Metabolic rate, latitude and thermal stability of roosts, but not phylogeny, affect rewarming rates of bats

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HIGHLIGHTS

- We examined ecological, behavioral and physiological drivers of rewarming rates of 45 bat species.
- After controlling for phylogeny, high basal metabolic rate was associated with rapid rewarming.
- Species that live at higher absolute latitudes, and in less thermally stable roosts, also rewarmed most rapidly.
- Results suggest some species rely on passive means to reduce costs of rewarming, but others rely on faster metabolism as an alternative.
- This reinforces the importance of local climate, physiology, and behaviour in the proliferation of heterothermic endotherms

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ABSTRACT

Torpor is an adaptation that allows many endotherms to save energy by abandoning the energetic cost of maintaining elevated body temperatures. Although torpor reduces energy consumption, the metabolic heat production required to arouse from torpor is energetically expensive and can impact the overall cost of torpor. The rate at which rewarming occurs can impact the cost of arousal, therefore, factors influencing rewarming rates of heterothermic endotherms could have influenced the evolution of rewarming rates and overall energetic costs of arousal from torpor. Bats are a useful taxon for studies of ecological and behavioral correlates of rewarming rate because of the widespread expression of heterothermy and ecological diversity across the >1200 known species. We used a comparative analysis of 45 bat species to test the hypothesis that ecological, behavioral, and physiological factors affect rewarming rates. We used basal metabolic rate (BMR) as an index of thermogenic capacity, and local climate (i.e., latitude of geographic range), roost stability and maximum colony size as ecological and behavioral predictors of rewarming rate. After controlling for phylogeny, high BMR was associated with rapid rewarming while species that live at higher absolute latitudes and in less thermally stable roosts also rewarmed most rapidly. These patterns suggests that some bat species rely on passive rewarming and social thermoregulation to reduce costs of rewarming, while others might rely on thermogenic capacity to maintain rapid rewarming rates in order to reduce energetic costs of arousal. Our results highlight species-specific traits associated with maintaining positive energy balance in a wide range of climates, while also providing insight into possible mechanisms underlying the evolution of heterothermy in endotherms.

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1. Introduction

Endothermic animals defend a constant, high body temperature (T_b) via endogenous, metabolic heat production, which can allow for sustained activity across a range of conditions. However, maintaining an elevated T_b is energetically expensive, especially during cold weather, and can become challenging during periods of low resource availability [1]. To offset these high energy demands, many endotherms employ facultative heterothermy or torpor [2]. Torpor is characterized by a controlled reduction of T_b, metabolic rate (MR), and other physiological functions, greatly reducing energy consumption [2]. Short bouts of torpor during certain parts of the day (i.e., daily torpor) and/or longer bouts at specific times of year (i.e., seasonal torpor or hibernation) allow endotherms to maintain positive energy balance across a range of environmental conditions and timescales, and can also result in other ecological benefits (reviewed by Geiser and Brigham [3]).

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Bouts of torpor can be separated into three phases: 1) the cooling phase, during which an individual decreases T_b and MR to a new, lower set-point, 2) the torpid phase, during which the individual defends a reduced T_b set-point and torpid metabolic rate (TMR, which can be less than 1% of resting MR) [4], and, 3) the warming or arousal phase, during which the individual actively terminates the torpor bout and rewarms to normothermic T_b. The arousal phase is considered one of the major disadvantages of heterothermy because rewarming from torpid to euthermic T_b is energetically expensive and requires extensive heat generation capacity [2,5]. High arousal costs negate potential energy savings of short torpor bouts, and therefore may represent an important factor in the cost-benefit tradeoff influencing torpor expression. Ultimately, these costs could represent a substantial portion of the long-term energy budget. Thus, mechanisms for mitigating costs of arousing from torpor are likely important drivers of the ecology, behavior, and life histories of heterothermic endotherms.

Somewhat counter-intuitively, rapid rewarming is less energetically demanding than rewarming slowly because the need to balance heat production with heat loss to the environment is less costly over shorter time intervals [6]. This predicts that selection should favor rapid rewarming or, alternatively, other energy-saving mechanisms such as social thermoregulation or passive rewarming to minimize arousal costs. If arousal from torpor depends on metabolic heat production, heterothermic species with greater thermogenic capacity (potentially higher basal and/or summit metabolic rate) should be capable of rewarming more rapidly [7]. However, the evolutionary relationship between MR and rewarming rate remains unclear. Geiser and Baudinette [7] found a nearly perfect correlation between BMR and rewarming rate across all mammalian taxa but few subsequent analyses exist and, those studies that do have found equivocal patterns within a single taxon or group [8,9].

