

RESEARCH ARTICLE

How hornbills handle heat: sex-specific thermoregulation in the southern yellow-billed hornbill

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ABSTRACT

At a global scale, thermal physiology is correlated with climatic variables such as temperature and aridity. There is also evidence that thermoregulatory traits vary with fine-scale microclimate, but this has received less attention in endotherms. Here, we test the hypothesis that avian thermoregulation varies with microclimate and behavioural constraints in a non-passerine bird. Male and female southern yellow-billed hornbills (*Tockus leucomelas*) experience markedly different microclimates while breeding, with the female sealing herself into a tree cavity and moulting all her flight feathers during the breeding attempt, becoming entirely reliant on the male for provisioning. We examined interactions between resting metabolic rate (RMR), evaporative water loss (EWL) and core body temperature (T_b) at air temperatures (T_a) between 30°C and 52°C in male and female hornbills, and quantified evaporative cooling efficiencies and heat tolerance limits. At thermoneutral T_a , neither RMR, EWL nor T_b differed between sexes. At $T_a > 40^\circ\text{C}$, however, RMR and EWL of females were significantly lower than those of males, by ~13% and ~17%, respectively, despite similar relationships between T_b and T_a , maximum ratio of evaporative heat loss to metabolic heat production and heat tolerance limits (~50°C). These sex-specific differences in hornbill thermoregulation support the hypothesis that avian thermal physiology can vary within species in response to fine-scale microclimatic factors. In addition, Q_{10} for RMR varied substantially, with $Q_{10} \leq 2$ in some individuals, supporting recent arguments that active metabolic suppression may be an underappreciated aspect of endotherm thermoregulation in the heat.

KEY WORDS: Thermoregulation, Microclimate, Sex-specific, Avian, Heat

INTRODUCTION

An enduring question in evolutionary physiology concerns the extent to which adaptation to local climate contributes to overall

physiological diversity (Gaston et al., 2009; Bozinovic et al., 2011; Muñoz and Bodensteiner, 2019). Traits related to thermal physiology can have important fitness consequences and be under strong selective pressure from the physical environments organisms occupy (Angilletta et al., 2010; Boyles et al., 2012). Thermal adaptation is evident in taxa ranging from plants to endotherms, and understanding how climate drives the evolution of thermal traits is vital for elucidating the processes determining the geographical distribution and range limits of species (Bozinovic et al., 2011; Keller and Seehausen, 2012). How these thermal traits correlate with climatic conditions has been studied at multiple levels of biological organisation and across varying spatiotemporal scales (Addo-Bediako et al., 2000; Sunday et al., 2011; Araújo et al., 2013; Bozinovic et al., 2014; White et al., 2007; Naya et al., 2013).

At the broadest spatial scale, thermoregulatory traits of both endo- and ectothermic animals vary with latitudinal gradients in climate. For instance, lower thermal limits in ectotherms decrease with latitude, whereas upper thermal limits do not, resulting in an increased thermal tolerance breadth in species occupying higher latitudes (Addo-Bediako et al., 2000; Sunday et al., 2011; Araújo et al., 2013). Global-scale analyses investigating correlations between climate and endotherm thermoregulation reveal that basal metabolic rate in birds is negatively correlated with air temperature (White et al., 2007). In addition, both maximum and summit metabolism, the maximum rate of resting metabolic thermogenesis during cold exposure, are also negatively correlated with prevailing air temperature (McKechnie and Swanson, 2010).


Thermal physiology can also be correlated with climatic conditions at finer spatial scales. More evidence for physiological adaptation reflecting climate is apparent from studies that have examined physiological variation along aridity gradients. Among insects, reptiles, birds and mammals, taxa and populations inhabiting arid areas display lower rates of evaporative water loss compared with their mesic counterparts (Addo-Bediako et al., 2001; Tieleman et al., 2003; Williams et al., 2004; Van Sant et al., 2012; Guillon et al., 2014; Cox and Cox, 2015; Belasen et al., 2017; Dupoué et al., 2017; Klüg-Baerwald and Brigham, 2017). Similarly, basal metabolic rates are negatively correlated with habitat aridity in endotherms (Lovegrove, 2000; Tieleman et al., 2003; Williams et al., 2004). At the intraspecific level, there is some evidence that avian resting metabolic and evaporative water loss rates and core body temperature (T_b) are lower in arid populations than mesic populations (Noakes et al., 2016; Trost, 1972; but see also Smit et al., 2013). The adaptive value of reduced metabolic and evaporative water loss rates in desert animals is thought to be related to increased water and energy savings in response to low and unpredictable availability of resources (Lovegrove, 2000, 2003; Tieleman et al., 2003).

Evidence that thermal physiology evolves in response to climate at multiple spatial scales raises the possibility of similar variation at an intra-population level in response to variation in the fine-scale

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microclimatic conditions individuals occupy. Indeed, most of the evidence that individuals within the same populations can occupy different microclimate space stems, indirectly, from literature investigating sex-specific differences in habitat use or behaviour (Cryan and Wolf, 2003; Lailvaux et al., 2003; Beal et al., 2014; Liwanag et al., 2018; Vidal et al., 2008; Cameron et al., 2018). For example, sex-specific habitat use is prevalent in ectothermic western Indian anoles (Butler et al., 2007). Furthermore, sex-specific habitat use amongst endotherms occurs in four species of North American ducks, where females occupy shallower and more vegetated sites compared with males (Bergan and Smith, 1989). Similarly, the great bustard (*Otis tarda*) exhibits sex-specific behavioural thermoregulation, with males retreating to shaded microsites in response to high temperatures earlier than females (Alonso et al., 2016). Birds exhibiting uniparental care [i.e. one sex entirely responsible for incubation and hatchling care, with the other responsible for food provisioning (Cockburn, 2006)] may experience sex-specific microclimates during breeding seasons. Consequently, males and females may experience substantially different thermal regimes (van de Ven et al., 2020).

