



# Efficient Evaporative Cooling and Pronounced Heat Tolerance in an Eagle-Owl, a Thick-Knee and a Sandgrouse

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Avian evaporative cooling and the maintenance of body temperature ( $T_b$ ) below lethal limits during heat exposure has received more attention in small species compared to larger-bodied taxa. Here, we examined thermoregulation at air temperatures ( $T_{air}$ ) approaching and exceeding normothermic  $T_b$  in three larger birds that use gular flutter, thought to provide the basis for pronounced evaporative cooling capacity and heat tolerance. We quantified  $T_b$ , evaporative water loss (EWL) and resting metabolic rate (RMR) in the ~170-g Namaqua sandgrouse (*Pterocles namaqua*), ~430-g spotted thick-knee (*Burhinus capensis*) and ~670-g spotted eagle-owl (*Bubo africanus*), using flow-through respirometry and a stepped  $T_{air}$  profile with very low chamber humidities. All three species tolerated  $T_{air}$  of 56–60°C before the onset of severe hyperthermia, with maximum  $T_b$  of 43.2°C, 44.3°C, and 44.2°C in sandgrouse, thick-knees and eagle-owls, respectively. Evaporative scope (i.e., maximum EWL/minimum thermoneutral EWL) was 7.4 in sandgrouse, 12.9 in thick-knees and 7.8 in eagle-owls. The relationship between RMR and  $T_{air}$  varied substantially among species: whereas thick-knees and eagle-owls showed clear upper critical limits of thermoneutrality above which RMR increased rapidly and linearly, sandgrouse did not. Maximum evaporative heat loss/metabolic heat production ranged from 2.8 (eagle-owls) to 5.5 (sandgrouse), the latter the highest avian value yet reported. Our data reveal some larger species with gular flutter possess pronounced evaporative cooling capacity and heat tolerance and, when taken together with published data, show thermoregulatory performance varies widely among species larger than 250 g. Our data for Namaqua sandgrouse reveal unexpectedly pronounced variation in the metabolic costs of evaporative cooling within the genus *Pterocles*.

**Keywords:** Charadriiformes, heat tolerance, hyperthermia, Pterocliiformes, Strigiformes, thermoregulation

## INTRODUCTION

Evaporative cooling and the maintenance of body temperature ( $T_b$ ) below environmental temperature is critical for terrestrial taxa that encounter operative temperatures (Bakken, 1976; Robinson et al., 1976) exceeding normothermic  $T_b$ , or when metabolic heat produced during activity must be dissipated to avoid  $T_b$  reaching lethal limits (e.g., Nilsson and Nord, 2018; Thompson et al., 2018). There is increasing evidence that among endotherms, evaporative cooling has evolved in tandem with organismal traits such as surface water dependence (Czenze et al., 2020) and roost microsite preferences (Maloney et al., 1999; Cory Toussaint and McKechnie, 2012; Czenze et al., in press). Gaining a better understanding of the upper limits to animals' evaporative cooling capacity and their exposure to extreme heat events has taken on new urgency in light of rapid global heating (IPCC, 2021), increasingly frequent heat-related mortality events (Welbergen et al., 2008; Ratnayake et al., 2019; McKechnie et al., 2021b) and large increases in risks of lethal dehydration or hyperthermia predicted for coming decades (Albright et al., 2017; Conradie et al., 2020).

On account of birds being predominantly small and diurnal and making limited use of thermally buffered diurnal refugia such as burrows, avian heat tolerance and evaporative cooling capacity has been the subject of long-standing interest among ecological physiologists (e.g., Dawson, 1954; Lasiewski and Seymour, 1972; Weathers, 1981). Maximum evaporative cooling capacity varies substantially among avian orders (reviewed by McKechnie et al., 2021a). Maximum ratios of evaporative heat loss/metabolic heat production (EHL/MHP) are consistently higher in taxa that use pronounced gular flutter (Dawson and Fisher, 1969; O'Connor et al., 2017) or have the capacity for rapid cutaneous evaporative water loss (Calder and Schmidt-Nielsen, 1967; Marder and Arieli, 1988; McKechnie et al., 2016a) compared to groups that rely on panting as their primary pathway of evaporative heat loss (Dawson, 1954; Weathers, 1981). Whereas fractional increases in EWL are broadly consistent across taxa, the metabolic heat production associated with panting means that evaporative cooling capacity and heat tolerance limits tend to be lower in passerines, which represent > 50% of extant birds, compared to some non-passerine orders (McKechnie et al., 2021a).

The number of species for which evaporative cooling capacity and heat tolerance limits have been quantified under standardised conditions has increased substantially in the last decade, facilitating direct comparisons among taxa (e.g., Whitfield et al., 2015; Smith et al., 2017; Smit et al., 2018). However, the body mass ( $M_b$ ) of most species investigated is <100 g, and much less is known about limits to heat tolerance in larger species. Two notable exceptions include the ~610-g brown-necked raven (*Corvus ruficollis*), in which Marder (1973) reported  $T_b = 44.7^\circ\text{C}$  and EHL/MHP = 1.67 at an air temperature ( $T_{\text{air}}$ ) of  $50^\circ\text{C}$ , and the ~1300-g MacQueen's bustard (*Chlamydotis macqueenii*) which maintains  $T_b < 43^\circ\text{C}$  even at  $T_{\text{air}}$  as high as  $55^\circ\text{C}$  (Tieleman et al., 2002). In the latter study, bustards showed no discernable increase in RMR above a lower critical limit of thermoneutrality of  $28.5^\circ\text{C}$ , but a ~10-fold

increase in evaporative water loss (EWL) above minimum thermoneutral levels.

In light of the limited data available for medium- and large-bodied birds and the negative allometric scaling of variables such as surface area – volume ratio and EWL as a fraction of total body water per unit time, we quantified upper limits to heat tolerance and evaporative cooling capacity in three medium-sized southern African species: Namaqua sandgrouse (*Pterocles namaqua*; ~170 g, Pterocliiformes), spotted thick-knee (*Burhinus capensis*; ~430 g, Charadriiformes) and spotted eagle-owl (*Bubo africanus*; ~670 g, Strigiformes). We employed the same standardised methods of a stepped  $T_{\text{air}}$  profile and very low chamber humidities used in several recent studies (e.g., Whitfield et al., 2015; Smith et al., 2017; Smit et al., 2018). Because these three orders are all known to employ gular flutter (Bartholomew et al., 1968; Thomas and Robin, 1977; Ward, 1990), we expected that maximum EHL/MHP and heat tolerance limits (HTL: maximum  $T_{\text{air}}$  tolerated before onset of severe hyperthermia) are relatively high in these species.

## MATERIALS AND METHODS

### Study Site and Species

The thermoregulatory capacity of three species representing three orders was assessed during the 2018–2019 austral summer (2 December – 4 February) at the 76-km<sup>2</sup> Black Mountain Mine Conservation Area ( $29^\circ18'S$ ,  $18^\circ51'E$ ) near Aggeneys, Northern Cape, South Africa. The Black Mountain Mine Conservation Area is situated within the Koa River Valley, which consists predominantly of red sand dunes with scattered shrubs (*Rhigozum trichotomum*) and large-seeded grasses (*Brachiaria glomerata*, *Stipagrostis amabilis*, *Stipagrostis ciliata*, and *Stipagrostis brevifolia*). In parts of the study site, the dunes are separated by unvegetated gravel plains. The climate is arid with mean annual precipitation of ~100 mm and daily maximum  $T_{\text{air}}$  that ranged from  $26.4$  to  $40.3^\circ\text{C}$  during the study period (Kemp et al., 2020). The study species varied in  $M_b$ , as well as foraging behaviour, water dependence and activity phase (Table 1). We captured eight Namaqua sandgrouse (hereafter, sandgrouse), 10 spotted thick-knees (hereafter, thick-knees), and seven spotted eagle-owls (hereafter, eagle-owls). Namaqua sandgrouse were captured at a regularly frequented drinking spot using mist nets. Eagle-owls were captured at night opportunistically using a modified spring trap baited with two live mice. Thick-knees were captured at night using a hand-held net and torch.

