that variation in the relative importance of these processes may explain some of the taxonomic patterns observed here. Calder and Schmidt-Nielson (Calder and Schmidt-Nielsen, 1967) categorised variation in respiratory EHL pathways among avian orders, as 1) a combination of panting and gular flutter, where gular flutter and panting/breathing rates are synchronised [e.g. Strigiformes, Columbiformes and Cuculiformes ((Bartholomew et al., 1968)], 2) strictly gular flutter where the frequency and amplitude of breathing cycles are generally significantly lower, and generally asynchronous, with gular flutter rates [e.g. some Pelicaniformes and some Caprimulgiformes (Bartholomew et al., 1968; Lasiewski and Bartholomew, 1966; Lasiewski and Seymour, 1972)], or 3) panting only [e.g. Passeriformes ((Calder and Schmidt-Nielsen, 1967))]. Bartholomew et al. (1968) further suggested that variation in the two primary modes of gular flutter may have consequences for the energetic costs of ventilating the buccal region, with species that maintain rapid gular flutter rates accompanied by slow breathing rates, Caprimulgiforms, and some Pelicanifroms, achieving very high evaporative cooling efficiencies, compared to species where panting frequency and amplitude is completely synchronised with gular fluttering.

The phylogenetic variation in modes of avian respiratory EHL pathways is interesting, and there is no clear explanation for the dichotomy between gular flutter and/or panting among avian taxa. One potential physiological cost of respiratory EHL (other than the supposed energetic cost) is an elevated risk of alkalosis during severe heat stress (Calder and Schmidt-Nielsen, 1966; Calder and Schmidt-Nielsen, 1968), and at least one field behavioural study demonstrated that reliance on panting could carry fitness costs when birds are exposed to high air temperatures (du Plessis et al., 2012). Some authors have argued that gular

fluttering reduces these risks of alkalosis, compared to panting, where CO_2 levels are likely to be higher over gas-exchange tissues (Schmidt-Nielsen, 1990). The prevalence of gular flutter in phylogenetically older taxa, such as Paleognathae and most basal Neognathae, suggests it is a plesiomorphic trait. This may indicate that the function of the hyoid bone, which ventilates the gular area, was modified in some clades of the Neognathae. Interestingly, Psitticiformes [parrots, which are closely related to Passeriformes, (Hackett et al., 2008) are the only avian taxon in which lingual flutter (movement of tongue) is known to supplement panting to ventilate the buccal area (Bucher, 1981; Bucher, 1985). However, to the best of our knowledge no studies have determined if the latter mode of respiratory EHL is any different in efficiency to panting alone.

In conclusion, substantial phylogenetic variation exists in avian heat tolerance and evaporative cooling capacity, an observation reiterated by our comparative analysis of representatives of five orders. These patterns may have far-reaching implications for the ecology and evolution of birds that routinely have to maintain T_b below environmental temperature. The consequences of this variation for water balance and risk of hyperthermia during very hot weather are strikingly illustrated by the observation that, among the species considered here at T_a ~50°C, rates of EWL expressed in term of body mass loss vary ~2-fold; from an equivalent of 2.3 % Mb h^{-1} in nightjars to 4.6 % Mb h^{-1} in starlings. Moreover, at the same T_a the T_b of starlings is ~3 °C higher than that of nightjars. Quantifying phylogenetic diversity in avian thermal physiology under hot conditions is critical for testing hypotheses regarding physiological adaptation, and predicting vulnerability to the higher T_a and more frequent and intense heat waves associated with anthropogenic climate change (Albright et al.). For instance, the 140-fold (from 0.0003 mW $°C^{-1}$ in nightjars to 0.0417 mW/ °C in starlings)

and 3.6-fold (0.093 g h^{-1} °C^{-1} in nightjars to 0.337 g h^{-1} °C^{-1} in rollers) variation on slopes of RMR and EWL vs T_a, respectively, among the five species suggests that, even within similarly-sized species, the consequences of the predicted 4 °C increase in extreme T_a maxima for the 21st Century will vary substantially. The current phylogenetic sampling of data on avian thermoregulatory patterns in the heat is poor, and a thorough review of the functional role these mechanisms play in the efficiency of evaporative cooling will require more avian taxa from unstudied orders.