One avian taxon that represents a fascinating model for testing hypotheses concerning sex-specific differences in thermal physiology arising from sex-specific microsite selection is the hornbills (Bucerotiformes: Bucerotidae). During the breeding season, female hornbills seal themselves into nest cavities as a protective mechanism against predators (Moreau and Moreau, 1941; Witmer, 1992; Kemp, 1995). Here, females are confined to the nest, undergo a complete wing and tail moult, and males are solely responsible for provisioning females and chicks for much of the breeding attempt (Klaassen et al., 2003; Kemp, 1969). For hornbills occupying hot environments with summer rainfall, females are sealed into cavities during the hottest time of the year and experience a combination of high air temperature, limited convective cooling and humidity above prevailing levels, which together pose substantial thermoregulatory challenges (Fig. 1; Gerson et al., 2019; van Dyk et al., 2019). In addition, the complete, simultaneous wing and tail feather moult undergone by females while confined in the nest cavity means that the heat load experienced by the females includes the metabolic cost of re-growing feathers (Buttemer et al., 2019). In addition, at high temperatures females experience restricted water and food supply because of decreased provisioning rates by male hornbills, reflecting trade-offs between behavioural thermoregulation and foraging (van de Ven et al., 2019, 2020); thus, females experience increased water demands for evaporative cooling combined with reductions in water and food supply.

Here, we investigated thermoregulation in southern yellow-billed hornbills (*Tockus leucomelas* Lichtenstein 1842) in the Kalahari Desert of southern Africa, where air temperatures can approach 40°C during the mid-summer breeding period (Fig. 1). Using a flow-through respirometry system, we measured resting metabolic rate (RMR), evaporative water loss (EWL) and core body temperature (T_b) over air temperatures (T_a), ranging from mild to hot. We hypothesised that highly asymmetric parental care in the hornbills, and the related sex-specific differences in microclimates, has led to the evolution of sex differences in thermal physiology under high heat loads. We predicted that compared with males under resting conditions, females: (1) have more efficient evaporative cooling characterised by lower RMR above the upper critical limit of thermoneutrality, ultimately resulting in higher maximum evaporative heat loss (EHL)/metabolic heat production (MHP) ratios [i.e. overall capacity for evaporative cooling and a significant predictor of heat tolerance limits (Czenze et al., 2020)], and (2) have higher heat tolerance limits. In addition, because there is evidence for temperature-independence of avian

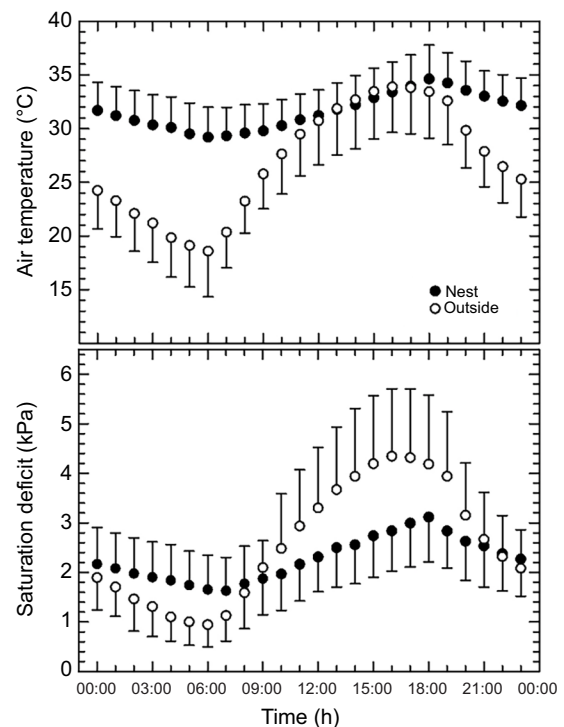


Fig. 1. Mean air temperature and saturation deficit in southern yellow-billed hornbill (*Tockus leucomelas*) tree-cavity nests compared with outside conditions. Means \pm s.d. for 1 h bins are shown for air temperature and saturation deficit within nests (black circles and error bars) and outside conditions (clear circles and error bars). Error bars for saturation deficit are flipped after 09:00 h. Nest temperatures were recorded using Thermochron iButtons (DS1923, Maxim, Sunnyvale, CA, USA, resolution=0.0625°C) at 5 min intervals. Corresponding ambient temperature and saturation deficit was obtained from a nearby weather station (Vantage Pro2, Davis Instruments, Hayward, USA).

RMR at high T_a in some species (Weathers, 1981) and there have been arguments that metabolic suppression is involved in endotherm thermoregulation in the heat (Song et al., 1997; Lovegrove et al., 2014; Reher et al., 2018), we also compared Q_{10} , a quantitative descriptor of the Arrhenius effect of temperature on metabolic rate, between male and female hornbills experiencing very high T_a values. An additional aim of the study was to quantify upper limits to heat tolerance and evaporative cooling capacity in a member of the Bucerotiformes, as this order is absent from the data currently available for arid-zone birds (McKechnie et al., 2021a).

MATERIALS AND METHODS

Study site and species

This study was conducted during the hornbills' breeding season, which usually spans from October to November (Kemp, 2005). All sampling took place between 1 October and 2 November 2018 in the southern Kalahari Desert at Radnor farm (26°6'23"S, 22°52'54"E) near Vorstershoop, North West Province, South Africa. The vegetation of this region is characterised as Molopo Bushveld, consisting of a mix of open woodland and closed shrubland dominated by *Vachellia erioloba* and *Boscia albitrunca* trees and *Lycium cinereum*, *Lycium hirsutum* and *Rhigozum trichotomum* shrubs (Rutherford et al., 2006). During the summer months, maximum air temperatures are typically between 33 and 37°C and the site receives ~221 mm precipitation (Fick and Hijmans, 2017).