Captured birds were placed in clean cloth bags and transported (approximately 45-min trip by vehicle) to an animal holding room where they were kept indoors in individual cages (0.8 m<sup>3</sup>). Individuals were kept in cages for no more than 12 h and provided with an *ad libitum* supply of water. In the case of the eagle-owls and thick-knees, experimental procedures took place the morning following capture,  $\geq 10$  h after birds last fed. In the case of sandgrouse, the time between capture and being placed in respirometry chambers was ~3 h.

**TABLE 1** | Taxonomy, body mass, activity period, major diet and water dependence of the three study species.

Species	Order/Family	Body mass (g) (sample size) [range]	Activity period	Diet	Drinks?
Namaqua Sandgrouse <i>Pterocles namaqua</i>	Pteroclitiformes/Pteroclitidae	165.8 ± 17.2 (8) [130–185]	Diurnal	Seeds	Yes
Spotted Thick-knee <i>Burhinus capensis</i>	Charadriiformes/Burhinidae	434.3 ± 34.4 (10) [362–484]	Nocturnal	Invertebrates	Yes
Spotted Eagle-Owl <i>Bubo africanus</i>	Strigiformes/Strigidae	667.9 ± 77.1 (7) [570–775]	Nocturnal	Vertebrates/invertebrates	Yes

Body masses were obtained by weighing birds immediately following capture, and activity, diet and drinking behaviour were obtained from species accounts in Hockey et al. (2005).

## Air and Body Temperature

A temperature-sensitive passive integrated transponder (PIT) tag (BioTherm13, Biomark, Boise, ID, United States) injected into each individual's abdominal cavity measured  $T_b$ . We calibrated these tags in a circulating water bath (model F34; Julabo, Seelbach, Germany) over a 35–50°C range against a thermistor probe (TC-100; Sable Systems, Las Vegas, NV, United States). We placed PIT-tagged individuals in a metabolic chamber placed next to an antenna connected to a transceiver system (HPR+; Biomark). The PIT tags we used for this study were from the same batch as the 23 tags calibrated by Freeman et al. (2020), who found that measured values deviated from actual values by  $0.28 \pm 0.23^\circ\text{C}$ . We inserted a thermistor probe (TC-100; Sable Systems) sealed with a rubber grommet through the approximate centre of the lid of each metabolic chamber to measure  $T_{\text{air}}$  during the gas exchange measurements.

## Gas Exchange

We measured EWL and carbon dioxide production ( $\dot{V}_{\text{CO}_2}$ ) using an open flow-through respirometry system. The metabolic chamber consisted of either a 12.8-L (sandgrouse) or 60-L (eagle owl and thick-knee) air-tight plastic container (previously shown to not adsorb water vapour; Whitfield et al., 2015). Plastic mesh platforms were placed in the metabolic chambers to ensure individuals were elevated at least ~10 cm above a ~1 cm layer of mineral oil. These plastic chambers were situated within a temperature-controlled custom-built aluminium box (~640 L). The  $T_{\text{air}}$  within the chamber was controlled via a Peltier device (AC-162 Thermoelectric Air Cooler; TE Technology, Traverse City, MI, United States) connected to a digital controller (TC-36-25-RS485 Temperature Controller; TE Technology) and mounted to the side of the box.

Atmospheric air was supplied by an oil-free compressor and passed through a membrane dryer (Champion CMD3 air dryer and filter; Champion Pneumatic, Princeton, IL, United States) to scrub water vapour before being split into baseline and experimental channels. A needle valve (Swagelok, Solon, OH, United States) regulated flow rate in the baseline channel and a mass flow controller (MC50 SLP, Alicat Scientific Inc., Tucson, AZ, United States), calibrated using a soap-bubble flow meter (Gilibator-2; Sensidyne, St. Petersburg, FL, United States), regulated flow rates in the experimental channel. To maximise air mixing, and minimise any potential convective cooling at higher flow rates, we positioned the air inlet near

the top of the metabolic chamber with an elbow joint facing upward and the air outlet below the mesh platform. Flow rates ( $13\text{--}65\text{ L min}^{-1}$ ) were adjusted to maintain humidity at a dewpoint  $< -5^\circ\text{C}$  within the chamber, depending on  $T_{\text{air}}$ ,  $M_b$  and individual behaviour, while still allowing for the accurate measurements of differences in water vapour and  $\text{CO}_2$  between incurrent and excurrent air. A respirometry multiplexer (model MUX3-110118M, Sable Systems) in manual mode and an SS-3 Sub-sampler (Sable Systems) subsampled excurrent air from the baseline and chamber air. Subsampled air was pulled through a  $\text{CO}_2/\text{H}_2\text{O}$  analyser (model LI-840A; LI-COR, Lincoln, NE, United States). The  $\text{CO}_2/\text{H}_2\text{O}$  analyser was regularly zeroed using nitrogen, and spanned for  $\text{CO}_2$  using a certified calibration gas ( $\text{CO}_2$  concentration = 2,000 ppm; Afrox, South Africa). We regularly zeroed the  $\text{H}_2\text{O}$  sensor of the LI-840A using nitrogen and spanned it using air with a known dewpoint produced by a dewpoint generator (DG-4; Sable Systems). The system's tubing was Bev-A-Line IV tubing (Thermoplastic Processes Inc.). An analogue-digital converter (model UI-3; Sable Systems) digitised voltage outputs from the thermistor probes and analysers, which were recorded every 5 s using Expedata software (Sable Systems).

## Experimental Protocol

Following Czenze et al. (2020) we placed individuals in chambers prior to measurements to habituate. Before measurements commenced, individuals were held without food for at least 1 h in the metabolic chamber, and together with the preceding period of fasted captivity ensured birds were likely postabsorptive when data collection started (Karasov, 1990).

Measurements took place during the day and individuals were exposed to a stepped series of progressively higher  $T_{\text{air}}$  values over which  $\dot{V}_{\text{CO}_2}$  and EWL were recorded. We started measurements with a baseline air subsample until water and  $\text{CO}_2$  readings were stable (~5 min). Chamber excurrent air was then subsampled once  $T_{\text{air}}$  had stabilised at the target value. We recorded data at this  $T_{\text{air}}$  until  $\dot{V}_{\text{CO}_2}$  and  $\text{H}_2\text{O}$  traces were stable for at least 10 min, with individuals spending approximately 20–25 min at the target  $T_{\text{air}}$  before we switched back to baseline air for another 5 min. Trials began at  $28^\circ\text{C}$  with  $4^\circ\text{C}$  increases until  $40^\circ\text{C}$  and then  $2^\circ\text{C}$  increases until a maximum of  $60^\circ\text{C}$  (the maximum  $T_{\text{air}}$  our thermistors could accurately measure). We monitored individuals continuously using an infrared video camera. Individuals were removed from the chamber when they reached their thermal endpoints [i.e., loss of coordination or balance, sudden and rapid decrease in EWL or resting metabolic

rate (RMR), rapid and uncontrolled increase in  $T_b$  to values  $>45^\circ\text{C}$ ] or displayed sustained escape behaviours like agitated jumping. In the case of the sandgrouse, measurements were terminated at  $T_{\text{air}}$  slightly above  $60^\circ\text{C}$ . Once an individual was removed from the chamber we quickly dabbed its underbelly with ethanol to facilitate rapid cooling and held it under chilled air produced by an air conditioner. Once the individual's  $T_b$  was stable ( $40\text{--}42^\circ\text{C}$ ), we offered it water using a syringe, and placed it back in its cage to rest, with *ad libitum* water available. The bird was later released at the site of capture. This experimental protocol has been used previously on birds and bats and individuals recaptured several days or weeks post-release showed no adverse effects (Kemp and McKechnie, 2019; Czenze et al., in press).