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Tables:

Table 1: Physiological variables (mean ± SD, n) related to thermoregulation at the air temperature (T_a) range we tested African cuckoo (*Cuculus gularis*; cuckoo), lilac-breasted roller (*Coracias caudatus*; roller), and Burchell's starling (*Lamprotornis australis*; starling). Minimum values represent lowest value for each individual at T_a < 35°C, or the upper critical limit of thermoneutrality (T_{uc}) for each species. Maximum values where obtained at the highest T_a tested; when n<3, we also show average values at the second highest T_a tested. We further indicate mean values for the highest T_a (n≥3), i.e. T_a = 46°C, shared by the three species.

Physiological variable	cuckoo	roller	starling
Body mass (g)	110.00 ± 0.02 (5)	94.50 ± 8.50	109.10 ± 9.30 (5)
Body temperature (T_b)			
Minimum T_b (°C)	40.04 ± 0.57 (6)	40.69 ± 0.82 (11)	40.65 ± 0.75 (5)
Inflection T_a (°C)	32.8	36.7	34.2
T_b vs T_a slope	0.2790	0.1613	0.1843
Maximum T_b (°C)	44.0 (2)	43.58 ± 0.84 (5)	42.81 ± 1.03 (3)
	43.7 (2)	43.40 ± 0.68 (2)	43.18 (2)
Maximum T_a (°C)	48 (2)	54 (5)	48 (3)
	50 (2)	56 (2)	50 (2)
T_b at onset of panting/gular flutter (°C)	41.56 ± 0.57 (5)	41.31 ± 0.53 (9)	41.46 ± 0.23 (5)
T_a at onset of panting/gular flutter (°C)	40.89 ± 0.79 (5)	41.18 ± 1.94 (9)	35.92 ± 4.45 (5)
Resting metabolic rate (RMR)			
Minimum RMR (W)	0.643 ± 0.198 (5)	0.69 ± 0.13 (8)	1.189 ± 0.311 (5)
T_{uc} (°C)	na	47.5	31
RMR slope (mW °C^{-1})	0.0224	0.0396	0.0417
Maximum RMR (W)	2.773 (1)	1.00 ± 0.24 (4)	1.538 ± 0.215 (3)
	0.955 (1)	1.07 ± 0.17 (2)	1.913 (2)
Max. RMR / Min. RMR	3.06 (1)	1.45 (4)	1.29 (3)
	1.24 (1)	1.55 (2)	1.61 (2)
Evaporative water loss (EWL)			
Minimum EWL (g h^{-1})	0.481 ± 0.144 (6)	0.85 ± 0.38 (8)	1.00 ± 0.67
Inflection T_a (°C)	42.7	44.7	33.3
EWL slope (g h^{-1} °C^{-1})	0.2383	0.3370	0.2306
Maximum EWL (g h^{-1})	2.72 (2)	4.81± (4)	3.33 ± 0.51 (3)
	3.09 (2)	5.41± (2)	4.97 (2)
Max. EWL / Min. EWL	5.66 (2)	5.66 (4)	3.33 (3)
	6.57 (2)	6.36 (2)	4.97 (2)
Minimum EHL/MHP	0.382 ± 0.157 (5)	0.72 ± 0.43 (8)	0.29 ± 0.29
Maximum EHL/MHP	0.80 (1)	3.35 ± 0.75 (4)	1.70 ± 0.280 (3)
	2.13 (1)	3.48 ± 0.85 (2)	1.90 (2)
EHL/MHP vs Ta slope	0.1590	0.1709	0.0704
Shared T_a = 46°C			
T_b (°C)	43.00 ± 0.95 (5)	41.96 ± 1.30 (8)	42.61 ± 0.36 (4)
RMR (W)	0.857 ± 0.111 (4)	0.830 ± 0.333 (8)	1.700 ± 0.378 (4)

EWL (g hr^{-1})	1.88 ± 0.28 (5)	1.86 ± 0.38 (8)	3.40 ± 1.15 (4)
EHL/MHP (W)	1.49 ± 0.24 (4)	1.57 ± 0.32 (8)	1.31 ± 0.20 (4)

Table 2: Mass-specific values for resting metabolic rate (RMR) and evaporative water loss (EWL) related to thermoregulation at the air temperature (T_a) range we tested African cuckoo (*Cuculus gularis*; cuckoo), lilac-breasted roller (*Coracias caudatus*; roller), and Burchell's starling (*Lamprotornis australis*; starling). Minimum values represent lowest value for each individual at T_a < 35°C, or the upper critical limit of thermoneutrality (T_{uc}) for each species. Maximum values where obtained at the highest T_a tested; when n<3, we also show average values at the second highest T_a tested. We also indicate mean values for the highest T_a (n≥3), i.e. T_a = 46°C, shared by the three species.

