Seasonal metabolic adjustments and partitioning of evaporative water loss in Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi*

by

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Declaration

I, Ingrid Ané Minnaar, declare that the thesis/dissertation, which I hereby submit for the degree MSc: Zoology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

14 September 2013

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Summary

Seasonal metabolic adjustments and partitioning of evaporative water loss in Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi*
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The capacity to thermoregulate over a wide range of T_{aS} is critical for maintaining homeostasis in endotherms. Several aspects of the thermoregulatory properties of bats remain poorly studied when compared to other mammals and birds. I examined two specific aspects of thermoregulation in bats: the seasonal variation of maximum metabolic heat production and the partitioning of total evaporative water loss (TEWL) into respiratory and cutaneous components. I measured basal metabolic rate (BMR) and summit metabolism (M_{sum}) in captive and wild Wahlberg's epauletted fruit bats, *Epomophorus wahlbergi*, during summer and winter. I measured metabolic rate using flow-through respirometry, and elicited M_{sum} by exposing bats to low temperatures in a helox (21% O_2, 79% He) atmosphere. BMR decreased by 22-25% during winter in both captive and wild bats, with the BMR of captive bats 9-13% lower than the wild individuals across seasons. M_{sum} was approximately seasonally stable in both captive and wild bats, but M_{sum} in captive individuals was 13-18% higher than their wild conspecifics during both seasons. The ratio between M_{sum} and BMR (i.e., metabolic expansibility) was greater in winter than during summer for both captive and wild bats. One likely explanation for the greater resting thermogenic capacity of the bats in captive individuals concerns their reduced activity levels; compared to wild, free-ranging bats, heat produced as a by-product of activity probably contributed far less to thermoregulation, apparently leading to an increase in resting heat production capacity in captive individuals.

At the other end of the thermal scale, knowledge of heat tolerance and the evaporative cooling mechanisms employed by bats in hot weather remains rudimentary. At high air temperatures (T_a), endotherms avoid overheating by dissipating heat via evaporative water loss. TEWL may be partitioned into cutaneous evaporative water loss (CEWL) and respiratory evaporative water loss (REWL). I quantified CEWL and REWL in *E. wahlbergi* at T_as of 10-40 °C using a latex mask. When T_a exceeded normothermic T_b, bats drastically increased their TEWL, metabolic rate and T_b. The relative contribution of CEWL to TEWL was the greatest at moderate T_as where it represented up to 80% of TEWL. REWL was the major route of evaporative cooling at the highest T_a: at T_a = 40 ºC, REWL represented 45% of TEWL. To avoid hyperthermia, *E. wahlbergi* greatly increased metabolic rate at high T_{aS} to avoid hyperthermia, further compounding the need to cool down. REWL is thought to be less efficient as than CEWL in offloading heat at high T_{aS} as panting increases metabolic heat, whereas CEWL occurs passively. There is a need for further studies to be conducted on the thermoregulatory capabilities of bats in varying environmental conditions, both intra- and interspecifically.

Disclaimer

Chapter 1 and 2 have been prepared for submission to different peer-reviewed journals. As a result, the format and styles vary between the chapters.

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Seasonal metabolic adjustments in Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) from southern Africa: differences between wild and captive individuals

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Summary

Variation in basal metabolic rate (BMR) and summit metabolism (M_{sum}) are important components of seasonal acclimatization in some endotherms. Whereas seasonal adjustments in BMR and M_{sum} have been extensively studied in temperate-zone birds, in so far as it could be established, knowledge on similar studies involving bats remain largely lacking. I measured BMR and M_{sum} during summer and winter in captive and wild Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) from southern Africa using flow-through respirometry, with M_{sum} elicited by cold exposure in a helox (21% O_2, 79% He) atmosphere. In captive and wild bats, BMR was 22-25% lower in winter, but M_{sum} remained approximately stable across seasons. During both summer and winter, the M_{sum} of captive bats was 13-18% higher compared to wild bats, and BMR was 9-13% lower. Metabolic expansibility (i.e., the ratio of M_{sum} to BMR) was unexpectedly high (overall mean ≈ 12), and was greater during winter than summer for both the captive and wild populations. I hypothesised that the divergent patterns of seasonal variation in M_{sum} between wild and captive bats reflect differences in the relative thermoregulatory contributions of metabolic heat generated as a by-product of activity. Specifically, the artificially reduced activity levels of the captive bats likely meant that they needed to increase resting thermogenic capacity to defend body temperature on winter nights. These results highlight the need for further studies examining how minimum and maximum resting metabolic rates vary with environmental factors, both among and within species.

Introduction

One way in which endotherms acclimatize to seasonal environmental changes is through adjustments in the upper and/or lower limits of normothermic metabolic rate (Swanson, 2010). Both minimum and maximum resting metabolic rates are influenced by environmental and physiological factors, including climate and migration (Lovegrove, 2005; McNab, 2008; reviewed in Swanson, 2010). Seasonal metabolic adjustments are thought to be correlated with factors such as the requirement for enhanced cold tolerance in winter (Swanson and Garland, 2008; Swanson and Liknes, 2006), and possibly the requirement for energy conservation in regions with cool, dry winters (Smit and McKechnie 2010).

Basal metabolic rate (BMR, the minimum normothermic resting metabolic rate of an endotherm), is the minimum metabolic rate required to maintain the essential physiological processes necessary for survival (Randall et al., 2002). BMR is measured during the inactive phase in non-growing animals that are resting, post-absorptive and non-reproducing, within their thermoneutral zone (i.e., the range of ambient temperatures where metabolic rate is at its minimum) (McNab, 1997; Randall et al., 2002).

Since the measurement conditions of BMR are standardised, BMR is often compared between species to elucidate sources of variation in baseline metabolic requirements (McNab, 2008). In resting mammals, approximately 70% of BMR is generated by internal organs (e.g., brain, heart, gut and kidney) (Taigen, 1983). Body mass is by far the largest source of interspecific BMR variation (McNab, 2008; Swanson, 2010). For instance, McNab (2008) collated BMR data for 639 mammal species, and found that 96.8% of the variation is attributable to body mass (M_b). Using BMR data from 84 species of bats McNab (2008) determined that for Chiroptera:

$$BMR = 0.052 \times {M_b}^{0.770}$$

This relationship between *BMR* (kJ hr^{-1}) and M_b (g) was strongly correlated r^2=0.928. Among species of similar body mass, however, large differences in BMR are sometimes evident, and numerous studies have demonstrated that BMR is influenced by phylogeny, habitat and diet (McNab, 2008; Swanson, 2010). Conversely, during activity, 75% of the increase in metabolic rate, on average, is attributable to the heat produced by muscle tissue (in Taigen, 1983).

Metabolic capacity and metabolic scope studies have mostly been conducted on birds and rodents from temperate-zone regions. In deer mice (*Peromyscus maniculatus*), maximum metabolic rate (MMR) and summit metabolism [M_{sum}; maximum resting metabolic rate during cold exposure (Swanson, 2010; but see Almeida and Cruz-Neto, 2011; Rezende et al., 2004; Sparti, 1992)] were shown to be positively correlated (Hayes and Chappell, 1990). Moreover, both MMR and M_{sum} vary between seasons. The hypertrophy of flight and/or heart mass brought on by winter acclimatization and/or migration usually results in an increase of M_{sum} in birds (Swanson, 2010). M_{sum} was also found to increase during winter in rodents and was higher in temperate than tropical species (Bozinovic and Rosenmann, 1989; Rezende et al., 2004; Sparti, 1992).

MMR and M_{sum} have been found to correlate positively with BMR in some studies (Koteja, 1987; Rezende et al., 2002; Rezende et al., 2004; Sparti, 1992; Taigen, 1983). It follows from the aerobic capacity model for the origin of endothermy that an increase in M_{sum} is associated with an increase in BMR, since the increase in the size of muscle and organ tissues results in an increase in maintenance costs (Hayes and Garland, 1995; reviewed in Swanson, 2010). Thus, a higher BMR is expected in winter when M_{sum} increases. However, the decreased availability of food and its lower ease of digestion, quality and energy content, can result in a lower BMR during winter (i.e., the food-habit hypothesis) (Cruz-Neto and Bozinovic, 2004; McNab, 2001; McNab, 2008).

Although Chiroptera is the second largest mammalian order, surprisingly few studies have examined MMR or M_{sum} in this group, and only one has investigated seasonal adjustments in BMR and M_{sum} (Almeida and Cruz-Neto, 2011). Thus far, bats have been found to have a higher than expected MMR compared to other mammals (most likely as the result of the high energy output of flight muscles) (Koteja, 1987). Almeida and Cruz-Neto (2011) found that neither BMR nor M_{sum} differed seasonally in three species of small insectivorous bats, but this may have been due to the small temperature variation between seasons at their study site, and/or the behavioural and physiological reduction in energy requirements, such as huddling and torpor.

In diurnal species, the rest phase typically coincides with the coldest part of the diurnal cycle, whereas this is not the case for nocturnal species. *A priori*, one might therefore expect that seasonal adjustments in BMR and M_{sum} would be less pronounced in nocturnal endotherms compared to diurnal species, since these seasonal adjustments generally appear to be related to cold tolerance and energy conservation during the rest phase. Smit and McKechnie (2010) showed that birds from high latitudes generally increase BMR in winter, whereas the opposite is the case for species from the tropics and sub-tropics. They postulated that because northern hemisphere birds experience colder winter air temperatures than subtropical species from the southern hemisphere, these temperate-zone birds need a greater thermogenic capacity so as to defend their normothermic body temperature; it follows from the 'energy demand hypothesis' that their maintenance metabolic rates would also be higher in order to support the higher demand of the organs involved in nutrition as well as their muscle tissue (Liknes et al., 2002).

