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RESEARCH ARTICLE

Summit metabolism and metabolic expansibility in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*): seasonal acclimatisation and effects of captivity

Ingrid A. Minnaar, Nigel C. Bennett, Christian T. Chimimba and Andrew E. McKechnie*

ABSTRACT

Summit metabolism (M_{sum}), the maximum rate of resting metabolic thermogenesis, has been found to be broadly correlated with climatic variables and the use of heterothermy in some endotherms. Far less is known about M_{sum} and metabolic expansibility [ME, the ratio of M_{sum} to basal metabolic rate (BMR)] in bats compared with many other endotherm taxa. We measured BMR and M_{sum} during winter and summer in captive and wild populations of a pteropodid from the southern subtropics, Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) in Pretoria, South Africa. The M_{sum} of fruit bats ranged from 5.178±0.611 W (captive, summer) to 6.006±0.890 W (captive, winter), and did not vary significantly between seasons. In contrast, BMR decreased by 17–25% in winter. The combination of seasonally stable M_{sum} but flexible BMR resulted in ME being significantly higher in winter than in summer, ranging from 7.24±1.49 (wild, summer) to 13.11±2.14 (captive, winter). The latter value is well above the typical mammalian range. Moreover, both M_{sum} and ME were significantly higher in captive bats than in wild individuals; we speculate this represents a phenotypic response to a reduction in exercise-associated heat production while in captivity. Our data for *E. wahlbergi*, combined with those currently available for other chiropterans, reveal that M_{sum} in bats is highly variable compared with allometrically expected values for other mammals.

KEY WORDS: Acclimatisation, Cold exposure, Helox, Phenotypic flexibility, Thermogenic capacity

INTRODUCTION

The lowest environmental temperature to which an endotherm can defend normothermic body temperature (T_b) is determined primarily by its maximum capacity for metabolic thermogenesis (Scholander et al., 1950). Summit metabolism (M_{sum}) is the maximum rate of resting metabolic thermogenesis in the absence of exercise-associated heat production (Swanson et al., 1996) [also referred to as cold-induced peak metabolic rate (Wiersma et al., 2007)]. Among mammals, metabolic expansibility [ME, the ratio of M_{sum} to basal metabolic rate (BMR); also referred to as factorial aerobic scope] is typically 4–8, but may be as high as 10–13 (Careau, 2013; Hinds et al., 1993). Most avian ME values are similar to those typical of mammals, with maximum reported values of 9.0–9.5 (Arens and Cooper, 2005; van de Ven et al., 2013a). Among mammals and birds, interspecific variation in heat production capacity is correlated with climate, with M_{sum} generally being higher in species inhabiting colder regions (Rezende et al., 2004; Swanson and Garland, 2009).

One endotherm order about which remarkably little is known in terms of resting heat production capacity is the Chiroptera. The first published estimate of M_{sum} in a bat of which we are aware was for the molossid *Tadarida brasiliensis*, in which mass-specific M_{sum} was equivalent to ~21× BMR (Canals et al., 2005). The very high ME value for *T. brasiliensis* contrasts with more recent data for three frugivorous phyllostomids (*Artibeus lituratus*, *Sturnira lilium* and *Carollia perspicillata*) in which ME ranged from 3.4 to 5.2 (Almeida and Cruz-Neto, 2011).

A priori, two broad predictions can be made regarding resting heat production capacity in bats. Negative correlations between M_{sum} and air temperature in mammals and birds (Rezende et al., 2004; Swanson and Garland, 2009), together with the diurnal rest phase of bats coinciding with the warmer part of the circadian cycle, lead to the prediction that selection for high M_{sum} in bats should be reduced in comparison with diurnal taxa that rely heavily on resting metabolic heat production for thermoregulation during their nocturnal rest phases. In other words, because bats are inactive during the warmer daytime, their requirements for non-activity-associated thermogenesis are likely to be more modest than those for nocturnally inactive taxa [although the very short daily foraging periods of some bats (e.g. Dechmann et al., 2011) raise the possibility that this might not always be the case]. Conversely, resting heat production capacity in rodents is correlated with torpor use, with ME negatively correlated with both minimum air temperature and torpid T_b (Careau, 2013). This link between ME and the metabolic machinery involved in rewarming from heterothermy leads to a second prediction in the opposite direction, namely that M_{sum} and ME should be comparatively high in bats that hibernate and/or use daily torpor.