Physiological variable	**Cuckoo**	**Roller**	**starling**
Resting metabolic rate (RMR)			
Minimum RMR (mW g^{-1})	5.85 ± 1.79 (5)	8.91 ± 1.11 (6)	10.76 ± 1.98 (5)
Maximum RMR (mW g^{-1})	25.37 (1) 8.62 (1)	10.55 ± 3.61 (3) 11.32 (2)	14.15 ± 3.45 (3) 16.39 (2)
Evaporative water loss (EWL)			
Minimum EWL (mg g^{-1} h^{-1})	4.45 ± 1.45 (6)	8.28 ± 2.82 (6)	8.92 ± 5.67 (5)
EWL slope (mg g^{-1} h^{-1} °C^{-1})	2.10	4.03	2.07
Maximum EWL (mg g^{-1} h^{-1})	30.09 (1) 27.14 (1)	52.91 ± 8.95 (3) 57.36 (	30.19 ± 3.36 (3) 42.41 (2)

Table 3: Slopes and upper and lower 95% confidence intervals (CI) for African cuckoos (cuckoo; *Cuculus gularis*, this study), Lilac-breasted rollers (roller; *Coracias caudatus,* this study), Burchell's starlings (starling; *Lamprotornis australis*, this study), laughing doves [dove; *Spilopelia senegalensis* (McKechnie et al. 2016b)], and Freckled nightjars [nightjar; *Caprimulgis tristigma* (O'Connor et al., 2017)] derived from linear mixed effects models. Non-overlapping 95% CI are indicated by different symbols.

Species	RMR (mW °C^{-1})	EWL (g h^{-1} °C^{-1})	EHL/MHP (mW/°C^{-1})	T_b (°C °C^{-1})
Cuckoo	0.0224 [0.0059, 0.0389][a]	0.2383 [0.2030, 0.2736][a]	0.1590 [0.1041, 0.2139] [a]	0.2790 [0.2408, 0.3172][a,b]
Roller	0.0396 [0.0096, 0.0696][a]	0.3370 [0.2802, 0.3938][b]	0.1709 [0.1342, 0.2076] [a]	0.1613 [0.1353, 0.1874][c,d]
Starling	0.0417 [0.0220, 0.0613][a]	0.2306 [0.1900, 0.2712][a]	0.0704 [0.0610, 0.0798][b]	0.1843 [0.1375, 0.2311][b,c,d]
Dove	0.0382 [0.0190, 0.0574][a]	0.1999 [0.1583, 0.2415][a]	0.0803 [0.0687, 0.0919][b]	0.2367 [0.1920, 0.2814][a,b]
Nightjar	0.0003 [-0.0015, 0.0021][b]	0.093 [0.0812, 0.1048][c]	0.224 [0.2044, 0.2436][a]	0.151 [0.1334, 0.1687][d]

Figure legends:

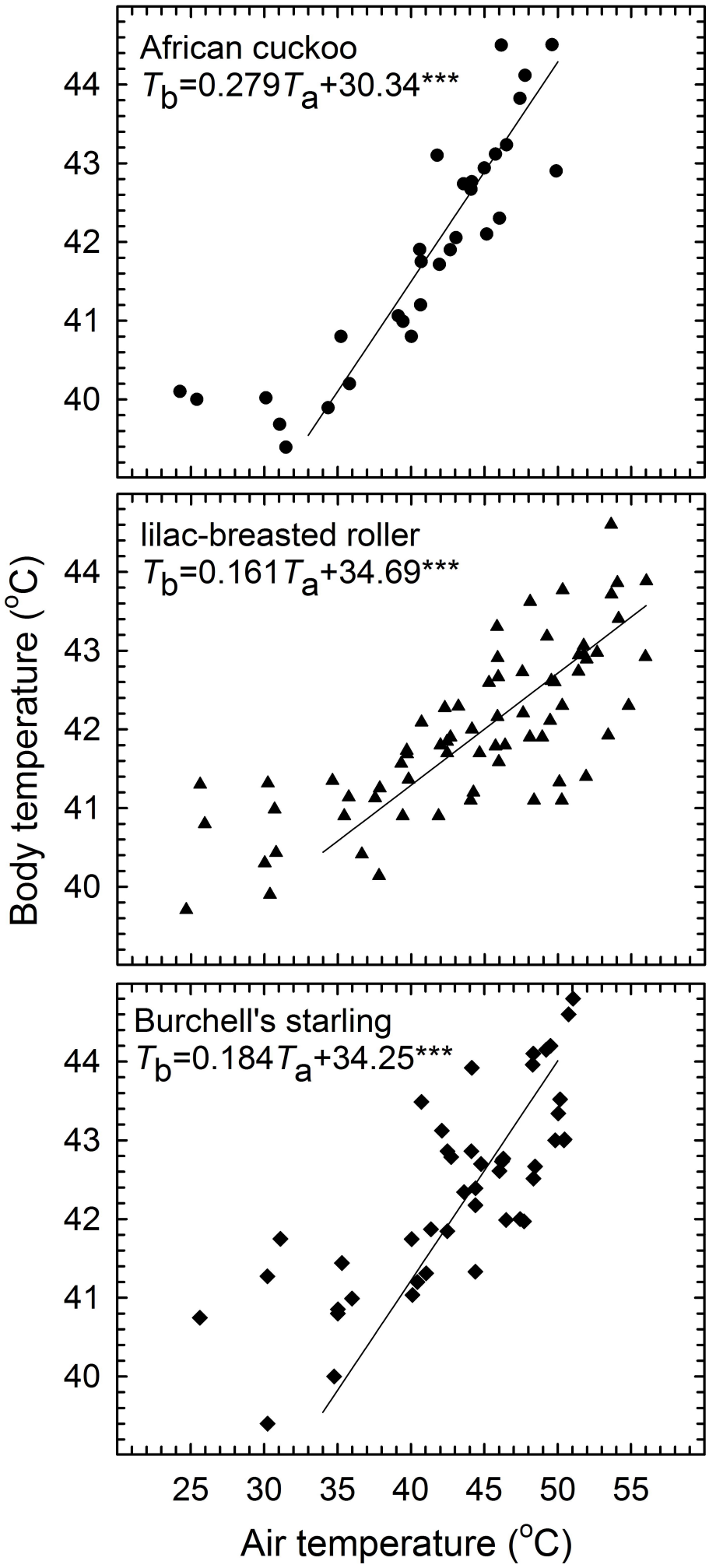
Figure 1: **Body temperature (T_b) in African cuckoos (*Cuculus gularis*; n=6), lilac-breasted rollers (*Coracias caudatus*; n=10), and Burchell's starlings (*Lamprotornis australis*; n=7) as a result of air temperature (T_a).** The trendlines represent relationships between T_b and T_a above an upper inflection T_a (see methods). The slopes and intercepts were calculated using linear mixed-effects models. Significant relationships are represented by asterisks: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