## Data Analysis

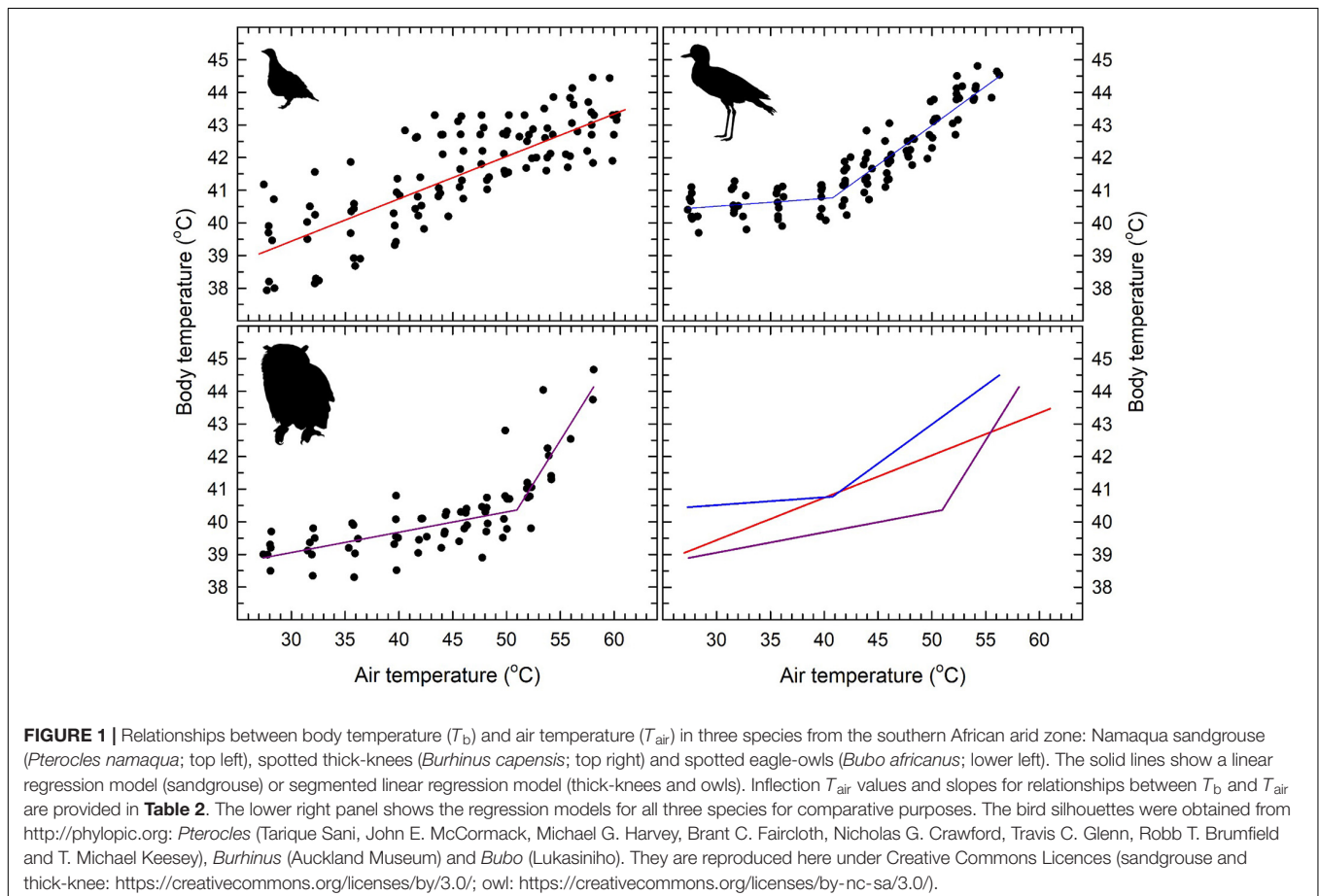
We used the R package *segmented* (Muggeo, 2008) to perform a broken stick regression analysis in R 3.5.2 (R Core Team, 2018) to identify inflection points for EWL, RMR,  $T_b$ , and EHL/MHP. To determine whether a broken-stick regression fit the data better than a simple linear model, we compared broken-stick models against generalised mixed-effect models created in R package *nlme* (Pinheiro et al., 2009) using ANOVA. If we retained a broken stick regression and significant inflections occurred, we analysed data below and above inflection points separately when

estimating slopes for the relationships of EWL, RMR, EHL/MHP, and  $T_b$  as functions of  $T_{\text{air}}$ . We created general linear mixed-effect models for each species using the R package *nlme* to test for an effect of  $T_{\text{air}}$  on each response variable above inflection points. We accounted for the repeated measures design of our study (i.e., measurements at multiple  $T_{\text{air}}$  values per individual) by including individual identity as a random factor in all analyses. Initial models contained  $T_{\text{air}}$ ,  $M_b$ , and the  $T_{\text{air}}:M_b$  interaction and model selection was performed using the “dredge” function in the *MuMIn* package (Bartoń, 2013). We selected the model with the highest rank among competing models using Akaike weights and Akaike information criterion values corrected for small sample size (AICc) (Burnham and Anderson, 2002). Body mass did not emerge as an important predictor for any of the response variables of any species. We assessed significance at  $\alpha < 0.05$  and values are presented as mean  $\pm$  SD.

## RESULTS

### Body and Air Temperature

Normothermic  $T_b$  at thermoneutrality varied by  $\sim 1.4^\circ\text{C}$  among species, from  $39.1 \pm 0.4$  ( $^\circ\text{C}$ ) in eagle-owls to  $40.5 \pm 0.4$  ( $^\circ\text{C}$ ) in thick-knees (Figure 1 and Table 2). All three species showed significant increases in  $T_b$  above normothermic levels when





**TABLE 2 |** Variables related to interactions between body temperature, metabolic heat production and evaporative heat dissipation as functions of air temperature in three species from the arid zone of southern Africa, quantified using flow-through respirometry: Namaqua sandgrouse (*Pterocles namaqua*), spotted thick-knees (*Burhinus capensis*) and spotted eagle-owls (*Bubo africanus*).

	Namaqua sandgrouse	Spotted thick-knee	Spotted eagle-owl
Body mass (g)	165.8 ± 17.2 (8)	434.3 ± 34.4 (10)	667.9 ± 77.1 (7)
<b>Body temperature</b>			
Min. $T_b$ (°C)	39.4 ± 1.2 (8)	40.5 ± 0.4 (9)	39.1 ± 0.4 (6)
Inflection $T_{air}$ (°C)	N/A	40.5 ± 0.05 (10)	51.1 ± 0.05 (7)
$T_b$ versus $T_{air}$ slope (°C)	0.13 <sup>a</sup>	0.24	0.53
Max. $T_b$ (°C)	43.2 ± 0.8 (7)	44.3 ± 0.4 (3)	44.2 (2)
Max. $T_{air}$ (°C)	60 (7)	56 (3)	58 (3) 60 (1)
$T_b$ at onset of gular flutter (°C)	41.5 ± 1.4 (9)	41.9 ± 0.4 (9)	39.4 ± 0.6 (5)
$T_{air}$ at onset of gular flutter (°C)	42.5 ± 4.7 (9)	44.5 ± 2.8 (9)	36.7 ± 4.1 (5)
95th percentile $T_b > T_{air}$ (°C)	42.6	41.2	40.8
<b>Resting metabolic rate</b>			
Min. RMR (W)	0.83 ± 0.14 (8)	2.03 ± 0.34 (3)	2.38 ± 0.55 (7)
$T_{uc}$ (°C)	N/A	47.3 ± 0.01 (10)	49.2 ± 0.02 (7)
RMR slope (mW °C <sup>-1</sup> )	N/A	417.6	314.0
Max. RMR (W)	1.14 ± 0.18 (8)	5.61 ± 1.56 (3)	4.49 ± 1.99 (3) 5.16 (1)
Max. RMR/min. RMR	1.37	2.76	1.96
<b>Evaporative water loss</b>			
Min. EWL (g h <sup>-1</sup> )	1.01 ± 0.66 (8)	1.83 ± 0.57 (10)	1.79 ± 0.65 (7)
Inflection $T_{air}$ (°C)	37.9 ± 1.70 (8)	42.7 ± 0.55	37.3 ± 1.55 (7)
EWL slope (g h <sup>-1</sup> °C <sup>-1</sup> )	0.28	1.51	0.74
Max. EWL (g h <sup>-1</sup> )	7.48 ± 1.90 (7)	23.52 ± 11.35 (5)	14.04 (2)
Max. EWL/min. EWL	7.41	12.85	7.80
Min. EHL/MHP	0.66 ± 0.44 (8)	0.56 ± 0.17 (10)	0.52 ± 0.2 (7)
EHL/MHP inflection $T_{air} - T_b$ (°C)	-0.2 ± 0.04 (8)	-2.3 ± 1.54 (10)	-5.2 ± 1.25 (7)
EHL/MHP slope (°C <sup>-1</sup> )	0.22	0.20	0.14
Max. EHL/MHP	5.49 ± 1.74 (7)	3.15 ± 0.79 (9)	2.75 ± 0.8 (6)