The lack of a consistent relationship between MR and rewarming rate could also reflect the fact that rewarming in many species is largely passive [10,11,12] and does not rely solely on metabolic heat production. For species employing passive strategies, the energetic costs associated with rewarming may be heavily influenced by behavioral and environmental factors that allow individuals to take advantage of external heat sources for passive rewarming [6,11,13]. The availability of solar radiation and fluctuations in ambient temperature (T_a) are important determinants of the energetic cost of rewarming [10,11,12,13]. Many heterothermic endotherms have limited exposure to sunlight and/or variation in natural T_a in their burrows, nests, or roosts (e.g., underground burrows, caves, well-insulated trees), minimizing opportunities for passive rewarming. In addition, variation in the rate at which heterothermic endotherms rewarm from torpor might also reflect local behavioral and physiological adaptation to different climatic regimes. Many other energetic and physiological traits vary along latitudinal gradients [14–17], including BMR, capacity for non-shivering thermogenesis, heterothermy index (i.e., continuous metric of heterothermy [18]) and thermoregulatory scope (i.e., range of body temperatures exhibited by a species, mean T_b — minimum T_b [17]). Species residing at higher latitudes demonstrate greater thermogenic capacity (i.e., higher MR [15] and greater non-shivering thermogenesis [16]) to cope with unpredictable, occasionally extreme changes in environmental conditions. Temperate heterotherms might also exhibit faster rewarming rates to reduce costs of arousal when T_a is cold and access to passive heat sources is limited.

Social thermoregulation is another behavioral strategy that could influence rewarming rate. Some heterotherms huddle with conspecifics to reduce heat loss and/or increase T_a of their immediate microclimate [19,20]. Huddling is common in species that hibernate, roost, or nest in enclosed burrows where opportunities for passive heating are reduced. If groups of individuals can share thermoregulatory costs or reduce heat loss by huddling, this could reduce selection pressure favoring rapid warming. On the other hand, solitary species or those

in smaller groups may depend on endogenous thermogenic capacity and rapid rewarming to keep the cost of arousal low [6,21].

Bats are a useful model taxon for comparative studies of heterothermy and rewarming. They are among the most ecologically diverse vertebrates and many species from both temperate and tropical regions employ daily and/or seasonal torpor, with some temperate species dependent on months of hibernation for over-winter survival [22]. Different bat species roost in a range of habitats, from caves and mines with high thermal stability, to tree hollows with moderate thermal stability to shedding bark or exposed foliage with high thermal variability. In addition, there is enormous variation in the potential for social thermoregulation among bats with some species roosting in large colonies of up to hundreds of thousands or millions of individuals and others roosting solitarily for most or all of their annual cycle [23]. This variation in ecology and behavior provides an excellent opportunity to test hypotheses about the evolution of rewarming rates in heterothermic endotherms.

We used comparative analyses to assess how physiological, behavioral and environmental variation affect rewarming rates of bats. First, we tested the hypothesis that metabolic rate is associated with rewarming rate in bats and predicted that species with higher BMR would exhibit faster maximum rewarming rates. Second, we tested the hypothesis that latitudinal variation in climate affects the evolution of rewarming rates. We predicted that bat species living at higher, temperate latitudes might be capable of faster maximum rewarming rates compared to low latitude species because of selective pressure imposed by colder, less predictable environmental conditions. Finally, we tested the hypothesis that roosting behavior affects the evolution of rewarming rates and predicted that species living in smaller groups and in less thermally stable roosts would exhibit the highest rewarming rates because these species have limited opportunities for passive rewarming and, therefore, should have faced selection pressure favoring rapid rewarming.

2. Methods

2.1. Field measurements of rewarming rate

We collected data for silver-haired (*Lasionycteris noctivagans*) and northern long-eared bats (*Myotis septentrionalis*) at the Sandilands Forest Discovery Centre in Manitoba, Canada (49.67°N, 95.90°W), from July 27th to August 1st, 2012 and 2014. Bats were captured using mist nets (12 m by 6 m), held in cloth bags and transported less than 1 km on foot to a nearby field laboratory. Here, bats were kept in their holding bags in a quiet room with screen-windows, under natural photoperiod (16 h light, 8 h dark) and natural T_a (minimum 11 °C–maximum 33 °C) for up to 24 h. At least two times a day, bats were provided with water, using a disposable pipette but were not fed because captivity was so brief.

