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

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Summary

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 to many other endotherm taxa. We tested the prediction that a non-heterothermic pteropodid fruit bat from the southern subtropics exhibits a relatively modest capacity for thermogenic heat production, by measuring M_{sum} and BMR during summer and winter in wild and captive populations of Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) in Pretoria, South Africa. The M_{sum} of the 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 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 compared to wild individuals; we speculate this represents a phenotypic response to reduced 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 to allometrically expected values for mammals.

Keywords: Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi*, metabolic rate, summit metabolism, helox, cold exposure, acclimatisation, phenotypic flexibility

Introduction

The lowest environmental temperature at 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}; (Swanson et al., 1996), also referred to as cold-induced peak metabolic rate (PMR_c), (Wiersma et al., 2007)] is the maximum resting metabolic rate, i.e., maximum metabolic thermogenesis in the absence of exercise-associated heat production. Among mammals, metabolic expansibility (ME, the ratio of M_{sum} to basal metabolic rate, BMR) 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 for mammals, with maximum reported values of 9-9.5 (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 taxon 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 ~21.4 times BMR (Canals et al., 2005). The very high ME value for *T. brasilensis* 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 may be made regarding resting heat production capacity in bats. The 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, leads 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 more modest than those for nocturnally inactive taxa [although the very short daily foraging periods of some bats (e.g., (Dechmann et al., 2011) raises the possibility that this might not always be the case]. On the other hand, however, resting heat production capacity in rodents is correlated with torpor use, with ME negatively correlated with both minimum air temperatures 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 heat production capacity 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 acclimatization in birds typically involves the up-regulation 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 BMR may either show no seasonal change (Almeida and Cruz-Neto, 2011; Coburn and Geiser, 1998) or winter increases (Downs et al., 2012). Seasonal acclimatization in mammalian M_{sum} has received far less attention; 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 tested the prediction that a non-heterothermic species has a relatively modest capacity for resting metabolic heat production above BMR compared to diurnal taxa, such as most birds. We also examined seasonal adjustments in BMR and M_{sum} in order to further investigate metabolic acclimatization in bats, and we 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/or the magnitude and direction of seasonal changes in these variables, differs between captive and wild, free-ranging bats.

Materials and Methods

Study animals

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). The captive population consisted of 10 adult *E. wahlbergi* [nine non-reproductive females, one male; mean ± SD body mass (M_b) = 84.1 ± 7.9 g at time of capture] that we captured using mist-nets (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 approximately 18 ºC and 5 ºC, respectively (South African Weather Service). Captive bats were housed in outdoor aviaries (each 5 m long x 2.5 m wide x 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 air temperature (T_a). The male bat was kept separately from the females. Bats were maintained on a diet of mixed fruit supplemented with vitamins and minerals (Barnard, 2009) and 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, and summer data between 9 and 18 December 2012. BMR and M_{sum} were measured in each individual within 24 hr of each other, with the order of measurements randomised. All measurements took place during the daytime. The captive fruit bats were used for a separate series of evaporative water loss measurements (not included here) between the winter and summer study periods.

Basal metabolic rate

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 & 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 the oil and to provide them 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 in 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) placed close to each chamber and attached to a PIT tag reader (Model FS2001F-ISO, Biomark, Boise, Idaho, USA) 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≈-50ºC) and CO_2 (< 5 ppm) 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 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, Johannesburg, South Africa). The O_2 analyser was spanned to 20.95 % using atmospheric air scrubbed of water vapour and CO_2 using Drierite and magnesium perchorate (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_as between 5 and 35 °C in increments of 5 °C. Bats experienced each T_a for at least six hours, and the order of T_a exposure was randomised. We then fitted a two-segment linear regression model to RMR vs 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, since this fell within the zone of thermoneutrality. Each bat spent at least 6 hr at this T_a. Bats were weighed before and after measurements to obtain an average body mass (M_b) that was used for metabolic rate calculations, and food was removed at least eight hours before metabolic measurements to ensure that bats were postabsorptive.