Ten female and nine male hornbills were captured using spring traps (60×60 cm) baited with superworms (*Zophobas morio*). Prior

to capture, individuals were monitored to ensure that they were not involved in a current breeding attempt; i.e. solitary males, females in a tree cavity, pairs displaying allopreening or allofeeding; and that females were not in moult (Kemp, 2005; Myers, 2000; Mills et al., 2005). Mean±s.d. body mass (M_b) for males and females was 242.3±10.7 g ($n=9$) and 198.9±21.9 g ($n=10$), respectively. Following capture, the birds were transported to a field laboratory in cotton bags where they were individually housed in cages (60×40×40 cm) and provided with an *ad libitum* supply of darkling beetle larvae *Zophobas morio* and water. Birds were sexed based on culmen length, tarsus length and M_b measurements (Kemp, 2005). Individuals were kept for no longer than 24 h prior to experimentation and were released on the same day.

Microclimate data

To test our assumption that females experience a more thermally challenging environment than males do when confined to their tree cavity nests, we analysed data collected from a semi-habituated population of hornbills at Kuruman River Reserve (26°58'S, 21°49'E) in the Northern Cape of South Africa. Occupied tree cavity nest temperatures (T_a) and humidity were recorded using Hygrochron iButtons (DS1923, Maxim, Sunnyvale, CA, USA, resolution=0.0625°C) at 5 min intervals. To prevent data anomalies arising from females coming into contact with the loggers, iButtons were attached to the upper internal surfaces of cavities. The temperature sensors of the iButtons were calibrated in a circulating water bath against a NiCr-NiAl thermocouple thermometer (Thermocouple HH21A, Omega Engineering, Stanford, USA). The NiCr-NiAl thermocouple thermometer (resolution=0.1°C) was calibrated against a mercury-in-glass thermometer with accuracy traceable to the National Institute of Standard Technology, MD, USA. Recordings were taken from three nests during the summer months of 2015, for a total of 45 nest-days. Corresponding T_a and humidity were obtained from a nearby weather station (Vantage Pro2, Davis Instruments, Hayward, USA). We acknowledge that T_a does not equate to operative temperature for males but provides an indication of the minimum operative temperature in the environment available to the birds if they seek deep shade during the hottest part of the day.

Gas exchange measurements

A flow-through respirometry system was used to measure rates of carbon dioxide production (ml min^{-1}) and rates of evaporative water loss (EWL; mg min^{-1}). A 60 litre airtight container functioned as the metabolic chamber. The chamber was fitted with an air inlet positioned near the top of the chamber and the outlet near the bottom to maximise mixing. A platform constructed from plastic mesh was positioned ~10 cm above a 1 cm layer of mineral oil, which prevented evaporation from excreta. The chamber was placed within a custom-built temperature-controlled chest (105×81×81 cm), constructed from polystyrene sheets positioned between the interior chipboard and exterior aluminium sheeting. The T_a within the chest was regulated by a digital controller (TC-720 Temperature Controller, TE Technology, Traverse City, MI, USA) that controlled a thermoelectric air cooler (TC 720 OEM, TE Technology). Atmospheric air, supplied by an oil-free compressor was pushed through a membrane dryer (Champion®CMD3 air dryer and filter, Champion Pneumatic, Quincy, IL, USA) and scrubbed of water vapour. Downstream of the membrane dryer, the airstream was split into a baseline and experimental channel. The flow rate in the baseline channel was regulated at ~1 l min^{-1} by a needle valve (Swagelok, Solon, OH, USA). In the experimental channel, the flow rate was regulated by a

mass flow controller (0–60 SLPM, Alicat Scientific Inc., Tucson, AZ, USA), calibrated against a flow rate calibrator (Gilibrator 2, Sensidyne, St Petersburg, FL, USA). Flow rates were selected to maintain absolute humidity levels within the chamber as low as possible (<1 kPa) while still maintaining an accurately measurable difference in CO_2 and H_2O concentration between the incurrent and excurrent air. Flow rates ranging from 2.3 to 57.5 l min^{-1} (25±17 l min^{-1}) were used, depending on T_a , exposure time and the activity of birds. Excurrent air from the chamber and baseline channel were sequentially sub-sampled using a respirometry multiplexer (MUX3-1101-18 mol l^{-1} , Sable Systems, Las Vegas, NV, USA) in manual mode. Sub-sampled air was pulled through a $\text{CO}_2/\text{H}_2\text{O}$ analyser (LI-840A, LI-COR, Lincoln, NE, USA) regularly zeroed using nitrogen, and spanned for CO_2 using an analytically certified gas with a known CO_2 concentration of 2000 ppm (AFROX, Johannesburg, South Africa) and for H_2O using a dewpoint generator (DG4, Sable Systems, Las Vegas, NV, USA). All tubing in the system was Bev-A-Line IV tubing (Thermoplastic Processes Inc., Warren, NJ, USA). Voltage outputs from the analysers and thermistor probes were digitised using an analogue-digital converter (UI3, Sable Systems, Las Vegas, NV, USA) and recorded with a sampling interval of 5 s using Expedata software (Sable Systems, Las Vegas, NV, USA).