As part of a concurrent respirometry study, every night up to four bats were placed in 100 mL transparent, acrylic chambers within a temperature-controlled cabinet set at 15 °C (i.e., well below the lower critical temperature) to encourage bats to enter torpor. The following day, between 13:00 and 22:00 individuals were removed from their chambers one at a time, and T_b was measured immediately (T_{b1}), by inserting a lubricated, 1 mm diameter thermocouple approximately 3 mm into the rectum until the reading was stable. After the first measurement, the bat was placed in a handling bag for approximately 5 min to minimize handling stress, after which a second T_b measurement was recorded (T_{b2}). Rewarming rate was calculated by subtracting T_{b1} from T_{b2} and dividing by the time interval between the two T_b measurements to give a rate in °C/min. Following T_b measurements, bats were weighed, provided water with a disposable pipette and released at the site of capture at dusk. These procedures and fieldwork were conducted under a Manitoba Conservation Wildlife Scientific Permit and were approved by the University of Winnipeg Animal Care Committee.

We used unpublished temperature radio-telemetry data for hoary bats (*Lasiurus cinereus*) and Eastern red bats (*Lasiurus borealis*) collected as part of a field study by McGuire et al. [24]. These data were not reported by McGuire et al. [24], but were collected at the same time, and using identical methods as their data for silver-haired bats. Briefly, bats were captured at Long Point, Ontario, Canada using mist nets. A small patch of fur was trimmed in the interscapular region and a temperature-sensitive radiotransmitter (± 0.1 °C, 0.38 g, <4% of body mass; Pip3, Lotek Wireless, Newmarket, Ontario, Canada) was affixed with ostomy bonding cement (Torbot; Cranston, RI, USA). Each bat was immediately released and later tracked to the day roost, where a datalogging receiver (SRX400; Lotek Wireless) recorded T_{sk} every ~30 s throughout the day. We extracted arousal rate from telemetry data, using T_{sk} timecourses to visually identify periods of arousal from torpor. We then calculated maximum rewarming rate from the steepest portion of the T_{sk} trace.

2.2. Rewarming rate database

We reviewed the literature and tabulated rewarming rates for 41 bat species from previously published studies and combined this pre-existing data with our own field measurements to create a database of 45 species (Table 1). When possible, we restricted our analysis to warming rates based on T_b instead of T_{sk}. However, a number of studies of bats have used external temperature-sensitive transmitters to record skin temperature (T_{sk}) in the lab and field. Acknowledging there can be differences between absolute values of T_b and T_{sk} [see [24–26]], we argue that T_{sk} is an adequate proxy for T_b in the context of rewarming; Willis and Brigham [26] showed that, when bats are rewarming at their maximum rate, the slopes of the lines are virtually indistinguishable, especially at warmer temperatures (i.e., our temperature range of 18–22 °C). We performed a calibration on seven silver-haired bats and found a tight correlation between rewarming rates calculated using T_{sk} vs. T_b ($R^2 = 0.86$, $F_{1,6} = 36.3$, $p < 0.001$). We used the maximum reported rewarming rate for each species or extracted the maximum rewarming rate from T_{sk} traces as described above [9]. Since both rewarming rates and differences between T_{sk} and T_b are affected by T_a [7,24,25,26], we only included data for bats recorded arousing from torpor when T_a was between 18 and 22 °C.

BMR values were available from the literature for 23 of the 45 species in our analysis (see Table 1). Body mass accounted for over 86% of

Table 1
Data used in our analysis including latitude representative of the geographic range, maximum colony size, stability of roost temperature on a three-point scale (i.e., roost score, see Methods section), body mass, basal metabolic rate (BMR) and rewarming rate for 45 bat species. Latitude was calculated based on centroid coordinate values (i.e., latitude and longitude) from geographic data obtained for each species' distribution. Colony size is the maximum colony size reported in the literature. Roost score represents the temperature stability of roosts on a three-point scale ranging from 0 (highly stable roosts; e.g., caves) to 2 (highly unstable roosts; e.g., foliage). Body mass values were taken from the original sources for the rewarming rate data.