$T_b$ , body temperature;  $T_{air}$ , air temperature; RMR, resting metabolic rate; EWL, evaporative water loss; EHL, evaporative heat loss; MHP, metabolic heat production; Max., maximum; Min., minimum. Values are presented as mean ± SD, with sample sizes in parentheses. Unless otherwise indicated, slopes are for relationships at  $T_{air}$  values above listed  $T_{air}$  inflection points. In some cases, single values are listed if they were substantially higher than averages.

<sup>a</sup>No inflection occurred and slope is for  $T_b$  over the entire range of  $T_{air}$ .

exposed to higher  $T_{air}$  (Figure 1). In thick-knees and eagle-owls, significant inflections above which  $T_b$  increased rapidly occurred at  $T_{air} = 40.7^\circ\text{C}$  and  $T_{air} = 50.9^\circ\text{C}$ , respectively (Table 2 and Figure 1). At  $T_{air}$  values above these inflection points,  $T_b$  increased significantly ( $t = 16.94$ ,  $P < 0.001$  and  $t = 7.36$ ,  $P < 0.001$ , respectively) and in a linear fashion in thick-knees and eagle-owls. In sandgrouse, on the other hand, no inflection was evident and a linear model provided the best fit for the relationship between  $T_b$  and  $T_{air} \geq 32.5^\circ\text{C}$  [the value we assumed to represent this species' lower critical limit of thermoneutrality (Figure 2)]. Over this  $T_{air}$  range,  $T_b$  increased significantly ( $t = 18.53$ ,  $P < 0.001$ ) to a maximum of  $43.2 \pm 0.8^\circ\text{C}$  ( $n = 7$ ). Mean maximum  $T_b$  values at very high  $T_{air}$  varied among the three species from  $43.2^\circ\text{C}$  to  $44.3^\circ\text{C}$  (Table 2). Heat tolerance limits (HTL, i.e., maximum  $T_{air}$  tolerated before thermal endpoints reached) varied from  $56^\circ\text{C}$  in thick-knees to  $60^\circ\text{C}$  in eagle-owls and Namaqua sandgrouse (Table 2), although HTL for the latter species may be even higher in some individuals.

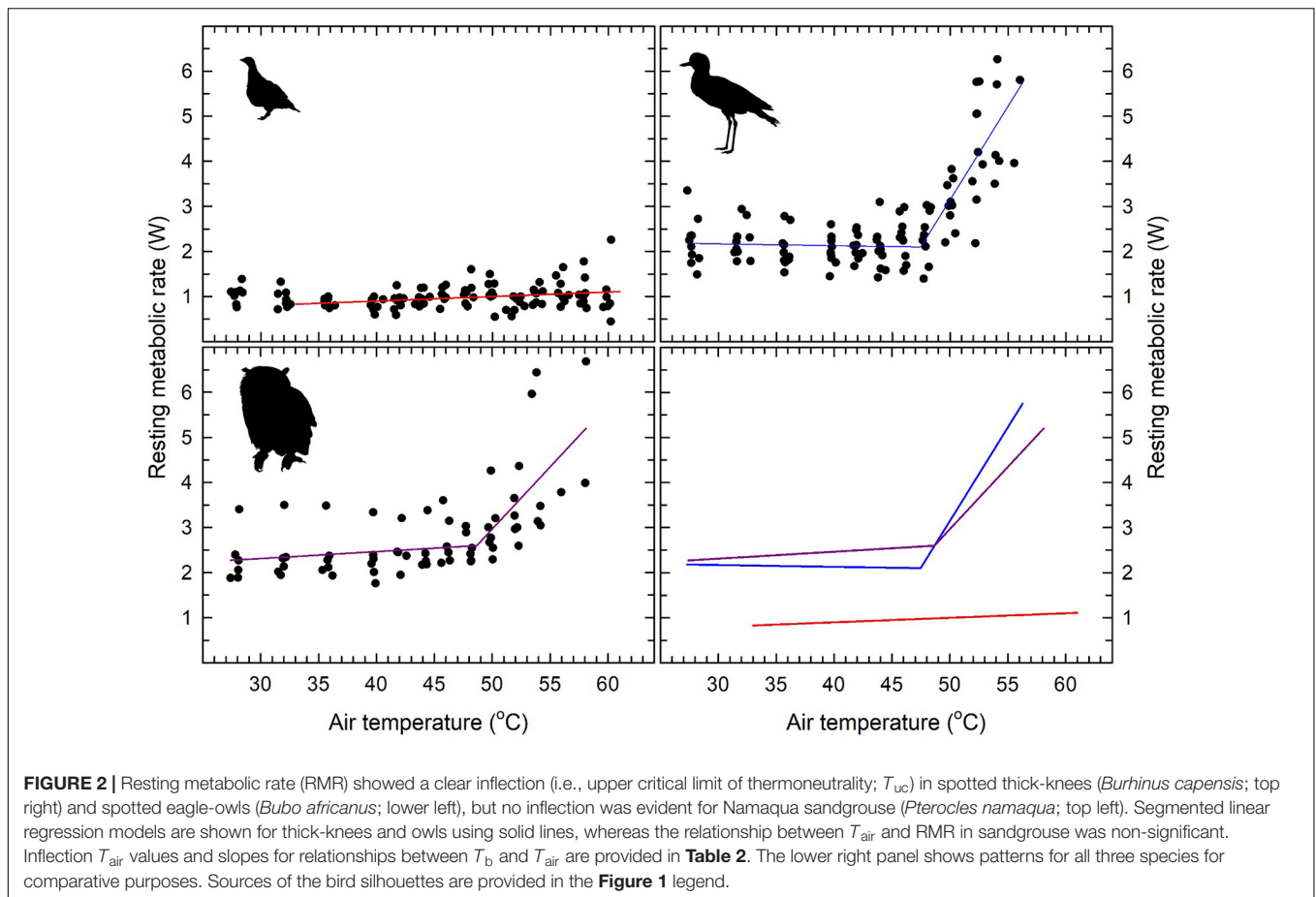
## Resting Metabolic Rate

Minimum thermoneutral RMR values varied from 0.83 W in Namaqua sandgrouse to 2.38 W in the eagle-owl (Table 2). Namaqua sandgrouse did not show a significant inflection in

RMR, which did not vary significantly with  $T_{air}$  (Figure 2). In contrast, significant inflections in RMR were evident for both thick-knees and eagle owls (Table 2), above which linear increases in RMR were significant ( $t = 9.15$ ,  $P < 0.001$  and  $t = 4.1$ ,  $P < 0.001$ , respectively; Figure 2). These inflection  $T_{air}$  values, which correspond to the upper critical limits of thermoneutrality ( $T_{uc}$ ), were  $T_{air} = 47.3^\circ\text{C}$  in thick-knees and  $T_{air} = 49.2^\circ\text{C}$  in eagle-owls. Maximum RMR at the highest  $T_{air}$  achieved was equivalent to 137%, 196%, and 276% of minimum thermoneutral values in Namaqua sandgrouse, eagle-owls and thick-knees, respectively (Figure 2). Mass-specific maximum RMR in thick-knees was ~85% higher compared to sandgrouse or eagle-owls (Table 3).