Air and body temperature measurements

A 3-mm diameter thermistor probe (Sable Systems, Las Vegas, NV, USA) was inserted through a hole sealed with a rubber grommet in the side of the metabolic chamber to measure T_a during gas exchange measurements. Core body temperature was continuously measured using temperature-sensitive passive integrated transponder (PIT) tags (BioTherm, Biomark, Biose, ID, USA) and a portable transceiver system linked to an antenna (HPR+, Biomark) placed adjacent to the respirometry chamber. The PIT tags were injected intraperitoneally, following Whitfield et al. (2015). Before implantation, PIT tags were calibrated against a digital thermocouple reader (RDXL12SD, Omega, Stamford, CT, USA) in a circulating water bath (F34, Julabo, Seelbach, Baden-Württemberg, Germany), over temperatures of 30–50°C. Temperatures measured by PIT tags deviated by 0.06±0.05°C ($n=20$) from actual values.

Experimental protocol

Each individual was weighed on an electronic balance (EJ-160, A&D, Tokyo, Japan) and held in the metabolic chamber, without food and water, for at least 1.75 h before commencing measurements. Birds were exposed to a stepped profile of increasing T_a between 30 and 52°C. At $T_a=30$ –40°C, T_a was increased in 5°C increments, whereas at $T_a>40$ °C, it was increased in 2°C increments. All individuals spent >10 min, and the majority 15–20 min, at each T_a value before data were recorded. Runs lasted for approximately 4 h, including the habituation time. Measurements commenced with a baseline air subsample until H_2O and CO_2 readings were stable (~5 min). Subsequently, the excurrent chamber air was sub-sampled when T_a had stabilised at the target value, followed by another 5 min baseline.

During measurements, behaviour and activity of individuals were continuously monitored using a CCTV video camera and an infrared light source. Trials were terminated, and individuals immediately removed from the chamber if their T_b exceeded 45°C and/or if they displayed a sudden decrease in EWL or RMR. Trials were also terminated if they displayed behavioural stress such as prolonged escape behaviour (i.e. agitated jumping, pecking and/or wing flapping), or if they exhibited a loss of coordination or balance. Birds that displayed a rapid decrease in EWL, RMR or increase in

$T_b > 45^\circ\text{C}$ were considered to have reached their thermal endpoints. Once removed, a cotton pad soaked in 99% ethanol was rubbed on the bird's body to augment rates of heat loss, following Whitfield et al. (2015). Once T_b stabilised at normothermic levels ($40\text{--}42^\circ\text{C}$), the bird was placed back into its cage at room temperature to rest with *ad libitum* water and food until their release at the site of capture. In almost all cases, the individuals lost less than 5% M_b through faeces and water during measurements and no individuals displayed any adverse effects after experimentation. This experimental protocol has been previously used and, in one instance with opportunistic monitoring for several weeks post-release, no adverse effects were observed (Kemp and McKechnie, 2019). All experimental protocols were approved by the University of Pretoria Animal Ethics committee (protocol EC023-18) and the South African National Biodiversity Institute's Research Ethics and Scientific Committee (protocol P18/12).

Data analysis

Using equations 9.5 and 9.6 from Lighton (2008), whole animal rates of CO_2 production (\dot{V}_{CO_2}) and rates of EWL were calculated from the lowest 5-min steady-state traces, assuming $0.803\text{ mg H}_2\text{O ml}^{-1}$ vapour. Evaporative heat loss (W) was calculated assuming a latent heat of vaporisation of water of $2.406\text{ J mg H}_2\text{O}^{-1}$ (Tracy et al., 2010). Given the high flow rates, oxygen consumption was not measured, thus we could not calculate the respiratory exchange ratio (RER). Birds were kept without food or water for a minimum of 1.75 h before measurements, with the allometrically predicted gut passage time for a 240 g bird, the largest individual in our study, being 95 min (Karasov, 1990). We hence assumed $\text{RER}=0.71$, representative of lipid metabolism in post-absorptive birds, and calculated RMR (W) from \dot{V}_{CO_2} using $27.8\text{ J ml}^{-1}\text{ CO}_2$ (Walsberg and Wolf, 1995). All statistical analyses were performed in R 3.5.2 (<https://www.r-project.org/>). Piecewise linear regression models were fitted separately to data from each sex to identify inflection points in EWL, RMR, T_b and EHL/MHP as a function of T_a , using the functions described by Muggeo et al. (2014); these functions account for pseudoreplication when identifying inflection points. Furthermore, a piecewise linear regression model was fitted to EHL/MHP as a function of $T_a - T_b$. We also calculated Q_{10} for the RMR of each individual using minimum T_b and RMR values at $T_a = 30\text{--}35^\circ\text{C}$ and maximum values at $T_a = 48\text{--}50^\circ\text{C}$ in order to evaluate the possibility of metabolic suppression at high T_a .

To test the prediction that male and female hornbills display differing thermoregulatory responses to increases in T_a , the male and female RMR, EWL, EHL/MHP and T_b data were pooled, with separate linear mixed-effects models fitted to data above and below the highest of the two sex-specific inflection points for each response variable using *lme4* (Bates et al., 2015). Model selection was conducted using the dredge function in the MuMIn package (<https://CRAN.R-project.org/package=MuMIn>). The global model contained T_a (or $T_a - T_b$), sex, M_b and the $T_a \times \text{sex}$ interaction. Model goodness of fit was assessed using Akaike information criterion values corrected for small sample size (AICc). Models with $\Delta\text{AICc} < 2$ were considered equally parsimonious, and a final model was constructed by averaging the coefficients of all models below this threshold (Burnham and Anderson, 2002). A single top model explained the variation in EWL, EHL/MHP and T_b , whereas for RMR two competing models provided similar support for data below and above the inflection point (Table S2). Since we were interested in the effect of sex on the response variable, we report conditional averages rather than the full averages, following

Symonds and Moussalli (2011) and Grueber et al. (2011). Multiple measurements from the same individuals required us to account for pseudo-replication by including bird identity as a random term. Body mass did not emerge as a significant predictor for any of the response variables (Table S2). We repeated the above procedure to test whether male and female hornbills displayed differing mass-specific rates of EWL (msEWL) and RMR (msRMR) above and below physiological thresholds (Fig. S1, Table S2). The Q_{10} of RMR in male and female hornbills was compared using a two-tailed *t*-test, as were differences between prevailing conditions and those within tree-cavities. Values are presented as means \pm s.d.