Nocturnal species (such as bats) are active during the coldest part of the 24-hr daily cycle. Thus, the metabolic heat they produce during their night time activity presumably contributes to their thermoregulation, by substituting for metabolic thermogenesis. On the

other hand, diurnal species (such as most birds) experience on average colder temperatures than bats from the same area during the same part of the year since the birds' rest phase is during the coldest part of the 24-hr daily cycle. We would therefore expect diurnal species to show greater winter increases in BMR and M_{sum} compared to nocturnal species. However, as far as it could be established, metabolic comparisons between diurnal and nocturnal species remain completely unexplored. The influence of captivity on BMR and M_{sum} has also been little explored. In birds, captive and wild individuals have been shown to vary in the scaling of BMR (McKechnie et al., 2006). The phenotypic plasticity in metabolic rate between these two groups was attributed to differences in food quantity and quality, as well as activity; the reduced activity levels of captive animals could change the relative importance of resting thermogenic heat capacity when compared to more active wild populations. The metabolic differences between a wild and captive population calls into question the validity of extrapolating metabolic rates measured in captive individuals to their conspecific wild counterparts.

For this study, I measured seasonal changes in BMR and M_{sum} in wild and captive Wahlberg's epauletted fruit bats, *Epomophorus wahlbergi* (Sundevall, 1846) from southern Africa, to establish patterns of seasonal metabolic acclimatization in this species, and to assess whether this varies between wild and captive individuals.

Materials and methods

Study animals

I mist-netted (Ecotone Ultra Thin Mist Nets, Gdynia, Poland) ten adult *E. wahlbergi* bats (nine females and one male) in March 2012 at the Pretoria National Botanical Garden in Gauteng, South Africa (25°44'S; 28°16'E). Bats were kept in outdoor aviaries (3 m long x 2 m wide x 2 m high) at the University of Pretoria's Experimental Farm during experiments (7 km from the capture site) which enabled them to acclimatize to natural environmental conditions. The male bat was kept separately from the female bats. Bats were maintained on a diet of mixed fruit supplemented with vitamins and minerals (Barnard, 2009) and water was provided *ad libitum*.

Free-ranging bats were captured on the University of Pretoria campus and kept overnight in the outdoor aviaries on the Experimental Farm. I caught six wild males and four wild females during both summer and winter. Experiments were conducted from end-July to end-August 2012 (austral winter) and during December 2012 (austral summer).

Basal metabolic rate

I determined the air temperature (T_a) at which to measure BMR during both seasons by ascertaining the thermoneutral zone. To calculate this, I exposed bats to a range of T_as and (MR) identified the T_a at which the mean MR was the lowest. Bats were weighed prior to and after measurements to obtain an average body mass (M_b) that was used for metabolic rate calculations. It was ensured that bats were postabsorptive by removing uneaten food at least eight hours before metabolic measurements (Genoud, 1993; Morris et al., 1994).

During BMR measurements, the bats were placed in 2.1-L airtight plastic chambers individually (Lock & Lock, Blacktown, NSW, Australia). To prevent evaporation from excrement affecting readings, I filled the bottom of the plastic chambers with a 1 cm-deep mineral oil layer. A plastic mesh platform and a three-sided plastic mesh enclosure were placed inside the chamber to prevent the bat from reaching the oil and to provide them with enough space to hang in a natural posture, respectively.

Body temperature (T_b) was measured with a temperature-sensitive passive integrated transponder (PIT) tag that was injected subcutaneously (Destron Fearing, St. Paul, MN, USA) into each bat's interscapular region. Subcutaneous temperature has been shown to be an adequate measure of core T_b in bats (Gorman et al., 1991). A loop antenna (Racket Antenna, Biomark, Boise, Idaho, USA) (that was attached to a PIT tag reader (Model FS2001F-ISO, Biomark, Boise, Idaho, USA) was placed over the plastic chamber to allow for the continual recording of T_b. Plastic chambers were fitted with inlet and outlet fittings that were placed at opposite ends of the chamber (with one at the upper end, and one lower down) to allow for adequate air mixing.

Chambers were placed inside a darkened, temperature-controlled cabinet (Model KMF 720, Binder, Tuttlingen, Germany) for at least 30 min prior to the start of recording to allow bats enough time to relax. The BMRs of two bats were measured within the same experimental run. T_a was measured by inserting a thermistor probe (Sable Systems, Las Vegas NV, USA) through a small hole in the lid of the airtight chamber that was sealed by the probe itself. A UI-2 digital analog convertor (Sable Systems) received signals from the thermistor probe.

BMR was measured with an open flow-through respirometry system. A compressor supplied atmospheric air which was scrubbed of water vapour (dewpoint≈-50 ºC) and CO_2 by an adsorption dryer (Ecodry K-MT 3, Parker Zander, Charlotte, North Carolina, USA). A

mass flow controller (Model FMA5520, Omega Engineering, Bridgeport, NJ, USA) supplied air to each chamber at flow rates of 1.1-1.5 L min^{-1}. I regularly calibrated the mass flow controller using a soap bubble flow meter (Baker and Pouchot, 1983). It took 6.4 min to reach 99% equilibrium within the chamber (Lasiewski et al., 1966). A TRM8 Respirometry Multiplexer (Sable Systems) and a SS-3 Sub-sampler (Sable Systems) sub-sampled air successively from the baseline for 1-10 min, followed by the first chamber and then the second chamber (each sampled for 7 to 20 minutes), finishing off with 5-10 min baseline reading. The experimental runs were terminated when bats obtained a stable T_b (between 2 and 5 hours). Subsampled air was drawn through a CO_2 analyser (CA-10a, Sable Systems), an O_2 analyser (FC-10B, Sable Systems) a dew point analyser (RH-300, Sable Systems).

Data were acquired and digitised using a Universal Interface II (Sable Systems). ExpeData software on a desktop PC was used to record the data.

Summit metabolism

I elicited M_{sum} by exposing bats to a cold environment in a helox (21% O_2, 79% He) atmosphere, using the sliding cold exposure protocol (Swanson et al., 1996). A helox atmosphere allows for M_{sum} to be elicited at a higher temperature than would normally be possible in an atmospheric air environment because helox has an increased thermal conductance (Rosenmann and Morrison, 1974). Thus, Helox also decreases the risk of freeze injury associated with extreme cold exposure (Rosenmann and Morrison, 1974).

M_{sum} measurement followed the same open flow-through respirometry as explained for the BMR measurements above. However, the bats were placed inside a modified 40-L fridge/freezer (ARB, Kilsyth, Victoria, Australia) during experiments and suspended iButtons (Maxim Integrated, San Jose, CA, USA) 1 cm above the bottom of the chamber to determine

T_a. I placed one bat at a time into a 1.3-L chamber which was the placed inside the modified fridge/freezer with a T_a around -5 °C. The bat was allowed to acclimate to the cold while pumping atmospheric air through the system at a flow rate of 2.5 L min^{-1}. After about 5 min, I switched to a helox atmosphere and ended the baseline recording as soon as a stable helox reading was achieved. As soon as this happened, I started the slide of T_a from -5 towards -20 °C (typically attained after approximately 1 hour). Runs were terminated when VO_2 reached a plateau and no longer increased with a decreasing T_a. It was verified that M_{sum} had been obtained by measuring T_b before and after M_{sum} measurements to ensure bats became hypothermic. T_b was obtained by scanning the bat's PIT tag with a handheld scanner (DTR-4, Destron Fearing, South St Paul, Minnesota, USA), after which a baseline reading was recorded. Bats were weighed before and after M_{sum} measurements and the average was used in MR calculations. Since M_{sum} measurements always followed BMR measurements, the M_b reading before M_{sum} experiments took place was affected by the weight loss experienced by bats during their BMR measurements.

Data analysis

I tested all data for normality and homogeneity of variances. I first performed a least-squares linear regression to determine if whole-organism BMR and M_{sum} were correlated with M_b during BMR and M_{sum} measurements, respectively. Performing a least-squares linear regression also allowed me to determine the relationship between BMR and M_{sum}, and M_b. BMR and M_{sum} were found to be correlated with their respective M_bs (see below). Thus, to avoid the confounding effects of mass, sex and only a single male in the captive group on BMR and M_{sum}, I used a mixed model analysis of variance (ANOVA) to analyse mass-specific metabolic rates.

I chose to use a mixed model ANOVA as I had a mixture of between-subject (i.e., captive bats vs. wild bats; males vs. females) and within-subject factors (i.e., bats that were measured during both seasons). In addition to the confounding effects of the above factors on BMR and M_{sum}, I further used mass-specific MR data in my mixed model ANOVA as the analysis only allows for qualitative variables as explanatory variables. Thus, it was not possible to use M_b as a covariate of whole-organism BMR and M_{sum}. Therefore, season (winter or summer), sex and group (wild or captive) were used as explanatory variables (and the interaction between them), with either mass-specific BMR or M_{sum} as the dependent variable for my mixed-model analyses. I also performed a mixed model ANOVA (using the same explanatory variables as listed above) for each of the following dependent variables: mass-specific metabolic expansibility (i.e., the ratio between M_{sum} and BMR; M_{sum}/BMR), and T_b, T_a and M_b for both BMR and M_{sum}. For post hoc analysis, I used Tukey's honest significance test. Since it was only possible to compare M_b for the BMR measurements to two-levels of interaction, an independent, two-tailed t-test was instead used to test for differences between wild males and wild females during summer and winter.

Values are presented as mean±s.d. I assumed a significance level of $P \leq 0.05$. For my data analysis, I used the XLSTAT 2013 statistical package.

Results

Body mass

M_b during BMR measurements was not significantly different between wild and captive bats or across seasons. However, sex had a significant main effect on M_b ($F_{1,36}$=45.8, P<0.0001). Male bats were heavier (112.46±8.90 g) than females (87.04±8.17 g) during BMR measurements (P<0.0001). Wild males were significantly heavier than wild females during summer (109.94±8.34 g vs. 90.81±11.62 g; t(8)=3.1, P=0.02) and winter (115.15±8.32 g vs. 88.20±5.55 g; t(8)=5.6, P=0.0005).

