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Bat thermoregulation in the heat: Limits to evaporative cooling capacity in three southern African bats

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ABSTRACT

High environmental temperatures pose significant physiological challenges related to energy and water balance for small endotherms. Although there is a growing literature on the effect of high temperatures on birds, comparable data are scarcer for bats. Those data that do exist suggest that roost microsite may predict tolerance of high air temperatures. To examine this possibility further, we quantified the upper limits to heat tolerance and evaporative cooling capacity in three southern African bat species inhabiting the same hot environment but using different roost types (crevice, foliage or cave). We used flow-through respirometry and compared heat tolerance limits (highest air temperature (T_a) tolerated before the onset of severe hyperthermia), body temperature (T_b), evaporative water loss, metabolic rate, and maximum cooling capacity (i.e., evaporative heat loss/metabolic heat production). Heat tolerance limits for the two bats roosting in more exposed sites, *Taphozous mauritanus* (foliage-roosting) and *Eptesicus hottentotus* (crevice-roosting), were $T_a = \sim 44$ °C and those individuals defended maximum T_b between 41 °C and 43 °C. The heat tolerance limit for the bat roosting in a more buffered site, *Rousettus aegyptiacus* (cave-roosting), was $T_a = \sim 38$ °C with a corresponding T_b of ~ 38 °C. These interspecific differences, together with a similar trend for higher evaporative cooling efficiency in species occupying warmer roost microsites, add further support to the notion that ecological factors like roost choice may have profound influences on physiological traits related to thermoregulation.

1. Introduction

Many endotherms routinely experience environmental temperatures above their normothermic body temperatures (T_b), conditions under which evaporative cooling becomes a critical thermoregulatory process (Dawson, 1954; Herreid and Schmidt-Nielsen, 1966). Maintaining a stable T_b is often challenging for small endotherms as they exchange heat with their environment rapidly due to their high surface area to volume ratios. Bats are among the smallest endotherms and there is a wealth of data regarding their responses to low ambient temperatures (T_a) (reviewed by Ruf and Geiser, 2015). However, fewer studies have examined bats' response to high T_a (Bonaccorso and McNab, 1997; Downs et al., 2012; Korine and Arad, 1993; McNab, 1989; McNab and Bonaccorso, 2001; Noll, 1979; Procter and Studier, 1970; Studier, 1970;

Studier et al., 1970; Studier and Wilson, 1970) and fewer still have examined responses at $T_a \geq 40$ °C (Bronner et al., 1999; Cory Toussaint and McKechnie, 2012; Cryan and Wolf, 2003; Licht and Leitner, 1967a, 1967b; Ochoa-Acuña and Kunz, 1999).

Evaporative cooling is the main avenue for heat dissipation in bats, and evaporation may constitute 80%–85% of overall water flux (Arad and Korine, 1993; Studier et al., 1970). Thus, similar to desert bird species, under hot conditions individuals may face frequent trade-offs pitting water conservation and dehydration tolerance against high evaporative cooling requirements to avoid lethal hyperthermia (Dawson and Bartholomew, 1968). However, unlike other small endotherms, the diurnal rest phase of bats may force some species to endure the hottest part of the day with no access to water other than that contained in the food in their gut when returning to roost. The challenges of high

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rest-phase T_a may be particularly severe for subtropical and tropical species that use relatively unbuffered roost sites like arboreal microsites, rock crevices or roofs (e.g., Bronner et al., 1999).

Yangochiroptera appear to tolerate substantially higher maximum T_b values (43–45 °C; Cory Toussaint and McKechnie, 2012; Licht and Leitner, 1967a, 1967b; Maloney et al., 1999) compared to Pteropodidae (37 – 40 °C; Bartholomew et al., 1970, 1964). There have also been suggestions that heat tolerance of small bats may be correlated with roost microclimate (Bronner et al., 1999; Cory Toussaint and McKechnie, 2012; Marom et al., 2006; Minnaar et al., 2014), and there is some evidence for such correlations among nocturnal rodents (Lovegrove et al., 1991; B. van Jaarsveld et al., unpublished data). The roosts occupied by *Mops condylurus*, for instance, reach 50°– 60 °C in summer and individuals exposed to $T_a = 45$ °C showed rapid increases in evaporative water loss (EWL) and tolerated $T_b = \sim 43$ °C (Maloney et al., 1999). Cory Toussaint and McKechnie (2012) proposed that the variation in heat tolerance found among sympatric species may be correlated with the thermal properties of the roosts used by these species, a notion broadly supported by their data for three southern African species and the limited data available in the literature (Cory Toussaint and McKechnie, 2012). Specifically, the authors found that *Nycteris thebaica*, which roost in large thermally buffered (i.e., cave-like) baobab tree cavities, were least tolerant of high T_a .