RESULTS

Microclimates

Daily maximum T_a recorded inside the nests ($\bar{x}=34.36 \pm 0.48^\circ\text{C}$, $n=45$ days) was similar to the corresponding value outside the nest ($\bar{x}=33.36 \pm 0.75^\circ\text{C}$, $n=40$ days; $t=1.14$, d.f.=83, $P=0.26$; Fig. 1). Minimum saturation deficit was significantly lower inside the nests ($\bar{x}=3.26 \pm 0.13\text{ kPa}$, $n=45$ days) compared with outside ($\bar{x}=4.21 \pm 0.24\text{ kPa}$, $n=40$ days; $t=3.36$, d.f.=83, $P<0.001$; Fig. 1).

Evaporative water loss

The inflection point in the relationship between EWL and T_a occurred at $T_a=40.5^\circ\text{C}$ for males and $T_a=39.7^\circ\text{C}$ for females, above which EWL increased with increasing T_a . Below the inflection point, EWL increased significantly with T_a ($t=5.56$, $P<0.001$; Fig. 2) but rates of EWL were minimal ($0.5 \pm 0.2\text{ g h}^{-1}$, $n=19$) and sex did not emerge as a significant predictor (Fig. 2, Table S1). At T_a values above the inflection, EWL increased linearly and significantly with T_a ($t=20.22$, $P<0.001$; Fig. 2), and differed between sexes ($t=2.36$, $P=0.03$; Fig. 2). Rates of EWL increased to a maximum of $6.2 \pm 1.6\text{ g h}^{-1}$ ($n=9$) at $T_a \sim 50^\circ\text{C}$ among females (with one female reaching 7.6 g h^{-1} at $T_a \sim 52^\circ\text{C}$) and a maximum of $7.8 \pm 1.3\text{ g h}^{-1}$ ($n=7$) at $T_a \sim 50^\circ\text{C}$ among males. Rates of EWL at $T_a \sim 50^\circ\text{C}$ were 17% lower in females compared with males; equivalent to $3.3 \pm 0.5\% M_b\text{ h}^{-1}$ ($n=7$) for males and $3.2 \pm 0.9\% M_b\text{ h}^{-1}$ ($n=10$) for females.

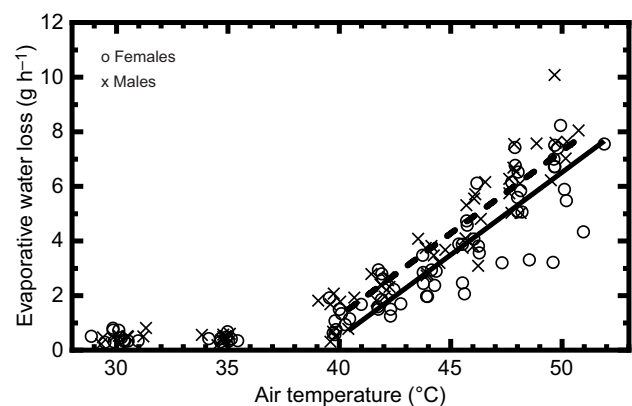


Fig. 2. Relationship between evaporative water loss (EWL) and air temperature (T_a) in southern yellow-billed hornbills (*Tockus leucomelas*). Data were obtained from 10 females (circles) and nine males (crosses) using open flow-through respirometry. Lines indicate linear mixed-effects regression models fitted to $T_a > 40.5^\circ\text{C}$, males (dashed): $\text{EWL} = 0.60T_a - 23.72$ and females (solid): $\text{EWL} = 0.60T_a - 22.93$.

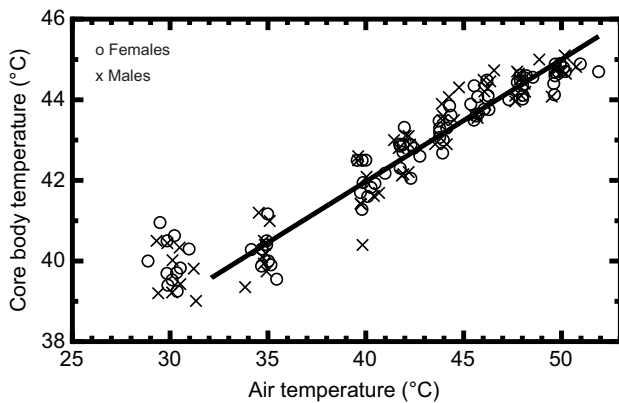


Fig. 3. Relationship between core body temperature (T_b) and air temperature (T_a) in southern yellow-billed hornbills (*Tockus leucomelas*). Data were obtained from 10 females (circles) and nine males (crosses) using open flow-through respirometry. Lines indicate linear mixed-effects regression models fitted to $T_a > 32.1^\circ\text{C}$, $T_b = 0.30T_a + 29.86$.