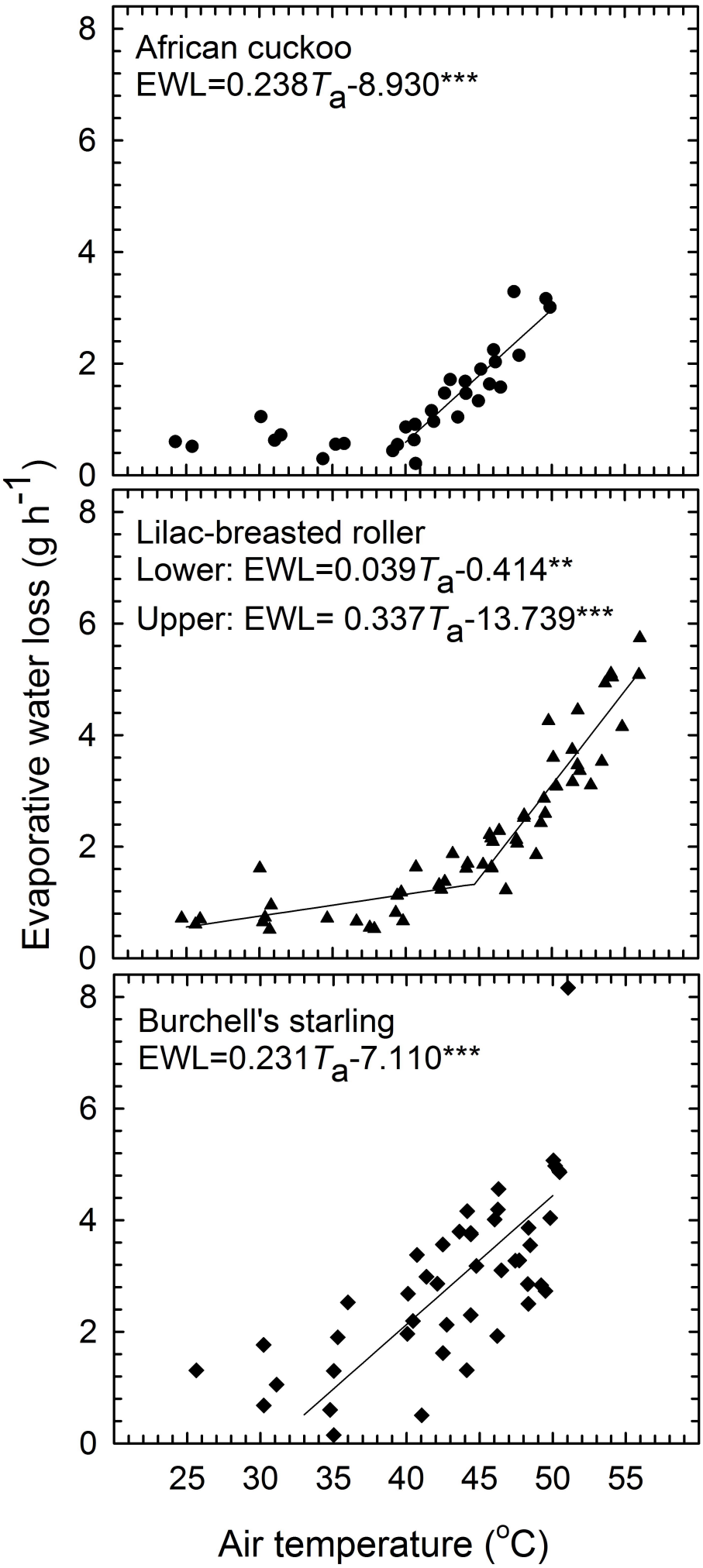
Figure 2: **Resting metabolic rate (RMR) in African cuckoos (*Cuculus gularis*; n=5), lilac-breasted rollers (*Coracias caudatus*; n=10), and Burchell's starlings (*Lamprotornis australis*; n=7) as a result of air temperature (T_a).** The trendlines represent relationships between T_b and T_a above an upper inflection T_a (see methods). The slopes and intercepts were calculated using linear mixed-effects models. Significant relationships are represented by asterisks: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