## Evaporative Water Loss

Gular flutter commenced at  $T_{air}$  between  $36.7^\circ\text{C}$  and  $44.5^\circ\text{C}$  and  $T_b$  between  $39.4^\circ\text{C}$  and  $42.4^\circ\text{C}$  (Table 2). Minimum EWL varied nearly 2-fold from  $1.01 \text{ g h}^{-1}$  in Namaqua sandgrouse to  $1.83 \text{ g h}^{-1}$  in thick-knees (Table 2 and Figure 3). All species showed clear inflection points above which EWL increased rapidly and linearly; these varied from  $T_{air} = 33.9^\circ\text{C}$  in Namaqua sandgrouse to  $T_{air} = 42.8^\circ\text{C}$  in thick-knees (Table 1 and Figure 3). Increases in EWL were significant for sandgrouse ( $t = 19.94$ ,  $P < 0.001$ ),



**TABLE 3** | Mass-specific resting metabolic rate (RMR) and evaporative water loss (EWL) at high air temperature in three species from the arid zone of southern African: Namaqua sandgrouse (*Pterocles namaqua*), spotted thick-knees (*Burhinus capensis*) and spotted eagle-owls (*Bubo africanus*).

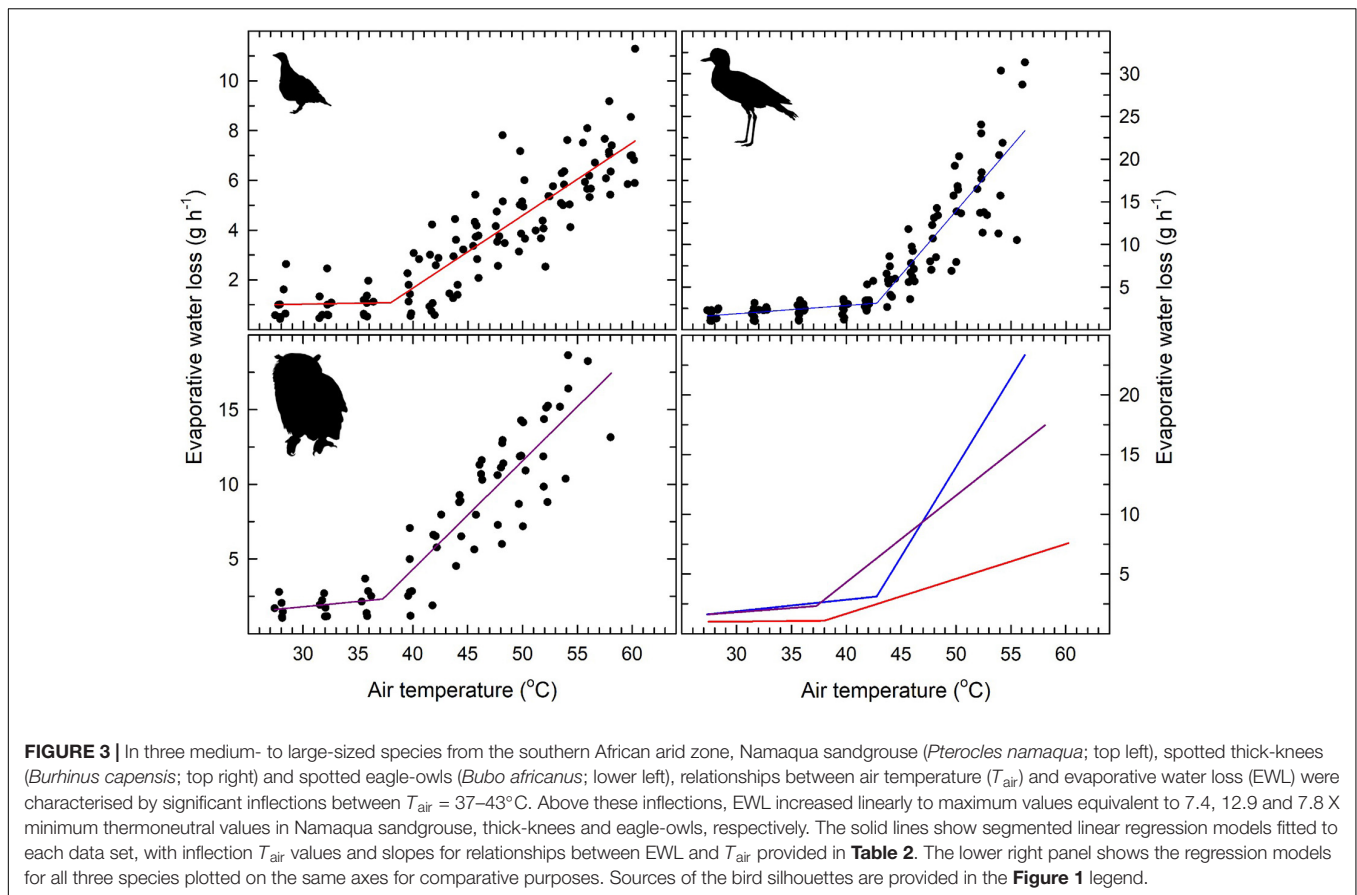
	Namaqua sandgrouse	Spotted thick-knee	Spotted eagle-owl
<b>RMR</b>			
Min. RMR ( $mW g^{-1}$ )	$5.06 \pm 0.78$ (8)	$4.69 \pm 0.55$ (9)	$3.57 \pm 0.74$ (7)
RMR slope ( $mW g^{-1} \text{ } ^\circ\text{C}^{-1}$ )	N/A	0.93	0.58
Max. RMR ( $mW g^{-1}$ )	$6.96 \pm 1.84$ (8)	$12.16 \pm 3.1$ (3)	$7.07 \pm 3.53$ (3) 9.06
<b>EWL</b>			
Min. EWL ( $mg h^{-1} g^{-1}$ )	$6.5 \pm 5.3$ (8)	$4.2 \pm 1.2$ (10)	$2.7 \pm 1.12$ (7)
EWL slope ( $mg h^{-1} g^{-1} \text{ } ^\circ\text{C}^{-1}$ )	1.8	3.4	1.39
Max. EWL ( $mg h^{-1} g^{-1}$ )	$45.2 \pm 10.8$ (7)	$51.0 \pm 24.2$ (3)	$26.05 \pm 13.5$ (6)

RMR, resting metabolic rate, EWL, evaporative water loss, EHL, evaporative heat loss, MHP, metabolic heat production, Max., maximum, Min., minimum. Values are presented as mean  $\pm$  SD, with sample sizes in parentheses.

thick knees ( $t = 13.67$ ,  $P < 0.001$ ), and eagle owls ( $t = 19.59$ ,  $P < 0.01$ ). Maximum rates of EWL ranged from  $7.48 g h^{-1}$  in Namaqua sandgrouse to  $23.52 g h^{-1}$  in thick-knees, with the highest mass-specific values in thick-knees (**Table 3**). The fractional increase in EWL (i.e., the ratio of maximum EWL to minimum EWL) ranged from  $\sim 7.41$  for Namaqua sandgrouse to  $\sim 12.85$  for thick-knees (**Table 2**).

The relationships between evaporative heat loss (EHL)/metabolic heat production (MHP) and  $T_{air}-T_b$  gradients (**Figure 4**), were characterised by significant inflections for all

species (**Table 2**). Above these inflection points, EHL/MHP increased linearly with increasing  $T_{air}-T_b$ , with relationships significant for sandgrouse ( $t = 15.63$ ,  $P < 0.001$ ), thick knees ( $t = 11.62$ ,  $P < 0.001$ ) and eagle owls ( $t = 11.30$ ,  $P < 0.001$ ). Maximum evaporative cooling efficiency (i.e., maximum EHL/MHP) ranged from 2.75 for eagle-owls to 5.49 in Namaqua sandgrouse (**Figure 4**). However, these EHL/MHP values were calculated assuming RER = 0.71 (i.e., lipid catabolism), and could be higher if individuals used carbohydrates (i.e., RER = 1.00). In this case, maximum EHL for eagle-owls and



Namaqua sandgrouse would shift upward to 3.66 and 7.30, respectively. Visual inspection of the data in **Figure 4**, however, does not support this possibility. Theoretically, evaporative heat loss and metabolic heat production should be equal (i.e., EHL/MHP = 1.0) when  $T_{air} = T_b$ . Currently EHL/MHP = 1.0 occurs at approximate  $T_{air} - T_b$  values of  $-1.5$ ,  $-2.0$ , and  $-4.5^\circ\text{C}$  for thick-knees, eagle-owls, and Namaqua sandgrouse, respectively. Recalculating EHL/MHP using  $RER > 0.71$  would shift these values even further below  $T_{air} - T_b = 0$ , suggesting our assumption that  $RER = 0.71$  is likely correct.