Comparisons of physiological variables related to heat tolerance are complicated by variation in methods among studies, including flow rates and hence humidity levels in metabolic chambers (e.g., van Dyk et al., 2019; Gerson et al., 2014; Powers, 1992). The need for data collected under standardised conditions to facilitate comparative analyses provided the stimulus for a recent series of papers that has revealed substantial variation among and within avian taxa in terms of heat tolerance and evaporative cooling capacity (McKechnie et al., 2016a, 2017; O'Connor et al., 2017; Smit et al., 2018; Whitfield et al., 2015). Here, we applied the same approach to elicit the upper limits of heat tolerance and evaporative cooling capacity in three southern African bat species occurring sympatrically in a hot environment to test the hypothesized role of roost microclimate in determining chiropteran heat tolerance limits and evaporative cooling capacity. We predicted that heat tolerance limits and evaporative cooling capacity would be lowest in a cave-dwelling species that is buffered from high T_a compared to species regularly exposed to high T_a .

2. Material and methods

2.1. Study species and site

Bats were captured in Goro Nature Reserve (S22°59'; E29°25') on the semi-arid northern slope of the Soutpansberg mountain range in Limpopo Province, South Africa. The Limpopo Valley was selected as the study area because it is one of the hottest parts of southern Africa, with summer T_a that routinely exceeds 40 °C (South African Weather Service). The Soutpansberg is comprised of Soutpansberg mountain bushveld, northern mistbelt forest, Soutpansberg summit sourveld, Makhado sweet bushveld and Musina mopane bushveld. We used mist nets to capture bats at the site during the austral summer in October– December 2016. Species were identified using a taxonomic key (Monadjem et al., 2010). Forearm length (to nearest 0.1 mm), and body mass (to nearest 0.5 g) of each bat was recorded.

Our study species were a frugivorous cave-dwelling pteropodid, *Rousettus aegyptiacus* (132.6 ± 16.6 g, n=6), an insectivorous foliage-roosting emballonurid, *Taphozous mauritanus* (24.4 ± 0.9 g, n=5), and an insectivorous crevice-roosting vespertilionid, *Eptesicus hottentotus* (13.6 ± 1.6 g; Table 1; Monadjem et al., 2010). All individuals were non-reproductive adults. All three species depend on surface water (Dengis, 1996; Monadjem et al., 2010; Skinner and Chimimba, 2005). Individual *E. hottentotus* and *T. mauritanus* were held overnight in cloth bags and provided with drinking water at dusk before measurements

Table 1

Summary of thermoregulatory performance for three southern African bats, *Eptesicus hottentotus*, *Taphozous mauritanus*, and *Rousettus aegyptiacus*. T_b = body temperature, T_a = air temperature, RMR = resting metabolic rate, T_{uc} = upper critical limit of thermoneutrality, EWL = evaporative water loss, EHL= evaporative heat loss, MHP = metabolic heat production. Minimum and maximum values for each variable are indicated as “Min.” or “Max.” respectively. Means, SD and sample sizes (in parentheses) are reported.

Variable	<i>Eptesicus hottentotus</i>	<i>Taphozous mauritanus</i>	<i>Rousettus aegyptiacus</i>
Body mass (g)	13.6 ± 1.6 (3)	24.4 ± 0.9 (5)	132.6 ± 16.6 (6)
Body temperature			
Min. T_b (°C)	30.7 (2)	36.0 ± 0.71 (3)	36.1 ± 0.64 (6)
		35.6 (1)	
Inflection T_a (°C)	33.0	33.3	31.7
T_b versus T_a slope	1.03	0.82	0.34
Max. T_b (°C)	41.1 ± 0.3 (3)	42.3 ± 0.6 (4)	38.15 ± 0.4 (6)
	42.08 (1)*	42.9 (1)*	
Max. T_a (°C)	41.8 ± 0.06 (3)	42.2 ± 0.6 (4)	38.06 ± 0.6 (6)
	43.1 (1)	44.6 (1)	
Resting metabolic rate			
Min. RMR (W)	0.07 (2)	0.24 ± 0.07 (5)	1.09 ± 0.59 (5)
T_{uc} (°C)	33.0	31.0	28.5
RMR slope (mW °C ⁻¹)	16.7	22.6	30.44
Max. RMR (W)	0.20 ± 0.03 (3)	0.45 ± 0.11 (4)	1.4 ± 0.26 (6)
	0.27 (1)		
Max. RMR/Min. RMR	3.11	1.88	1.34
Evaporative water loss			
Min. EWL (g h ⁻¹)	0.06 ± 0.01 (3)	0.18 ± 0.06 (5)	0.46 ± 0.19 (5)
Inflection T_a (°C)	40.5	36.7	34.2
EWL slope (g h ⁻¹ °C ⁻¹)	0.18	0.12	0.23
Max. EWL (g h ⁻¹)	0.37 ± 0.07 (3)	0.74 ± 0.6 (4)	1.51 ± 0.56 (6)
	0.70 (1)	0.86 (1)	
Max. EWL/min. EWL	7.54	4.24	3.28
Min. EHL/MHP	0.45 ± 0.20 (3)	0.53 ± 0.21 (5)	0.29 (2)
Max. EHL/MHP	1.21 ± 0.15 (3)	1.04 ± 0.56 (4)	0.75 ± 0.19 (6)
	1.71 (1)	1.44 (1)	

*A single individual *E. hottentotus* and *T. mauritanus* tolerated $T_a > 42$ °C, and those maximum values are also shown.

commenced the following morning. *Rousettus aegyptiacus* were housed individually in cages constructed from wooden frames and shade cloth. They were provided with fruit until 2 h before measurements and *ad libitum* water before the start of measurements. All experimental protocols were approved by the Animal Ethics Committee of the University of Pretoria (protocol EC022-16), and the Research Ethics and Scientific Committee of the National Zoological Garden (P16-13).