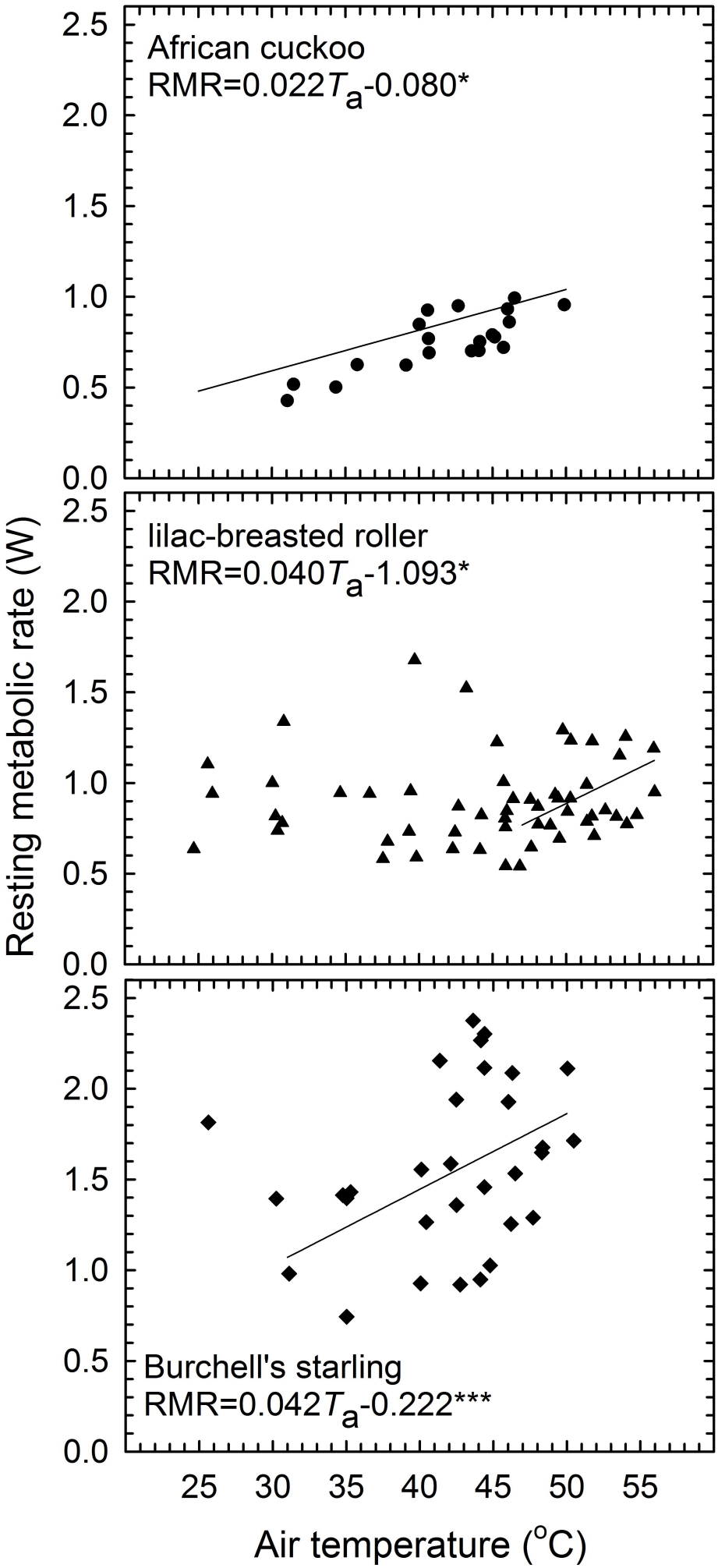
Figure 3: **Evaporative water loss (EWL) in African cuckoos (*Cuculus gularis*; n=6), lilac-breasted rollers (*Coracias caudatus*; n=10), and Burchell's starlings (*Lamprotornis australis*; n=7) as a result of air temperature (T_a).** The trendlines represent relationships between T_b and T_a above an upper inflection T_a (see methods). The slopes and intercepts were calculated using linear mixed-effects models. For lilac-breasted rollers two significant relationships are shown (25 °C < T_a < 44.7 °C; and T_a > 44.7 °C). Significant relationships are represented by asterisks: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