Summit metabolism

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, decreasing the risk of freeze injury, as rates of heat loss are ~3-fold higher in helox (Rosenmann and Morrison, 1974). For measurements of M_{sum} bats in 1.3-L chambers chambers (Lock & Lock, Blacktown, NSW, Australia) were placed in a 40-L portable fridge/freezer (ARB, Kilsyth, Victoria, 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 ºC, thus we measured chamber temperature during M_{sum} measurements using an 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 approximately 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, following which the sliding cold exposure protocol was initiated by setting the fridge/freezer's setpoint to its minimum (-18 °C, resulting in a chamber cooling rate of ~10 °C hr^{-1}) and excurrent helox was subsampled using the same setup as used 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, South St Paul, Minnesota, USA). 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. Respiratory exchange ratios (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, 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. Since 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 used 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 general linear model (GLM) 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. Since 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 GLMs 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 GLMs, but without including M_b as a covariate. Denominator degrees of freedom for fixed effects were estimated following Satterthwaite (1946).

To compare M_{sum} in *E. wahlbergi* to that of other mammalian species, we used the M_{sum} and M_b values reported by Hinds et al. (1993) and values for rodents collated by 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. Since 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).

Results

Body mass

Among female fruit bats, 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 *vs* captive; $F_{1,14.14}$ = 0.849, P = 0.372). Among wild bats, M_b did not vary significantly across seasons ($F_{1,15.15}$ = 1.148, P = 0.300). 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.

Basal metabolic rate

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 during neither season in captive bats (winter: $F_{1,9}$ = 3.518, P = 0.097; summer: $F_{1,9}$ = 0.264, P = 0.621; Figure 1). When analysed in a GLM with M_b as a covariate, BMR was significantly lower in winter compared to summer ($F_{1,16.87}$ = 11.906, P = 0.003) and was significantly higher in wild bats compared to 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, of 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 in wild compared to captive bats ($F_{1,22.10}$ = 7.932, P = 0.010; Table 1).