Core body temperature and thermal endpoints

An inflection in T_b occurred at $T_a = 32.0^\circ\text{C}$ for females and $T_a = 32.1^\circ\text{C}$ for males. At T_a above the inflection point, T_b increased linearly and significantly with T_a ($t = 41.11$, $P < 0.001$; Fig. 3), and sex did not emerge as a significant predictor (Fig. 3, Table S1). Below the inflection point, T_a had no effect on T_b ($t = 1.28$, $P = 0.22$; Fig. 3) and sex did not emerge as a significant predictor (Table S1, Fig. 3). At $T_a \sim 30^\circ\text{C}$, mean normothermic T_b values were $39.9 \pm 0.6^\circ\text{C}$ ($n = 19$). Females reached thermal endpoints at $T_a = 50.6 \pm 1.4^\circ\text{C}$ with corresponding $T_b = 45.0 \pm 0.3^\circ\text{C}$ ($n = 10$). Males reached thermal endpoints at $T_a = 50.4 \pm 1.2^\circ\text{C}$ ($n = 9$), at which $T_b = 44.8 \pm 0.3^\circ\text{C}$ ($n = 9$). Nine females reached $T_a = 50^\circ\text{C}$ and one female reached $T_a = 51^\circ\text{C}$; whereas seven males reached 50°C and two males reached 48°C .

Resting metabolic rate

Inflections in RMR occurred at $T_a = 39.2^\circ\text{C}$ for males and at $T_a = 38.8^\circ\text{C}$ for females, which we interpret as the upper critical limits of thermoneutrality. The onset of panting corresponded closely with these inflection points, occurring at $T_a = 37.3 \pm 2.5^\circ\text{C}$ ($n = 9$) in males and at $T_a = 38.6 \pm 2.6^\circ\text{C}$ ($n = 10$) in females. Two

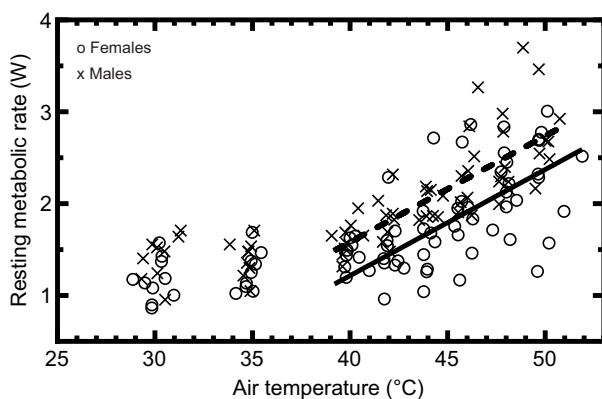


Fig. 4. Relationship between resting metabolic rate (RMR) and air temperature (T_a) in southern yellow-billed hornbills (*Tockus leucomelas*). Data were obtained from 10 females (circles) and nine males (crosses) using open flow-through respirometry. Lines indicate linear mixed-effects regression models fitted to $T_a > 39.2^\circ\text{C}$, males (dashed): $\text{RMR} = 0.12T_a - 3.04$ and females (solid): $\text{RMR} = 0.12T_a - 3.40$.

competing models similarly supported data below and above the inflection point (Table S1). Below the inflection point, a minimum RMR of $1.3 \pm 0.2 \text{ W}$ ($n = 18$) occurred at $T_a \sim 30^\circ\text{C}$ and did not differ between sexes (averaged model: $z = 1.90$, $P = 0.05$; Fig. 4). Above the inflection point, RMR increased linearly and significantly with T_a (averaged model: $z = 14.28$, $P < 0.001$; Fig. 4) and was lower in females (averaged model: $z = 2.43$, $P = 0.01$; Fig. 4). At $T_a \sim 50^\circ\text{C}$, RMR of males and females was $2.7 \pm 0.4 \text{ W}$ ($n = 7$) and $2.3 \pm 0.6 \text{ W}$ ($n = 9$), respectively, equivalent to a 13% difference. Individuals' Q_{10} values for RMR varied widely, from 1.68 in a female to 8.05 in a male; however, the mean values for males (5.03 ± 2.36 , $n = 9$) and females (4.09 ± 1.37 , $n = 10$) did not differ ($t = 1.05$, d.f. = 12.53, $P = 0.31$).

Evaporative heat loss/metabolic heat production

An inflection in EHL/MHP occurred at $T_a = 37.1^\circ\text{C}$ for males and $T_a = 37.3^\circ\text{C}$ for females. When EHL/MHP was plotted against $T_a - T_b$, an inflection point was identified at $T_a - T_b = -4.0^\circ\text{C}$ for males and $T_a - T_b = -4.1^\circ\text{C}$ for females. Above the inflection point EHL/MHP increased linearly and significantly with $T_a - T_b$ ($t = 23.27$, $P < 0.001$; Fig. 5) and sex did not emerge as a significant predictor (Fig. 4, Table S1). Below the inflection point, the null model provided the best fit (Fig. 5, Table S1). The y-intercept of the linear model for T_a values above the inflection was 0.95, indicating that 95% of MHP is dissipated by evaporative cooling when $T_b = T_a$. At T_a below the inflection point, the minimum ratio of EHL/MHP was 0.2 ± 0.1 ($n = 18$) at $T_a \sim 30^\circ\text{C}$ and increased to a mean maximum value of 1.8 ± 0.2 ($n = 18$) at $T_a \sim 50^\circ\text{C}$ (with one female reaching a value of 2.0 at $T_a \sim 50^\circ\text{C}$).