As is the case for BMR, there is increasing evidence that M_{sum} is not fixed within individuals, but is adjusted in response to environmental cues. Many endotherms respond to seasonal variation in energy requirements and/or food availability by means of acclimatisation involving changes in both M_{sum} and/or BMR, with the direction and magnitude of these changes varying widely among and within species (Lovegrove, 2005; Swanson, 2010). In north-temperate climates, winter acclimatisation in birds typically involves the upregulation of both M_{sum} and BMR (reviewed by McKechnie and Swanson, 2010; Swanson, 2010), whereas in subtropical habitats avian BMR is generally lower in winter than in summer (Smit and McKechnie, 2010). Among mammals, winter decreases in body mass are generally associated with proportional reductions in BMR in species smaller than 100 g, whereas intermediate-sized (0.1–10 kg) species typically show winter increases in BMR (Lovegrove, 2005). The data currently available for bats indicate that

Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa.

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

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List of symbols and abbreviations

BMR	basal metabolic rate
M_b	body mass
ME	metabolic expansibility
M_{sum}	summit metabolism
NST	non-shivering thermogenesis
RER	respiratory exchange ratio
RMR	resting metabolic rate
T_a	air temperature
T_b	body temperature
T_{cl}	cold limit temperature
T_{lc}	lower critical limit of thermoneutrality
$\dot{V}_{CO_2}$	carbon dioxide production
$\dot{V}_{O_2}$	oxygen consumption

BMR may either show no seasonal change (Almeida and Cruz-Neto, 2011; Coburn and Geiser, 1998) or increase in winter (Downs et al., 2012). Seasonal acclimatisation in mammalian M_{sum} has received far less attention; Lovegrove (Lovegrove, 2005) found limited evidence for winter increases in non-shivering thermogenesis (NST) capacity among small mammals, and most other studies have similarly focused on NST rather than M_{sum} (e.g. Chen et al., 2012; Zhu et al., 2012).

In this study, we addressed several questions concerning maximum resting thermogenic capacity and seasonal metabolic adjustments in bats inhabiting seasonal subtropical habitats. First, we measured BMR, M_{sum} and ME in a pteropodid not known to use torpor or hibernation, in order to compare these variables with those of other endotherms, in particular diurnal taxa such as most birds. The substantial overlap between avian and mammalian ME values suggests that such comparisons may be informative, even though they involve two classes in which endothermy evolved independently and which differ in the relative importance of shivering thermogenesis versus NST (Bicudo et al., 2001; Cannon and Nedergaard, 2004). We also examined seasonal adjustments in BMR and M_{sum} in order to further investigate metabolic acclimatisation in bats, and compared our results with published data to investigate whether intraspecific variation exists among conspecific populations occupying different areas. Finally, we examined whether BMR and/or M_{sum}, and the magnitude and direction of seasonal changes in these variables, differ between captive and wild free-ranging bats. Comparative analyses often include data from captive as well as wild populations, but the potential effects of captivity on metabolic variables are rarely considered. Factors including reduced exercise and greater food availability in captive environments could conceivably influence the metabolic machinery of bats in a variety of ways. Our study species was Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi* (Sundevall 1846), which is widespread in southeastern Africa (Monadjem et al., 2010). The available data suggest that this species shows at least some flight activity throughout the night (Fenton et al., 1985).

RESULTS

Body mass

Among female fruit bats, body mass (M_b) averaged 87.9±7.6 g in winter and 86.2±8.9 g in summer, and did not vary with season ($F_{1,20.97}$=1.049, P=0.318) or population (i.e. wild versus captive; $F_{1,14.14}$=0.849, P=0.372). Among wild fruit bats, M_b did not vary significantly across seasons ($F_{1,15.15}$=1.148, P=0.300). Overall, males were significantly heavier than females ($F_{1,14.89}$=26.648, P<0.001), with males averaging 110.1±7.9 g and females 88.6±8.4 g.

BMR

BMR was significantly related to M_b in wild bats in winter ($F_{1,9}$=8.244, P=0.021) but not in summer ($F_{1,9}$=2.849, P=0.130), and was not related to M_b during either season in captive bats (winter: $F_{1,9}$=3.518, P=0.097; summer: $F_{1,9}$=0.264, P=0.621; variance estimates for individual identity: σ^2=0.0006974, $\sigma^2_{residual}$=0.0194817; Fig. 1). When analysed with M_b as a covariate, BMR was significantly lower in winter than in summer ($F_{1,16.87}$=11.906, P=0.003) and was significantly higher in wild bats than in captive individuals ($F_{1,20.53}$=6.533, P=0.019; Table 1). Among captive bats, mean BMR during winter was equivalent to 83.4% of summer BMR; among wild bats the equivalent value was 75.2%. During winter and summer the BMR of captive female bats (N=9) was equivalent to 83.3% and 73.6%, respectively, that of wild females (N=4). Normothermic T_b measured during the BMR measurements did not differ with season ($F_{1,25.59}$=2.113, P=0.158), but was significantly lower (on average, by 1.08°C in winter and 0.49°C in summer) in wild than in captive bats ($F_{1,22.10}$=7.932, P=0.010; Table 1).