2.2. Air and body temperature measurements

We measured T_a within the respirometry chamber using a thermistor probe (Model TC-100, Sable Systems, Las Vegas, NV, USA) inserted through a small hole in the chamber that was sealed with a rubber grommet. Body temperature was measured using temperature-sensitive passive integrated transponder (PIT) tags (BioTherm, Biomark, Boise, ID, USA). We injected the PIT tag subcutaneously between the scapulae of each individual. A PIT tag reader and portable transceiver system (model FS2001, Destron Fearing, St. Paul, MN, USA) were used to continuously record T_b of each bat during gas exchange measurements. We calibrated a subsample of PIT tags in a circulating water bath over temperatures ranging from 20 to 50 °C against a Cu-Cn thermocouple (IT-18, Physitemp Instruments Inc., Clifton NJ, USA).

2.3. Gas exchange measurements

We used an open flow-through respirometry system to measure carbon dioxide production (V_{CO_2}) and EWL during measurements. We

used metabolic chambers that consisted of either a 4 L (*T. mauritanus* and *E. hottentotus*) or 8 L (*R. aegyptiacus*) airtight container outfitted with a plastic mesh platform elevated ~ 10 cm above a ~ 1 cm layer of mineral oil to trap excreta. The inner walls and top of the chamber were also covered in mesh so the bats could crawl and hang in their natural roosting posture. These chambers have been previously shown to not absorb water vapour (Whitfield et al., 2015). The chambers were placed in a controlled-temperature box constructed from an ice chest (~ 100 L). The T_a within the chambers was regulated using a Peltier device (model AC-162, TE Technology Inc., Traverse City, MI, USA) and a custom-built controller. Air was supplied via a pump with a maximum capacity of approximately 30 L min⁻¹ (model DOA-P13- BN, Gast Air Pumps, Benton Harbour, MI, USA) before passing through silica gel and Drierite to remove water vapour. Thus, dry air was supplied to the baseline and chamber channels. A needle valve (Swagelok, Solon, OH, USA) was used to regulate the flow rate in the baseline channel, and mass flow controller (MFC; model MC-10SLPM-D/5m, Alicat Scientific Inc., Tucson AZ, USA), calibrated using a soap-bubble flow meter (Gilibrator 2, Sensidyne, St Petersburg, FL, USA), regulated the experimental flow rates. The air inlet was placed close to the top of each metabolic chamber with an elbow joint facing the top (to minimize any potential convective cooling at higher flow rates) and the air outlet below the mesh platform to maximize air mixing. Flow rates ranged from 2.5–11.5 L min⁻¹ (*R. aegyptiacus*), 2.5–9.5 L min⁻¹ (*T. mauritanus*), and 2.5–11.3 L min⁻¹ (*E. hottentotus*), depending on T_a , and individual behavior. All flow rates were adjusted to maintain water vapour pressures as low as possible (i. e., <5 ppt) within the chamber while allowing for the accurate measurements of differences in water vapour and CO₂ between incurrent and excurrent air.

Excurrent air from the chamber and baseline air were subsampled sequentially using a respirometry multiplexer (model MUX3-1101-18M, Sable Systems) in manual mode and an SS-3 Sub-sampler (Sable Systems). Subsampled air was pulled through a CO₂/H₂O analyzer (model LI-840A, LI-COR, Lincoln, NE, USA), which was regularly zeroed using nitrogen, and spanned for CO₂ using a certified calibration gas with a known CO₂ concentration of 2000 ppm (AFROX, Johannesburg, South Africa). The H₂O sensor of the LI-840A was regularly zeroed using nitrogen and spanned using a dewpoint generator (DG-4, Sable Systems, Las Vegas NV). All tubing in the system was Bev-A-Line IV tubing (Thermoplastic Processes Inc., Warren, NJ, USA). Voltage outputs from the analyzers and thermistor probes were digitized using an analog-digital converter (model UI-3, Sable Systems, Las Vegas NV, USA) and recorded with a sampling interval of 5 s using Expedata software (Sable Systems).

2.4. Experimental protocol

Data were collected during the diurnal rest-phase following the methods described by Whitfield et al. (2015), where animals were exposed to a stepped profile of progressively higher T_a . This approach is conceptually analogous to the sliding cold exposure protocol widely used for measurements of summit metabolism (Swanson et al., 1996), and was used because it is not feasible to maintain animals at very high T_a for periods typical of steady-state measurements at lower T_a values.