Species	Latitude (°)	Colony Size	Roost Score	Mass (g)	BMR (mL O2/h)	Source for BMR	Rewarming Rate (°C/min)	Source for Rewarming Rate
Antrozous pallidus	33.69	139	2	28.0	21.20	[48]	1.00	[63]
Asellia tridens	22.70	5000	0	15.0	–	–	0.42	[64]
Chaerephon pumila	−0.29	300	2	18.0	–	–	1.05	[9]
Chalinolobus gouldii	−26.01	40	2	12.0	25.20	[49]	1.40	[64]
Chalinolobus picatus	−28.40	15	0	6.0	–	–	1.40	[64]
Corynorhinus rafinesquii	33.90	118	1	10.5	–	–	0.83	[9]
Corynorhinus townsendii	37.65	300	0	10.5	–	–	1.52	[63]
Eptesicus fuscus	38.50	700	2	16.0	17.00	[50]	1.50	[50]
Eptesicus serotinus	41.33	20	2	25.0	43.10	–	1.30	[65]
Glossophaga soricina	5.20	1000	1	9.5	21.60	[51]	0.35	[66]
Hipposideros armiger	22.45	1000	0	53.0	32.86	[52]	0.51	[52]
Hipposideros speoris	16.50	1200	1	9.0	–	–	0.35	[64]
Lasionycteris noctivagans	43.80	50	2	10.5	–	–	2.70	This study
Lasiurus borealis	38.51	1	2	13.0	–	–	1.78	This study
Lasiurus cinereus	27.79	1	2	25.0	–	–	2.14	This study
Macroglossus minimus	−1.67	1	2	16.0	21.00	[53]	0.31	[53]
Miniopterus schreibersii	39.61	10,000	0	15.0	25.42	[27]	1.40	[64]
Mops condylurus	−25.02	700	2	36.0	31.09	[54]	0.80	[64]
Mormopterus loriae	−16.10	300	2	8.5	–	–	0.90	[64]
Mormopterus planiceps	−30.00	150	2	9.5	–	–	1.40	[64]
Myotis adversus	4.12	300	2	8.0	–	–	1.20	[64]
Myotis californicus	35.96	52	2	5.0	–	–	1.29	[63]
Myotis evotis	43.85	30	2	7.3	–	–	0.67	[67]
Myotis lucifugus	49.73	3000	1	7.6	9.30	[55]	0.80	[68]
Myotis myotis	44.92	1000	1	25.0	25.00	[56]	1.50	[64]
Myotis nattereri	48.08	200	2	8.0	–	–	1.05	[64]
Myotis septentrionalis	47.15	60	1	8.2	–	[27]	1.99	This study
Myotis thysanodes	34.86	300	1	6.5	17.40	[57]	0.91	[63]
Nyctalus noctula	47.63	234	2	27.0	39.69	[27]	1.58	[64]
Nyctimene albiventer	−5.15	1	2	28.0	26.30	[58]	0.60	[69]
Nyctophilus bifax	−18.00	7	2	10.1	13.10	[59]	0.75	[70]
Nyctophilus geoffroyi	−26.20	20	2	7.8	10.99	[60]	1.34	[71]
Nyctophilus gouldii	−30.40	20	2	8.8	10.39	[61]	0.61	[72]
Pipistrellus hesperus	31.80	12	2	4.0	–	–	1.20	[73]
Pipistrellus pipistrellus	42.22	300	1	6.0	12.10	[27]	0.95	[64]
Plecotus auritus	51.09	30	1	12.0	11.20	[62]	0.70	[64]
Rhinolophus ferrumequinum	36.86	300	0	18.0	46.50	[27]	0.75	[64]
Rhinolophus hipposideros	40.39	100	1	6.0	–	–	1.05	[64]
Rhinolophus megaphyllus	−22.10	2000	0	8.0	25.84	[9]	0.84	[64]
Rhinopoma hardwickei	21.80	5000	0	10.0	–	–	0.50	[64]
Rhinopoma microphyllum	20.23	5000	0	19.0	–	–	0.30	[64]
Tadarida brasiliensis	2.83	200,000	0	12.0	15.30	[48]	0.73	[63]
Tadarida teniotis	38.03	100	2	30.0	31.52	[27]	0.78	[64]
Taphozous australis	17.30	100	0	22.0	–	–	0.57	[64]
Taphozous melanopogon	13.92	4000	0	26.0	–	–	0.15	[64]

the variation in BMR for these species ($F_{1,21}$ = 21.7, p = 0.0001, r^2 = 0.86). Therefore, to account for effects of body mass on BMR we used the residuals of this linear regression between body mass and BMR as a predictor variable in subsequent analyses (hereafter, mass-corrected BMR).

