Avian thermoregulation in the heat: evaporative cooling capacity in an archetypal desert specialist, Burchell's sandgrouse (*Pterocles burchelli*)

Running title

Sandgrouse heat tolerance

Andrew E. McKechnie[1,*]

Ben Smit[2]

Maxine C. Whitfield[1]

Matthew J. Noakes[1]

William A. Talbot[3]

Mateo Garcia[3]

Alexander R. Gerson[4]

Blair O. Wolf[3]

[1]DST-NRF Centre of Excellence at the Percy FitzPatrick Institute, Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

[2]Department of Zoology, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa

[3]UNM Biology Department, University of New Mexico, MSC03-2020, Albuquerque, NM 87131-0001, USA

[4]Department of Biology, University of Massachusetts, Amherst, MA 01003, USA

*Author for correspondence (aemckechnie@zoology.up.ac.za)

Keywords

body temperature; critical thermal maximum; evaporative water loss; hyperthermia; Pterocliformes; upper critical limit of thermoneutrality

Summary statement

Sandgrouse are the archetypal arid-adapted birds. We show that evaporative cooling in an African sandgrouse is surprisingly inefficient, and suggestive of heavy reliance on respiratory rather than cutaneous heat dissipation.

Abstract

Sandgrouse (Pterocliformes) are quintessential examples of avian adaptation to desert environments, but relatively little is known about the limits to their heat tolerance and evaporative cooling capacity. We predicted that evaporative cooling in Burchell's sandgrouse (*Pterocles burchelli*) is highly efficient and provides the basis for tolerance of very high air temperature (T_a). We measured body temperature (T_b), resting metabolic rate (RMR) and evaporative water loss (EWL) at T_a between 25 °C and ~58 °C in birds exposed to successive increments in T_a. Normothermic T_b averaged 39.0 °C, lower than typical avian values. At $T_a > 34.5$ °C, T_b increased linearly to a maximum of 43.6 °C at $T_a = 56$ °C. The upper critical limit of thermoneutrality (T_{uc}) was $T_a = 43.8$ °C, closely coinciding with the onset of panting and gular flutter. Above the T_{uc}, RMR increased 2.5-fold to 2.89 W at $T_a = 56$ °C, a fractional increase far exceeding that of many other species under comparable conditions. Rates of EWL increased rapidly at $T_a > 42.9$ °C to 7.84 ± 0.90 g h^{-1} at $T_a = 56$ °C, an 11-fold increase above minimal levels. Maximum evaporative cooling efficiency (ratio of evaporative heat loss to metabolic heat production) was 2.03, but could be as high as 2.70 if our assumption that the birds were metabolising lipids is incorrect. Thermoregulation at very high T_a in *P. burchelli* was characterised by large increases in RMR and EWL, and is much less efficient than in taxa such as columbids and caprimulgids.

Introduction

Deserts represent some of the most inhospitable and physiologically challenging habitats occupied by terrestrial organisms. Extremes of air temperature combined with desiccating conditions and highly unpredictable water and food resources impose severe constraints on the maintenance of energy and water balance (Dawson and Bartholomew, 1968; Dawson and Schmidt-Nielsen, 1964; Serventy, 1971). The challenges of avoiding lethal hyperthermia are especially pronounced in birds because of their small body size, typically diurnal activity and limited use of thermally-buffered microsites such as burrows; the adaptations that permit birds to occupy even the hottest deserts remain one of the most enduring fields of inquiry among ecological and evolutionary physiologists (Austin, 1976; Dawson and Bartholomew, 1968; Keast, 1960; Maclean, 1984; Williams and Tieleman, 2005). Thermoregulation under extremely hot conditions, when intense solar heat loads may result in birds

experiencing operative temperatures 15 °C or more above normothermic body temperature (T_b; Bakken, 1976; Robinson et al., 1976; Wolf and Walsberg, 1996b), involves consequential trade-offs between hyperthermia avoidance via evaporative heat dissipation and dehydration avoidance through the conservation of body water.

Sandgrouse (Pterocliformes) are the quintessential arid-adapted avian taxon. Consisting of 16 species distributed across the Afrotropics and Palaearctic, sandgrouse possess a suite of physiological and behavioural traits with clear functional links to desert environments (de Juana, 1997), such as the unique feather morphology and behaviours that allow adult birds to transport water in their belly-feathers to their chicks (Cade and Maclean, 1967; Maclean, 1983; Meade-Waldo, 1896). Sandgrouse have a pronounced capacity to tolerate extremely hot conditions (Thomas, 1984; Thomas and Maclean, 1981; Thomas and Robin, 1977). Several of these workers have noted that the onset of heat dissipation behaviours such as panting occurs at much higher air temperature (T_a) in sandgrouse than in most other birds. Moreover, behavioural responses of sandgrouse to $T_a > T_b$ include ptiloerection and huddling behaviour, a behaviour that may reduce environmental heat loads and which becomes significantly more pronounced when birds are dehydrated (Thomas and Maclean, 1981).

In laboratory studies of thermoregulation, four species of sandgrouse were found to have comparatively low resting metabolic rates, high upper critical limits of thermoneutrality, and pronounced evaporative cooling capacities (Hinsley, 1992; Hinsley et al., 1993; Marder et al., 1986). However, with the exception of the study by Marder et al. (1986), in which spotted sandgrouse (*Pterocles senegallus*) were exposed to $T_a = 60$ °C for 120 min, little is known about the upper limits to heat tolerance and evaporative cooling in this group of birds.

We investigated the heat tolerance limits and evaporative cooling capacity of sandgrouse in the context of their distinctive behaviour and ecology by measuring T_b, resting metabolic rate (RMR) and evaporative water loss (EWL) during acute heat stress in Burchell's sandgrouse (*Pterocles burchelli*), a species endemic to the arid regions of southern Africa (Lloyd, 2005). We predicted that *P. burchelli* has a pronounced capacity for evaporative cooling and can tolerate higher T_a values than many other arid-zone species, and furthermore that evaporative cooling in this species is very efficient, with comparatively shallow increases in EWL and RMR with increasing T_a above normothermic T_b. Because sandgrouse begin panting only at very

high T_a (Thomas and Maclean, 1981; Thomas and Robin, 1977), we also predicted that an inflection point in RMR indicating the upper critical limit of thermoneutrality (T_{uc}) occurs only at a T_a greater than normothermic T_b.

Materials and methods

Study species and site

The study took place 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 temperatures ranging from approximately 20°C to 43°C (Whitfield et al., 2015). Seventeen Burchell's sandgrouse (*Pterocles burchelli* Sclater 1922) with a mean body mass (M_b) of 192.9 ± 12.2 g SD were caught early in the morning on the property Gemsbok (27°06' S, 22°04' E) using mist nets set up around waterholes where the birds regularly drink. Following capture, birds were transported in cloth bags approximately 40 km by road to a field laboratory set up at Leeupan Ranch (26°58´S, 21°50´E; see Whitfield et al. 2015). The study took place during the austral summer in late January 2013.

Sandgrouse were used for measurements the same day they were captured. They were held in cages constructed of shade cloth with grain (crushed maize, millet and sunflower seeds) and water available *ad libitum*, but were never observed to feed while in captivity. Birds were always offered water before experimental measurements, and a feeding tube attached to a syringe was used to introduce 5 - 10 mL of water directly into the crop if they were unwilling to drink, which was the case for most individuals. Measurements typically lasted 2-3 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 they were released at the site of capture.