Bats were maintained at each T_a for a period of at least 10 min, but more typically 20–30 min, until T_b , V_{CO_2} and EWL stabilized at constant values maintained for at least 5 min. Data were collected starting at T_a = ~20 °C (*R. aegyptiacus*) or T_a = ~25 °C (*T. mauritanus* and *E. hottentotus*), with T_a subsequently increased in ~2 °C increments. Experimental runs were ended when an animal showed heat stress (i.e., agitation, problems maintaining posture, or a trajectory of increasing body temperature exceeding 42 °C toward lethal T_b of 44 °C; Licht and Leitner, 1967b, 1967a).

2.5. Statistical analyses

Data were acquired and analysed using Expedata software (Sable Systems, Las Vegas NV, USA). We corrected for analyzer drift and lag using the relevant algorithms in Expedata software (Sable Systems, Las Vegas NV, USA). Equations 9.5 and 9.6 from Lighton (2008) were used to calculate V_{CO_2} and evaporative water loss (EWL) from the lowest stable 5-min periods of CO₂ and water vapour at a given T_a , assuming 0.803 mg H₂O mL⁻¹ vapour. We selected the lowest 5-min average of V_{CO_2} at each T_a to represent resting values.

We calculated whole-animal V_{CO_2} , resting metabolic rate (RMR), EWL and evaporative cooling capacity (expressed as evaporative heat loss/metabolic heat production [EHL/MHP]) as described by Whitfield et al. (2015). To convert V_{CO_2} to RMR, we assumed a respiratory exchange ratio (RER) of 0.71, representative of post-absorptive lipid metabolism (Withers, 1992) and converted rates of V_{CO_2} to metabolic rate (W) using 27.8 J mL⁻¹ CO₂. To determine inflection points where T_b , EWL, RMR started to increase with increasing T_a , we fitted segmented linear regression models using the *segmented* package (Muggeo, 2009) in R (R 3.5.2; R Core Team, 2018) for each species. Linear mixed effects models were fitted to the data with the R package *nlme* (Pinheiro et al., 2009) to determine the relationship between each physiological variable and T_a . We accounted for pseudoreplication (due to multiple measurements from individuals) by including individual identity as a random factor in all analyses. We assessed significance at $p < 0.05$ and values are presented as mean ± s.d. unless specifically stated.

There remains debate about whether segmented linear models or polynomial models are more appropriate for increases in EWL at T_a approaching or exceeding normothermic T_b . We opted to use segmented models, for the reasons outlined by Whitfield et al. (2015). These authors found negligible differences in model fit, and argued that segmented linear models are preferable in terms of facilitating comparisons among and within species.

3. Results

3.1. Body temperature

Each species showed approximately stable normothermic T_b with an inflection point that ranged from 31.7 °C ± 1.5 (SE) (*R. aegyptiacus*) to 33.3 °C ± 0.7 (SE) (*T. mauritanus*), above which T_b increased linearly (Table 1, Fig. 1). The slope of T_b vs. T_a above the inflections point was substantially shallower for *R. aegyptiacus* (df=20.0, t=16.6, r² =0.94, slope = 0.34) compared to either *T. mauritanus* (df=21.4, t=14.3, r² =0.93, slope = 0.82), or *E. hottentotus* (df=22.2, t=17.3, r² =0.93, slope = 1.03; Table 1). *Rousettus aegyptiacus* also had the lowest mean heat tolerance limit (T_a = 38.06 ± 0.6 °C) and lowest mean maximum T_b (38.15 ± 0.4 °C). Individuals of both *E. hottentotus* and *T. mauritanus* tolerated ~44 °C and defended maximum T_b between 41 °C and 43 °C (Table 1).

3.2. Resting metabolic rate

At $T_a < \sim 30$ °C there was considerable variation in RMR; however, all individuals exhibited similar activity so this likely represents individual variation when exposed to these temperatures. This variation decreased substantially at higher temperatures and RMR for all three species increased significantly with T_a above their respective inflection points with *R. aegyptiacus* = 28.5 °C ± 2.0 (SE), *T. mauritanus* = 31.0 °C ± 0.9 (SE), and T_a = 33 °C ± 1.4 (SE) in *E. hottentotus* (Table 1). Above the inflection point, RMR increased to maximum values equivalent to 134% (*R. aegyptiacus*), 188% (*T. mauritanus*), and 311% (*E. hottentotus*) of minimal values (Table 1; Fig. 2).