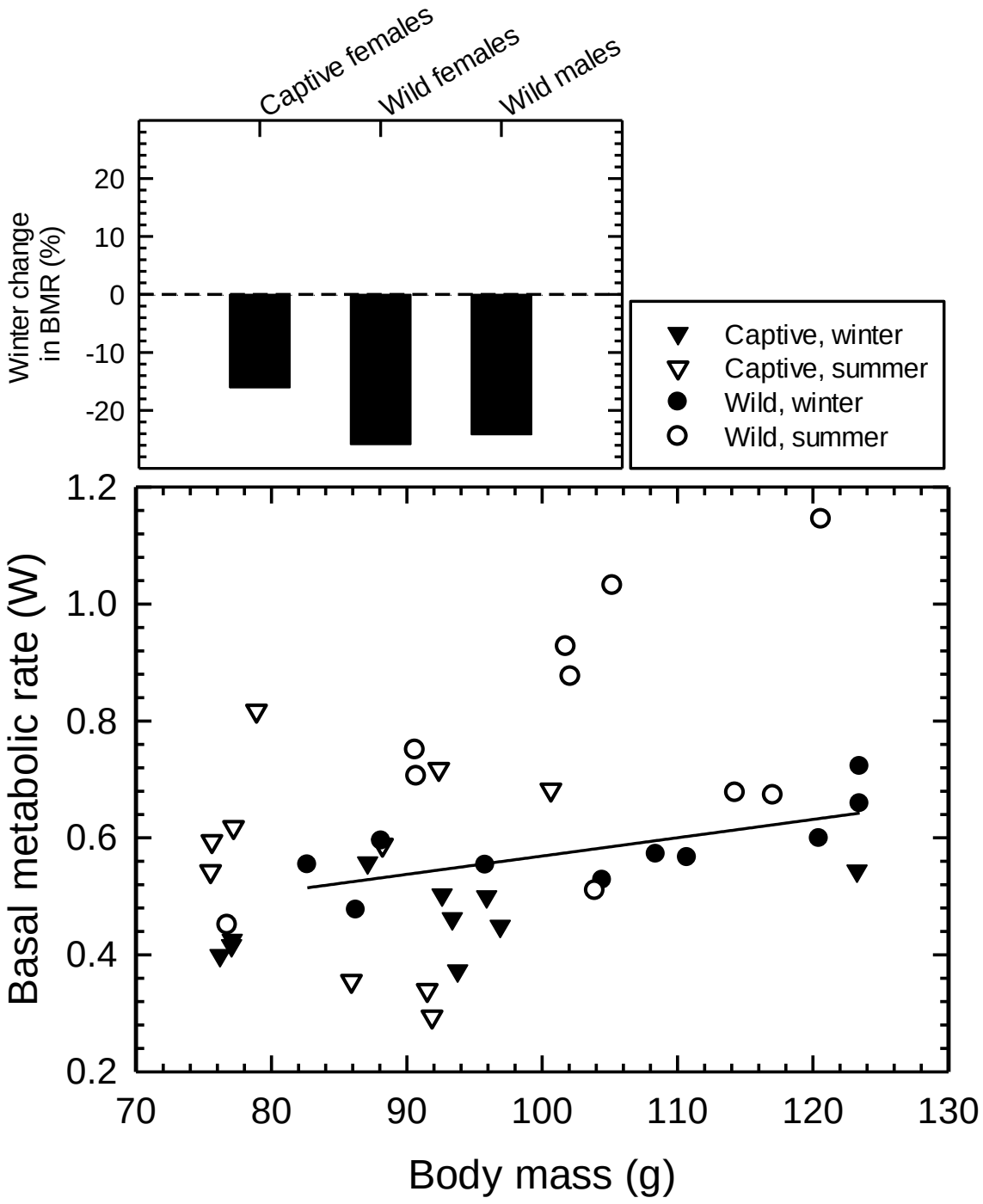

Figure 1. Basal metabolic rate (BMR) as a function of body mass (M_b) in wild and captive populations of Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) during summer and winter. The upper panel shows the mean percentage change in BMR during winter compared to summer for captive females, wild females and wild males (no value shown for single captive male). 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, $F_{1,9}$ = 8.244, P = 0.021).

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. In all instances, n =10.

Variable	Captive population		Wild population	
	Winter	**Summer**	**Winter**	**Summer**
T_b (ºC)	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} (ºC)	-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

Summit metabolism and metabolic expansibility

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; Figure 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 ($F_{1,23.12}$ = 8.408, P = 0.008). During winter, the M_{sum} of captive females (n = 9) was equivalent to 130.8 % of 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