M_{sum} and ME

M_{sum} was significantly related to M_b in wild bats during both winter ($F_{1,9}$=9.452, P=0.015) and summer ($F_{1,9}$=67.572, P<0.001), and during winter in the captive bats ($F_{1,9}$=7.163, P=0.028) but not summer ($F_{1,9}$=1.689, P=0.230; variance estimates for individual identity: σ^2=0.2949, $\sigma^2_{residual}$=0.3162; Fig. 2). In contrast to BMR, M_{sum} did not vary significantly with season ($F_{1,26.22}$=1.879, P=0.182). However, M_{sum} was significantly higher in captive bats

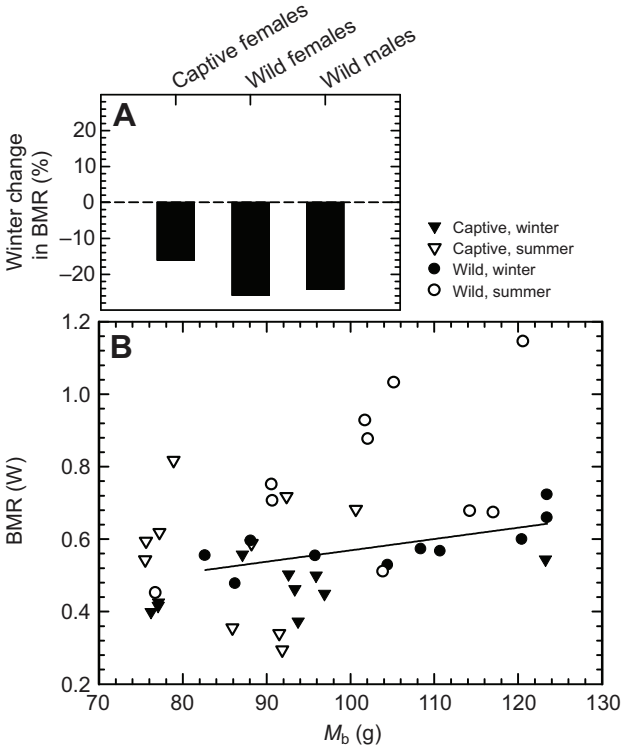

Fig. 1. Basal metabolic rate (BMR) as a function of body mass (M_b) in a wild and captive population of Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) during summer and winter. (A) The mean percentage change in BMR during winter compared with summer for captive females, wild females and wild males (no value shown for single captive male). (B) Linear regression models yielded a significant fit only in the case of the winter data for wild bats (solid line: BMR=0.256+0.003M_b; r^2=0.508).

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Table 1. Body temperature (T_b), basal metabolic rate (BMR), temperature at cold limit (T_{cl}) in helox, summit metabolism (M_{sum}) and the ratio of M_{sum} to BMR in wild and captive populations of Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) in Pretoria, South Africa, during summer and winter

	Captive population		Wild population	
Variable	Winter	Summer	Winter	Summer
T_b (°C)	35.45±0.90	35.46±0.54	34.37±0.70	34.97±0.88
BMR (W)	0.463±0.062	0.555±0.174	0.582±0.068	0.775±0.220
Helox T_{cl} (°C)	−10.03±2.97	−9.19±1.48	−7.71±4.13	−3.35±4.04
M_{sum} (W)	6.006±0.890	5.178±0.611	5.786±1.579	5.404±1.121
M_{sum}/BMR	13.11±2.14	10.52±4.62	9.99±2.71	7.24±1.49

Data are means ± s.d. In all instances, N=10.

($F_{1,23.12}$=8.408, P=0.008). During winter, the M_{sum} of captive females (N=9) was equivalent to 130.8% that of wild females (N=4), with the corresponding value for summer being 110.0%. The cold limit temperature (T_{cl}) at which M_{sum} was reached also varied significantly with season ($F_{1,28.54}$=8.886, P=0.006) and between the wild and captive populations ($F_{1,22.72}$=10.582, P=0.004; Table 1).

Values of ME (i.e. M_{sum}/BMR) varied significantly among seasons ($F_{1,18.36}$=8.190, P=0.010) and between captive and wild populations ($F_{1,18.88}$=11.624, P=0.003), being significantly higher in winter than in summer, and higher in captive bats than in wild bats (Fig. 3). Mean ME values ranged from 7.24±1.49 (wild, summer) to 13.11±2.14 (captive, winter). Among wild bats, ME did not differ between sexes ($F_{1,14.99}$=2.889, P=0.110).