During M_{sum} measurements, group ($F_{1,32}$=14.3, P=0.001) had a significant main effect on M_b ($F_{1,3}$=14.3, P=0.001). Wild bats weighed more than captive bats (99.63±12.95 g vs. 86.61±11.26 g). However, the main effect of group on M_b was influenced by the interaction between group and season ($F_{1,32}$=4.0, P=0.05). Post hoc analyses revealed that the wild bats were heavier than captive bats during summer (99.91±12.35 g vs. 84.85±8.16 g; P<0.0001) and winter (103.05±14.80 g vs. 90.30±13.46 g; P<0.0001). Captive bats were heavier during winter (90.30±13.46 g) than summer (84.85±8.16 g; P<0.0001), but wild bats did not differ in their M_b across seasons (P=0.15).

M_b during M_{sum} measurements was also affected by sex ($F_{1,32}$=32.2, P<0.0001), which was influenced by its interaction with season ($F_{1,32}$=22.4, P<0.0001). Post hoc analysis showed that wild males were heavier than females during winter (113.53±7.82 g vs. 87.34±5.31 g; P<0.0001) and summer (106.60±8.36 g vs. 89.88±10.62 g; P<0.0001), wild males were heavier in in winter than summer (113.53±7.82 g vs.106.60±8.36 g; P<0.0001), but wild females were heavier during summer than winter (89.88±10.62 g vs. 87.34±5.31 g; P=0.002).

Body and air temperature

Whether a bat was wild or captive had a significant effect on normothermic T_b ($F_{1,32}$=9.2, P=0.005). This result was influenced by both sex ($F_{1,32}$=6.3, P=0.02) and further influenced by season ($F_{1,32}$=7.9, P=0.01). Post hoc analysis showed that, overall, captive individuals had significantly higher normothermic T_b than wild individuals (35.45±0.72 °C vs. 34.67±0.83 °C; P<0.0001). Captive females had a higher normothermic T_b than wild females overall (35.30±0.49 °C vs. 34.89±0.86 °C; P=0.004), but wild females and wild males did not differ in their normothermic T_b (P=0.2). Seasonally, wild males had a higher normothermic T_b during summer (34.90±0.80 °C) than winter (34.15±0.69 °C; P=0.002).

T_b at the end of M_{sum} measurements was significantly lower than T_b at the start of the M_{sum} measurements for both wild bats and captive bats during summer and winter (t(9)≥4.4, P≤0.002), confirming that bats became hypothermic. Season ($F_{1,32}$=6.6, P=0.02) and group ($F_{1,32}$=13.9, P=0.001) significantly affected T_b during M_{sum} experiments. Season and group also significantly interacted with one another ($F_{1,32}$=7.0, P=0.01). Post hoc analysis revealed that, overall, captive individuals (34.25±2.03 °C) had a higher T_b following M_{sum} experiments than wild individuals (30.61±3.29 °C; P<0.0001). Seasonally, T_b during M_{sum} measurements was higher in winter (32.86±3.42 °C) than in summer (32.00±3.14 °C; P<0.0001). Captive bats had a higher T_b during M_{sum} measurements than wild bats in summer (34.27±1.45 °C vs. 29.73±2.69 °C; P<0.0001) and winter (34.23±2.56 °C vs. 31.49±3.73 °C; P<0.0001). Wild bats had a significantly higher T_b during M_{sum} measurements in winter (31.49±3.73 °C) than summer (29.73±2.69 °C; P<0.0001), whereas the T_b during M_{sum} measurements for captive bats did not differ seasonally (P=0.08).

The helox temperature at which M_{sum} occurred was significantly affected by the group to which the bat belonged ($F_{1,32}$=11.4, P=0.002) and season ($F_{1,32}$=9.6, P=0.004). The main effect that group and season had on the helox temperature at which M_{sum} occurred was influenced by their interaction ($F_{1,32}$=4.3, P=0.05). Post hoc analysis revealed that, overall, M_{sum} occurred in captive individuals at a lower helox temperature (-9.6±2.32 °C) than wild individuals (-5.53±4.56 °C; P<0.0001), and the helox temperature at which M_{sum} occurred was lower in winter (-8.87±3.70 °C) than summer (-6.27±4.21 °C; P<0.0001). Captive bats had a lower helox temperature at which M_{sum} occurred than wild bats during summer (-9.19±1.47 °C vs. -3.35±4.04 °C; P<0.0001), but not winter (P=0.06). Wild bats had a lower helox temperature at which M_{sum} occurred during winter than summer (-7.71±4.13 °C vs. -3.35±4.04 °C; P<0.0001), unlike captive bats (P=0.4).

Respiratory exchange ratio

The respiratory exchange ratio (RER) during BMR measurements was 1.04±0.37 in summer and 0.82±0.08 in winter. Captive bats had a RER value of 1.00±0.34 during BMR and wild bats had an RER of 0.86±0.21. During M_{sum}, the RER was 0.64±0.05 for captive individuals and 0.68±0.06 for wild individuals.

Basal and summit metabolic rate

BMR and M_b were positively correlated with a least-squares regression equation of BMR (mW) = 113.86+5.00*M_b (r^2=0.165). Season had a significant main effect on mass-specific BMR ($F_{1,32}$=13.1, P=0.001). Post hoc analysis showed that BMR in summer was

approximately 31% higher than BMR in winter (7.06±2.04 mW g^{-1} vs. 5.38±0.69 mW g^{-1}; P<0.0001) (Fig. 1).

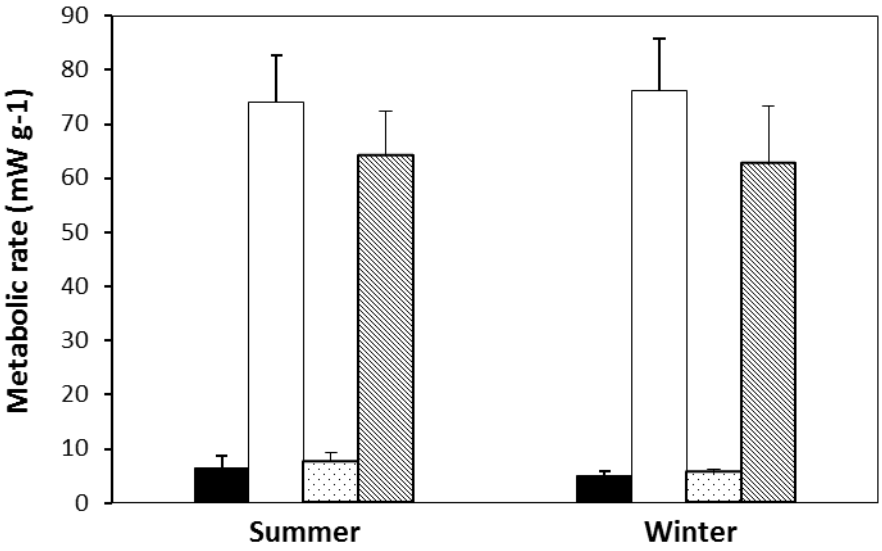

Figure 1. Metabolic rate (mW g^{-1}) in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) from southern Africa across seasons. The solid bars denote the captive bats; solid black represents BMR and solid white represents M_{sum}. The shaded bars denote the wild bats; the dot pattern represents BMR and the stripe pattern represents M_{sum}.

M_{sum} and M_b were positively correlated with a least-squares regression equation of M_{sum} (mW) = 1192.57+56.22*M_b (r^2=0.397).

Group was the only factor that had a significant main effect on mass-specific M_{sum} ($F_{1,32}$=16.3, P=0.000). The effect of group was influenced by its interaction with sex ($F_{1,32}$=4.2, P=0.05). Post hoc analysis revealed that captive bats had an M_{sum} (75.12±8.85 mW g^{-1}) 15.5% higher than that of wild bats (63.49±9.18 mW g^{-1}; P<0.0001), overall (Fig. 1). The M_{sum} of captive females was 22% higher than wild females (76.02±8.79 mW g^{-1} vs. 58.00±7.75 mW g^{-1}; P<0.0001). Wild males had a mass-specific M_{sum} that was 11% greater

than that of wild females (66.48±9.10 mW g^{-1} vs. 58.00±7.75 mW g^{-1}; P=0.01), despite the males' greater M_b.

A least-squares linear regression showed that mass-specific BMR did not correlate with M_{sum} in this study.

Both group ($F_{1,32}$=10.4, P=0.003) and season ($F_{1,32}$=4.5, P=0.04) significantly affected metabolic expansibility (i.e., M_{sum}/BMR). Post hoc analysis revealed that the ratio of M_{sum} to BMR was greater in the captive bats (13.96±4.32) than in the wild bats (10.16±2.81; P<0.0001) (Fig. 2). Metabolic expansibility was also greater during winter (13.20±3.06) than summer (10.93±4.69; P=0.000) (Fig. 2).

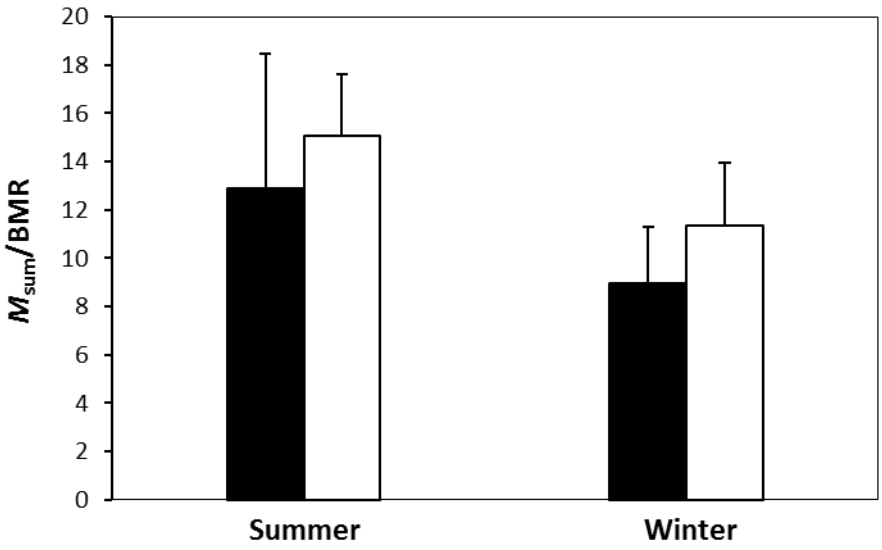

Figure 2. Metabolic expansibility (M_{sum}/BMR) in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) from southern Africa across seasons. The solid black bars denote the captive bats and the solid white bars denote the wild bats.