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

Air temperature (T_a) and body temperature (T_b) were measured using the same methods and equipment described by Whitfield et al. (2015). Briefly, T_b was measured using calibrated temperature-sensitive passive integrated transponder (PIT) tags (Biomark, Boise, ID, USA) injected intraperitoneally into the abdominal cavity. During gas exchange measurements, T_b was monitored using a reader and transceiver system (model FS2001, Biomark, Boise, ID, USA). Carbon dioxide production ($\dot{V}_{CO_2}$) and EWL were measured over the T_a range of 25-58°C, also using the same experimental setup as described by Whitfield et al. (2015) for their measurements during the 2013 season. Sandgrouse 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 15 and 75 L min^{-1} depending on the experimental T_a in order to keep chamber humidity below 5 ppt so that humidity within the chamber remained similar to those the birds experience naturally. As was the case in the study by Whitfield et al. (2015), we found that increasing flow rate during the course of measurements (and thereby reducing chamber humidity) usually kept birds calm even at very high T_a.

Experimental protocol

Experiments took place during the day using the same protocol as described by Whitfield et al. (2015), with birds exposed to progressively higher T_a using a stepped profile with 5°C increments at T_a between 25°C and 40°C, and 2°C increments at T_a between 40 °C and 58°C. Birds spent a minimum of 10 min, and on average ~30 min, at each T_a value. Birds were continually monitored during measurements using a video camera and an infrared light source (Whitfield et al., 2015). As was the case in the study by Whitfield et al. (2015), measurements were terminated when a bird a) was obviously stressed (sustained escape behaviour such as agitated jumping, pecking and/or wing flapping), or b) showed signs of extreme heat stress such as loss of coordination or balance; a sudden decrease in EWL, RMR and/or an uncontrolled increase in T_b. In the last instance, the bird was considered to have reached its upper limit of heat tolerance, and the T_a associated with the onset of these signs of heat stress and/or T_b approaching 45°C was considered the thermal endpoint for that individual. Any bird that reached its thermal endpoint was removed from the chamber

and held in front of an air-conditioner producing chilled air, with a cotton pad soaked in ethanol rubbed on the bird's body in order to aid in heat loss (Whitfield et al. 2015).

Data analyses

Data were analysed following Whitfield et al. (2015). We present whole-animal values, although in the case of EWL we calculated the slope of mass-specific EWL vs T_a to facilitate comparisons with the allometric equation presented by McKechnie and Wolf (2010). Rates of EWL were converted to rates of evaporative heat loss (W) assuming a latent heat of vaporisation of water of 2.406 J mg^{-1} at 40 °C (Tracy et al., 2010). We suspect that birds were postabsorptive at the time of measurements, but were unable to confirm this. Birds were captured early in the morning, with measurements typically starting at mid-morning the same day. Although food was offered, they were never observed feeding in captivity. Each bird's crop was palpated before measurements and in all instances appeared to be empty. Hence, we assumed a respiratory exchange ratio (RER) = 0.71, representative of lipid metabolism in post-absorptive birds (Walsberg and Wolf, 1995), and converted rates of $\dot{V}_{CO_2}$ to metabolic rate (W) using 27.8 J mL^{-1} CO_2 (Withers, 1992).

Broken-stick linear regression models fitted in the *R* package *segmented* (Muggeo, 2009) were used to identify inflection points in physiological variables, and linear mixed effects models that included individual identity as a random factor were fitted in the *R* package *nlme* (Pinheiro et al., 2009). In the case of RMR, values at T_a = 25 °C were excluded when we fitted a model to identify the inflection point, as visual inspection of the data suggested that this T_a value fell below the lower critical limit of thermoneutrality (Figure 1). Values are presented as means ± SD.
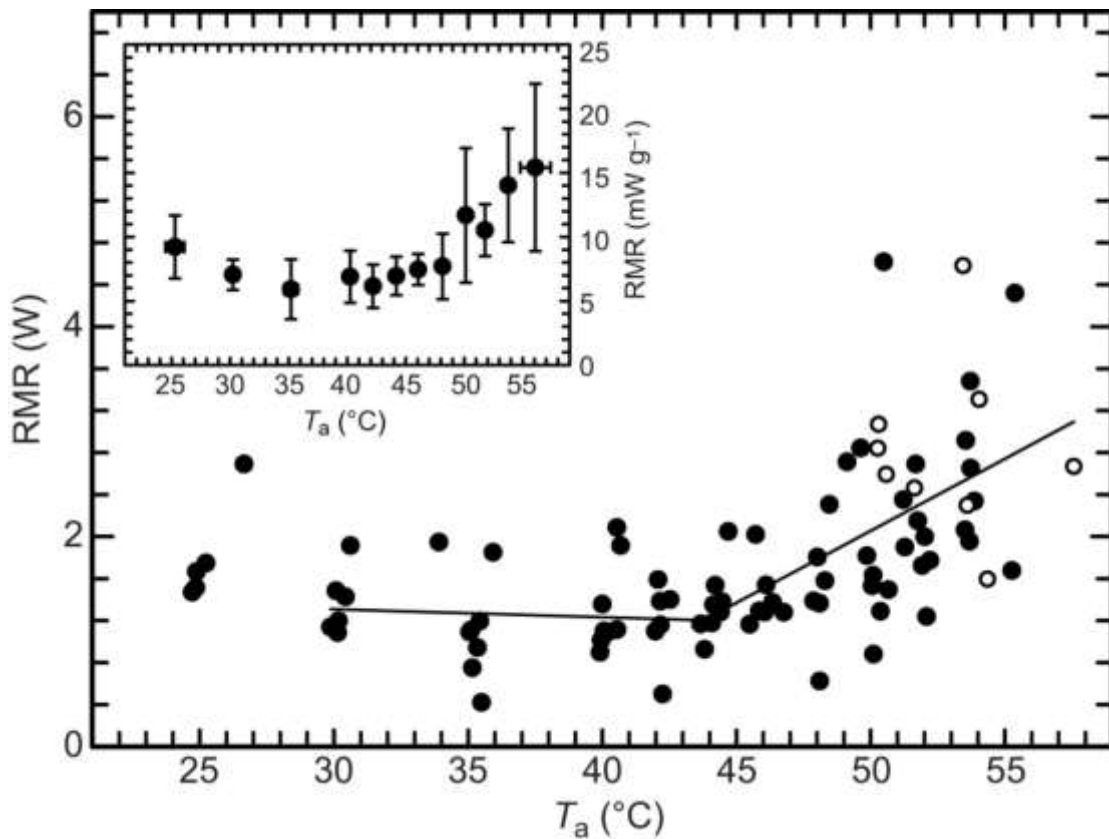
Figure 1. The resting metabolic rate of Burchell's sandgrouse (*Pterocles burchelli*) showed an inflection point at an air temperature (T_a) of 43.8 °C, corresponding to the upper critical limit of thermoneutrality for this species. Data for individuals that reached their thermal endpoint are shown as clear circles. The solid line is the segmented linear regression model that provided the best fit; the relationship between RMR and $T_a > 43.8$ °C is RMR = $0.137T_a$ - 4.800 (r^2 for segmented regression model = 0.414). The RMR data measured at $T_a = \sim 25$ °C were excluded from the regression model-fitting procedure, as this T_a appeared to fall below the thermoneutral zone. The inset graph shows mean ± SD mass-specific RMR for T_a bins of 2 °C. For each bin n = 5 - 12, except at $T_a = \sim 56$ °C, where n = 3.

Results

Body temperature and thermal endpoints

The normothermic T_b of *P. burchelli* ranged from 38.80 ± 0.68 °C (n = 5) at $T_a = \sim 25$ °C to 39.07 ± 0.67 °C (n = 8) at $T_a = \sim 35$ °C (Figure 2), with an overall average T_b of 39.00 ± 0.65 °C (n=5-8) in the range 25 °C ≤ T_a ≤ 35 °C. An inflection point in T_b occurred at $T_a = 34.5$ °C, above which T_b increased linearly and significantly (t = 21.48, P < 0.001) to a maximum of 43.61 ± 1.16 °C (n = 3) at $T_a = \sim 56$ °C. The single highest T_b value (i.e., mean T_b over a 5-min period) was 44.78 °C, measured in an individual experiencing $T_a = 53.60$ °C. There was no significant relationship between T_a and the rate of T_b increase during the last 5 min at each T_a value (t = 0.23, P =

0.820). However, mean rates of increase tended to be higher at $T_a > 40$ °C (0.02 – 0.05 °C min^{-1}) than at $T_a < 40$ °C (0.002 - 0.01 °C min^{-1}).