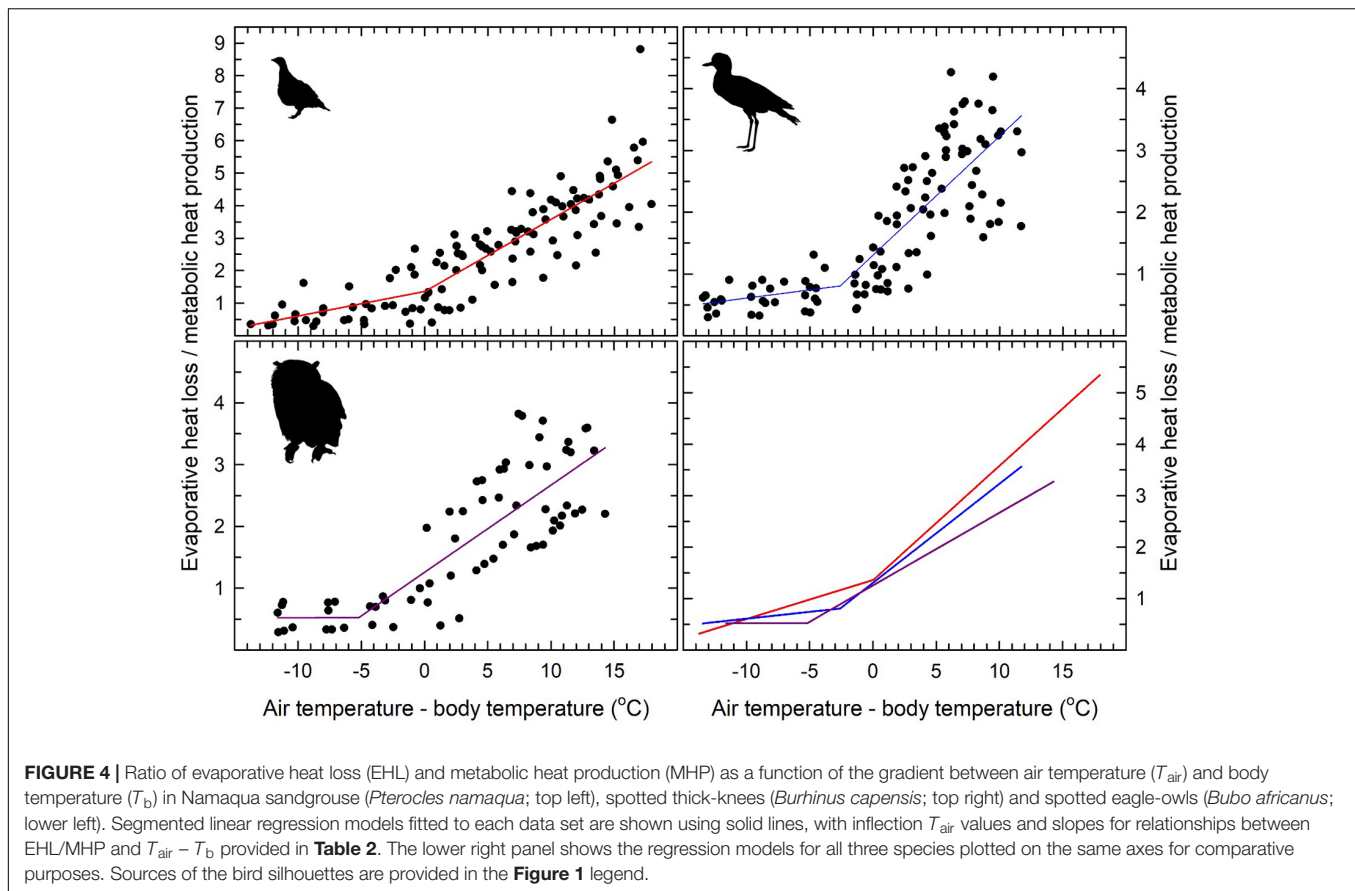
## DISCUSSION

In the three species we examined here, rapid increases in EWL facilitated the defence of  $T_b$  below lethal limits even at  $T_{air} > 55^\circ\text{C}$ . Although these experimental  $T_{air}$  values are well above the range our study species ever experience naturally, our data provide insights into the avoidance of lethal hyperthermia at midday operative temperatures in unshaded microsites, where intense solar radiation may result in microclimates substantially hotter than  $T_{air}$  alone (Bakken, 1976; Robinson et al., 1976). Together with maximum EHL/MHP values of 2.8–5.5, the high heat tolerance limits of these species support the notion that gular flutter provides the basis for pronounced evaporative cooling and heat tolerance, although quantifying the contribution of gular

flutter was precluded here by us not partitioning respiratory and cutaneous EWL. We found less support for the idea that patterns of thermoregulation at high  $T_{air}$  remain similar within genera and families, with patterns of thermoregulation in *P. namaqua* and *B. africanus* differing to varying extents from those of previously-studied sandgrouse and owls, respectively. We discuss these differences in more detail below.

## Patterns of Thermoregulation

Relationships between  $T_b$  and  $T_{air}$  conformed to typical avian patterns in thick-knees and eagle-owls, although the inflection  $T_{air}$  for eagle-owls is well above the range of  $30\text{--}40^\circ\text{C}$  typical of most species investigated to date (McKechnie et al., 2017; McWhorter et al., 2018; Smit et al., 2018; Czenze et al., 2020). The eagle-owl inflection is also substantially higher than those reported for two smaller owls;  $T_{air} = 37.3^\circ\text{C}$  in 101-g western screech-owls (*Megascops kennicottii*) and  $T_{air} < 30^\circ\text{C}$  in 40-g elf owls (*Micrathene whitneyi*; Talbot et al., 2018). In contrast to the thick-knees and eagle-owls, no significant inflection occurred in the relationship between  $T_b$  and  $T_{air}$  in Namaqua sandgrouse, a pattern qualitatively similar to those reported for *P. namaqua* and double-banded sandgrouse (*P. bicinctus*) held in outdoor enclosures during summer in the Namib Desert (Thomas and Maclean, 1981), as well as black-bellied sandgrouse (*P. orientalis*) at  $T_{air}$  between 10 and  $45^\circ\text{C}$  (Hinsley et al., 1993).



Maximum  $T_{\text{b}}$  at thermal endpoints is within the range documented for non-passerines (McKechnie et al., 2021a), with values  $>44^{\circ}\text{C}$  for thick-knees and eagle-owls near the upper end of this range. The heat tolerance limits of all three species are higher than those of most non-passerines and all passerines, with the values of  $58^{\circ}\text{C}$  and  $60^{\circ}\text{C}$  in eagle-owls and Namaqua sandgrouse, respectively, comparable to those of some caprimulgids and columbids, the avian taxa with the highest documented heat tolerances (Marder and Arieli, 1988; O'Connor et al., 2017; Talbot et al., 2017).