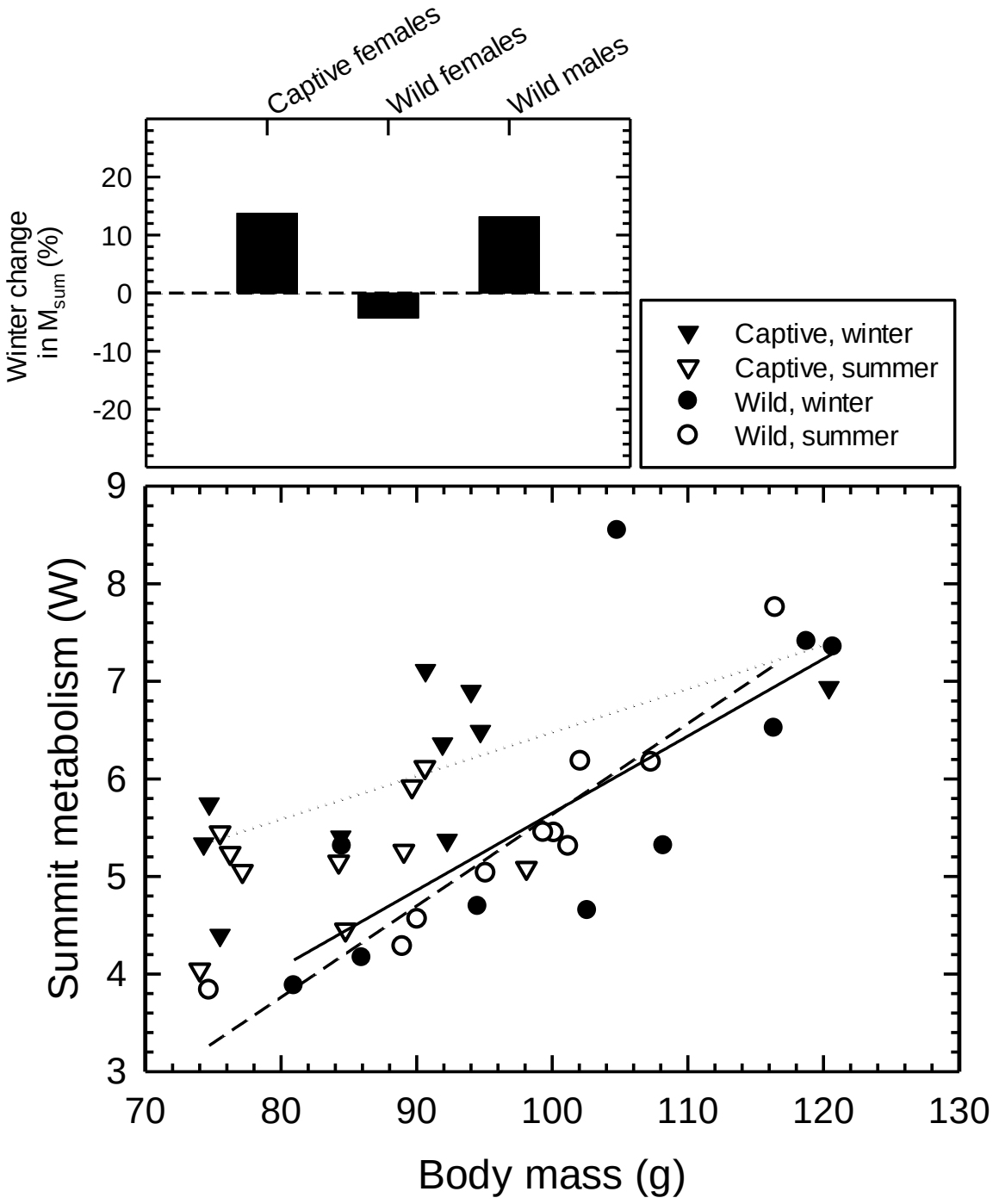

Figure 2. Summit metabolism (M_{sum}) as a function of body mass in wild and captive populations of Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) during summer and winter. The upper panel shows the mean percentage change in BMR during winter compared to summer for captive females, wild females and wild males (no value shown for single captive male). Linear regression models yielded significant fits as follows: wild, winter (solid line: M_{sum} = -2.255 + 0.079M_b; r^2 = 0.542, $F_{1,9}$ = 9.452, P = 0.015), wild, summer (dashed line: M_{sum} = -3.725 + 0.094M_b; r^2 = 0.894, $F_{1,9}$ = 67.572, P < 0.001), captive, winter (dotted line: M_{sum} = 2.0193 + 0.045M_b; r^2 = 0.472, $F_{1,9}$ = 7.164, P = 0.028).