All individuals that reached thermal endpoints did so at $T_a > 50$ °C (Figure 2). The T_b of individuals that reached their thermal endpoint was 44.15 ± 0.41 °C, significantly higher ($F_{1,18} = 7.44$, P = 0.014) than that of individuals that did not (42.91 ± 0.89 °C).
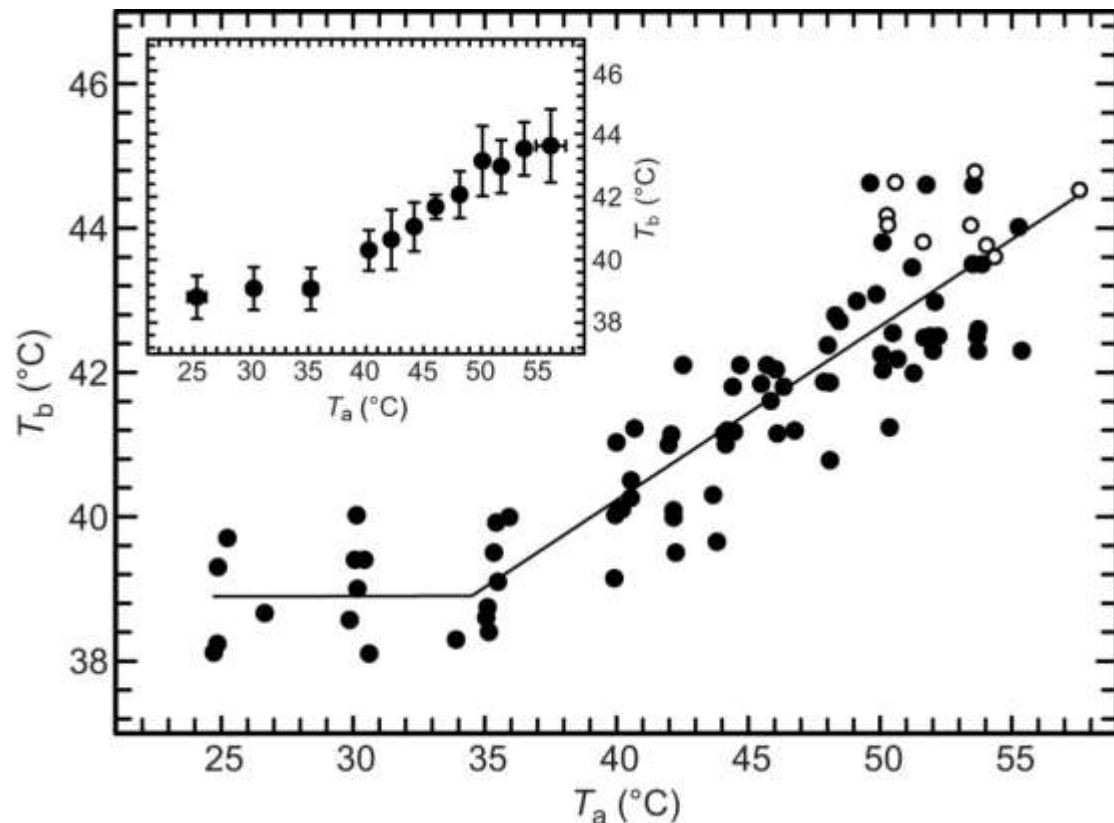

Figure 2. Body temperature (T_b) of Burchell's sandgrouse (*Pterocles burchelli*) as a function of air temperature (T_a). Data for individuals that reached their thermal endpoint are shown as clear circles. The solid line is the segmented linear regression model that provided the best fit; the relationship between T_b and $T_a > 34.5$ °C is $T_b = 0.241T_a + 30.58$ (r^2 for segmented regression model = 0.818). The inset graph shows mean ± SD T_b for T_a bins of 2 °C. For each bin n = 5 - 12, except at T_a =~56 °C, where n = 3.

Resting metabolic rate

The minimum RMR within the thermoneutral zone was 1.162 ± 0.515 W (n = 8) at T_a = ~35 °C (Figure 1). The inflection point above which RMR increased occurred at T_a = 43.8 °C, which we interpret as the T_{uc} (Figure 1). At $T_a > T_{uc}$, RMR increased linearly and significantly (t = 6.19, P < 0.001) to a maximum of 2.887 ± 1.335 W (n = 3) at T_a = ~56 °C, equivalent to 2.5 X minimum thermoneutral RMR. Visual inspection of the data suggests that RMR began to increase with decreasing T_a at T_a =

~25 °C (Figure 1), but there were insufficient data at $T_a < 30$ °C to fit a segmented regression model and reliably estimate the lower critical limit of thermoneutrality.

The estimated T_{uc} of 43.8 °C corresponded closely with the onset of panting and gular flutter, which commenced at $T_a = 44.4 \pm 1.7$ °C (n = 8). Observations of the sandgrouse during measurements revealed that both panting and gular flutter occurred. However, since the surveillance camera was positioned for monitoring overall behaviour during measurements, it was often not possible to distinguish between panting and gular flutter (e.g., when birds were facing away from the camera), and so we were unable to quantify these heat dissipation behaviours separately.
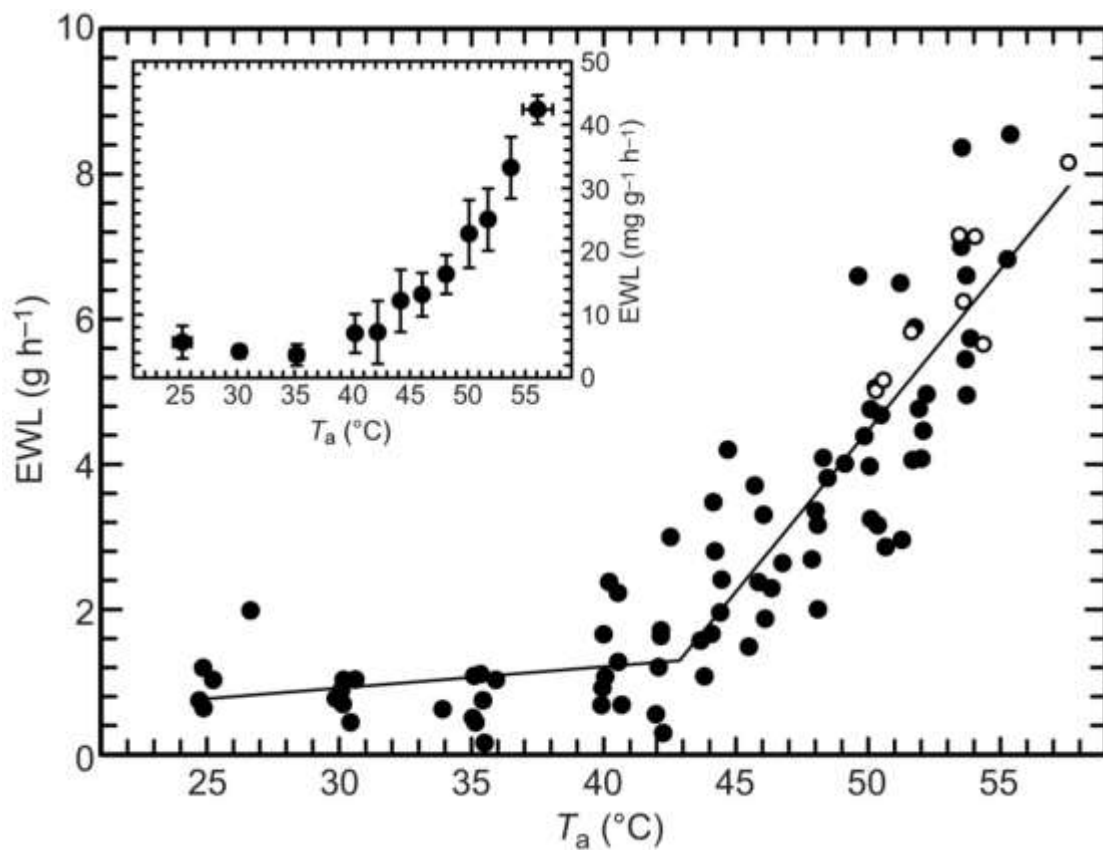

Figure 3. Evaporative water loss (EWL) in Burchell's sandgrouse (*Pterocles burchelli*) increased rapidly above an inflection point at an air temperature (*T_a*) of 42.9 °C. Data for individuals that reached their thermal endpoint are shown as clear circles. The solid line is the segmented linear regression model that provided the best fit; the relationship between EWL and $T_a > 42.9$ °C is EWL = $0.445T_a - 17.76$ (r^2 for segmented regression model = 0.843). The inset graph shows mean ± SD mass-specific EWL for T_a bins of 2 °C. For each bin n = 5 - 12, except at $T_a = \sim 56$ °C, where n = 3.