DISCUSSION

No sex differences were evident in T_b , RMR or EWL at thermoneutral T_a . At higher temperatures, however, female hornbills used significantly less water ($\sim 17\%$) and energy ($\sim 13\%$) than males to defend similar T_b values and to achieve similar maximum EHL/MHP values. These differences broadly support our first prediction, that evaporative cooling is more efficient in female hornbills compared with males; although the maximum EHL/MHP values are similar, absolute water and energy requirements are lower in females compared with males. As rates of EWL and RMR scale allometrically with M_b (White and Seymour, 2003; McKechnie and Wolf, 2010), a possible

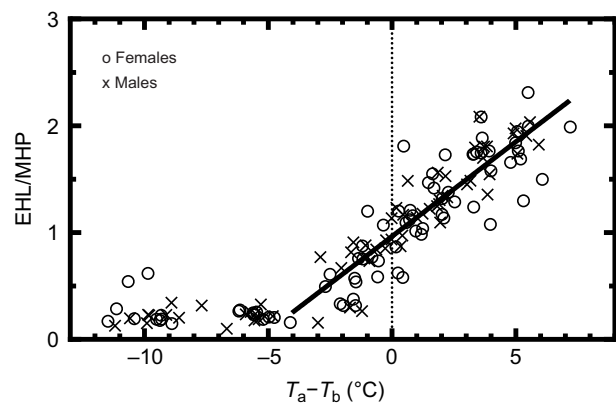


Fig. 5. Relationship between evaporative heat loss/metabolic heat production (EHL/MHP) and the air temperature-body temperature gradient ($T_a - T_b$) in southern yellow-billed hornbills (*Tockus leucomelas*). Data were obtained from 10 females (circles) and nine males (crosses) using open flow-through respirometry. Line indicates linear mixed-effects regression model fitted to $T_a - T_b > -4.04^\circ\text{C}$, $\text{EHL/MHP} = 0.18(T_a - T_b) + 0.96$.

explanation is that the sex-specific rates of EWL and RMR in hornbills are driven by sexual dimorphism in M_b . However, M_b did not emerge as a significant predictor for any of the variables we tested. In addition, given a negative relationship of mass-specific EWL and RMR to M_b (McKechnie and Wolf, 2010; White and Seymour, 2003), one would expect female hornbills to display greater mass-specific EWL and RMR compared with males. Yet, there was no significant effect of sex on mass-specific rates of EWL and RMR (Fig. S1, Table S2), further suggesting that sex-specific differences in thermoregulation in the hornbills are not attributable to sexual dimorphism in M_b . Our second prediction was not supported; relationships between T_b and T_a were indistinguishable between sexes, providing no support for the notion that females have greater heat tolerance limits, and maximum EHL/MHP did not differ between sexes.

The heat tolerance limit of $\sim 50^\circ\text{C}$ in male and female hornbills is lower than values of $54\text{--}62^\circ\text{C}$ reported for similarly sized non-passerine species, galah (*Eolophus roseicapilla*; McWhorter et al., 2018), Burchell's sandgrouse (*Pterocles burchelli*; McKechnie et al., 2016a) and crested pigeon (*Ocyphaps lophotes*; McKechnie et al., 2016b). Although male and female hornbills displayed similar heat tolerance limits under laboratory conditions, two aspects of the species' ecology not evaluated in this study may translate into sex-specific differences in heat tolerance limits and maximum evaporative cooling demands under natural conditions. First, although incarcerated female hornbills experience more thermally challenging conditions (i.e. the combined effect of T_a and humidity) compared with males over time scales of hours to days (Fig. 1), male hornbills potentially experience transient operative temperatures approaching or even exceeding 50°C when foraging on the ground in full sun. Furthermore, T_a is only an approximation of minimum operative temperature experienced by males. Second, females undergo complete flight feather moult while in the nest, which might enhance non-evaporative heat dissipation when $T_a < T_b$, although any such effect would presumably be offset by the increase in MHP associated with moult (e.g. Buttemer et al., 2019). To elucidate whether sex-specific differences in heat tolerance limits are also manifested in this species under natural conditions, future studies should (1) examine free-ranging T_b limits in relation to operative temperatures and (2) evaluate the possibility that the effects of humidity on evaporative cooling at high environmental temperatures differ between males and females, particularly when the latter are sealed in breeding cavities.

Maximum T_b during acute heat exposure also did not differ between sexes, with maxima of $\sim 45^\circ\text{C}$ for both males and females broadly consistent with those reported for most species examined to date (Dawson, 1954; Gerson et al., 2019). Tolerance of unusually high T_b in a central American passerine has been suggested to be functionally linked to impeded EHL in humid environments (Weathers, 1981), the reasoning on which our prediction of more pronounced facultative hyperthermia in female hornbills was based. The observation of Weathers (1981), as well as those of a handful of other authors reporting avian tolerance of $T_b > 45^\circ\text{C}$ (Arad and Marder, 1982; Dmi'el and Tel-Tzur, 1985; Freeman et al., 2020), reiterates the need to determine the upper limits to hyperthermia tolerance in many more species, and to evaluate the relative roles of deleterious temperature effects on cell membrane fluidity, transport pathways across membranes and other aspects of macromolecule function (Adolph, 1947; Roti Roti, 2008) and oxygen supply limitation (Teague et al., 2017; Pörtner et al., 2017) in determining these limits.

Expressed as percentages of M_b , maximum rates of EWL were similar in male and female hornbills, suggesting similar risks of

lethal dehydration on extremely hot days. However, the single-parent provisioning employed by this species means that, under natural conditions, breeding females may often be under greater dehydration risk arising from mismatches between supply and demand as a consequence of male foraging decisions. Behavioural observations on breeding hornbills reveal that, during very hot weather, males are unable to meet the energetic requirements of females, resulting from a decreased foraging efficiency under high heat loads, thereby exposing female hornbills to a higher risk of lethal dehydration (van de Ven et al., 2020). Indeed, such mismatches are thought to result in nest abandonment and potentially death if the female leaves her nest in a flightless state (van de Ven, 2017). In addition to direct hyperthermia-related mortality, dehydration is responsible for lethal effects of high T_a values and is expected to be a critical factor in determining the potential for avian species to persist in arid regions under anticipated global heating (McKechnie and Wolf, 2010; Albright et al., 2017; Conradie et al., 2019).