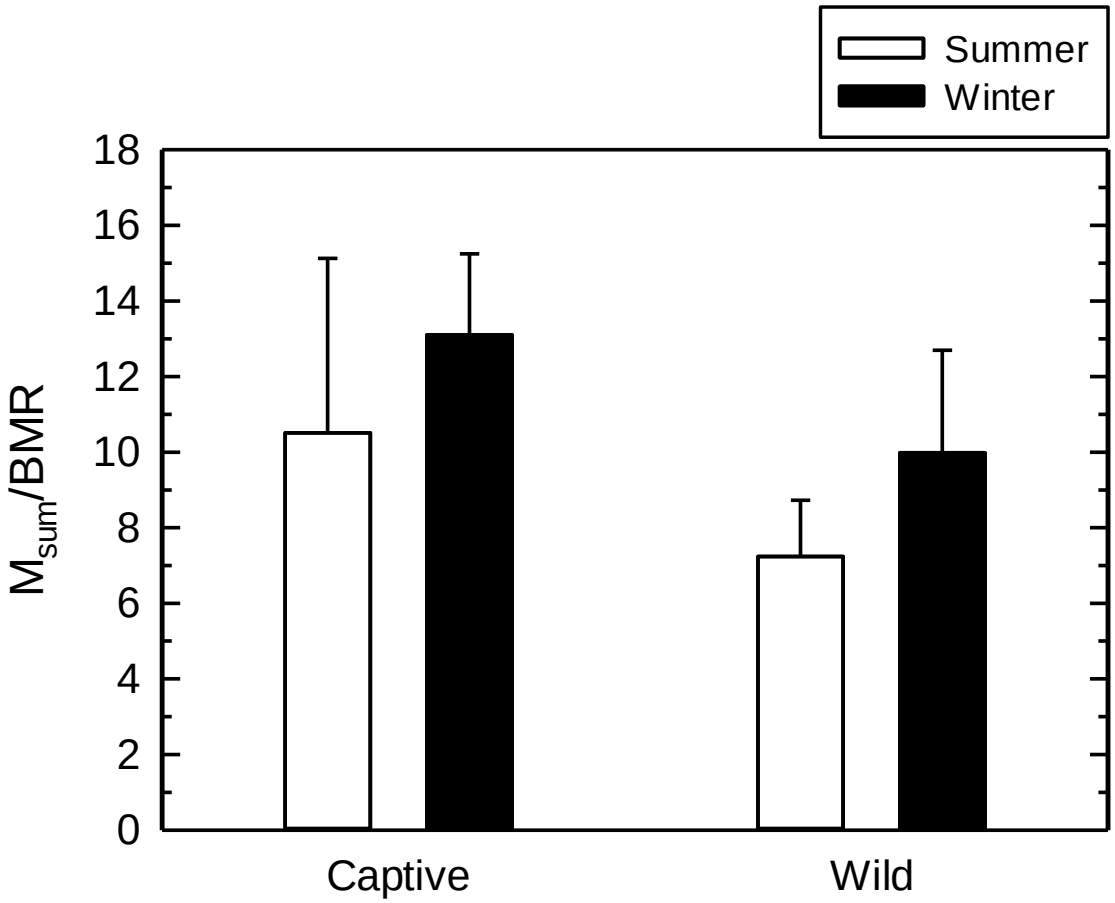

Figure 3. Mean metabolic expansibility [ratio of summit metabolism (M_{sum}) to basal metabolic rate (BMR)] in captive and wild populations of Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*). Error bars indicate standard deviations.

significantly higher in winter compared to summer, and higher in captive bats compared to wild bats (Figure 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 ($F_{1,14.99} = 2.889$, $P = 0.110$).

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 (i.e., M_{sum}/BMR) 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 RER being below the theoretically expected range of 0.71 – 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, since our RER values for BMR, 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). The latter 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 thus reiterate the need for further studies of the substrates 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. McKechnie, 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} (Figure 4). The M_{sum} measured in *Tadarida brasiliensis* by