Evaporative water loss

Rates of EWL were minimal at T_a = ~30 °C and ~35 °C, with values of 0.808 ± 0.225 g h^{-1} (n = 6) and 0.710 ± 0.343 g h^{-1} (n = 8), respectively. An inflection point in EWL occurred at T_a = 42.9 °C, above which EWL increased with increasing T_a to a maximum of 7.842 ± 0.903 g h^{-1} (n=3) at T_a = ~56 °C, equivalent to an 11.0-fold increase over the value at T_a = ~35 °C (Figure 3). Evaporative water loss at T_a = ~56 °C was equivalent to 4.30 ± 0.23 % M_b hr^{-1}. Above the inflection T_a, the relationship between mass-specific EWL (MS_EWL; mg g^{-1} h^{-1}) and T_a was described by the relationship MS_EWL = 2.354T_a − 94.523 (r^2 = 0.745).
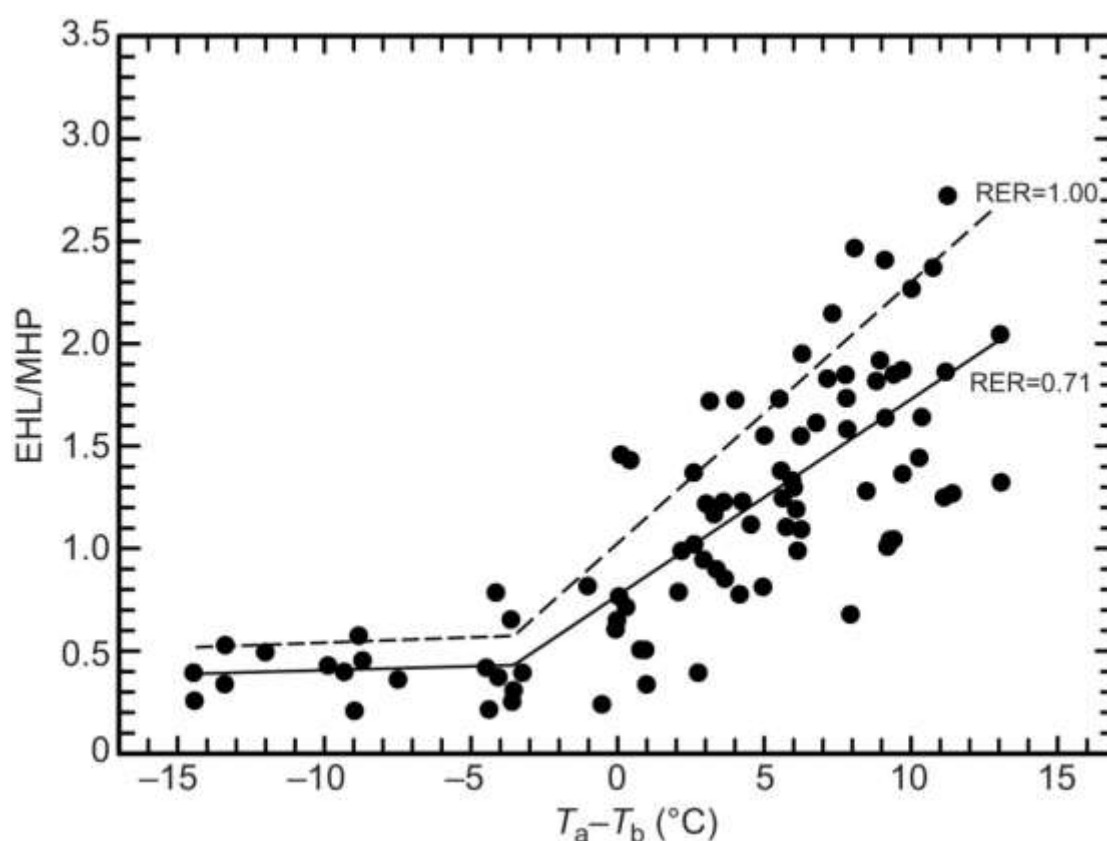

Figure 4. The relationship between the ratio of evaporative heat loss (EHL) and metabolic heat production (MHP) in Burchell's sandgrouse (*Pterocles burchelli*) as a function of the gradient between air temperature (T_a) and body temperature (T_b). Values were calculated assuming a respiratory exchange ratio (RER) of 0.71, corresponding to lipid metabolism. The solid line is the segmented linear regression model that provided the best fit; the relationship between EHL/MHP and T_a–T_b > -3.5 °C is EHL/MHP = 0.096(T_a-T_b) + 0.771 (r^2 for segmented regression model = 0.636). The dashed line is the regression model when the data were recalculated assuming RER = 1.00, corresponding to carbohydrate metabolism. In this case, the relationship is EHL/MHP = 0.128(T_a-T_b) + 1.025.

The mean ratio of evaporative heat loss (EHL) to metabolic heat production (MHP) remained below 1.00 at all $T_a < 44$ °C, increased to 1.139 ± 0.302 (n=8) at T_a = ~44 °C, and reached a maximum value of 2.029 ± 0.699 (n =3) at T_a = ~56 °C. When EHL/MHP was plotted as a function of the T_a-T_b gradient (Figure 4), an inflection point was evident at T_a-T_b = -3.54 °C. Above this inflection point, the y-intercept of the linear regression model was 0.77; in other words, on average 77 % of metabolic heat production was dissipated by evaporation when $T_b = T_a$.

Discussion

Thermoregulation at high T_a in *P. burchelli* is characterised by relatively low normothermic T_b, a relatively high T_{uc} that closely coincides with the onset of panting and gular flutter, rapid increases in both EWL and RMR with increasing T_a, and a maximum evaporative cooling capacity within the range typical for other birds investigated to date.

Body temperature

The T_b of *P. burchelli* was relatively stable at T_a below normothermic T_b. In black-bellied sandgrouse (*P. orientalis*), in contrast, the day-time cloacal T_b of birds in respirometry chambers was linearly related to T_a between 12 °C and 43 °C, with predicted T_b of 39.2 °C and 40.6 °C at T_a = 25 °C and 35 °C, respectively (Hinsley et al., 1993). Thomas and Maclean (1981) measured cloacal T_b of double-banded (*P. bicinctus*) and Namaqua (*P. namaqua*) sandgrouse held in cardboard boxes placed in outdoor aviaries at a site in the Namib Desert. Compared to *P. burchelli*, both these species maintained higher T_b at T_a = ~35 °C (~39.0°C) [*P. bicinctus*: 40.0 ± 0.6 °C (n = 11); *P. namaqua*: 40.4 ± 0.4 °C (n = 5); Thomas and Maclean, 1981]. The active-phase T_b of six spotted sandgrouse (*P. senegallus*) at T_a = 27 °C was 41.4 ± 0.6 °C, measured in birds in the hand using a hypodermic needle probe inserted into the lower intestinal region (Marder et al., 1986).