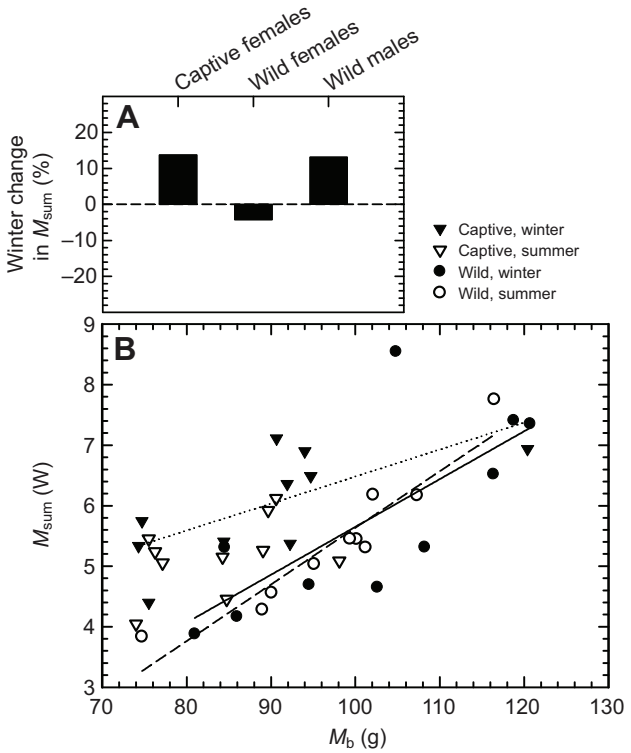

Fig. 2. Summit metabolism (M_{sum}) as a function of M_b in a wild and captive population of *E. wahlbergi* during summer and winter. (A) The mean percentage change in M_{sum} during winter compared with summer for captive females, wild females and wild males (no value shown for single captive male). (B) Linear regression models yielded significant fits as follows: wild, winter (solid line: M_{sum}=–2.255+0.079M_b; r^2=0.542); wild, summer (dashed line: M_{sum}=–3.725+0.094M_b; r^2=0.894); captive, winter (dotted line: M_{sum}=2.019+0.045M_b; r^2=0.472).

DISCUSSION

Our data reveal considerable phenotypic flexibility in the upper and lower limits of resting metabolic rate in a subtropical pteropodid fruit bat, with BMR varying seasonally and both BMR and M_{sum} differing significantly between wild and captive individuals. The flexibility in BMR and M_{sum} was manifested as ME values that varied widely across seasons and between wild and captive populations. Observed ME ranged from ~7, within the typical mammalian range, to >13, well above the typical range (Careau, 2013; Hinds et al., 1993).

Some potential error is added to our M_{sum} estimates on account of the mean respiratory exchange ratio (RER) of 0.669±0.058 during measurements being below the theoretically expected range of 0.71 to 1.00, corresponding with metabolism of lipids and carbohydrates, respectively (Withers, 1992). It is unlikely that these low RER values are an artefact of experimental error, as our mean RER value for BMR (0.837±0.091), which was measured on the same days as M_{sum}, fell well within the expected range. We are not aware of published thermal equivalence data suitable for converting respiratory gas exchange to metabolic rate when RER <0.71, and hence assumed RER=0.71 when estimating M_{sum}. Values of RER below the expected range of 0.71–1.00 have been reported by several workers (reviewed by Walsberg and Hoffman, 2005). These authors also pointed out that accepted RER and thermal equivalence values are based largely on data obtained from medium- to large-bodied domesticated taxa during the first half of the 20th century, and may not necessarily be expected to apply universally to species from phylogenetically diverse groups operating under a wide variety of thermogenic requirements and exercise intensities. Our data

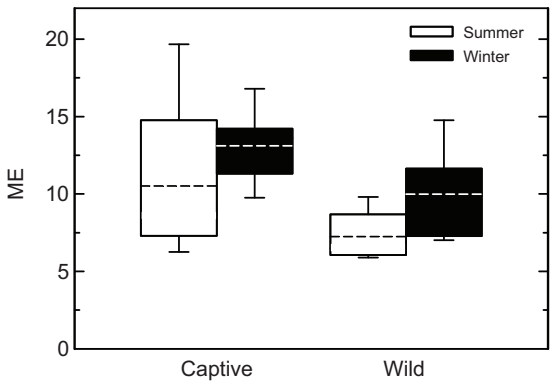

Fig. 3. Metabolic expansibility (ME) in a captive and wild population of *E. wahlbergi*. ME is the ratio of M_{sum} to BMR. Means are indicated by dashed lines, each box indicates the 10th and 25th percentiles, and the error bars indicate the 75th and 90th percentiles.