Discussion

BMR in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) from southern Africa was higher in summer than in winter. This difference was not driven by T_b, as there was no seasonal difference in normothermic T_b. Furthermore, captive bats had a higher normothermic T_b than wild bats, but this was most likely not due to sex difference as captive females had a higher normothermic T_b than wild females and in turn these females did not differ in their normothermic T_bs from wild males. Wild males defended a higher normothermic T_b during summer than winter. During BMR measurements, male bats weighed more than female bats overall and wild males were heavier than wild females in summer and winter.

M_{sum} did not differ seasonally. $T_{b,}$ however, was higher in winter than in summer during M_{sum} measurements. The helox temperature at which M_{sum} occurred was lower in winter than summer. This is indicative of bats tolerating a lower T_a during winter, which was facilitated by a higher T_b during winter measurements or perhaps lower thermal conductance in winter compared to summer. A least-squares linear regression showed that mass-specific BMR did not correlate with M_{sum}, thus the aerobic capacity model for the origin of endothermy was not supported in this study.

Captive bats had a higher M_{sum} than wild bats, overall. It can be argued that this difference is due to differences in captive individuals and wild individuals rather than being driven by the skewed sex ratio in the captive group, as the M_{sum} of captive females was higher than wild females. Captive bats weighed less than wild bats in both summer and winter during M_{sum} experiments, so the difference in M_{sum} observed between captive and wild bats could be due to the greater mass of the wild bats. Captive individuals also had a higher T_b during M_{sum} experiments than wild individuals in summer and winter. Overall, M_{sum} occurred

in captive individuals at a lower helox temperature than wild individuals, indicating that captive bats tolerated cold better than wild bats due to their higher thermogenic capacity. Captive bats had a lower helox temperature at which M_{sum} occurred than wild bats during summer only. This suggests that the overall difference in tolerable helox temperature between captive and wild bats was mostly driven by summer differences in cold tolerance. Wild bats tolerated cold better during winter than summer due to their higher T_b during M_{sum} measurements in winter. On the other hand, captive bats did not differ seasonally, nor did their T_b during M_{sum} measurements. Captive bats were heavier during winter than summer M_{sum} experiments, whereas wild bats did not differ in their M_b across seasons. Wild males had an M_{sum} that was greater than wild females, even though the wild males were heavier than the wild females during winter and summer in the M_{sum} experiments. It is possible that wild males had a greater thermogenic capacity than wild females. During M_{sum} experiments, wild males were heavier in winter than summer, but wild females were heavier during the summer than winter.

Bats tolerated a greater environmental temperature range, and thus colder temperatures, during winter compared to summer. Captive bats were more cold tolerant than wild bats overall, as metabolic expansibility was greater in the captive bats.

The captive and wild bats differed in their BMR, M_{sum} and metabolic expansibility. Firstly, the wild bats possessed a higher BMR than the captive bats. A presumed increase in aerobic capacity brought on by an increase in muscle tissue (due to the relatively higher exercise activity of the wild bats), could explain the increase in BMR (i.e., the increased maintenance cost of the muscle tissue) (Hayes and Garland, 1995; reviewed in Swanson, 2010). However, M_{sum} was higher in the captive bats and not the wild bats.

Alternatively, diet differences could potentially explain why wild bats displayed a higher BMR than the captive bats. A diet higher in caloric value is thought to result in a

relatively higher BMR (McNab, 2008), but since it is unlikely the wild bats consumed a better diet than the captive individuals (which were fed high caloric fruit diets and the wild bats were observed to have digestion difficulties during winter as they excreted green, loose guano when they were mist-netted in winter as opposed to summer), this explanation does not seem likely. Another more plausible explanation for the difference in BMR between captive individuals and wild individuals is the amount of unsaturated fat consumed by the bats. Lower levels of unsaturated fat have been shown to increase MMR (and thus M_{sum}) (Pierce et al.; 2005). Seeing that a higher MMR often corresponds to a higher BMR value (Koteja, 1987; Rezende et al., 2002; Rezende et al., 2004; Taigen, 1983), it would make sense for wild bats that did not receive the supplementary fatty acids the captive bats did, to have the higher BMR value. However, BMR and M_{sum} were not correlated for the bats in this study.

The functional relationship between M_{sum} and BMR remains unclear (Almeida and Cruz-Neto, 2011; Swanson, 2010). I found that there was no correlation between BMR and M_{sum} in the bats in my study. BMR differences may therefore be due to differences in food availability, as this is often the reason for intraspecific variation in BMR (Tieleman et al., 2003). During winter, when food is generally less easy to digest and/or the thermoregulatory demands are high, gut and other digestive organ mass increases are observed in birds (Karasov, 1990; Swanson, 2010), driving increases in BMR. The increase in BMR could however, simply be explained by the mass difference between male and female bats. Since there was a disproportionate amount of females in the captive group, the presence of more males in the wild group greatly influenced the M_b of the wild group, especially when you consider that wild males weighed more than wild females.

An increase in organ mass typically causes corresponding increases in BMR due to the elevated maintenance costs of the larger organs (Swanson, 2010). In addition to gut mass, the relative sizes of the kidney, liver and heart have been shown to influence BMR (Swanson,

2010), so an increase in mass of the latter organs could account for the elevated BMR in summer. On the other hand, the summer and winter RER values during the BMR measurements indicate that a mixture of carbohydrates and lipids were the preferred fuel source; in summer carbohydrates were relied on more as a fuel source, whereas fat was used more in winter, another indication that perhaps the higher carbohydrate content of the summer diet of the bats contributed to the higher BMR value.

Whereas there was a seasonal difference in BMR, there was no difference in M_{sum} across the seasons. A seasonal difference in M_{sum} values is usually expected if a species prepares for migration by increasing their flight muscle mass and/or heart mass (Swanson, 2010), or if it over winters in cold, high latitude habitats characterized by very low environmental temperatures. Yet, a seasonal difference would still be expected if bats were more active during summer. Perhaps bats were equally active in summer and winter, and/or had a similar capacity for shivering thermogenesis.

The increased M_{sum} of captive bats could also have been due to an increased shivering thermogenesis capacity and, at the same time, have not resulted in an increased BMR (if we consider that in this study M_{sum} and BMR do not influence each other). This would seem unlikely though as activity was limited within the confines of the enclosure of the captive bats.

Metabolic expansibility was also greater in captive than wild bats, reflecting the relatively higher M_{sum} and relatively lower BMR. This implies that the captive bats in this study were more tolerant of low environmental temperatures. This makes sense if we consider the possibility that the heat produced via activity substitutes for thermoregulatory heat to a greater degree in wild bats than in captive individuals (Humphries and Careau, 2011). It is possible that they were able to tolerate a lower food supply as they had lower maintenance costs (as indicated by their lower BMR) and had a higher cold tolerance as a

result of their increased M_{sum} values (and because they were able to tolerate a lower helox temperature than wild bats). The ratio between M_{sum} and BMR was also higher during winter since bats were better able to tolerate low T_as during winter, indicating phenotypic plasticity in cold tolerance between the cold and warm seasons.

According to the BMR equation developed by McNab (2008), a BMR value of 1.75 kJ hr^{-1} was expected, if the average M_b recorded during BMR measurements (95.94 g) was taken. However, I found a much higher value of 2.48 kJ hr^{-1} (a 29% increase from the expected BMR) for *E. wahlbergi* in this study. The equation determined by McNab (2008) was based on BMR data from mostly insectivorous bats. Thus, the greater observed BMR for this species could be due to its high-caloric diet of fruit, amongst many other plausible explanations.

Downs et al. (2012) measured BMR in *E. wahlbergi* and found that winter BMR was higher than summer BMR by 17.9%. I found the opposite relationship between BMR and season for *E. wahlbergi*, with BMR increasing in summer compared to winter by 31%. This difference between studies could be due to large intraspecific variation in the magnitude and direction of seasonal BMR adjustments, as has been found to occur in some birds (van de Ven et al., 2013). Downs et al. (2012) captured *E. wahlbergi* in Pietermaritzburg, KwaZulu-Natal Province in South Africa, a region that is lower in altitude, warmer and wetter than Pretoria where the *E. wahlbergi* in the present study was sampled from. The intraspecific variation could be due to the large climatic differences experienced by the two different *E. wahlbergi* groups (van de Ven et al., 2013). In three species of fruit-eating phyllostomid bats from Brazil, Almeida and Cruz-Neto (2011) found that neither BMR nor M_{sum} differed seasonally in these insectivorous bats, which was corroborated by the M_{sum} findings in the present study, but not in its BMR results. However, the metabolic expansibility values I observed in *E. walhbergi* are much higher than those found by the latter authors; *Artibeus*

lituratus was shown to have a mean metabolic expansibility of 5.15, *Carollia perspicillata* had a mean metabolic expansibility of 4.82 and *Sturnira lilium* had a mean metabolic expansibility of 3.63. *Epomophorus wahlbergi* had a comparatively greater metabolic expansibility during summer (10.93±4.69) and winter (13.20±3.06). The bats in my study tolerated a greater environmental temperature range, and thus colder temperatures, during winter compared to summer. Moreover, they tolerated cold better during both seasons compared to the three phyllostomids from Brazil. The narrow metabolic expansibility of the Brazilian bats compared to *E. wahlbergi* could be due to environmental differences associated with the respective proximity to the equator. The study site in Brazil where the phyllostomid bats were caught (22°21'S; 47°28'W) is closer to the equator than the site in South Africa where I sampled (25°44'S; 28°16'E), resulting in a narrower range of environmental temperatures experienced by the bats. This would negate the need for a greater cold tolerance compared to the Afrotropical bat which can experience more pronounced seasonal variation in air temperature, although direct comparison is precluded by the much larger M_b of *E. wahlbergi* compared to the species examined by Almeida and Cruz-Neto (2011). When compared to birds, the bats in the present study had a higher metabolic expansibility when compared to most species (in Swanson, 2010). This is unexpected as the heat produced as a result of flying during the night should enable bats to rely on activity instead of thermoregulatory heat production to tolerate cold, and thus they should have a narrower metabolic expansibility compared to diurnal species. Evidently, more intra- and interspecific research needs to be done on fruit-eating bats from different latitudes and from different climates, as well as between nocturnal and diurnal species.