The active-phase normothermic T_b values we recorded in *P. burchelli* are lower than those of four other sandgrouse species. These differences may represent genuine interspecific variation, but we suspect that they instead arise from methodological differences among studies. Data for the four species listed above were all collected using cloacal probes (or hypodermic needle probes in the case of *P. senegallus*), whereas our data were collected using PIT tags injected into the

abdominal cavity. Sandgrouse are notorious for being nervous and highly-strung study subjects, and in several cases cloacal measurements of T_b have proven impossible on account of the birds' behaviour (Hinsley, 1992; Hinsley et al., 1993). In light of the nervous disposition of these birds, we suspect the lower T_b of *P. burchelli* compared to the species investigated previously reflects the fact that in our study birds were unrestrained within respirometry chambers, and did not have cloacal thermocouples inserted and secured to their plumage. It is striking that the highest mean T_b recorded in sandgrouse at moderate T_a (41.4 ± 0.6 °C in *P. senegallus*) was associated with a measurement protocol likely to be highly stressful, with birds held in the hand and a hypodermic needle probe inserted into their intestinal region (Marder et al., 1986).

The active-phase normothermic T_b of *P. burchelli* (~39.0°C) is lower than typical avian values; Prinzinger et al. (1991) reported a mean active-phase T_b for all birds of 41.02 ± 1.3 °C, and T_b = 39 °C is well below the typical range for taxa other than ratites (Clarke and Rothery, 2008). However, in view of the potential methodological variation discussed above, it is not possible at this stage to rigorously compare the T_b values of sandgrouse in general to those of other taxa.

At T_a approaching or exceeding normothermic T_b, the T_b of *P. burchelli* increased linearly to maximum values in the 43-45 °C range, a response qualitatively similar to other species exposed to the same heat tolerance experimental protocol employed in the present study (Smith et al., 2015; Whitfield et al., 2015). Compared to the T_b values reported in other sandgrouse at high T_a, those we measured in *P. burchelli* were relatively high. For instance, at T_a = ~50 °C, the mean T_b of 43.1 °C in *P. burchelli* compares with 41.6 °C in *P. bicinctus* and 41.0 °C in *P. namaqua* (Thomas and Maclean, 1981), and 42.6 °C in *P. senegallus* (Marder et al., 1986).

The T_b values (44-45 °C) associated with thermal endpoints in *P. burchelli* are in the same range as those reported for three southern African ploceid passerines (Whitfield et al., 2015) and southern African and Australian doves (McKechnie et al. in review), but slightly lower than those of one North American quail and two doves (45-46.1 °C; Smith et al., 2015). The significantly higher T_b in sandgrouse that reached thermal endpoints compared to those that did not is consistent with the argument that these T_b values are close to the birds' critical thermal maxima. As was the case for the passerines examined by Whitfield et al. (2015), patterns of T_b in *P. burchelli* appear to conform approximately to generalized biphasic models of heat

stroke during acute heat stress (Leon, 2006), where initial increases in T_b of 2-4 °C are followed by the regulation of approximately constant hyperthermic T_b, after which thermoregulatory breakdown is associated with rapid, unregulated increases in T_b toward lethal levels. A visual examination of the trajectory of T_b during acute heat stress in *P. burchelli* reinforces this impression, as T_b appears to reach a plateau at T_a > 50 °C (Figure 2 inset).

Resting metabolic rate

The T_{uc} of 43.8 °C we observed in *P. burchelli* is higher than typical avian T_{uc} values (e.g., Calder and Schmidt-Nielsen, 1967; Dawson and Bennett, 1973; McNab and Bonaccorso, 1995; Tieleman and Williams, 2002; Weathers, 1981). The fact that in *P. burchelli* the thermoneutral zone extends to several degrees above normothermic T_b confirms the findings of workers who previously investigated the thermal physiology of sandgrouse: Hinsley and her colleagues were unable to reliably define daytime T_{uc} values for *P. orientalis*, *P. alchata* or *P. bicinctus* based on measurements of RMR over a T_a range extending up to ~43 °C (Hinsley, 1992; Hinsley et al., 1993). Moreover, these results are also consistent with behavioural data indicating that heat dissipation behaviours commence only at T_a well above T_b (Thomas and Maclean, 1981; Thomas and Robin, 1977).

The inflection in RMR that defined the T_{uc} of *P. burchelli* coincided very closely with the mean T_a at which birds began to pant and/or gular flutter within respirometry chambers. This observation suggests strongly that the increase in RMR at high T_a primarily reflects the energetic cost of these processes, and that patterns of RMR at high T_a in this species conform well to the classic Scholander-Irving model of endothermic homeothermy (Scholander et al., 1950a; Scholander et al., 1950b). A combination of panting and gular flutter has also been reported in other sandgrouse species (Marder et al., 1986).

Contrary to our prediction that evaporative cooling in *P. burchelli* is highly efficient, fractional increases in RMR at T_a > T_{uc} were relatively large: RMR increased by ~150 % above thermoneutral values at T_a = 56 °C. This increase is substantially greater than those of 30 %, 35 % and 60 % in three ploceid passerines that reached T_as of 48-54 °C under similar experimental conditions (Whitfield et al., 2015), or the corresponding increases in RMR at T_as of 56–62 °C in Gambel's quail

(*Callipepla gambelli*) and six species of columbids from North America, Australia and southern Africa (Smith et al., 2015; McKechnie et al. in review).

Increases in avian RMR at T_a above the TNZ presumably comprise the effect of increasing T_b on metabolism plus the energetic cost of evaporative cooling mechanisms. Within the TNZ of *P. burchelli*, the Q_{10} for RMR (calculated from mean RMR and T_b values at $T_a = 35$ °C and $T_a = 44$ °C) was 2.2. Assuming the same Q_{10} at $T_a > T_{uc}$, we estimate that just 17 % of the increase in RMR between $T_a = 46$ °C and 54 °C was attributable to increases in T_b. This relatively small contribution of temperature effects is consistent with the argument that increases in RMR above the TNZ primarily reflect the energetic cost of evaporative cooling pathways such as panting. In general, the temperature dependence of avian RMR during hyperthermia remains unclear; whereas the Q_{10} for RMR in *P. burchelli* is similar to those expected for biological processes in general (Withers, 1992), data for other species suggest lower Q_{10} values, which in some cases may be close to 1 (i.e., independent of T_b; see for example Table 3 of Smith et al., 2015).

The minimum daytime RMR of the 193-g *P. burchelli* is similar to the corresponding values reported for other similar-sized sandgrouse: 0.731 W (*P. bicinctus*; 197 g) to 0.981 W (*P. alchata*; 243 g; Hinsley, 1992; Hinsley et al., 1993). As our measurements took place during the day (i.e., active phase), we could not estimate the basal metabolic rate of this species.

Evaporative water loss

Minimal EWL at thermoneutrality in *P. burchelli* (0.710 g hr^{-1} at $T_a = $ ~35 °C) is equivalent to 187 % of the value predicted by Williams' (1996) conventional equation for arid-zone birds, and 183 % of the value predicted by his phylogenetically-independent equation for all birds. Compared to the predictions of Williams' conventional equation, in other sandgrouse species EWL at $T_a = 25$ °C varies from close to expected to well above expected (*P. orientalis*: 98 %; *P. alchata*: 166 %). Thus, there appears to be no clear pattern among the sandgrouse for which data are currently available in terms of how EWL at moderate T_a compares to allometrically-expected values.