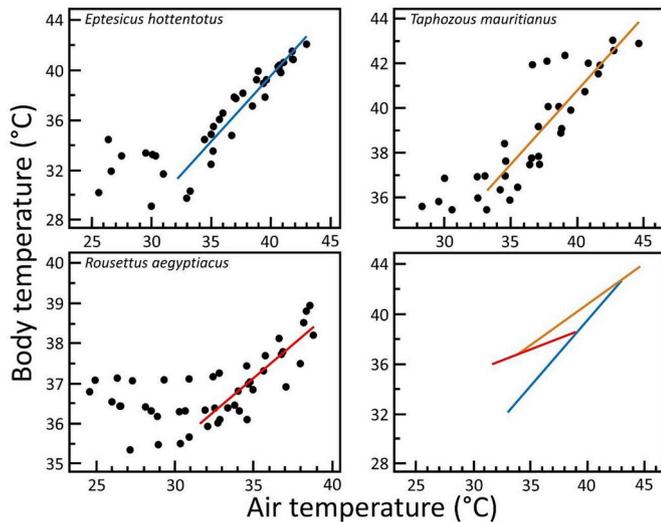


Fig. 1. Relationship between body temperature and air temperature for three southern African bats, *Eptesicus hottentotus* ($13.6 \pm 1.6g$), *Taphozous mauritanus* ($24.4 \pm 0.9g$), and *Rousettus aegyptiacus* ($132.6 \pm 16.6g$). Note that the scaling of the x and y-axes varies among panels. For each species, the solid line is the upper portion of the segmented linear regression model that provided the best fit. The bottom right panel shows the regression models for all three species plotted together for comparative purposes.

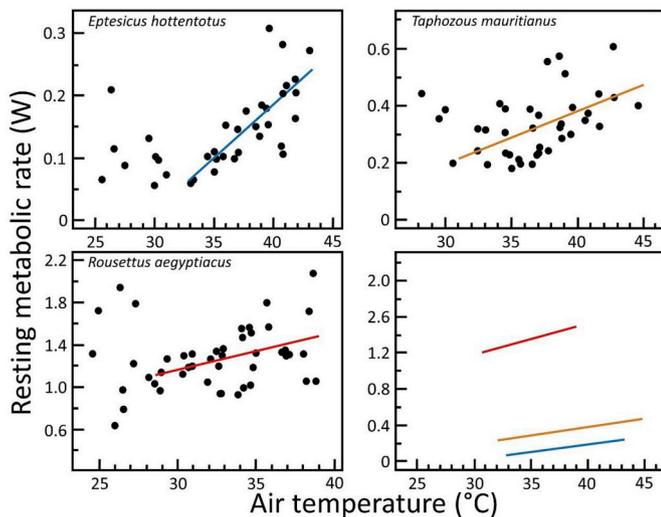


Fig. 2. Relationship between resting metabolic rate and air temperature for three southern African bats, *Eptesicus hottentotus* ($13.6 \pm 1.6g$), *Taphozous mauritanus* ($24.4 \pm 0.9g$), and *Rousettus aegyptiacus* ($132.6 \pm 16.6g$). Note that the scaling of the x and y-axes varies among panels. For each species, the solid line is the upper portion of the segmented linear regression model that provided the best fit. The bottom right panel shows the regression models for all three species plotted together for comparative purposes.

3.3. Evaporative water loss

In all three species, EWL remained approximately constant with increasing T_a and then increased rapidly and linearly above an inflection point that ranged from 34.2 ± 0.7 (SE) (*R. aegyptiacus*) to 40.5 ± 0.1 (SE) (*E. hottentotus*; Table 1, Fig. 3). Minimum rates of evaporative water ranged from 0.06 g h^{-1} (*E. hottentotus*) to 0.45 g h^{-1} (*R. aegyptiacus*; Table 1). The fractional increases in EWL (i.e., the ratio of maximum values to minimum values) were ~ 3.3 (*R. aegyptiacus*), ~ 4.2 (*T. mauritanus*), and ~ 7.5 (*E. hottentotus*). Maximum evaporative cooling capacity, expressed as maximum evaporative heat loss/metabolic

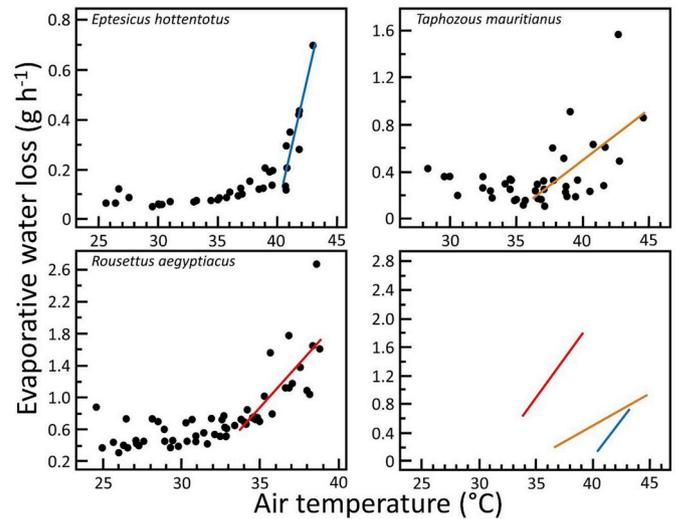


Fig. 3. Relationship between evaporative water loss and air temperature for three southern African bats, *Eptesicus hottentotus* ($13.6 \pm 1.6g$), *Taphozous mauritanus* ($24.4 \pm 0.9g$), and *Rousettus aegyptiacus* ($132.6 \pm 16.6g$). Note that the scaling of the x and y-axes varies among panels. For each species, the solid line is the upper portion of the segmented linear regression model that provided the best fit. The bottom right panel shows the regression models for all three species plotted together for comparative purposes.

heat production (EHL/MHP), ranged from 0.75 (*R. aegyptiacus*) to 1.3 (*E. hottentotus*).