The mechanisms and exact causes for the variation in BMR and M_{sum} are difficult to delineate due to the lack of knowledge of metabolic adjustment in bats. Thus, adding to the

body of knowledge of how minimum and maximum metabolic rates vary between and within species on a physiological and environmental basis is greatly needed.

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Chapter 2

Partitioning of evaporative water loss into respiratory and cutaneous pathways in Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi*

Partitioning of evaporative water loss into respiratory and cutaneous pathways in Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi*

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Abstract

When environmental temperature (T_a) exceeds body temperatures (T_b), endotherms avoid hyperthermia by increasing their rate of evaporative water loss (EWL). Small endotherms, such as bats, experience a faster rate of EWL at high T_as due to their greater surface area to volume ratio compared to larger endotherms. Further, water loss characteristics at low T_as potentially have important ecological consequences. Currently, little is known about the partitioning of total EWL (TEWL) into respiratory EWL (REWL) and cutaneous EWL (CEWL) in bats. I quantified CEWL and REWL, as well as metabolic rate and T_b in Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi*. Fruit bats drastically increased their TEWL, MR and T_b as T_a approached and exceeded normothermic T_b. Bats mainly used CEWL at moderate T_as, but REWL was the most important means of evaporative cooling at the highest T_a. CEWL represented 80% of TEWL at a T_a of 30 ºC, but decreased to 45% of TEWL at T_a = 40 ºC. Unlike microchiropterans, fruit bats did not show a decrease in CEWL at low to moderate T_as, illustrating a phylogenetic difference. *E. wahlbergi* incurred large metabolic costs to avoid hyperthermia at high T_as. Heat dissipation by means of REWL in hot weather is not as efficient compared to CEWL since panting involves muscle action, further compounding evaporative cooling efforts compared to CEWL. Therefore, CEWL may be more efficient for heat dissipation at high temperatures as it occurs passively. Elucidating evaporative cooling mechanisms in endotherms, as well as their plasticity, is important in understanding their ability to tolerate heat; a pressing topic in light of global warming.

Introduction

Evaporative heat loss is a significant component of the energy fluxes that occur between terrestrial endotherms and their environments, and has far-reaching implications for many aspects of their ecology and evolution. When environmental temperature exceeds body temperature (T_b), the only way in which terrestrial endotherms can avoid hyperthermia is through evaporative heat dissipation (Randall et al. 2002). Rates of evaporative water loss (EWL) typically increase rapidly with increasing environmental temperature as the latter approaches and exceeds T_b; in small birds, for instance, an increase in air temperature (T_a) from 40 to 48 °C may be associated with 7-fold increase in EWL (Wolf and Walsberg 1996), and in bats (10 to 29 g), rates of EWL at $T_a \approx$ 42-45 °C are typically 4 to 21-fold higher than at $T_a \approx$ 25 °C (Maloney et al. 1999; Cory Toussaint and McKechnie 2012).

Evaporative heat dissipation is of particular significance to species that routinely encounter hot conditions while roosting. Some bats in tropical and subtropical latitudes, for example, are regularly subjected to daytime roost temperatures in excess of 40 °C (Herreid 1963; Licht and Leitner 1967a; Bronner et al. 1999). Under these conditions, the augmentation of rates of EWL via mechanisms such as panting is thought to be important (in Carpenter 1986).

The avoidance of hyperthermia by means of increased EWL, however, represents a trade-off; under very hot conditions the rapid rates of EWL required to avoid hyperthermia may expose bats to a risk of dehydration associated with the loss of substantial fractions of their body water pool (Studier et al. 1970). Small endotherms are thought to be more vulnerable to dehydration at high T_a than larger species, on account of their higher surface area to volume ratios (Randall et al. 2002). The Angolan free-tailed bat (*Mops condylurus*), for instance, was found to drastically increase evaporative heat loss at $T_a >$ 40 °C, a daytime

temperature it frequently experiences in roosts, suggesting that individuals of this species may become quite dehydrated while roosting (Bronner et al. 1999; Maloney et al. 1999). Bats, arguably, face greater EWL challenges than other mammals and birds of similar size as their furless, membranous wings greatly add to their cutaneous surface area and thus to EWL (Herreid and Schmidt-Nielsen 1966).

At T_as below the thermal neutral zone (TNZ), EWL is typically approximately independent of T_a (Herreid et al. 1968; Geiser 2004; Muñoz-Garcia et al. 2012). Rates of EWL are significant determinants of energy and water balance during normothermy, as well as during heterothermy (daily torpor or hibernation), when bats may decrease T_b by more than 30 °C below normothermic levels (Geiser 2004). Heterothermy greatly decreases rates of EWL as metabolic rate (MR) and T_b may be far below normothermic values, however, cumulative water losses still greatly impact their water balance (McNab 2002). Bats are thought to overcome water deficits by arousing from torpor/hibernation to drink water (Thomas and Geiser 1997; Willis et al. 2011). However, arousal periods account for >75% of the total energy expenditure during hibernation so, in order to restore water balance bats incur massive energy costs (Thomas and Geiser 1997).

In bats, EWL represents up to 80-85% of total water loss (Studier 1970; Arad and Korine 1993; Basset et al. 2009), with total EWL (TEWL) being the sum of cutaneous EWL (CEWL) and respiratory EWL (REWL). In comparison to birds (reviewed by Wolf & Walsberg 1996) very little is known about the partitioning of TEWL into cutaneous and respiratory pathways in bats. It is thought that bats lose evaporative water predominantly via REWL due to their relatively large lung size, large lung volumes and alveolar surface areas (Bassett et al. 2009).

Chew and White (1960) estimated rates of REWL in the pallid bat, *Antrozous pallidus* from respiratory gas exchange assumptions. By subtracting REWL from TEWL, they

concluded that EWL in the pallid bat was predominated by CEWL at T_a = 25-27 °C, and that increases in TEWL when the wings were extended and not folded occurred as a result of an increase in metabolism (i.e., REWL) rather than exposed wing surface area (i.e., CEWL). However, the applicability of the latter results has been questioned by Herreid and Schmidt-Nielsen (1966), who asserted that there was an error in Chew and White's (1960) calculation, and that the T_a used in their study was not high enough to determine differences in water economy between species. Moreover, bats were restrained in a stretched position, which most likely increased the effect of stress on the metabolic and EWL results. Vogel (1969) was the first to attempt to partition TEWL by simultaneously measuring EWL from the head (REWL) and trunk (CEWL) in *Rhinopoma hardwickei*, *Myotis myotis* and *Rhinolophus ferrumequinum.* She found that *R. hardwickei* lost water predominantly via respiration, whereas *Myotis* and *Rhinolophus* mainly used CEWL; these interspecific differences became more pronounced as temperature increased. The method used to section off water loss from the head and the body could have increased the stress experienced by the bats during the experiment, and the CEWL from the head (including eyes and ears) contributed to the estimated REWL, thus overestimating the latter. Laburn and Mitchell (1975) attempted to partition EWL in *Rousettus aegyptiacus* by placing a plastic bag over the body of the animal whilst leaving its head exposed. However, this technique most likely increased the T_b of the bat and decreased the water vapour pressure difference between the bat and its environment, thereby inflating REWL and underestimating CEWL (Bassett et al., 2009). Most recently, Muñoz-Garcia et al. (2012) partitioned TEWL in the insectivorous Kuhl's pipistrelle (*Pipistrellus kuhlii*) using a mask system, and found that REWL decreased with resting MR, and that CEWL was less precisely regulated when *P. kuhlii* was in deep torpor compared to during normothermy or shallow torpor . However, bats in the latter study were restrained during measurements, and the associated stress may have affected the relative contributions

of REWL and CEWL. Moreover, restrained bats (i.e., those wearing a mask) in the latter study had significantly higher TEWL than unrestrained individuals not wearing a mask, supporting the contention that observed REWL / CEWL ratios were affected by stress arising from the experimental setup.

Notwithstanding the above studies, our knowledge of EWL partitioning in bats, and how this varies with T_a, remains rudimentary. In addition to being of scientific interest, a better understanding of inter- and intraspecific variation in the relative importance of REWL and CEWL could be useful in the context of a pressing environmental issue currently facing bats. Our understanding of the capacity of small endotherms to avoid hyperthermia during extremely hot weather, and the implications of EWL partitioning for understanding heat tolerance and evaporative cooling capacity, is critical in the context of predicting the impacts of more frequent and intense heat waves on bats in hot, tropical regions, as exemplified by the massive bird and bat fatalities have resulted from recent heat waves (>40 °C) in Australia and India (Welbergen et al. 2008; Towie 2009; Priyadarshi 2012). Bats that roost in trees are particularly vulnerable to extreme T_as as they do not have the microclimatic buffer that caves provide (Geiser 2004) and are exposed to solar radiation during the day.

I measured total, cutaneous and respiratory EWL in Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi* (Sundevall, 1846), across a range of T_as, including values above those currently experienced at the capture site. I predicted that at low T_a, CEWL would be the main pathway of EWL. The major avenue of EWL at high T_a is more difficult to discern, however, as fruit bats pant, lick their skin and fan their wings in hot weather (I.A. Minnaar, personal observation), this suggests that both REWL and CEWL are important.

Material and Methods

Study animals

I captured ten adult Wahlberg's epauletted fruit bats, *Epomophorus wahlbergi*, (nine females, one male) at the Pretoria National Botanical Garden in Gauteng, South Africa (25°44'S; 28°16'E) during March 2012 using mist nets (Ecotone Ultra Thin Mist Nets, Gdynia, Poland). I housed the male and female bats separately in outdoor aviaries (each 3 m long x 2 m wide x 2 m high) at the University of Pretoria's Experimental Farm during experiments (7 km from the capture site) between March and December 2012. Bats were maintained on the diet recommended by Barnard (2009), consisting of a mixture of fruits such as banana, apple, pear and papaya supplemented with vitamins and minerals. Food was placed in hanging baskets, with water provided *ad libitum*. The EWL experiments were conducted between September and November 2012 (austral spring). Approval of experiments was granted by the University of Pretoria's UP Animal Ethics Committee (AEC) (EC021-12). Bats weighed 84.1±7.9 g during the EWL experiments.