The inflection point in EWL in *P. burchelli* occurred at $T_a = $ ~43 °C, nearly 4 °C above normothermic T_b and at a much higher value than would be expected for a 193-g bird (35.1 °C; McKechnie and Wolf 2010 – see ESM). This high inflection in
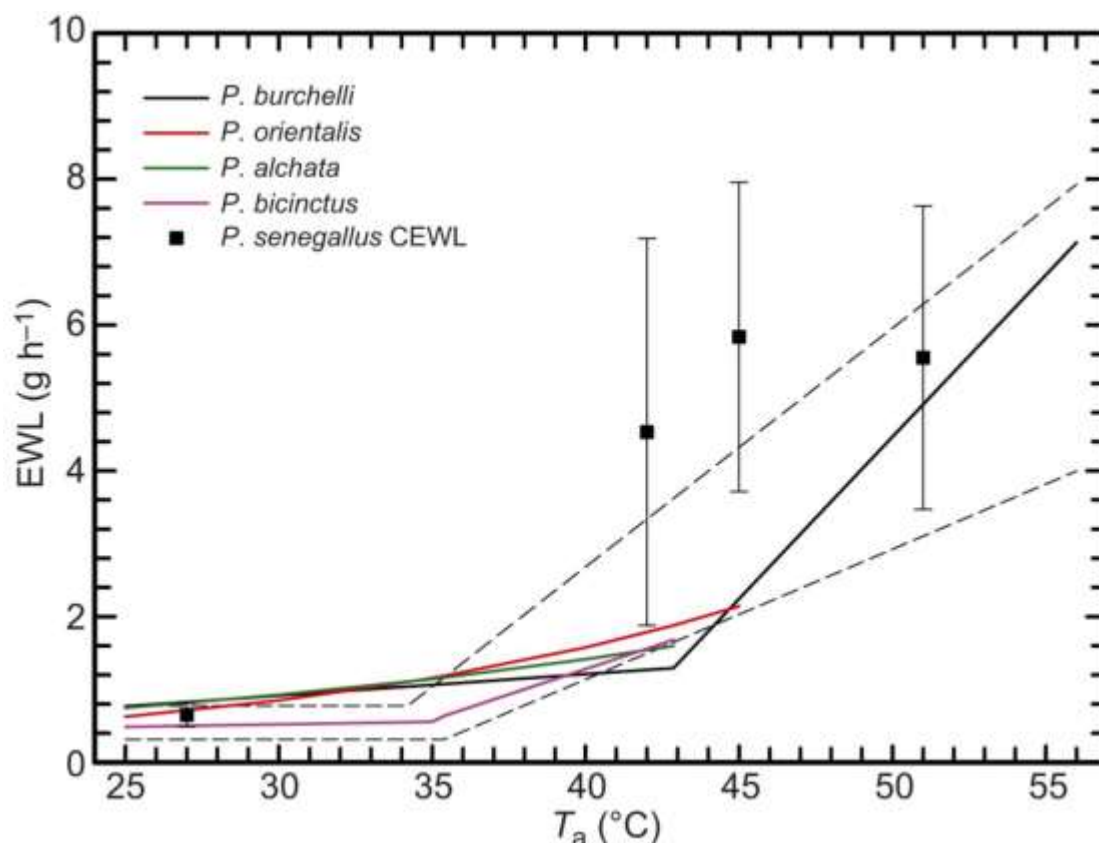
Figure 5. Evaporative water loss (EWL) as function of air temperature (T_a) in four species of sandgrouse. Data are shown for *Pterocles burchelli* (black line; present study), *P. orientalis* (red line; Hinsley et al., 1993), *P. alchata* (green line; Hinsley et al., 1993) and *P. bicinctus* (pink line; Hinsley, 1992). Also shown are cutaneous evaporative water loss (CEWL) rates measured in *P. senegallus* (squares; mean ± SD; Marder et al., 1986). The area between the dashed lines shows the range of EWL predicted for birds with body masses (M_b) between 150 g and 500 g (the approximate M_b range of sandgrouse; de Juana, 1997). These rates of EWL are based on predicted rates at moderate T_a (conventional equation for arid-zone birds; Williams, 1996), predicted inflection points at which EWL begins to rapidly increase (McKechnie and Wolf, 2010), and the predicted slopes of EWL *vs* high T_a (McKechnie and Wolf, 2010).

EWL is surprising, but consistent with the relatively modest increases in EWL above baseline levels observed in other sandgrouse at T_as of 40-45 °C (Hinsley, 1992; Hinsley et al., 1993; Figure 5). A high inflection T_a for EWL could also explain why the relationships between EWL and T_a in *P. orientalis* and *P. alchata* were best described by logarithmic curves (Hinsley et al., 1993), although Hinsley (1992) documented an inflection point at $T_a = 35$ °C in *P. bicinctus*.

      Above $T_a = 43$ °C, the slope of mass-specific EWL *vs* T_a in *P. burchelli* was much steeper than predicted by a recent analysis of the scaling of avian EWL (McKechnie and Wolf 2010 – see ESM). The observed slope of 2.354 mg g^{-1} hr^{-1} °C^{-1}

is equivalent to 224 % of the expected slope for a 193-g bird, at odds with our expectation that this species should show a relatively shallow slope and hence small increases in EWL. In sharp contrast, the slopes for *P. orientalis*, *P. alchata* and *P. bicinctus* were all well below (29 – 70%) allometrically-expected values (McKechnie and Wolf, 2010). However, this comparison likely reflects the much higher T_as to which birds were exposed in the present study compared to previous work; Hinsley (1992) and Hinsley et al. (1993) exposed birds to maximum T_a of 42-45 °C, temperatures at which EWL in *P. burchelli* was only just beginning to increase (Figure 5). The lower maximum T_a in the latter two studies is probably the major reason for this difference in slopes.

The maximum evaporative cooling capacity of *P. burchelli* (i.e., the maximum EHL/MHP ratio) was estimated as 2.029, with a y-intercept of 0.770 for EHL/MHP *vs* T_a (Figure 4). These calculations are based on an assumed RER = 0.71, corresponding to lipid metabolism. However, if we recalculate RMR assuming RER = 1.00 [i.e., a thermal equivalence of 20.9 J mL^{-1} CO_2 (Withers 1992), in which case estimated RMRs are 24.8 % lower than reported above], the maximum EHL/MHP increases to 2.699, and the y-intercept increases to 1.025 (Figure 4). The latter value is almost exactly that expected in theoretical grounds (the defence of a T_b setpoint equal to T_a requires that 100 % of metabolic heat production is dissipated evaporatively). Whereas we are confident that the sandgrouse were postabsorptive during measurements and hence likely metabolising lipids, the latter result raises the possibility that this assumption is incorrect. Previous work on the thermal physiology of sandgrouse does not shed any light; EHL/MHP in *P. orientalis* was well below 1.0 when T_a was approximately equal to T_b, but was approximately equal to 1.0 in *P. alchata* and *P. bicinctus* (Hinsley, 1992; Hinsley et al., 1993). This uncertainty regarding maximum EHL/MHP and the y-intercept illustrates a largely unavoidable limitation of our approach to measuring avian heat tolerance at extremely high T_a: the high flow rates required to keep chamber humidity low preclude accurate measurements of oxygen consumption ($\dot{V}_{O_2}$) and hence RER. The potential error associated with converting $\dot{V}_{CO_2}$ to metabolic rate in the absence of accurate RER measurements is almost three-fold greater than is the case for $\dot{V}_{O_2}$ (Walsberg and Wolf, 1995).

One factor critical in interpreting relationships between avian EWL and RMR at high T_a is the primary avenue of evaporative heat dissipation. Birds generally rely on either respiratory evaporative water loss (REWL) or cutaneous evaporative water loss (CEWL) as the primary mode of evaporative heat dissipation when $T_a > T_b$ (Hoffman and Walsberg, 1999; Marder and Arieli, 1988; McKechnie and Wolf, 2004; Ro and Williams, 2010; Tieleman and Williams, 2002; Wolf and Walsberg, 1996a). A comparison of passerines and columbids suggests that the primary mode of evaporative heat dissipation has important consequences for heat tolerance and evaporative cooling capacity, with CEWL-dominated cooling allowing for more efficient cooling and the capacity to handle higher maximum T_a compared to REWL-dominated cooling (McKechnie et al. in review).