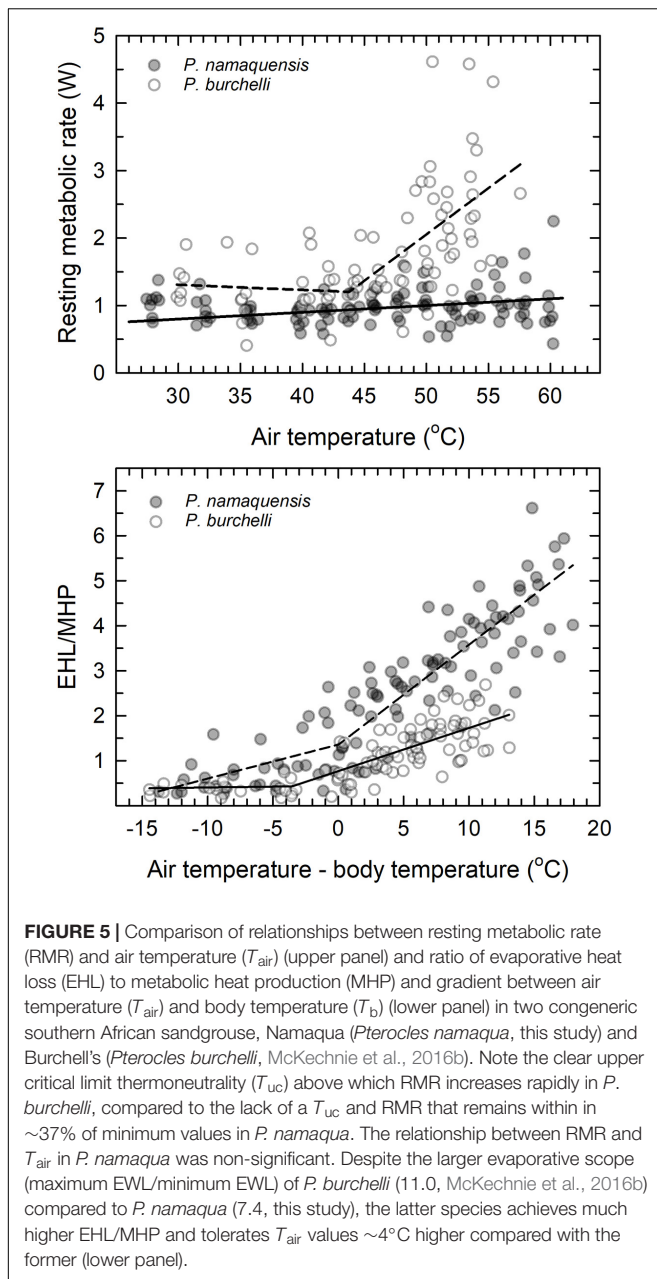
The linear increases of EWL with  $T_{\text{air}} > T_{\text{b}}$  also followed patterns typical of birds, although evaporative scope (i.e., maximum EWL/minimum EWL) was nearly twice as high in thick-knees (12.9) compared to Namaqua sandgrouse (7.4) or eagle-owls (7.8). Moreover, maximum rates of EWL for thick-knees ( $23.5 \text{ g h}^{-1}$  at  $T_{\text{air}} = 56^{\circ}\text{C}$ ) exceed by a substantial margin the highest rates of EWL reported for the much larger ( $\sim 3$ -fold) MacQueen's bustard (Tieleman et al., 2002). Evaporative water loss rates of thick-knees were equivalent to  $5.4\% M_{\text{b}} \text{ h}^{-1}$ , higher-than-expected fractional values more similar to those typically seen in small passerines (Wolf and Walsberg, 1996; McKechnie et al., 2017; Czenze et al., 2020).

Increases in RMR associated with evaporative heat dissipation at high  $T_{\text{air}}$  varied substantially. In Namaqua sandgrouse, maximum RMR at  $T_{\text{air}} = 60^{\circ}\text{C}$  was only 37% higher than minimum thermoneutral values, a pattern similar to those

observed in some caprimulgids (O'Connor et al., 2017), columbids (McKechnie et al., 2016a,b) and at  $T_{\text{air}} \leq 55^{\circ}\text{C}$  in MacQueen's bustard (Tieleman et al., 2002). Among other sandgrouse species, no daytime  $T_{\text{uc}}$  was evident in *P. orientalis* nor *P. bicinctus* at  $T_{\text{air}}$  values below  $\sim 43^{\circ}\text{C}$  (Hinsley, 1992; Hinsley et al., 1993), suggesting measurements may not have reached  $T_{\text{air}}$  high enough to elicit a  $T_{\text{uc}}$  in either of these species. The very high  $T_{\text{uc}}$  of eagle-owls ( $T_{\text{air}} = 49^{\circ}\text{C}$ ) is similar to the  $T_{\text{uc}} = 46.4^{\circ}\text{C}$  reported for western screech owls (Talbot et al., 2018) and other taxa, including lilac-breasted roller (*Coracias caudatus*:  $T_{\text{uc}} = 47.5^{\circ}\text{C}$ ; Smit et al., 2018) and several caprimulgids and columbids (Smith et al., 2015; McKechnie et al., 2016a; Talbot et al., 2017).

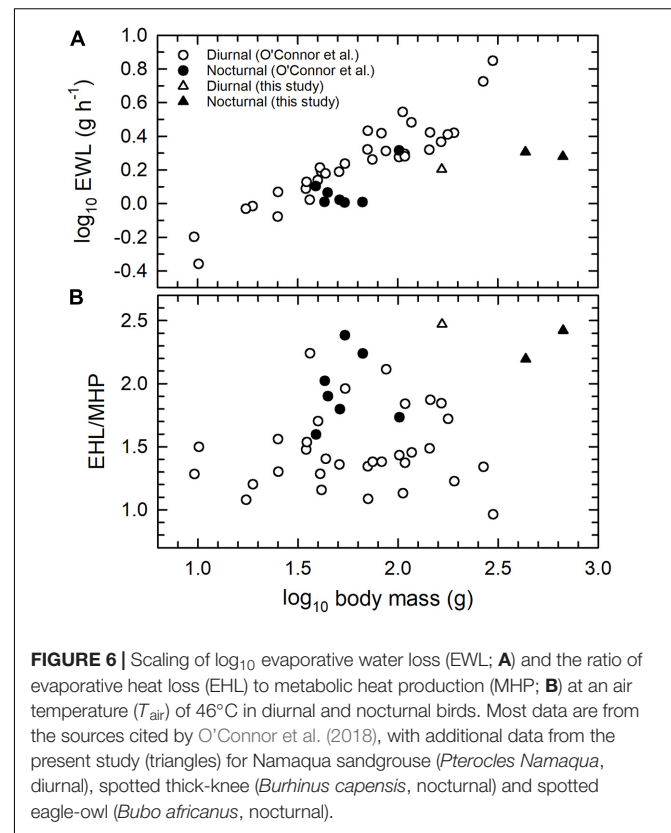
With the exception of maximum EHL/MHP = 3.4 in lilac-breasted rollers, the values we report here are the highest yet documented among birds other than caprimulgids and columbids (reviewed by McKechnie et al., 2021a). In particular, maximum EHL/MHP = 5.5 in Namaqua sandgrouse is the highest avian value measured to date, exceeding values of 4.7 and 5.2 previously reported for Namaqua doves (*Oena capensis*; McKechnie et al., 2016a) and Rufous-cheeked nightjars (*Caprimulgus rufigena*; O'Connor et al., 2017), respectively. We are not aware of published data on maximum EHL/MHP in Charadriiformes, but our value of 3.2 for thick-knees raises the possibility that the capacity for evaporative cooling may also be pronounced in this order (e.g., Grant, 1982).