Physiological measurements

Rates of EWL, MR and T_b were measured using an open flow-through respirometry system. Bats were placed individually in 4-L airtight plastic chambers (Lock & Lock, Blacktown**,** NSW, Australia) with a 1-cm layer of mineral oil at the bottom of each chamber to prevent evaporation from urine and faeces affecting estimates of EWL. A plastic mesh platform was placed 10 cm above the mineral oil to prevent the bat from coming into contact with the oil.

A three-sided plastic mesh frame was placed inside the plastic container to provide adequate opportunities for the bat to hang in a natural posture.

I measured CEWL and REWL using a mask system (Tieleman and Williams 2002; Muñoz-Garcia et al. 2012). Masks were constructed using liquid latex painted onto a mould made of modelling clay, the dimensions of which were determined by measuring male and female *E. wahlbergi* skulls. I used Velcro® strips to keep the mask in place over the bat's muzzle (Muñoz-Garcia et al. 2012) (Figure 1). Slits for the eyes and ears were cut into the mask, so that evaporation from the latter contributed to CEWL. Gaps between the sides of the mask and the bat's face allowed chamber air to be drawn into the mask, with a piece of flexible Tygon tubing connected to the apex of the mask used to draw air out of it.

Figure 1. Photo of *Epomophorus wahlbergi* wearing the mask used during evaporative water loss experiments. Masks were constructed out of latex. Velcro® strips keep the mask in place over the bat's muzzle.

Masked bats in airtight chambers were placed inside a darkened, temperature-controlled cabinet (PTC-1, Sable Systems, Las Vegas, NV, USA). Chamber temperature was measured using a thermistor (Sable Systems, Las Vegas NV, USA) inserted through the lid via a small hole (which was sealed by the probe). Signals from the thermistor were received by a digital analog convertor (UI-2, Sable Systems, Las Vegas NV, USA). Atmospheric air provided by a compressor was scrubbed of water vapour (dewpoint ≈ - 50 ºC) and CO_2 by an

adsorption dryer (Ecodry K-MT 3, Parker Zander, Charlotte, North Carolina, USA), and supplied to the chamber at rates of 3-10 L min^{-1} by a mass flow controller (Model FMA5520, Omega Engineering, Bridgeport, New Jersey, USA) regularly calibrated against a soap bubble flow meter (Baker and Pouchot 1983). Air was drawn from the mask at flow rates of 1.5-2.1 L min^{-1} using an air pump and rota-meter (calibrated as above), with the remainder of the incurrent air to the chamber exiting via an outlet fitting. These flow rates ensured that the water vapour partial pressure in the chamber and mask remained below 0.4 and 1.7 kPa (equivalent to dewpoints of -5 and 15 °C), respectively, and that mask [O_2] remained above 20.4%. The time required for air to reach 99% equilibrium within the chamber ranged from 1.8 to 6.1 min (Lasiewski et al. 1966).

Inlet and outlet fittings were placed at the bottom and top of the chamber respectively to maximise air mixing. During measurements, I could easily verify from the gas concentrations in the chamber that air from the mask did not escape into the chamber. On rare occasions that bats removed the mask during measurements, the data were discarded and the measurements repeated. Air was sub-sampled sequentially from a baseline channel, the chamber and the mask using a TR-RM8 Respirometry Multiplexer (Sable Systems) and SS-3 Subsampler (Sable Systems). CEWL was determined from the water vapour pressure reading in the chamber (taking the chamber incurrent and excurrent flow rates into account; the chamber excurrent flow rate was corrected by subtracting the mask excurrent flow rate, which pulled air from the chamber through the mask). REWL was determined from the water vapour pressure from the mask (taking the mask excurrent flow rate into account) and using the chamber water vapour pressure reading as the baseline reading.

Sub-sampled air was then pulled through a Sable Systems RH-300 dew point analyser, a CO_2 analyser (CA-10a, Sable Systems) and an O_2 analyser (FC-10B, Sable Systems).

I measured T_b with temperature-sensitive passive integrated transponder (PIT) tags (Destron Fearing, St. Paul, MN, USA). These were injected subcutaneously into each bat's interscapular region. Subcutaneous temperature is representative of core T_b in bats (Gorman et al. 1991). T_b data were continuously received via a loop antenna (Racket Antenna, Biomark, Boise, Idaho, USA) attached to a receiver and data logger (Model FS2001F-ISO, Biomark, Boise, Idaho, USA). The antenna was placed next to the bat on the outside of the airtight chamber.

Data from the gas analysers, temperature sensor and PIT tag receiver were acquired and digitised using a Universal Interface II analog-digital convertor (Sable Systems) and recorded using ExpeData (Sable Systems) software on a desktop PC.

Experimental protocol

Over a two-week period in September 2012, I habituated the bats to wearing the masks. Initially, they wore the masks for 30 min at a time inside the plastic metabolic chambers, but following the habituation protocol they eventually wore masks for up to five hours during measurements. Experiments took place during the day (i.e., the rest phase of the bats). By removing all uneaten food by 22h00, I ensured that bats were postabsorptive during experiments the following day as they had been without food for at least eight hours before experiments started (Genoud 1993; Morris et al. 1994).

After fitting each bat with a mask, I induced the bat to hang from the three-sided mesh cage before lowering the bat and cage into the metabolic chamber. The chamber was placed inside the darkened temperature-controlled cabinet for at least 30 min before the start of recording to allow the bat time to calm down. I sub-sampled baseline air for 1-10 min at the start of the run (depending on how long the reading would take to stabilise). I then

sequentially sub-sampled from the mask and the chamber, switching between the two channels when a stable reading was reached (typically 7-15 min). Air from the chamber reading was again sub-sampled after each mask reading to confirm that the mask was still in place and had not become dislodged. I ended each run with a 5 min baseline reading. This cycle was repeated until the bat reached a stable T_b. This would take anywhere between 2 and 5 hours, except at 38 °C and 40 °C where trials did not run for longer than 1 hour, and bats were given water at the end of experiments. Bats were weighed before and after measurements, with the body mass (M_b) value used for calculations taken as the average of these two values.

I measured EWL and MR at T_a values of 11.4±0.4, 20.7±0.1, 30.4±0.1, 34.9±0.7, 37.7±0.3 and 40.0±0.5 ºC (hereafter referred to as 10, 20, 30, 35, 38 and 40 ºC, respectively. One bat was measured at a time at a randomly chosen T_a. However, I conducted measurements at 38 °C and 40 °C only after all other T_as, as these are above the T_a range experienced by wild bats in Pretoria. Measurements were obtained from all ten bats at each T_a value, with each bat resting for at least two days between successive experiments.

Data analysis

I estimated oxygen consumption ($\dot{V}_{O_2}$) using equation 9.4, carbon dioxide production ($\dot{V}_{CO_2}$) using equation 9.5, the rate of EWL using equation 9.6 and the excurrent flow rate using equation 9.3 from Lighton (2008).

MR was estimated from $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. Basal MR (BMR) and EWL values were calculated as the 1-min average of the lowest stable value. I determined T_b by averaging the 1 min lowest-value period identified from the MR and EWL readings. I determined body surface area (A_b) using the method described by Marom et al. (2006), and the area of the head

covered by the mask (A_m) was determined by supposing it to be a truncated cone. Digital calipers were used when taking body and head measurements. To correct CEWL estimates for the area covered by the mask, I subtracted the estimated rate of CEWL from this area from REWL, and added it to the total CEWL. The three radii were determined as follows: *a* was equivalent to half the length of the bat's body, *b* was determined as half of the width of the bat's body and *c* was measured as half the height of the bat's chest (Marom et al. 2006).

The respiratory exchange ratio (RER) was calculated as the ratio of $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$. Bats had an average RER of 0.74 during experiments, indicating a predominance of lipid metabolism. However, some values fell below the 0.71-1.00 range, in which case I assumed an RER value of 0.71 when converting oxygen consumption to MR.

The dry heat transfer coefficient (C_{dry}) was determined using equation 5 from Willis and Cooper (2009):

$$C_{dry} = (\mathrm{MHP} - \mathrm{EHL})/(T_b - T_a)$$

Where *MHP* (metabolic heat production) is MR converted to joules and *EHL* (evaporative heat loss) is EWL converted to joules. Thermal conductance values were corrected for body and head surface area following Dawson and Schmidt-Nielsen (1966).

Data were analysed using XLSTAT 2013 statistical software. I performed repeated-measures analyses of variance (RM-ANOVA) for data comparison. I used the HSD test for post hoc comparisons. I used Spearman's rank order correlation coefficient (ρ) to examine the significance of correlations, since I used the same individuals for each experiment (and thus the results were not independent) (Zar 1996). The significance level was set at $P \leq 0.05$; values are presented as means ± standard deviation (s.d.).

Results

Body temperature and metabolic rate

T_b remained relatively stable at approximately 36 °C at T_a = 10-30 °C, but increased steadily at higher T_as to $T_b \approx 41$ °C at $T_a \approx 40$ °C (Figure 2), with one individual reaching T_b = 42.9 °C at T_a = 40.7 °C. T_b was positively correlated with T_a (ρ=0.76, P<0.0001). At $T_a \approx 40$ °C, I observed bats panting and licking their snouts and muzzles when I removed them from the metabolic chambers.