To the best of our knowledge, the only investigation of the relative importance of REWL vs CEWL in sandgrouse is that of Marder et al. (1986), who combined measurements of the diffusive resistance of breast skin in spotted sandgrouse (*P. senegallus*) with temperature data to estimate area-specific rates of CEWL. However, when these CEWL estimates for *P. senegallus* are converted to whole-animal rates (using a predicted surface area of 408 cm^2; Walsberg and King, 1978), they prove puzzling. Marder et al. (1986) reported an almost 7-fold increase in CEWL between $T_a = 27$ °C and $T_a = 42$ °C, to a value that is between 2- and 3-fold higher than total evaporative water loss (TEWL) in other sandgrouse at comparable T_a values (Figure 5). Estimated CEWL for *P. senegallus* then increases to values around 3-fold higher than TEWL for congeners at $T_a = 45$ °C, before decreasing slightly at $T_a = 51$ °C (Marder et al., 1986; Figure 5). Because of these quantitative discrepancies between CEWL in *P. senegallus* and TEWL in other sandgrouse, we suspect that Marder et al. (1986) may have greatly overestimated rates of cutaneous evaporation.

On the basis of their reported rates of CEWL in *P. senegallus*, Marder et al. (1986) argued that sandgrouse are similar to columbids in that CEWL is the primary mode of heat dissipation at very high T_a. However, our data for *P. burchelli* suggest the opposite; the close match between the upward inflection in RMR at the T_{uc} and the onset of panting and gular flutter, together with the rapid increases in RMR with increasing T_a to rates equivalent to ~2.5 X thermoneutral RMR, comprise the pattern expected if respiratory evaporation is the primary mode of heat dissipation at $T_a > T_b$.

Conclusions

Several authors have noted that sandgrouse tolerate heat loads among the most extreme faced by any birds (reviewed by Thomas, 1984), and the behavioural and physiological mechanisms sandgrouse use to thermoregulate under very hot conditions have received considerable attention (Dixon and Louw, 1978; Thomas and Maclean, 1981; Thomas and Robin, 1977). Our data on heat tolerance and evaporative cooling in *P. burchelli* reveal that, during acute heat stress, this species can tolerate T_a up to ~56 °C. They also suggest that sandgrouse may have lower normothermic T_b than many other birds, and likely cannot tolerate T_b exceeding 45 °C. Increases in EWL and RMR occur at higher T_a than typical for birds, with the thermoneutral zone extending to 43.8 °C in *P. burchelli*, also the approximate T_a at which panting and gular flutter commence. These findings may explain the observations of several authors who found that heat dissipation behaviours in sandgrouse are delayed to T_a well above T_b (Thomas, 1984; Thomas and Maclean, 1981; Thomas and Robin, 1977). These high inflection points for EWL and RMR may also be related to ptiloerection and huddling, behaviours characteristic of sandgrouse at $T_a > T_b$ (Thomas and Maclean, 1981).

We exposed sandgrouse to T_a much higher than was the case in most previous studies (Hinsley, 1992; Hinsley et al., 1993). One unexpected pattern to emerge is that the metabolic costs of thermoregulation at $T_a > T_{uc}$ are substantial in *P. burchelli*; RMR at the highest T_a tolerated was equivalent to ~250 % of minimal thermoneutral values, a much greater fractional increase than in passerines or columbids (Smith et al., 2015; Whitfield et al., 2015; McKechnie et al. in review). Moreover, the slope of the relationship between EWL and T_a was much steeper than expected. These observations were surprising because, *a priori*, we expected sandgrouse to possess very efficient evaporative cooling mechanisms. These data also suggest that REWL is probably the major avenue of evaporative heat loss at high T_a, contrary to the arguments of Marder et al. (1986). Sandgrouse hence appear to lack evaporative cooling mechanisms comparable in efficiency to those found in taxa such as columbids and caprimulgids. In these groups, increases in metabolic rate at $T_a > T_b$ are very small, with RMR often remaining indistinguishable from thermoneutral values until $T_a > 50$ °C (Dawson and Fisher, 1969; Smith et al., 2015; A.E. McKechnie et al in review; R.S. O'Connor et al. unpublished data).

We can only speculate as to why similarly efficient CEWL-dominated cooling seems not to have evolved in sandgrouse. One possibility is that the water demands

for CEWL-dominated cooling are not sustainable for birds that typically forage far from water and visit water sources to drink only once a day, usually in the early morning (de Juana, 1997) and in the case of *P. burchelli* typically 2-3 hr after sunrise (Lloyd, 2005). When foraging during the heat of the day, sandgrouse therefore likely experience strong trade-offs between activity, heat storage and evaporative water loss. We hypothesise that evaporative cooling dominated by REWL facilitates delaying the initiation of rapid evaporative heat dissipation. According to this reasoning, as T_a exceeds T_b, sandgrouse initally minimise heat loss via mechanisms such as ptilo-erection and huddling, and only commence gular flutter and panting when T_a is well above T_b. It is perhaps relevant that columbids with very high evaporative cooling capacities, such as Namaqua doves (*Oena capensis*) in which EHL/MHP ratios can approach 5 (McKechnie et al, in review), are seldom seen far from water during the hottest part of the day (Dean, 2005).

In summary, the heat tolerance of Burchell's sandgrouse is impressive, with individuals tolerating T_a up to ~56 °C when exposed to acute heat stress under laboratory conditions. However, tolerance of very high T_a involves large fractional increases in rates of evaporation and metabolism, making evaporative cooling in this species considerably less efficient than in groups such as columbids and caprimulgids. The high rates of evaporative water loss required for thermoregulation under extremely hot conditions, combined with maximum T_b limits that are not above the typical avian range, may partly explain the strong dependence of sandgrouse on often-distant water sources.

Competing interests

The authors declare no competing financial interests.

Author contributions

B.O.W. and A.E.M. designed the study. B.S., M.C.W., M.J.N, W.A.T., M.G. and B.O.W. collected data. A.R.G., A.E.M. and M.C.W. analysed the data. A.E.M. and B.O.W. wrote the manuscript.

Abbreviations

CEWL cutaneous evaporative water loss

EHL evaporative heat loss

EWL evaporative water loss

M_b body mass

MHP metabolic heat production

RER respiratory exchange ratio

REWL respiratory evaporative water loss

RMR Resting metabolic rate

T_a air temperature

T_b body temperature

T_{uc} upper critical limit of thermoneutrality

$\dot{V}_{CO_2}$ carbon dioxide production

$\dot{V}_{O_2}$ oxygen consumption

References

Austin, G. T. (1976). Behavioral adaptations of the verdin to the desert. *Auk* **93**, 245-262.

Bakken, G. S. (1976). A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data to field ecology. *Journal of Theoretical Biology* **60**, 337-384.

Cade, T. J. and Maclean, G. L. (1967). Transport of water by adult sandgrouse to their young. *Condor* **69**, 323-343.

Calder, W. A. and Schmidt-Nielsen, K. (1967). Temperature regulation and evaporation in the pigeon and the roadrunner. *American Journal of Physiology* **213**, 883-889.

Clarke, A. and Rothery, P. (2008). Scaling of body temperature in mammals and birds. *Functional Ecology* **22**, 58-67.

Dawson, W. R. and Bartholomew, G. A. (1968). Temperature regulation and water economy of desert birds. In *Desert biology*, (ed. G. W. Brown). New York: Academic Press.

Dawson, W. R. and Bennett, A. F. (1973). Roles of metabolic level and temperature regulation in the adjustment of western plumed pigeons (*Lophophaps ferruginea*) to desert conditions. *Comparative Biochemistry and Physiology* **44A**, 249-266.

Dawson, W. R. and Fisher, C. D. (1969). Responses to temperature by the spotted nightjar (*Eurostopodus guttatus*). *Condor* **71**, 49-53.