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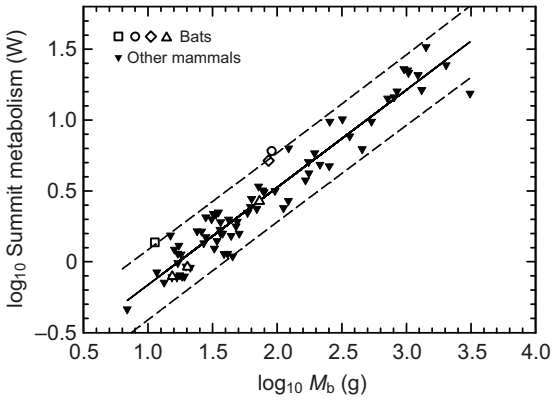

Fig. 4. Summit metabolism in bats compared with other mammals. Data were obtained from Hinds et al. (Hinds et al., 1993) and the meta-analysis for rodents by Careau (Careau, 2013) (see electronic supplementary material of latter paper for original sources). Rates of oxygen consumption were converted to metabolic rate assuming respiratory exchange ratio (RER)=0.71, i.e. metabolism of lipids. Data for the following bat species (open symbols) are indicated: *Tadarida brasiliensis*, square (Canals et al., 2005); *Artibeus lituratus*, *Sturnira lilium* and *Carollia perspicillata*, upward-pointing triangles (Almeida and Cruz-Neto, 2011); *Epomophorus wahlbergi* captive, winter, circle; and captive, summer, diamond (present study). The dashed lines indicate the 95% prediction intervals for the linear regression.

reiterate the need for further investigation of the substrates that endotherms metabolise during cold stress, as well as direct measurements of heat production. Unexpectedly low RER values are also associated with M_{sum} in elephant shrews (Macroscelidea) (M. L. Thompson, A.E.M., N. C. Bennett and N. Mzilikazi, unpublished data).

Estimated M_{sum} in *E. wahlbergi* was much higher than expected on the basis of available mammalian data. Our observed M_{sum} values are equivalent to 172% (summer, captive) to 191% (winter, captive) of the values predicted by a conventional analysis of the scaling of mammalian M_{sum} (Fig. 4). The M_{sum} measured in *T. brasiliensis* (Canals et al., 2005) is similarly high (183% of expected; Fig. 4). The M_{sum} of *T. brasiliensis* and that of captive *E. wahlbergi* during winter both fall outside the upper 95% prediction interval for a linear regression fitted to the currently available mammalian data (Fig. 4). In contrast, M_{sum} in three phyllostomids (Almeida and Cruz-Neto, 2011) (values averaged across seasons) is much closer to allometrically predicted values (82–99%; Fig. 4). Although the small number of chiropteran species in which M_{sum} has been measured precludes meaningful investigations of the phylogenetic or environmental correlates of interspecific variation, the wide range of M_{sum} values relative to those expected on the basis of M_b is striking. The high M_{sum} and ME we observed in both wild and captive *E. wahlbergi* do not support the prediction that nocturnal mammals inhabiting subtropical latitudes have only modest capacities for resting metabolic heat production compared with species inhabiting cold, north-temperate climates. Among the bat species for which estimates of M_{sum} do exist, *T. brasiliensis*, *C. perspicillata*, *S. lilium* and *A. lituratus* are all known to use torpor (Audet and Thomas, 1997; Hirshfeld and O'Farrell, 1976; Studier and Wilson, 1970), whereas torpor has not been documented in *E. wahlbergi* or any other pteropodid with M_b>50 g (Stawski et al., 2014). Thus, there is no clear link between the use of torpor and high M_{sum} in Chiroptera (Fig. 4), as the two data points that fall outside of the upper 95% prediction interval represent one species that uses torpor (*T. brasiliensis*) and one that does not (*E. wahlbergi*).

In *E. wahlbergi*, M_{sum} did not vary significantly across seasons. In the only other study of seasonal variation in M_{sum} in bats of which we are aware, Almeida and Cruz-Neto (Almeida and Cruz-Neto, 2011) similarly found no significant seasonal change in the M_{sum} of *A. lituratus*, *S. lilium* and *C. perspicillata*. Their study, like ours, involved bats living in subtropical habitats characterised by comparatively mild air temperature minima during winter. It may be that bats that maintain normothermic T_b in seasonally colder environments increase their M_{sum} in winter, although the data needed to evaluate this possibility are not currently available. Among endotherms in general, a link between seasonal adjustments in M_{sum} and cold tolerance is implied by negative correlations between M_{sum} and minimum air temperature among rodents (Careau, 2013) and birds (Swanson and Garland, 2009), as well as the observation that avian winter enhancements in cold tolerance are typically associated with seasonal increases in M_{sum} (Swanson and Bozinovic, 2011; Swanson and Garland, 2009).

One unexpected pattern to emerge during our study concerns the effect of captivity on M_{sum} and ME, which were significantly higher in the captive fruit bats than in the wild individuals. The higher ME of captive bats reflected a combination of lower BMR and higher M_{sum} compared with wild conspecifics. One possible explanation for the higher M_{sum} relates to the reduced activity levels of bats when confined to an aviary compared with free-ranging conditions; we might expect that heat generated as a by-product of flight contributed far less to thermoregulation in captive individuals than in the wild bats. This notion is supported by empirical evidence and theoretical predictions that activity-thermoregulatory heat substitution is common in flying endotherms, particularly at intermediate air temperatures (Humphries and Careau, 2011). Hence, we speculate that the increased capacity for resting thermogenesis observed in our captive bats was a response to a reduction in exercise-associated thermogenesis.