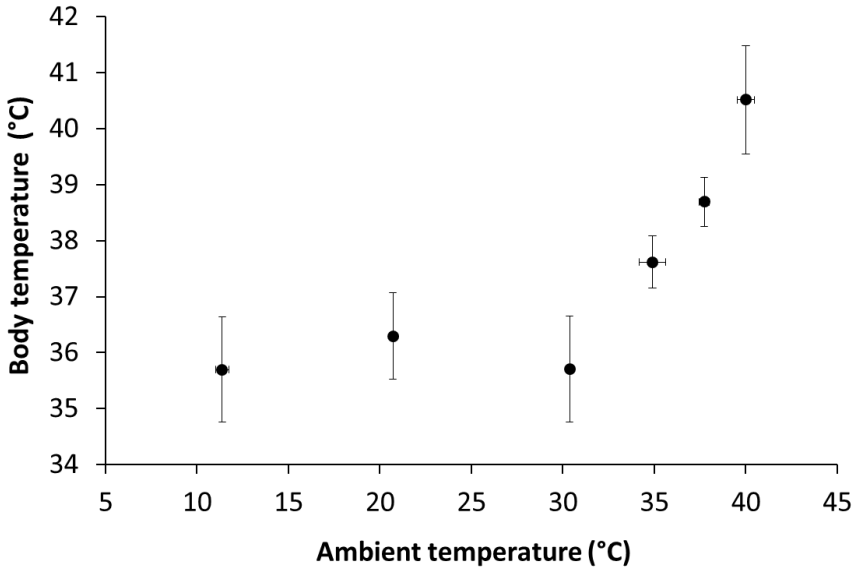

Figure 2. Body temperature of *Epomophorus wahlbergi* across a range of air temperatures (10 to 40 °C). Body temperature increases rapidly at high air temperatures. Values are presented as means±s.d.

MR decreased with increasing T_a between 10 °C and 30 °C, but increased at T_a = 30-40 °C (Figure 3). MR rapidly increased at high T_a.

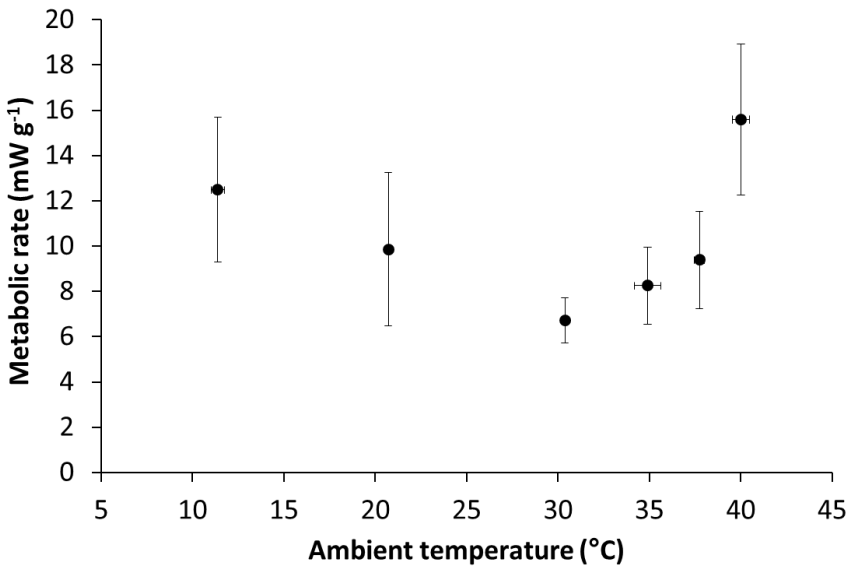

Figure 3. Metabolic rate of *Epomophorus wahlbergi* as a function of air temperature (10 to 40 °C). Metabolic rate sharply increases at $T_a \approx 40$ °C. Values are presented as means±s.d.

Evaporative water loss

TEWL increased with increasing T_a (ρ=0.92, P<0.0001), with pronounced increases at $T_a >$ 35 °C (Figure 4). TEWL was 19.93±5.22 mg g^{-1} hr^{-1} at $T_a \approx$ 40 °C, more than twice the corresponding value at $T_a \approx$ 38 °C, and 5-fold that at $T_a \approx$ 30 °C (Figure 4).

CEWL was positively correlated with T_a (ρ=0.87, P<0.0001), with the fraction of TEWL represented by CEWL increasing with $T_a \approx$ 10-30 °C and then decreasing at higher T_a, (Figure 4). The fractional contribution of CEWL to TEWL was highest at $T_a \approx$ 30 °C (79.9±8.4%) and lowest at $T_a \approx$ 40 °C (45.1±14.1%), with the latter being the only T_a value at which CEWL represented <50% of TEWL. On average, across all T_as investigated, CEWL contributed 64.0±19.4% of TEWL.

Surface-specific CEWL (ssCEWL) increased with increasing T_a from 0.02±0.01 mg g^{-1} h^{-1} cm^{-2} at $T_a \approx$ 10 °C to 0.10±0.05 mg g^{-1} h^{-1} cm^{-2} at $T_a \approx$ 40 °C (ρ=0.84, P<0.0001).

Bats used respiratory water loss as the major avenue of evaporative cooling at $T_a \approx 40$ °C (equivalent to 55.0±14.1% of TEWL) (Figure 4). REWL was positively correlated with T_a (ρ=0.68, P<0.0001). REWL increased 15-fold from $T_a \approx 30$ °C (0.74±0.26mg^{-1} g^{-1} hr^{-1}) to 40 °C (11.24±4.82 mg^{-1} g^{-1} hr^{-1}), and 3-fold from $T_a \approx 38$ °C (3.46±2.26 mg^{-1} g^{-1} hr^{-1}) to $T_a \approx 40$ °C (11.24±4.82 mg^{-1} g^{-1} hr^{-1}) (Figure 4).

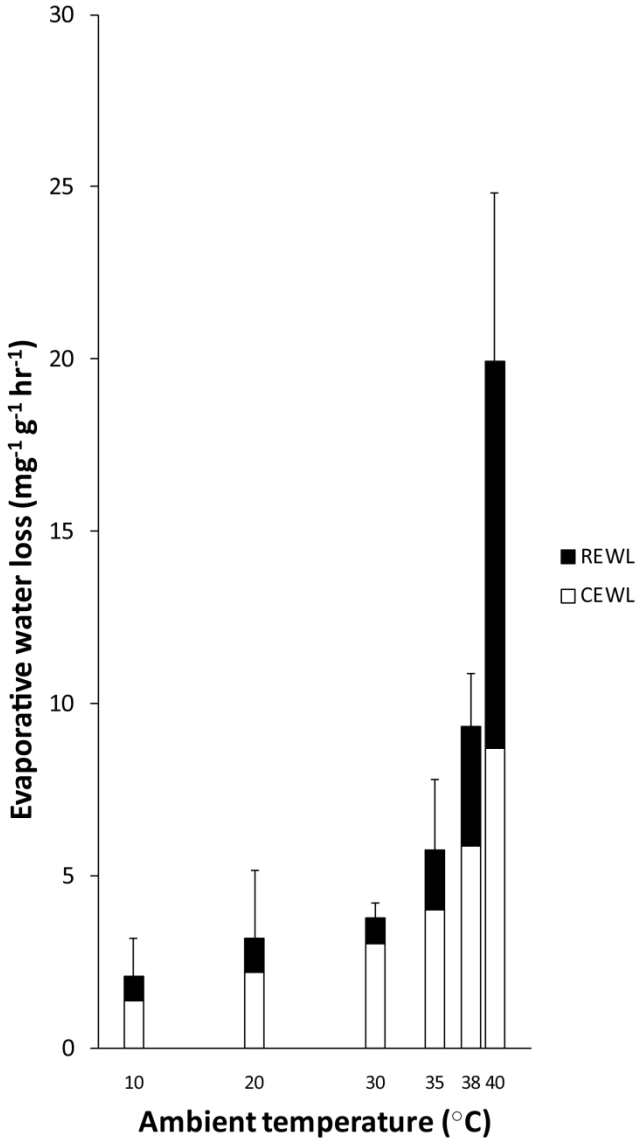

Figure 4. Total evaporative water loss of *Epomophorus wahlbergi* experimentally exposed to a range of air temperatures (10 to 40 °C). Total evaporative water loss is partitioned into cutaneous and respiratory evaporative water loss. At $T_a \approx 40$ °C, total evaporative water loss increases 10-fold from 10 °C. Values are presented as means; error bars are s.d. values of total evaporative water loss.

Thermal conductance

The mean body surface area of bats with their wings folded was A_b=70.92±12.06 cm^2, and the mean area of the head covered by the mask was A_m=21.10±5.28 cm^2. This gave the mean of the total area that contributed to CEWL as A_t=92.02±14.01 cm^2.

C_{dry} increased with increasing T_a, from 0.006±0.002 mW °C^{-1} cm^{-2} at $T_a \approx$ 10 °C to 0.17±0.15 mW °C^{-1} cm^{-2} at $T_a \approx$ 40 °C, and it was positively correlated with T_a (ρ=0.68, P<0.0001). C_{dry} increases when T_a exceeds normothermic T_b (Figure 5).

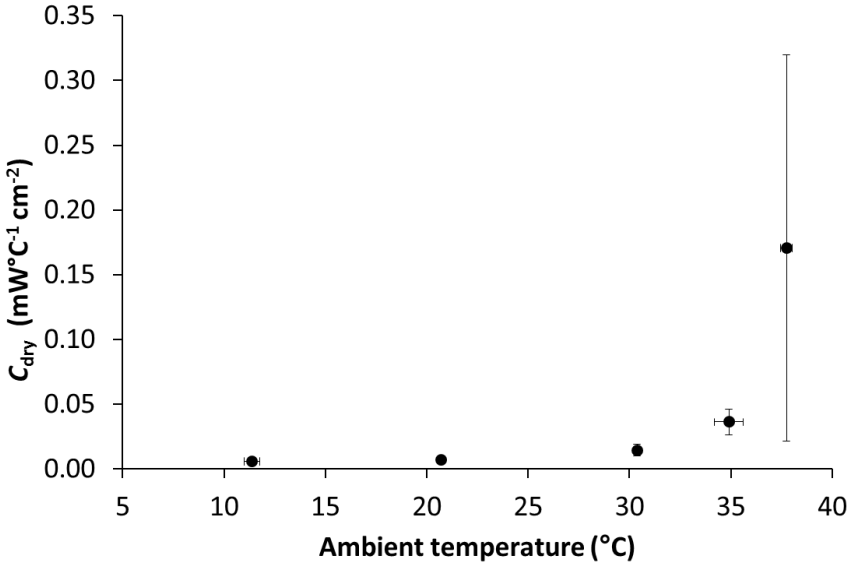

Figure 5. Dry heat transfer coefficient of *Epomophorus wahlbergi* as a function of air temperature (10 to 38 °C). The dry heat transfer coefficient increases when T_a approaches and surpasses normothermic T_b ($T_a \approx$ 38 °C). Values are presented as means±s.d.