Dawson, W. R. and Schmidt-Nielsen, K. (1964). Terrestrial animals in dry heat: desert birds. In *Handbook of physiology: adaptation to the environment.*, (ed. D. B. Dill), pp. 481-492. Washington, D.C.: American Physiological Society.

de Juana, E. (1997). Family Pteroclidae (sandgrouse). In *Handbook of the birds of the world. Vol. 4. Sandgrouse to cuckoos.*, eds. J. del Hoyo A. Elliot and J. Sargatal). Barcelona: Lynx Edicions.

Dean, W. R. J. (2005). Namaqua Dove. In *Roberts birds of southern Africa.*, eds. P. A. R. Hockey W. R. J. Dean and P. G. Ryan). Cape Town: The Trustees of the John Voelcker Bird Book Fund.

Dixon, J. and Louw, G. (1978). Seasonal effects on nutrition, reproduction and aspects of thermoregulation in the Namaqua sandgrouse (Pterocles namaqua). *Madoqua* **11**, 19-29.

Hinsley, S. A. (1992). Bioenergetics of a desert specialist, the double-banded sandgrouse, and the problems of stress induced by experimental conditions. *Comparative Biochemistry and Physiology* **102A**, 433-439.

Hinsley, S. A., Ferns, P. N., Thomas, D. H. and Pinshow, B. (1993). Black-bellied sandgrouse (*Pterocles orientalis*) and pin-tailed sandgrouse (*Pterocles*

alchata): closely related species with differing bioenergetic adaptations to arid zones. *Physiological Zoology* **66**, 20-42.

Hoffman, T. C. M. and Walsberg, G. E. (1999). Inhibiting ventilatory evaporation produces an adaptive increase in cutaneous evaporation in mourning doves *Zenaida macroura*. *Journal of Experimental Biology* **202**, 3021-3028.

Keast, A. (1960). Bird adaptations to aridity on the Australian continent. *Proceedings of the XII International Ornithological Congress*, 373-375.

Leon, L. R. (2006). The thermoregulatory consequences of heat stroke: are cytokines involved? *Journal of Thermal Biology* **31**, 67-81.

Lloyd, P. (2005). Burchell's Sandgrouse. In *Roberts birds of southern Africa.*, eds. P. A. R. Hockey W. R. J. Dean and P. G. Ryan). Cape Town: The Trustees of the John Voelcker Bird Book Fund.

Maclean, G. (1984). Avian adaptations to the Kalahari environment: A typical continental semidesert. *Koedoe* **27**, 187-193.

Maclean, G. L. (1983). Water transport by sandgrouse. *BioScience* **33**, 365-369.

Marder, J. and Arieli, U. (1988). Heat balance of acclimated pigeons *Columba livia* exposed to temperatures of up to 60°C T_a. *Comparative Biochemistry and Physiology* **91A**, 165-170.

Marder, J., Gavrieli-Levin, I. and Raber, P. (1986). Cutaneous evaporation in heat-stressed Spotted Sandgrouse. *Condor* **88**, 99-100.

McKechnie, A. E. and Wolf, B. O. (2004). Partitioning of evaporative water loss in white-winged doves: plasticity in response to short-term thermal acclimation. *Journal of Experimental Biology* **207**, 203-210.

McKechnie, A. E. and Wolf, B. O. (2010). Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. *Biology Letters* **6**, 253-256.

McKechnie, A.E., Whitfield, M.C., Smit, B., Gerson, A.R., Smith, E.K., Talbot, W.A., McWhorter, T.J. and Wolf, B.O. (in review) Avian thermoregulation in the heat: primary avenue of evaporative heat dissipation influences heat tolerance and cooling capacity. [submitted to *Journal of Experimental Biology*].

McNab, B. K. and Bonaccorso, F. J. (1995). The energetics of Australasian swifts, frogmouths and nightjars. *Physiological Zoology* **68**, 245-261.

Meade-Waldo, E. G. B. (1896). Sand grouse breeding in captivity. *Zoologist* **1896**, 298-299.

Muggeo, V. M. R. (2009). *Segmented*: an R package to fit regression models with broken-line relationships.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and RDevelopmentCoreTeam. (2009). *nlme*: Linear and nonlinear mixed effects models. R package version 3.1-94.

Prinzinger, R., Preßmar, A. and Schleucher, E. (1991). Body temperature in birds. *Comparative Biochemistry and Physiology* **99A**, 499-506.

Ro, J. and Williams, J. B. (2010). Respiratory and cutaneous water loss of temperate-zone passerine birds. *Comparative Biochemistry and Physiology A* **156**, 237–246.

Robinson, D. E., Campbell, G. S. and King, J. R. (1976). An evaluation of heat exchange in small birds. *Journal of Comparative Physiology B* **105**, 153-166.

Scholander, P. F., Hock, R., Walters, V. and Irving, L. (1950a). Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation and basal metabolic rate. *Biological Bulletin* **99**, 259-271.

Scholander, P. F., Hock, R., Walters, V., Johnson, F. and Irving, L. (1950b). Heat regulation in some arctic and tropical mammals and birds. *Biological Bulletin* **99**, 237-258.

Serventy, D. L. (1971). Biology of desert birds. In *Avian Biology Vol I*, eds. D. S. Farner and J. R. King), pp. 287-339. New York: Academic Press.

Smith, E. K., O'Neill, J., Gerson, A. R. and Wolf, B. O. (2015). Avian thermoregulation in the heat: resting metabolism, evaporative cooling and heat tolerance in Sonoran Desert doves and quail. *Journal of Experimental Biology* **218**, 3636-3646.

Thomas, D. (1984). Adaptations of desert birds: sandgrouse (Pteroclididae) as highly successful inhabitants of Afro-Asian arid lands. *Journal of Arid Environments* **7**, 157-181.

Thomas, D. and Maclean, G. (1981). Comparison of physiological and behavioral thermoregulation and osmoregulation in 2 sympatric sandgrouse species (Aves, Pteroclididae). *Journal of Arid Environments* **4**, 335-358.

Thomas, D. H. and Robin, A. P. (1977). Comparative studies of thermoregulatory and osmoregulatory behaviour and physiology of five species of sandgrouse (Aves : Pterocliidae) in Morocco. *Journal of Zoology, London* **183**, 229-249.

Tieleman, B. I. and Williams, J. B. (2002). Cutaneous and respiratory water loss in larks from arid and mesic environments. *Physiological and Biochemical Zoology* **75**, 590-599.

Tracy, C. R., Welch, W. R., Pinshow, B. and Porter, W. P. (2010). Properties of air: a manual for use in biophysical ecology. 4th Ed. *The University of Wisconsin Laboratory for Biophysical Ecology: Technical Report.*

Walsberg, G. E. and King, J. R. (1978). The relationship of the external surface area of birds to skin surface area and body mass. *Journal of Experimental Biology* **76**, 185-189.

Walsberg, G. E. and Wolf, B. O. (1995). Variation in the respirometry quotient of birds and implications for indirect calorimetry using measurements of carbon dioxide production. *Journal of Experimental Biology* **198**, 213-219.

Weathers, W. W. (1981). Physiological thermoregulation in heat-stressed birds: consequences of body size. *Physiological Zoology* **54**, 345-361.

Whitfield, M. C., Smit, B., McKechnie, A. E. and Wolf, B. O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *Journal of Experimental Biology* **218**, 1705-1714.

Williams, J. B. (1996). A phylogenetic perspective of evaporative water loss in birds. *Auk* **113**, 457-472.

Williams, J. B. and Tieleman, B. I. (2005). Physiological adaptation in desert birds. *BioScience* **55**, 416-425.

Withers, P. C. (1992). Comparative animal physiology. Fort Worth: Saunders College Publishing.

Wolf, B. O. and Walsberg, G. E. (1996a). Respiratory and cutaneous evaporative water loss at high environmental temperatures in a small bird. *Journal of Experimental Biology* **199**, 451-457.

Wolf, B. O. and Walsberg, G. E. (1996b). Thermal effects of radiation and wind on a small bird and implications for microsite selection. *Ecology* **77**, 2228-2236.