4. Discussion

The three bats we investigated here all showed typical mammalian increases in T_b , RMR and EWL when environmental temperatures approached or exceeded normothermic T_b . Although our sample sizes were low, particularly for the small insectivorous species, patterns of interspecific variation in heat tolerance and evaporative cooling capacity broadly supported our prediction concerning how these traits vary among species occupying roosts that differ in the extent to which they buffer occupants from high air temperatures. Upper T_a and T_b limits were lowest in *R. aegyptiacus* and this cave-roosting pteropodid was noticeably less heat tolerant than either of the small insectivorous species, reaching T_b maxima of just $\sim 38 \text{ }^\circ\text{C}$ before showing signs of severe heat stress.

One limitation of our study is that we were not able to directly measure temperatures in the roosts occupied by our study individuals. However, our assumption that the roost temperatures experienced by the cave-roosting *R. aegyptiacus* are substantially lower than those experienced by tree- or crevice-roosting *T. mauritanus* and *E. hottentotus* during hot weather is supported by numerous studies of cave-roosting bats. Caves occupied by *R. aegyptiacus* in Kenya varied only between $25.5 \text{ }^\circ\text{C}$ and $27 \text{ }^\circ\text{C}$ and, near Table Mountain, cave temperature ranged from $7.0 \text{ }^\circ\text{C}$ to $12.3 \text{ }^\circ\text{C}$, lower and more stable than mean daily T_a measured at the Cape Town Airport, which ranged from $\sim 9 \text{ }^\circ\text{C}$ to $17 \text{ }^\circ\text{C}$ (Barclay et al., 2017). Further, in caves inhabited by the obligate cave-roosting *Miniopterus schreibersii* and *Rhinolophus capensis* (Monadjem et al., 2010), mean temperatures over the course of a year ranged from $13.9 \text{ }^\circ\text{C}$ to $19.5 \text{ }^\circ\text{C}$ with absolute maximum and minimum temperatures of 9.9 and $22.3 \text{ }^\circ\text{C}$, whereas outside maximum T_a exceeded roost temperatures by as much as $17.7 \text{ }^\circ\text{C}$, (Brown and Bernard, 1994). Crevice roosts occupied by *Pipistrellus hesperidus* and *Tadarida aegyptiaca* in the same area as where the latter study took place often exceeded outside T_a (Z.J. Czenze unpublished data). In a comparative study of three sympatric bat species in the same region as the present study, *Nycteris thebaica* (the least heat-tolerant species) roosted in a thermally-buffered baobab tree cavity that remained $5 \text{ }^\circ\text{C}$ cooler than

maximum T_a (Cory Toussaint and McKechnie, 2012). Conversely, *T. mauritanus* and *Sauromys petrophilus* were more heat tolerant and roosted in crevices where temperatures sometimes exceeded T_a on very hot days (Cory Toussaint and McKechnie, 2012).

Direct comparisons among our three study species are potentially confounded by both phylogeny and body mass. Moreover, comparing the heat tolerance of *R. aegyptiacus* to that of other Yinpterochiroptera are challenging as comparable data on thermoregulation in the heat (e.g., $>36^\circ\text{C}$) are available for only one tree-roosting pteropodid from southern Africa (*Epomophorus wahlbergi* ~84 g; Minnaar et al., 2014) and, to some extent, four tree-roosting Australian and New Guinean pteropodids (*Dobsonia minor* ~87 g, *Pteropus poliocephalus* ~680 g, *Pteropus scapulatus* ~360 g, *Syconycteris australis* ~20 g; Bartholomew et al., 1964; Bartholomew et al., 1970).

The mean resting T_b we recorded for *R. aegyptiacus* (36.1°C) is similar to values previously reported for this species ($35.2 - 36.5^\circ\text{C}$; Korine and Arad, 1993; Noll, 1979) and other pteropodids ($33.8 - 38.3^\circ\text{C}$; Bartholomew et al., 1964; Bonaccorso and McNab, 1997; Downs et al., 2012; Jones, 1972; McNab, 1989; McNab and Armstrong, 2001; McNab and Bonaccorso, 2001; Ochoa-Acuña and Kunz, 1999) However, the mass-specific BMR (8.2 mW g^{-1}) we report here is higher than previously recorded values from the same species ($4.6 - 5.2 \text{ mW g}^{-1}$; Korine and Arad, 1993; Noll, 1979). Our study involved free-ranging individuals held overnight, whereas Korine and Arad (1993) held individuals in captivity for 2–3 weeks, and Noll's (1979) measurements involved captive individuals acclimated to 15°C or 30°C for six weeks. These differences, as well as variation among these studies in terms of experimental protocols (e.g., exposure to a single T_a compared to multiple ramped T_a) may, we suspect, be part of the reason for these differences in BMR values.