The lack of seasonal changes in the M_b of *E. wahlbergi* in this study contrasts with the significantly higher M_b (by ~15%) in winter reported for a captive population of the same species (Downs et al., 2012). The latter population originated from a site near the east coast of South Africa that is more mesic than our study area. There is no obvious explanation for why these conspecific populations differed in their seasonal patterns of M_b. However, the fact that both captive and wild bats in our study showed a markedly different pattern to the captive individuals investigated by Downs et al. (Downs et al., 2012) suggests that this variation is probably not related to any difference between the studies in terms of the conditions under which bats were maintained in captivity. In contrast, three species of frugivorous phyllostomids in southeastern Brazil had significantly lower M_b in winter than in summer, although in two of these species (*A. lituratus* and *C. perspicillata*) the fractional change in M_b between seasons was <5% (Almeida and Cruz-Neto, 2011).

We found that *E. wahlbergi* had significantly lower BMR during winter than summer, by ~17% in captive individuals and ~25% in wild individuals. These seasonal changes in the BMR of fruit bats in Pretoria contrast with those reported for a conspecific population captured and held in captivity in Pietermaritzburg (29°37′S 30°23′E). The latter population increased mass-specific BMR by ~22% and whole-animal BMR by ~40% during winter compared with summer (Downs et al., 2012), seasonal changes in the opposite direction to those shown by conspecifics in the present study. Moreover, the BMRs of the fruit bats held in captivity by Downs et al. (Downs et al., 2012) were substantially higher than those of the captive population we investigated here; assuming the same RER as the mean value during our measurements, summer BMR was

0.603 W (109% of the summer BMR we observed), whereas winter BMR was 0.846 W (183% of the corresponding value in our study). The BMRs predicted for *E. wahlbergi* on the basis of the phylogenetically independent scaling relationship of Cory Toussaint and McKechnie (Cory Toussaint and McKechnie, 2012) are 0.461 W (captive population) and 0.519 W (wild population). Observed values in our study ranged from 100.4% (captive, winter) to 149.4% (wild, summer) of those predicted.

The contrast in the direction of seasonal changes in BMR between the fruit bats in the present study and those investigated by Downs et al. (Downs et al., 2012) highlights the large variation that can exist in seasonal metabolic responses within species. The magnitude of the among-population differences in *E. wahlbergi* is similar to that recently documented in a bird; two southern red bishop (*Euplectes orix*) populations showed contrasting seasonal changes in both BMR and M_{sum}, with birds at a warmer coastal site showing no significant seasonal variation in BMR, whereas birds from a colder inland site increased BMR by 58% in winter (van de Ven et al., 2013b). However, whereas the two study sites in that study differed by ~10°C in winter minimum air temperature (T_a), Pretoria and Pietermaritzburg are climatically similar, with average minimum and maximum temperatures across all months differing by at most 2°C between them, and with mean annual precipitation differing by ~20% (Pretoria: 703 mm; Pietermaritzburg: 832 mm; South African Weather Service). Hence, there is no obvious climatic difference to which the large differences in seasonal BMR responses can be linked.

Our data reveal that captivity can have a substantial effect on metabolic parameters in pteropodid fruit bats. Although seasonal variation in both BMR and M_{sum} were qualitatively similar in the captive and wild populations, BMR was significantly lower and M_{sum} and ME significantly higher in captive fruit bats. These results suggest that (a) we should be cautious about assuming that metabolic data from captive populations can be extrapolated to wild conspecifics, and (b) synthetic analyses of metabolic rates should distinguish between data from wild and captive populations. Among birds, metabolic scaling exponents differ significantly between wild-caught and captive-raised populations (McKechnie et al., 2006), and our results here raise the possibility that similar variation may exist among bats and other mammals.

In conclusion, the high and flexible M_{sum} of *E. wahlbergi* highlights how little we know about the upper limits to resting heat production in bats. The limited data currently available for bats include ME values from ~3 (below the typical mammalian range) to ~21 (far above the typical mammalian range), suggesting that bats may prove a useful model taxon for identifying the factors driving the evolution of M_{sum} in endotherms.

MATERIALS AND METHODS

Study animals

The captive population comprised 10 adult *E. wahlbergi* (nine non-reproductive females, one male; mean ± s.d. M_b=84.1±7.9 g at time of capture) that we captured using mistnets (Ecotone Ultra Thin Mist Nets, Gdynia, Poland) at the Pretoria National Botanical Gardens, Pretoria, South Africa (25°44′S, 28°16′E). Pretoria has a mild, subtropical climate, with mean daily minimum temperatures during the warmest (January) and coldest (July) months of ~18°C and 5°C, respectively (South African Weather Service). Captive bats were housed in outdoor aviaries (each 5 m long×2.5 m wide×2.5 m high) at the University of Pretoria's Experimental Farm during experiments (3 km from the capture site), and hence experienced natural cycles of T_a. The male bat was housed separately from the females. Bats were maintained on a diet of mixed fruits supplemented with vitamins and minerals (Barnard, 2009); water was provided *ad libitum*.