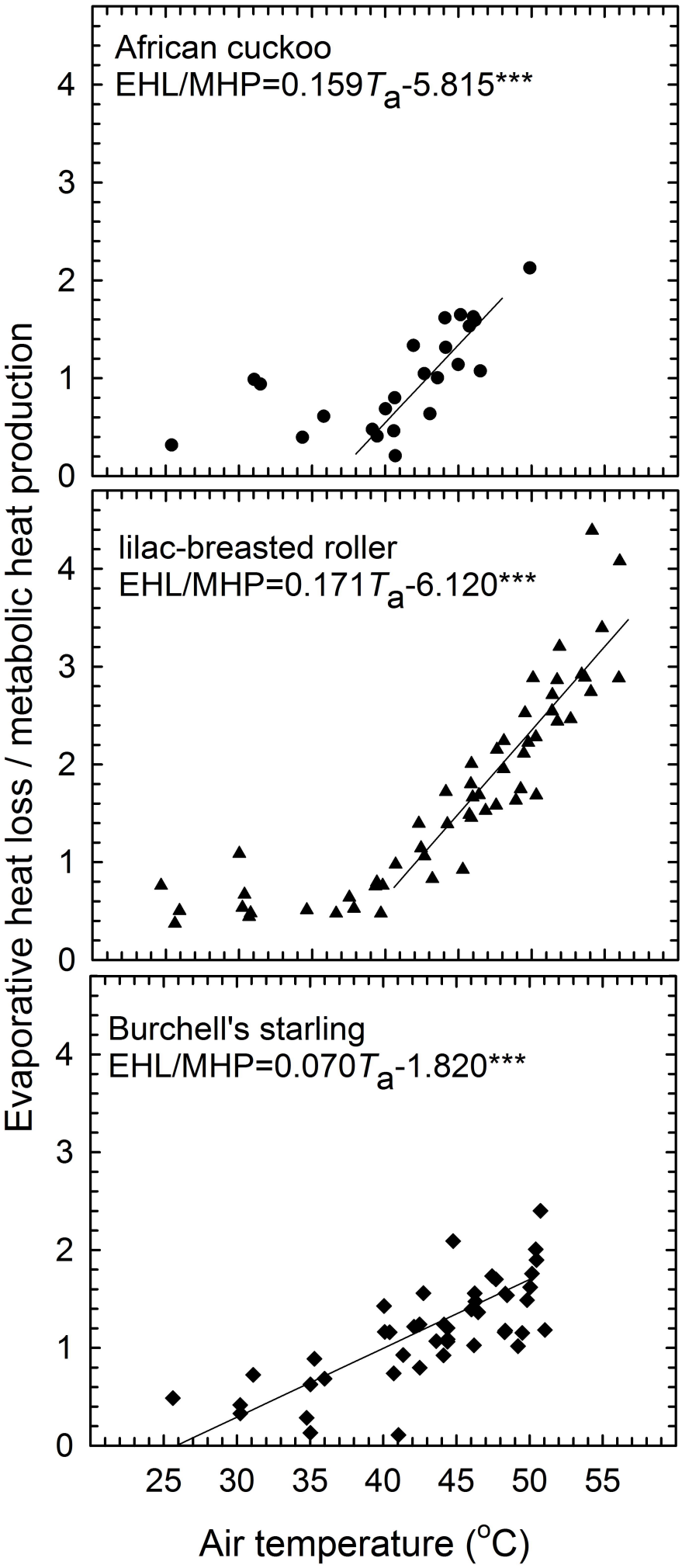
Figure 4: **Ratio of evaporative heat loss (EHL) to metabolic heat production (MHP) in African cuckoos (*Cuculus gularis*; n=6), lilac-breasted rollers (*Coracias caudatus*; n=10), and Burchell's starlings (*Lamprotornis australis*; n=7) as a result of air temperature (T_a).** The trendlines represent relationships between T_b and T_a above an upper inflection T_a (see methods). The slopes and intercepts were calculated using linear mixed-effects models. Significant relationships are represented by asterisks: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