The mean maximum T_b we recorded for *R. aegyptiacus* is nearly identical to previous studies (Arad and Korine, 1993; Noll, 1979), but nearly 2°C lower than the corresponding value of $\sim 40.5^\circ\text{C}$ of *E. wahlbergi* (Minnaar et al., 2014). The pteropodids, *D. minor*, *P. poliocephalus*, *S. australis*, *P. hypomelanus* were also comparatively more heat tolerant and, when exposed to $T_a = 39 - 40^\circ\text{C}$, all species maintained T_b between $39 - 40^\circ\text{C}$, and the T_b of *P. scapulatus* reached $40 - 42^\circ\text{C}$ at T_a s between $40 - 44^\circ\text{C}$ (Bartholomew et al., 1970, 1964; Ochoa-Acuña and Kunz, 1999)

The fractional increase in EWL exhibited by *R. aegyptiacus* (~ 3.3) was similar to individuals from Israel (~ 3.6 ; Korine and Arad, 1993), but lower than those of *E. wahlbergi* (~ 8) or *P. poliocephalus* (~ 5.2 ; Bartholomew et al., 1970; Minnaar et al., 2014). Further, the increase in metabolic rate above baseline values was similar for *R. aegyptiacus* (134%) compared to previous studies ($\sim 140\%$; Korine and Arad, 1993), but lower compared to the four Australasian species (151%–240%; Bartholomew et al., 1964; Bartholomew et al., 1970; Minnaar et al., 2014), suggesting that *R. aegyptiacus* has a more limited capacity for panting. The maximum ratio of cooling capacity (evaporative heat loss/metabolic heat production) of *R. aegyptiacus* (0.75) was substantially lower than values reported for well-hydrated conspecifics in Israel (~ 1.8 ; Korine and Arad, 1993) or *D. minor* (~ 2 ; Bartholomew et al., 1970, 1964), suggesting that *R. aegyptiacus* from the Soutpansberg mountain range are unable to cool themselves when $T_b > T_a$ or offload even 100% of metabolic heat production. Although there is some physiological variation between populations of *R. aegyptiacus* (Arad and Korine, 1993; Korine and Arad, 1993; Noll, 1979), the difference in cooling capacity is striking. In fact, the limited cooling capacity of *R. aegyptiacus* in our study is more similar to water restricted individuals, which were only able to dissipate $\sim 40\%$ of MHP (Korine and Arad, 1993). Although all individuals were held for $<24 \text{ hr}$ and provided with *ad libitum* fruit and water until 2 h before trials, we cannot exclude the possibility that individuals were dehydrated prior to measurements resulting in their limited cooling capacity.

The inflections for EWL (37.3°C) and RMR (35°C) for *E. wahlbergi* are considerably higher than those of *R. aegyptiacus* (Minnaar et al.,

2014). These authors argued that *E. wahlbergi*'s sudden increase in resting metabolic rate and EWL indicates that these bats attempt to avoid hyperthermia. It appears that *R. aegyptiacus* is even more averse to hyperthermic T_b and, compared to other frugivorous pteropodids, has a very modest capacity to defend normothermic T_b during hot conditions. Although *E. wahlbergi* and *R. aegyptiacus* are largely sympatric, our data suggest that differences in their thermoregulatory performance at high T_a are functionally linked to their roost microclimate. Although BMR varies with diet in endotherms (Cruz-Neto and Bozinovic, 2004; Muñoz-García and Williams, 2005), this observation supports the notion that cave-dwelling bats have evolved limited evaporative cooling capacity on account of their cool, typically humid environment where selection for heat tolerance is relaxed (Altringham, 2011). In contrast, *Epomophorus wahlbergi* and Australasian pteropodids typically roost in tree canopies (Bonaccorso et al., 2002; Monadjem et al., 2010) and are therefore more exposed to higher T_a values and conditions more likely to favour efficient evaporative cooling capacity.

Compared to the BMRs of bats in general, the BMR of *R. aegyptiacus* was equivalent to $\sim 173\%$ of their allometrically predicted values (Cory Toussaint and McKechnie, 2012), but falls within the range of other pteropodids (Bartholomew et al., 1964; Bonaccorso and McNab, 1997; Downs et al., 2012; McNab, 1989; McNab and Armstrong, 2001; McNab and Bonaccorso, 2001; Minnaar et al., 2014). The BMR of *T. mauritanus* was equivalent to 140% of predicted values, and that of *E. hottentotus* only 64% of predicted values (Cory Toussaint and McKechnie, 2012). This variation in BMR relative to allometrically-expected values among three species at a single site emphasizes the substantial variation in bat energetics that is likely independent of body mass, and underscores the need for further studies elucidating the sources of this variation (e.g., Cruz-Neto et al., 2001; McNab, 2003).