Additional bats (hereafter referred to as the wild population) were captured on the University of Pretoria campus and kept for 1–2 days in the outdoor aviaries (different aviary to the captive population) during late July/early August 2012 (winter measurements) and again in December 2012 (summer measurements). During both seasons, we caught six males and four females, with three individuals recaptured and used for measurements during both seasons. Winter data were obtained between 28 July and 29 August 2012, while summer data were collected between 9 and 18 December 2012. BMR and M_{sum} were measured in each individual within 24 h of each other, with the order of measurements randomised. All measurements took place during the daytime rest phase.

BMR

Metabolic rates were estimated from rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$). To measure BMR, we placed bats individually in 2.1 l airtight plastic chambers (Lock and Lock, Blacktown, NSW, Australia) fitted with inlet and outlet ports at opposite ends of the chamber. To prevent evaporation from urine and faeces affecting readings, a 1 cm layer of mineral oil was placed at the bottom of each chamber. A plastic mesh platform and a three-sided plastic mesh enclosure were placed inside the chamber to prevent the bat from coming into contact with the oil and to provide it with enough space to hang in a natural posture, respectively. 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 measurements.

We measured T_b using temperature-sensitive passive integrated transponder (PIT) tags (Destron Fearing, St Paul, MN, USA), injected subcutaneously into each bat's interscapular region. Subcutaneous temperature has been shown to be an appropriate measure of core T_b in bats (Gorman et al., 1991). A loop antenna (Racket Antenna, Biomark, Boise, ID, USA) placed close to each chamber and attached to a PIT tag reader (Model FS2001F-ISO, Biomark) allowed us to record T_b continuously. Air temperature within each chamber was measured using a thermistor probe (Sable Systems, Las Vegas, NV, USA) inserted through a small hole in the lid and sealed with a rubber grommet.

A compressor supplied atmospheric air scrubbed of water vapour (dewpoint approximately –50°C) and CO_2 (<5 ppm) by an adsorption dryer (Ecodry K-MT 3, Parker Zander, Charlotte, NC, USA). A mass flow controller (Model FMA5520, Omega Engineering, Bridgeport, NJ, USA) supplied air to each chamber at constant flow rates of 1.1–1.5 l min^{-1}. We regularly calibrated the mass flow controller using a soap bubble flow meter (Baker and Pouchot, 1983). The 99% equilibrium times (Lasiewski et al., 1966) for our system were 6.4–8.8 min. Excurrent air was subsampled using an SS-3 Subsampler (Sable Systems), which pulled the subsampled air through a water vapour analyser (RH-300, Sable Systems), a CO_2 analyser (CA-10a, Sable Systems) and an O_2 analyser (FC-10B, Sable Systems). The water vapour and CO_2 analysers were regularly zeroed using nitrogen (Afrox, Johannesburg, South Africa), and spanned using the oxygen dilution method (Lighton, 2008) and a certified span gas with 2000 ppm CO_2 (Afrox). The O_2 analyser was spanned to 20.95% using atmospheric air scrubbed of water vapour and CO_2 using Drierite and magnesium perchlorate (Merck, Modderfontein, South Africa), respectively. Voltage outputs from the analysers and thermistors were acquired and digitised using an analog–digital convertor (UI-2, Sable Systems) and recorded in ExpeData software on a desktop PC. We measured BMR in two bats at a time, using a respirometry multiplexer (TRM8, Sable Systems) to sequentially subsample air successively from a baseline channel (10 min), followed by one chamber and then a second (20 min each), before an additional baseline reading (10 min).

Before measuring BMR, we determined the lower critical temperature (T_{lc}) to ensure that measurements took place at thermoneutrality. We measured resting metabolic rate (RMR) and T_b in six individuals at each T_a between 5 and 35°C in increments of 5°C. Bats experienced each T_a for at least 6 h, and the order of T_a exposure was randomised. We then fitted a two-segment linear regression model to RMR versus T_a data for each season, and identified the inflection point representing T_{lc}. BMR was measured at T_a=30°C during both seasons, as this fell within the zone of thermoneutrality. Each bat spent at least 6 h at this T_a. Bats were weighed

prior to and after measurements to obtain an average M_b that was used for metabolic rate calculations, and food was removed at least 8 h before metabolic measurements to ensure that bats were post-absorptive.