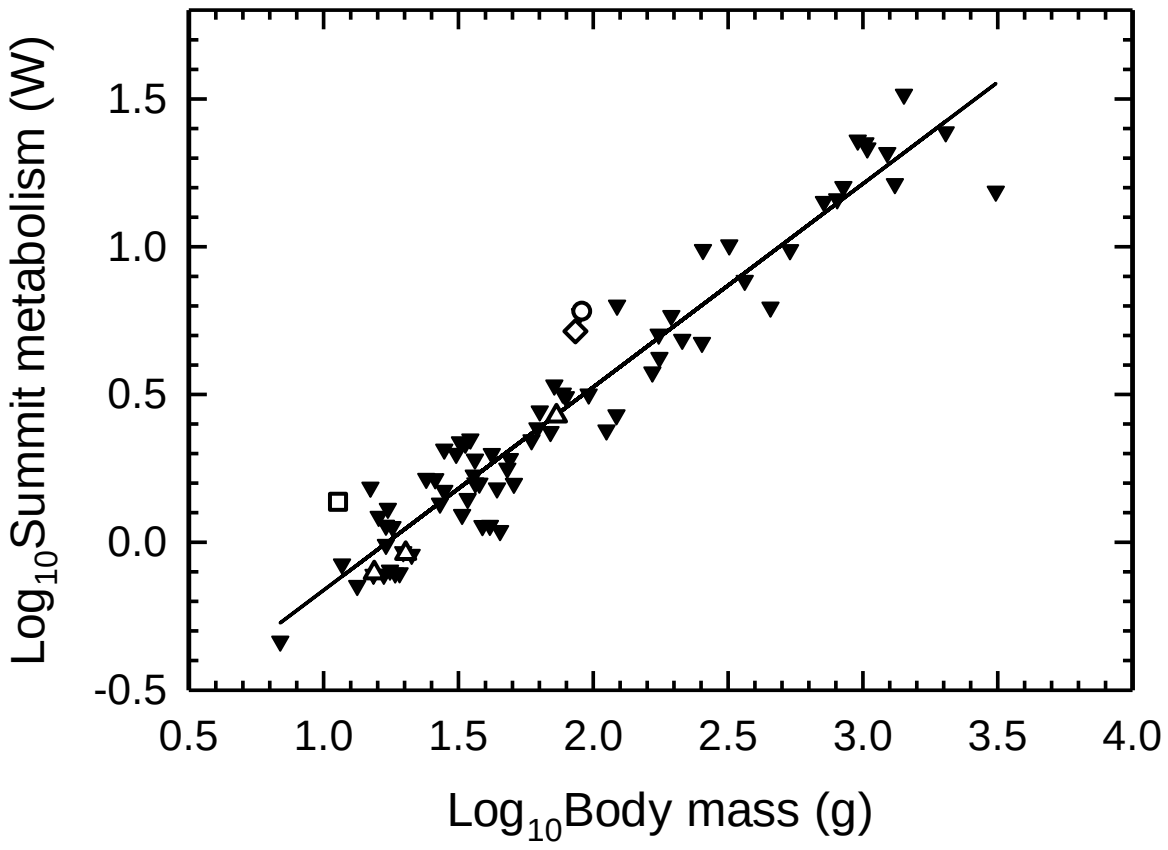

Figure 4. Summit metabolism in bats (clear symbols) compared with other mammals (filled downward-pointing triangles). Data (oxygen consumption) were obtained from (Hinds et al., 1993) and the meta-analysis for rodents by (Careau, 2013); see electronic supplementary material of latter paper for sources). Rates of oxygen consumption were converted to metabolic rates (Watts) assuming RER = 0.71, i.e., metabolism of lipids. Data for the following bat species 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].

Canals et al. (2005) is similarly high (183 % of expected; Figure 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 %; Figure 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 relatively 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 to species inhabiting cold, north-temperate climates.

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 (2011) similarly found no significant seasonal effect on M_{sum} in *Artibeus lituratus, Sturnira lilium* and *Carollia perspicillata*. Thus, the limited data available for bats are not consistent with the view that variation in M_{sum} among species/populations and within individuals is generally correlated with cold tolerance. M_{sum} is negatively correlated with minimum air temperature among rodents (Careau, 2013) and birds (Swanson and Garland, 2009). Moreover, avian winter enhancements in cold tolerance are typically associated with seasonal increases in M_{sum} (Swanson and Bozinovic, 2011; Swanson and Garland, 2009). Intraspecific variation in avian M_{sum} and seasonal adjustments of M_{sum} within individuals are also often negatively correlated with environmental temperatures (e.g., O'Connor, 1996; van de Ven et al., 2013b).

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 compared to their wild counterparts. The higher ME of captive bats reflected a combination of lower BMR and higher M_{sum} than wild individuals. One possible explanation for the higher M_{sum} concerns the reduced activity levels of bats when confined to an aviary compared to free-ranging conditions; we might expect that heat generated as a by-product of flight contributes far less to thermoregulation in captive individuals compared to the wild bats. Hence, we speculate that the increased capacity for resting thermogenesis we observed in 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 by 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 (see below). On the other hand, three species of frugivorous phyllostomids in southeastern Brazil had significantly lower M_b in winter compared to summer (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 (29º37'S 30º23'E). The latter population increased mass-specific BMR by ~22 % and whole-animal BMR by ~40 % during winter

compared to summer, seasonal changes in the opposite direction to those shown by bats in the present study. Moreover, the BMRs of the fruit bats held in captivity by Downs et al. (2012) were substantially higher than those of the captive population we investigated here; assuming the same RER value as the mean 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 (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. (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 found 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 the latter study differed by ~10 °C in winter minimum T_a, Pretoria and Pietermaritzburg are climatically similar, with average minimum and maximum temperatures across all months differing by at most 2 ºC between them, 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 phenotypically 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 includes 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 exploring the factors driving the evolution of maximum resting metabolic rate in endotherms.

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