Discussion

In *E. wahlbergi*, CEWL was the major avenue of EWL at T_as below thermoneutrality, but REWL became progressively more important at T_as approaching and exceeding T_b, with REWL representing the majority of EWL at $T_a \approx 40$ °C, These results reveal that this species relies heavily on respiratory heat loss under hot conditions. The metabolic cost of thermoregulation increased sharply at high T_a, with MR at $T_a \approx 40$ °C being higher than that at $T_a \approx 10$ °C.

The relationship between TEWL and T_a seen in *E. wahlbergi* follows the same pattern of rapid increases of evaporative water loss observed in other endotherms at T_a values that approach or exceed normothermic T_b. In small Microchiroptera from the northern hemisphere, TEWL increased 2 to 10-fold from $T_a > 30$ °C to $T_a \approx$ 40-43 °C (Herreid and Schmidt-Nielsen 1966; Carpenter and Graham 1967; Licht and Leitner 1967b; Cryan and Wolf 2003; Marom et al. 2006). The smallest bat for which EWL has been measured to date at $T_a \approx 40$ °C, *Pipistrellus kuhlii* ($M_b \approx 6.99$ g), increased TEWL by 1.5 times from $T_a \approx 30$ °C to $T_a \approx 40$ °C (Muñoz-Garcia et al. 2012). In small microchiropteran bats from the southern hemisphere, TEWL increased by 4 to 12-fold at $T_a \approx$ 40-45 °C to $T_a \approx 25$ °C (Hosken and Withers 1997; Hosken and Withers 1999; Maloney et al. 1999; Cory Toussaint and McKechnie 2012). Interestingly, a bat from a hot, semi-arid region in South Africa, *Sauromys petrophilus* ($M_b \approx 11$ g) was observed to have a maximum TEWL at $T_a \approx 42$ °C of 23.7±7.4 mg g^{-1} hr^{-1}, a 21-fold increase from the TEWL observed at $T_a \approx 25$ °C (Cory Toussaint and McKechnie 2012). In *Dobsonia minor*, a large megachiropteran from the Southern Hemisphere ($M_b \approx 87$ g), TEWL increased around 5-fold from $T_a \approx$ 25-30 °C to $T_a \approx 40$ °C (Bartholomew et al. 1970). Male *R. aegyptiacus* (Megachiroptera) ($M_b \approx 118$ g), captured in the north-eastern region of South Africa, sharply increased their TEWL at $T_a \approx 40$ °C by 2-

fold from the TEWL at $T_a \approx$ 25-30 °C (Laburn and Mitchell 1975). I am unaware of any other studies that have measured TEWL at T_as above the normothermic T_a in small and large Chiroptera. The megachiropteran bats in my study, *E. wahlbergi*, also showed a sharp increase in TEWL at $T_a \approx$ 40 °C compared to lower T_a. They increased their TEWL 5-fold from $T_a \approx$ 30 °C to $T_a \approx$ 40 °C. This was at the upper range observed in large bats, but it should be noted that only two species have been measured so far, so further studies need to be conducted to unearth any patterns between large bats and small bats, and between bats from different taxonomic groups.

As expected, CEWL was the main avenue of evaporative heat loss at T_as below the normothermic T_b of *E. wahlbergi*. The bats in my study relied more on REWL at $T_a \approx$ 40 °C. The same pattern was observed in *R. aegyptiacus* (Laburn and Mitchell 1975), but REWL may have been overestimated in the latter study (see Introduction). *Rhinopoma hardwickei*, an insectivorous bat, cooled itself primarily through REWL, which increased with T_a (Vogel 1969). On the other hand, *Myotis myotis* and *Rhinolophus ferrumeuinum* predominanlty used CEWL, which also increased as T_a increased (Vogel 1969). Muñoz-Garcia et al. (2012) found that CEWL was predominantly used for evaporative cooling from $T_a \approx$ 5-40 °C in the microchiropteran *Pipistrellus kuhlii*. In the latter species, ssCEWL increased from $T_a \approx$ 20 °C, with the maximum ssCEWL reported at $T_a \approx$ 40 °C (Muñoz-Garcia et al. 2012). In the present study, ssCEWL increased from $T_a \approx$ 10 °C, a lower T_a than that observed in the above-mentioned study.

The differing patterns of evaporative water loss partitioning employed by the chiropteran species studied to date could reflect broad phylogenetic variation, and/or adaptation driven by environmental variables. Currently, too few data exist on EWL partitioning in bats to evaluate this possibility. Considerably more data are available for birds, and indicate large differences between avian orders in terms of the primary avenue of

evaporative heat dissipation at high T_a. Passerines, for instance, rely predominantly on REWL for avoiding hyperthermia (Wolf and Walsberg 1996; Tieleman and Williams 2002), but other taxa, most notably the Columbiformes, instead dramatically increase their CEWL as T_a increases (Hoffman and Walsberg 1999; McKechnie and Wolf 2004). The partitioning of TEWL into cutaneous and respiratory components may have important consequences for maximum evaporative cooling capacity, and hence the tolerance of extreme heat. Cutaneous heat dissipation may be a more efficient means of thermoregulating in hot weather, since it does not require the muscle action associated with panting and/or gular flutter: in white-winged doves, heat-acclimated individuals with higher CEWL had significantly lower metabolic rates at $T_a \approx 45$ ºC compared to cool-acclimated individuals in which a greater proportion of TEWL occurred via respiratory losses (McKechnie and Wolf 2004).

The fact that in *E. wahlbergi* RMR at $T_a \approx 40$ ºC is higher than that at $T_a \approx 10$ ºC suggests that the avoidance of hyperthermia incurs a significant metabolic cost, and these bats may not be well-equipped to tolerate maximum air temperatures 2-5 ºC higher than current maxima. Since global air temperature is predicted to rise by 1.1 to 6.4 °C towards the end of the century from global warming (IPCC 2011), *E. wahlbergi* will most likely need to phenotypically adjust their heat tolerance capacity or behaviourally avoid hot environments.

In this study, bats no longer defended a set point T_b at $T_a > 35$ °C; they became hyperthermic and T_b increased by up to 7 ºC above normothermic levels . At $T_a \approx 38$ °C and 40 °C, bats conformed to T_a, thereby decreasing the T_b-T_a gradient as T_a increased. Facultative hyperthermia is a strategy used in hot environments where an animal conserves water by reducing the amount of evaporative heat loss necessary to maintain the gradient between T_b and T_a (Boyles et al. 2011). During experiments, some of the bats experienced T_bs above and around 42 °C, indicating that they may even tolerate, and display facultative hyperthermia, at T_as around 42 °C and 43 °C. In *Taphozous mauritianus* and *S. petrophilus*,

microchiropteran bats from a semi-arid region in South Africa, some individuals showed T_bs of 44.9 ºC and 46.5 ºC, respectively, at $T_a \approx 42$ ºC (Cory Toussaint and McKechnie 2012).

Whereas REWL had a greater effect on evaporative cooling at $T_a \approx 40$ °C than CEWL, water loss across the skin contributed the most to EWL at lower T_as. The contribution of CEWL to TEWL decreased with decreasing T_a from $T_a \approx 30$ °C illustrating phylogenetic differences in water partitioning strategies. More data are needed to investigate phylogenetic differences in EWL partitioning during varying climatic conditions.

Flying fox fatalities have been observed in Australia and India at T_as > 40 °C (Welbergen et al. 2008; Priyadarshi 2012). Bats were either unable to increase their TEWL above a certain limit, or they were unable to compensate for the water loss they experienced and thus became dehydrated. To further elucidate the relationship between TEWL and T_a at high T_a, bats need to be exposed to even higher T_as (i.e., those predicted from climate change models) at varying levels of hydration (see Korine and Arad 1993). Relative humidity can also greatly impact the thermoregulatory ability of bats (Herreid 1967; Licht and Leitner 1967b). In my study, bats were subjected to dry air, so the EWL rates observed in my study were most likely the upper limits of the bats' EWL capabilities. It would be informative to expose these bats to ecologically relevant (i.e., higher) humidities to evaluate how it impacts their thermoregulatory capabilities. Further acclimating bats to cold and warm T_as will determine the possible role phenotypic plasticity may play and whether *E. wahlbergi*, when heat-acclimated, will be able to phenotypically alter their evaporative cooling capacity at high temperatures to better cope with climate change (e.g., McKechnie and Wolf 2004).

Global temperature rises are predicted to occur as a result of human-induced climate change. Bats that roost in microsites that are not well buffered against climatic conditions (such as tree canopies) will be relatively more susceptible to high temperatures. The vulnerability of fruit bats to temperature extremes is further exacerbated by their slow

reproductive rates, possibly resulting in a slow adaptive response to climate change (Sherwin et al. 2012). Bats are not able to concentrate their urine to reduce water loss, so rely on free-standing water sources that are close to their roosts to compensate for their water loss (Adam and Hayes 2008). If water resources become scarce due to climate change, bats may be reproductively compromised (Adams 2010), and if fruit bats are unable to evolutionarily cope with the suspected temperature rise, they will have to move to more suitable environments to outpace global warming. Alternatively, man-made water holes could be provided to offset the water loss experienced under man-induced climate change (see Parris and Hazell 2005) or fruit bat roosts with adequate shade could be constructed close to known roosting sites. Investigating water loss strategies of fruit bats and how they will cope with global warming is essential as their demise could have drastic knock-on effects for the plants that they pollinate and whose seeds they disperse.

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