M_{sum}

We elicited M_{sum} by exposing bats to a cold environment in a helox (21% O_2, 79% He) atmosphere using a sliding cold exposure protocol (Swanson et al., 1996). A helox atmosphere allows M_{sum} to be reached at a much higher temperature than in air, as rates of heat loss are ~3-fold higher in helox, decreasing the risk of freeze injury (Rosenmann and Morrison, 1974). For measurements of M_{sum}, bats in 1.3 l chambers (Lock and Lock, Blacktown, NSW, Australia) were placed in a 40 l portable fridge/freezer (ARB, Kilsyth, VIC, Australia) modified by drilling holes through the lid for incurrent and excurrent tubing. The thermistors we used to measure T_a during BMR measurements do not function below ~5°C, thus we measured chamber temperature during M_{sum} measurements using a calibrated iButton (Maxim Integrated, San Jose, CA, USA) suspended 1 cm above the floor of the chamber. Atmospheric air was supplied to each chamber at a flow rate of 2.5 l min^{-1} for ~5 min after the bat was placed in the chamber. Thereafter, helox was supplied to the chamber at the same flow rate, controlled by a mass flow controller (Model FMA5520, Omega Engineering, Bridgeport, NJ, USA) calibrated as above, but with helox rather than air. The chamber temperature remained at ~0°C until approximately stable $\dot{V}_{O_2}$ was achieved (typically 5–15 min). Thereafter, baseline [O_2], [CO_2] and water vapour readings were obtained by pulling subsampled helox through the analysers, after which the sliding cold exposure protocol was initiated by setting the fridge/freezer's set point to its minimum (−18°C, resulting in a chamber cooling rate of ~10°C h^{-1}) and excurrent helox was subsampled using the same setup as for BMR. Measurements continued until $\dot{V}_{O_2}$ reached a plateau and no longer increased with decreasing T_a. We verified that M_{sum} had been achieved and the bat had become hypothermic by measuring T_b immediately upon removal from the chamber, using a handheld PIT tag scanner (DTR-4, Destron Fearing). After the removal of each bat, a second baseline reading was obtained by flowing helox through the analysers.

Data analysis

We estimated BMR and M_{sum} from traces of $\dot{V}_{O_2}$ by calculating the lowest and highest 5 min averages, respectively. We estimated $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ using equations 9.3, 9.4 and 9.5 from Lighton (Lighton, 2008). Values of RER were calculated as $\dot{V}_{CO_2}/\dot{V}_{O_2}$, and rates of gas exchange were converted to metabolic rates (W) using the thermal equivalence data in table 4.2 in Withers (Withers, 1992). During BMR measurements, RER averaged 0.837±0.091, indicating a mix of carbohydrate and lipid metabolism (Withers, 1992). During M_{sum} measurements, however, RER averaged 0.669±0.058, below the typical range of 0.71–1.00. As no published thermal equivalence data are available for values below 0.71, in instances where RER fell below the usual range we assumed RER=0.71 for estimating metabolic rates.

Assumptions concerning normality and homoscedascity were verified using Shapiro–Wilk tests and Levene's tests, respectively. The single male in the captive population precluded an analysis of sex effects among seasons and populations in a single model, and we analysed M_b in females using a linear mixed model with season and population as fixed effects and individual as a random effect. We also tested for seasonal changes and sex effects on M_b in wild bats using a similar model with season and sex as fixed effects. As M_b did not vary significantly across seasons in captive females or wild individuals of either sex, we pooled male and female data for further analyses. We tested for significant effects of M_b on BMR or M_{sum} within each season/population combination by fitting least-squares linear regression models. Because metabolic rates were significantly related to M_b in some season/population combinations but not others (see below), we tested for seasonal effects and differences between captive and wild bats using linear mixed models with either BMR or M_{sum} as the response variable, and season and population as fixed factors, individual as a random effect and M_b as a covariate. Normothermic T_b and ME were also analysed using linear mixed models, but without including M_b as a covariate. Denominator degrees of freedom for fixed effects were estimated as described elsewhere (Satterthwaite, 1946).

To compare M_{sum} in *E. wahlbergi* with that of other mammalian species, we used previously reported M_{sum} and M_b values (Hinds et al., 1993) and values for rodents collated by Careau (Careau, 2013) (see the electronic supplementary material of the latter for original sources). Both these studies reported M_{sum} as rates of oxygen consumption, which we converted to metabolic rates (W) assuming RER=0.71. We fitted a conventional least-squares linear regression to these data. As we used these data to merely illustrate the wide range of M_{sum} shown by bats, rather than testing a specific hypothesis regarding deviations from expected values, we did not calculate phylogenetically independent regressions or prediction intervals (Garland and Ives, 2000).

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Competing interests

The authors declare no competing financial interests.

Author contributions

A.E.M. and I.A.M. designed the study. I.A.M. collected and analysed data. I.A.M., A.E.M., N.C.B. and C.T.T. wrote the manuscript.

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