The sex differences in EWL and RMR at high T_a we report here for hornbills could conceivably arise in several ways. One is facultative hyperthermia, an often-cited mechanism for water conservation among desert birds (Gerson et al., 2019). However, relationships between T_b and T_a were indistinguishable for male and female hornbills, suggesting that facultative hyperthermia is not responsible for sex-specific differences in this species. Similar conclusions were reached concerning intraspecific variation in white-browed sparrow-weavers (*Plocepasser mahali*), even though a desert population had significantly higher heat tolerance limits in summer compared with two populations from cooler habitats (Noakes et al., 2016). Second, evaporative heat dissipation by females could involve lower metabolic heat production, for instance via enhanced cutaneous water loss (Menon et al., 1989; Tieleman and Williams, 2002; Webster and Bernstein, 1987; Williams, 1996), more efficient counter-current heat exchange across respiratory surfaces (Schmidt-Nielson et al., 1970) or more efficient panting via differences in the elastic properties and resonant frequency of the respiratory tract (Crawford and Kampe, 1971; Richards, 1970). A third possibility concerns sex differences in non-evaporative heat dissipation across the surface of the beak, which is smaller in females compared with males (Kemp, 2005). However, van de Ven et al. (2016) found no sex differences in heat dissipation via the beak in this species among individuals exposed to T_a between 15°C and 45°C , and it is unlikely this avenue of heat dissipation explains the sex differences we observed at $T_a > 39^\circ\text{C}$.

The notion of active suppression of metabolic rates (i.e. hypometabolism) under conditions of high heat load has received increased attention given recent evidence of 'hyperthermic daily torpor' in some small tropical mammals (Lovegrove et al., 2014; Reher et al., 2018). In Fig. 3, female RMR at T_a between 45°C and 50°C appears considerably more variable than that of males, with a number of female values remaining similar to the thermoneutral values even at T_a approaching 50°C . The Q_{10} values ≤ 2 for some individuals in our study support the notion that metabolic suppression may sometimes occur, although we could detect no significant sex difference. Nevertheless, the possibility that females could use metabolic suppression under humid conditions deserves further investigation, as does the apparent lack of temperature effects on metabolism under some conditions in both birds and mammals (Weathers, 1981; Tieleman and Williams, 1999; O'Connor et al., 2017; Lovegrove et al., 2014; Reher et al., 2018).

There is increasing evidence for fine-scale intraspecific variation in the thermal physiology of endotherms (Tieleman

et al., 2009; Glanville et al., 2012; McClennand et al., 2016; Noakes and McKechnie, 2019), which may arise from phenotypic flexibility, developmental plasticity (*sensu* Piersma and Drent, 2003), epigenetic transmission or genotypic adaptation (Merilä and Hendry, 2014). Far less is known about the contributions of these sources of variation to intraspecific variation in the heat tolerance of endotherms compared with that of ectotherms (Ketola et al., 2012; Xue et al., 2019), although recent work suggests that differences in heat tolerance among white-browed sparrow-weaver populations arise from phenotypic flexibility rather than genotypic adaptation (Noakes and McKechnie, 2019). The present study involved summer-acclimatised hornbills studied at the same time of year as this species breeds, and a future study testing whether these sex differences persist in winter-acclimatised individuals would be informative in terms of whether this variation arises from phenotypic flexibility.

Similar T_b values yet lower rates of EWL and RMR of female hornbills at high T_a compared with males likely confers adaptive benefits related to limited energy and water supply for females during hot weather, arising from the strong negative effects of hot weather on male foraging efficiency and hence nest provisioning rates (van de Ven et al., 2019, 2020), as opposed to avoidance of lethal hyperthermia. Resource availability and environmental heat loads are proposed to be important drivers in the evolution of endothermic thermoregulation (Brown et al., 2004; Angilletta et al., 2010; Speakman and Król, 2010; Boyles et al., 2011). Recent work on this species has revealed strong temperature-driven trade-offs between thermoregulation and foraging in male hornbills (van de Ven et al., 2019) and strong negative effects of high T_a during the breeding season on fitness components, including increased probability of nest abandonment, reduced likelihood of fledging and reduced fledgling mass (van de Ven et al., 2020). These sub-lethal fitness costs of chronic exposure to hot weather in hornbills and other arid-zone species (du Plessis et al., 2012; Cunningham et al., 2013; Kemp et al., 2020) are anticipated to result in substantial population declines and range contractions during the 21st century in response to global heating in the Kalahari (Conradie et al., 2019; Kemp et al., 2020).

In conclusion, differences in thermoregulation at T_a exceeding normothermic T_b , evident as lower water and energy requirements by female than male hornbills, supports the hypothesis that thermal physiology may evolve from sex-specific microclimates experienced during the breeding season. These findings, taken together with increasing evidence for substantial variation in evaporative cooling capacity and heat tolerance among related species co-occurring at a single site (Czenze et al., 2020) and in conspecific populations occurring along climatic gradients (Trost, 1972; Noakes et al., 2016) emphasise that much remains to be learned about adaptive variation in avian thermoregulation in the heat.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: B.v.J., N.C.B., A.E.M.; Methodology: B.v.J., N.C.B., S.J.C., A.E.M.; Formal analysis: B.v.J., R.K., T.M.F.N.V.; Resources: N.C.B., S.J.C., A.E.M.; Data curation: B.v.J., Z.J.C., R.K., T.M.F.N.V.; Writing - original draft: B.v.J., Z.J.C., S.J.C., A.E.M.; Writing - review & editing: B.v.J., N.C.B., Z.J.C., R.K., T.M.F.N.V., S.J.C., A.E.M.; Supervision: N.C.B., S.J.C., A.E.M.; Project administration: N.C.B., S.J.C., A.E.M.; Funding acquisition: N.C.B., S.J.C., A.E.M.

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Data availability

Data are available in the Dryad digital repository (McKechnie et al., 2021b): bcc2fzqbq.

Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.232777.supplemental>

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