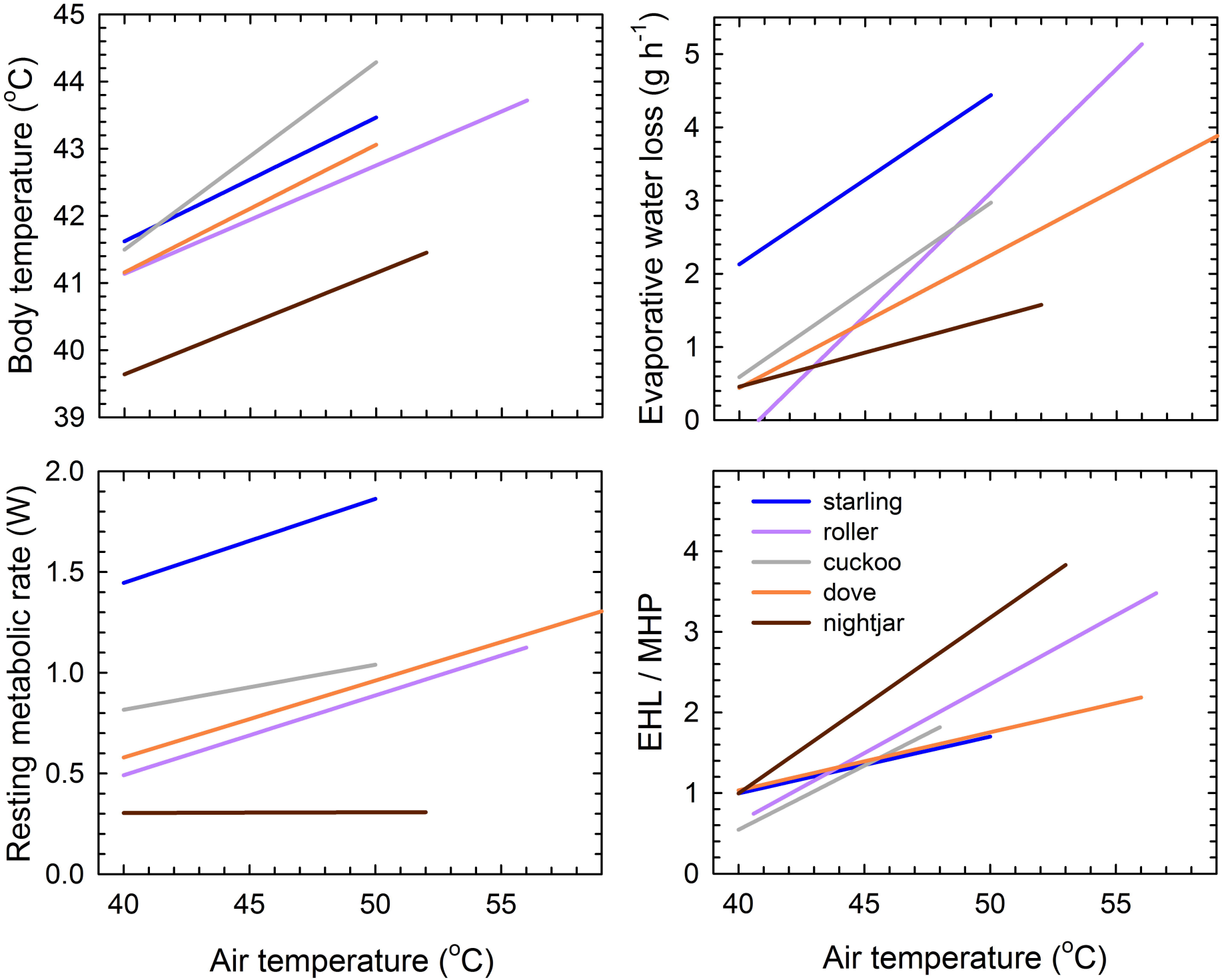
Figure 5: Physiological responses as a result of temperature in five desert species making use of varying modes of respiratory heat dissipation. These include African cuckoos (cuckoo; *Cuculus gularis*, this study) and laughing doves [dove; *Spilopelia senegalensis* (McKechnie et al. 2016b)] make use of gular flutter probably synchronised with panting. Lilac-breasted rollers (roller; *Coracias caudatus,* this study) and Burchell's starlings (starling; *Lamprotornis australis*, this study) make use of panting only. Freckled nightjars [nightjar; *Caprimulgis tristigma* (O'Connor et al. in press)] make use of rapid gular, but probably asynchronously to breathing rates. The slopes and 95% confidence levels of the relationship for each species are displayed in Table 3).


African cuckoo
T_b=0.279T_a+30.34***
lilac-breasted roller
T_b=0.161T_a+34.69***
Burchell's starling
T_b=0.184T_a+34.25***
Body temperature (°C)
Air temperature (°C)
40
41
42
43
44
25
30
35
40
45
50
55


African cuckoo
EWL=0.238T_a-8.930***
Lilac-breasted roller
Lower: EWL=0.039T_a-0.414**
Upper: EWL= 0.337T_a-13.739***
Burchell's starling
EWL=0.231T_a-7.110***
Evaporative water loss (g h^{-1})
Air temperature (°C)
0
2
4
6
8
25
30
35
40
45
50
55


African cuckoo
RMR=0.022T_a-0.080*
lilac-breasted roller
RMR=0.040T_a-1.093*
Burchell's starling
RMR=0.042T_a-0.222***
Resting metabolic rate (W)
Air temperature (°C)
0.0
0.5
1.0
1.5
2.0
2.5
25
30
35
40
45
50
55


African cuckoo
EHL/MHP=0.159T_a-5.815***
lilac-breasted roller
EHL/MHP=0.171T_a-6.120***
Burchell's starling
EHL/MHP=0.070T_a-1.820***
Evaporative heat loss / metabolic heat production
Air temperature (°C)
0
1
2
3
4
25
30
35
40
45
50
55


Body temperature (°C)
45
44
43
42
41
40
39
Evaporative water loss (g h-1)
5
4
3
2
1
0
Resting metabolic rate (W)
2.0
1.5
1.0
0.5
0.0
EHL / MHP
4
3
2
1
0
starling
roller
cuckoo
dove
nightjar
40
45
50
55
Air temperature (°C)
Air temperature (°C)