The maximum T_a and T_b of *T. mauritanus* and *E. hottentotus* were between $3 - 6^\circ\text{C}$ higher than *R. aegyptiacus*. The T_b of *T. mauritanus* and *E. hottentotus* increased rapidly above their respective inflection points with *E. hottentotus* reaching a T_b of 42.1°C and *T. mauritanus* $T_b = 42.9^\circ\text{C}$ at $T_a = 43.1$ and 44.6°C , respectively. These values fall within the maximum T_b range recorded in other yangochiropterans ($41 - 43^\circ\text{C}$; Cryan and Wolf, 2003; Henshaw and Folk Jr., 1966; Maloney et al., 1999). Further, *T. mauritanus* in a previous study reached $T_b = 42.9^\circ\text{C}$ at $T_a = 42^\circ\text{C}$, identical to the value we recorded here (Cory Toussaint and McKechnie, 2012). The maximum T_b values of insectivorous bat species investigated in the present study and previous work, place bats within the highest range of mammalian T_b and within the apparent high-temperature $40 - 45^\circ\text{C}$ ceiling common to most forms of multicellular life (Heinrich, 1977). Other small mammals that operate within this range include rodents, especially those occurring in hot environments such as deserts (reviewed by McKechnie and Wolf, 2019)

The minimum EWL exhibited by these all species fall within the range of previously recorded values of species from arid and mesic areas (Muñoz-García et al., 2016). The two yangochiropterans we investigated here increased EWL rapidly at $T_a = 36.7$ and 40.5°C . The fractional increase in EWL for *T. mauritanus* was 4.2 and nearly twice that for *E. hottentotus* (7.5); however, these values are much lower than other crevice and foliage-roosting African bat species ($\sim 10 - 21$; Maloney et al., 1999; Marom et al., 2006; Cory Toussaint and McKechnie, 2012), although the latter authors argued that heterothermy at lower T_a values below thermoneutrality may lead to inflated ratios of maximum to minimum EWL (Cory Toussaint and McKechnie, 2012). Puzzlingly, maximum fractional increases in evaporative water loss for *T. mauritanus* were nearly 3 X lower in our study compared to the values reported for this species by Cory Toussaint and McKechnie (2012). We can offer no explanation for this difference, but speculate that differences in experimental protocol such as chamber humidity may be involved. This among-study variation reiterates the need for standardized techniques for quantifying thermal physiology variables at high T_a similar to those recently developed for birds (e.g., McKechnie et al., 2016b; Smit et al., 2018; Talbot et al., 2017; Whitfield et al., 2015).

Extreme heat events are increasing in both intensity and frequency (IPCC, 2014) as a result of anthropogenic climate change, and $T_a \geq 42^\circ\text{C}$ has been identified as the major predictor of mass mortality among Australian pteropodids (Welbergen et al., 2008; Ratnayake et al., 2019). The risks of acute dehydration and hyperthermia during extreme heat events are increasing rapidly for arid-zone birds (Albright et al., 2017), as are the risks of sublethal fitness effects of exposure to sustained hot weather (Conradie et al., 2019). Although direct comparisons are confounded by differences in body mass, phylogeny and possibly diet, it appears that *R. aegyptiacus* has the lowest heat tolerance of the three species investigated here. The low heat tolerance limits of cave-dwelling species suggest that even small increases in temperature could be detrimental. This is likely true for species that inhabit cave-like roosts such as *N. thebaica*, which exhibited the lowest heat tolerance among sympatric crevice-roosting species (Cory Toussaint and McKechnie, 2012). Indeed, cave warming has already started to occur, creating shifts in the distribution of resident biota (Chevaldonné and Lejeune, 2003). Therefore, cave-roosting species like *R. aegyptiacus* that are not relatively heat-tolerant may be at greater risk of direct negative effects of higher T_a than currently thought.

In conclusion, heat tolerance and evaporative cooling capacity show considerable interspecific variation among bats, and heat tolerance limits appear to be related, in part, to roost microclimate. Bats roosting in more exposed sites tolerate higher T_a and maintain T_b below hyperthermic levels, whereas cave-roosting species do not appear to have the same capacity. We caution against a generalized interpretation of our results as although it follows the predictions and finding of Cory Toussaint and McKechnie (2012), more rigorous testing using multiple species per roost type is required to support the pattern reported here. This would be especially informative for obligate cave-roosting insectivorous species (e.g., members of *Miniopterus*, *Myotis*, *Hipposideros*, *Rhinolophus*). Further, although the role of diet on BMR (e.g., Cruz-Neto and Bozinovic, 2004; Muñoz-García and Williams, 2005) is well known, its effect on heat tolerances remains unstudied. Future studies may focus on species inhabiting the same cave but with different prey items. Ideally, all future studies should be conducted at one site over one season, and include similarly sized species that represent multiple taxa. This type of investigation will help control the confounding factors of phenotypic flexibility, body mass and phylogenetic inertia.

CRediT authorship contribution statement

Z.J. Czenze: Formal analysis, Writing - original draft. S. Naidoo: Formal analysis. A. Kotze: Supervision, Writing - review & editing. A.E. McKechnie: Supervision, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2020.102542>.

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