## Interspecific Variation Between Sandgrouse

The data currently available suggest patterns of thermoregulation in the heat are phylogenetically conserved within avian orders. The large differences in patterns of thermoregulation at very high  $T_{air}$  between *P. namaqua* and the congeneric Burchell's sandgrouse (*P. burchelli*, McKechnie et al., 2016b) were, therefore, surprising. Whereas RMR increased sharply to values equivalent to  $\sim 250\%$  of minimum thermoneutral levels in *P. burchelli*, the RMR of *P. namaqua* remained within 37% of minimal levels (Figure 5). Consequently, maximum EHL/MHP was substantially higher in *P. namaqua* (5.5 at  $T_{air} = 60^\circ\text{C}$ ) compared to *P. burchelli* (2.0–2.7 at  $T_{air} = 56^\circ\text{C}$ ). The



rapid increases in RMR and modest maximum EHL/MHP of *P. burchelli* bear a strong resemblance to the pattern typical of passerines, which lack gular flutter or rapid cutaneous water loss and rely on panting for evaporative heat dissipation. In contrast, the corresponding patterns for *P. namaqua* resemble the extremely efficient cooling characteristic of columbids (Smith et al., 2015; McKechnie et al., 2016a) and caprimulgids (O'Connor et al., 2017; Talbot et al., 2017). The sandgrouse provide the first indication that fundamental differences in evaporative cooling processes can occur within an order, or even a single genus.

We speculate the differences between these two species reflect the more arid distribution of *P. namaqua*, which likely experiences stronger selection to minimise energy and water requirements. The distribution of *P. burchelli* is centred on the Kalahari basin, spanning arid savanna in the south to mopane (*Colophospermum mopane*) woodlands in the north. Much of the distribution of *P. namaqua*, on the other hand, is more arid, including the Nama Karoo and hyperarid Namib Desert. With mean annual precipitation of  $\sim 100 \text{ mm y}^{-1}$ , our present study site is considerably more arid than any part of the range of *P. burchelli* (Lloyd, 2005). The methods used to quantify thermoregulatory responses in both species were identical, but we cannot rule out possibilities such as a greater stress response in *P. burchelli* compared to *P. namaqua*. However, in this scenario we would expect higher  $T_b$  under thermoneutral conditions in *P. burchelli* on account of stress-induced hyperthermia

**TABLE 4** | Comparison of body temperature ( $T_b$ ), evaporative water loss (EWL), resting metabolic rate (RMR) and ratio of evaporative heat loss (EHL) to metabolic heat production (MHP) at air temperatures ( $T_{air}$ ) of approximately 50°C in six species with body mass ( $M_b$ ) > 250 g: MacQueen's bustard (*Chlamydotis macqueenii*,  $n = 7$ ), spotted eagle-owl (*Bubo africanus*), brown-necked raven (*Corvus ruficollis*,  $n = 11-16$ ), chukar (*Alectoris chukar*), spotted thick-knee (*Burhinus capensis*) and galah (*Eolophus roseicapilla*,  $n = 7$ ).

Species	$M_b$ g	$T_b$ °C	EWL g h <sup>-1</sup>	RMR W	EHL/MHP	Source
MacQueen's Bustard	1245	41.5	8.64	3.26	1.77	Tieleman et al. (2002)
Spotted Eagle-Owl	668	40.6	11.29	2.96	2.61	Present study
Brown-necked Raven	610	44.7	17.84	7.19	1.67	Marder (1973)
Chukar	475	43.4	7.35	2.73	1.82	Marder and Bernstein (1983)
Spotted Thick-knee	434	42.9	14.35	3.05	3.02	Present study
Galah	266	42.3	7.19	2.96	1.62	McWhorter et al. (2018)

In the case of *C. ruficollis*, data were collected at  $T_{air} = 48^\circ\text{C}$ .

(Cabanac and Briese, 1992; Cabanac and Aizawa, 2000; Nord and Folkow, 2019). The similarity of both normothermic  $T_b$  (*P. burchelli*: 39.0°C and *P. namaqua*: 39.4°C) and maximum  $T_b$  (*P. burchelli*: 43.6°C and *P. namaqua*: 43.2°C) argues against the notion that the divergent responses of these congeners reflect an artefact of stress responses to the experimental conditions.

## Functional Links With Nocturnality in Thick-Knees and Owls

The high daytime environmental temperatures nocturnal birds endure in hot habitats, combined with their lack of drinking during the day, led O'Connor et al. (2018) to hypothesise that nocturnal species have evolved more economical evaporative cooling and reduced EWL to minimise dehydration risk during the day. An analysis of data for 32 diurnal and 7 nocturnal species (two owls, four caprimulgids and one owlet-nightjar) provided weak support: slopes of EWL between  $T_{air} = 40$  and 46°C were significantly lower in nocturnal species, but neither log<sub>10</sub> EWL nor EHL/MHP at  $T_{air} = 46^\circ\text{C}$  differed significantly with activity period (O'Connor et al., 2018). Our data for thick-knees and eagle-owls generally support O'Connor et al.'s (2018) hypothesised link between nocturnality and more economical evaporative cooling, and specifically the predictions of lower EWL (Figure 6A) and higher EHL/MHP (Figure 6B) in nocturnal species, as well as these authors' contention that their results reflected a small sample size for nocturnal species.

## Thermoregulation in the Heat: Interspecific Variation

The data we present here reveal that representatives of three non-passerine orders can achieve rapid evaporative heat dissipation and tolerance of  $T_{air} = 55-60^\circ\text{C}$ , similar to the performance of smaller species in highly heat tolerant taxa such as Caprimulgiformes and Columbiformes. Most species in which heat tolerance has been investigated so far are smaller than 100 g, and interspecific variation in interactions between evaporative heat loss and metabolic heat production at  $T_{air} > 45^\circ\text{C}$  in larger species has received little attention (Table 4). The contrast between MacQueen's bustards (Tieleman et al., 2002) and brown-necked ravens (Marder, 1973) is particularly striking (Table 4). Despite being twice as large, bustards' EWL and RMR were equivalent to <50% of corresponding values in ravens,

whereas EHL/MHP was similar and  $T_b$  was 3.2°C higher in ravens (Table 4). Although the relative humidity experienced by ravens (<30%, Marder, 1973) may have been higher than for bustards (<22%, Tieleman et al., 2002), we suspect these differences primarily reflect the ravens' reliance on panting and the constraints imposed by this relatively inefficient avenue of evaporative cooling (McKechnie et al., 2021a). Spotted eagle-owls (present study) maintained the lowest  $T_b$  at high  $T_{air}$  of any of the six species, via a combination of intermediate rates of EWL and low RMR (Table 4).

The pronounced interspecific variation in EWL at  $T_{air} = 50^\circ\text{C}$  reveals that vulnerability to lethal dehydration during extreme heat events differs considerably among the six species listed in Table 4. Assuming birds can tolerate losing water equivalent to 15% of  $M_b$  [following Albright et al. (2017) and others], survival times at  $T_{air} = 50^\circ\text{C}$  vary from 4.53 h (spotted thick-knee) and 5.13 h (brown-necked raven) to 8.88 h (spotted eagle-owl) and 21.61 h (MacQueen's bustard). That estimated survival times for these species are >4 h at this extremely high  $T_{air}$  emphasizes the effect of  $M_b$  on the risk of lethal dehydration during extreme heat events; larger birds are at considerably lower risk of mortality via dehydration tolerance limits being exceeded (McKechnie and Wolf, 2010).

## CONCLUSION

Our investigation of heat tolerance in three phylogenetically diverse birds with  $M_b$  of 170–680 g reveals pronounced evaporative cooling capacities and tolerance of extremely high  $T_{air}$  during acute heat exposure. These data add to evidence that many non-passerine taxa possess substantially more efficient evaporative cooling and greater heat tolerance compared to passerines (reviewed by McKechnie et al., 2021a). Pronounced heat tolerance in many non-passerines, including the three species in the present study, appears to be functionally linked to the capacity for gular flutter, or in the case of the columbids, rapid cutaneous evaporative cooling. The loss of gular flutter during avian evolution left passerines and several other taxa with much more limited evaporative cooling capacity, which is thought to have contributed to the evolution of passerines' pronounced use of facultative hyperthermia (Gerson et al., 2019).

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics Committee, University of Pretoria (protocol UP: EC057-18) and the Research Ethics and Scientific Committee of the South African National Biodiversity Institute (P18/37).

## AUTHOR CONTRIBUTIONS

RK, ZC, BW, MF, and AM designed the study. ZC, MF, RK, and BvJ collected and analysed the data. ZC, MF, and AM led the

writing of the manuscript. All authors contributed to the article and approved the submitted version.

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