We assessed ecological and behavioral predictors of rewarming rate using three metrics: latitude, maximum colony size, and roost stability. We identified maximum colony size and roost preferences from the literature [9,23] or from the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (version 2015.2). Due to a lack of direct measurements of roost temperature in the literature, we classified species according to roost stability on a three-point, categorical scale [9]: 0 representing highly stable microclimates (e.g., caves and mines), 1 representing less thermally stable roosts (e.g., tree hollows and buildings) or species that roost in caves for part of their annual cycle but also switch to trees or other structures, and 2 representing thermally unstable roosts characterized by large fluctuations in T_a (e.g., foliage, shedding bark). We obtained geographic data through IUCN Red List of Threatened Species in the form of two-dimensional shapefiles (i.e., geospatial vector data of points on a map). We calculated the centroid coordinate (latitude, longitude) for each species-specific shapefile, and then calculated the weighted latitude (i.e., larger shapefile vectors were weighted more heavily) for all species in our dataset using the 'maptools' package (version 0.8-29, [28]) in R (v3.1.1; [29]). An absolute value of latitude for each species was taken to provide a measure of distance from the equator without differentiating between northern and southern hemispheres. Body mass values for each species were obtained from the original sources of rewarming rate data and we used this variable as a predictor in subsequent analyses.

2.3. Analyses

All analyses were conducted in R [29]. Prior to analysis, we tested for normality and equality of variance for all variables and log10 transformed variables when appropriate. Significance was assessed at α = 0.05.

We used two analytical approaches to account for common ancestry. We first analyzed our dataset using a Phylogenetic Generalized Least-Squares model (PGLS; [30,31]) in R, using the 'ape' (v. 3.1.2; [32]) and 'caper' (v. 3.1.2; [33]) packages. We obtained data on the phylogenetic distances between bat species from the phylogenetic tree provided by Agnarsson et al. [34]. In PGLS models the value of the phylogenetic signal (λ) was estimated using maximum likelihood to optimally adjust the degree of phylogenetic correlation in the data. PGLS models allow for flexibility of λ, which can range from 0 to 1, where 0 indicates no phylogenetic signal and 1 represents a very strong phylogenetic signal [35]. Values of 1 are similar to phylogenetic independent contrasts (PIC; [36]) and assume a 'Brownian motion' model where trait data are fully explained by phylogeny. Values of 0 are similar to ordinary least squares models (e.g., linear regression) and assume no phylogenetic signal. Intermediate values of λ, calculated using PGLS models, indicate that phylogeny is corrected for but do not follow a full 'Brownian motion' evolutionary model [37]. Therefore, the use of PGLS models and maximum-likelihood estimation of λ allowed us to optimize the phylogenetic correction for our dataset [37].

Initial PGLS analyses generated low λ values (0.00 to 0.40), indicating that phylogeny was not a strong predictor of rewarming rate [38]. Therefore, we conducted a second analysis using a series of non-phylogenetically-corrected general additive models (GAMs), to account for non-linear relationships between rewarming rate and colony size that we observed in our preliminary analysis. We included colony size (log_{10} transformed) as a non-linear term in our models and absolute latitude, roost preference score and mass-corrected BMR residuals (log_{10} transformed; due to negative values we added a constant to every residual value before transforming) as linear terms. We evaluated the relative support for each analysis using the Akaike Information Criteria

corrected for small sample sizes (AIC_c). The model with the lowest AIC_c value was considered to be most appropriate for our data [39].

Since metabolic data were only available for a subset of the species, we conducted two separate sets of analyses: a global analysis with all 45 species, excluding BMR, as well as a partial analysis, including only the 23 species for which BMR data were available. The partial PGLS model included mass-corrected BMR, absolute latitude, colony size, and roost score as predictors of rewarming rate. The global analyses included body mass, absolute latitude, colony size, and roost score as predictors of rewarming rate. For each series of models we removed variables using model decomposition based on AIC_c values. All models within $\Delta AIC_c \leq 2$ are reported, as they were considered to have substantial support [39]. In total, we compared results from 30 candidate models (15 for PGLS, 15 for GAM). We quantified AIC_c weights (w_i), which sum to one across all models and represent the probability that each model provides the best fit compared to all other candidate models. From these AIC_c weights, we also calculated the variable weights (vw) of each predictor variable included in our models (i.e., mass-corrected BMR, mass, latitude, colony size, roost score) to assess the relative importance of each predictor (i.e., the predictor with the largest vw is estimated to be the most important) [39].

3. Results

We found maximum rewarming rates for 41 bat species from eight families in the literature and added new data for four species based on our own measurements (Fig. 1). Mean maximum rewarming rate was 1.02 ± 0.53 °C/min but was highly variable, ranging from 0.15 to 2.7 °C/min (Table 1). The most parsimonious global PGLS model included only latitude (Table 2), while other top models included latitude, roost score and mass, explaining between 8% and 32% of variation in rewarming rate. The most parsimonious partial PGLS model included all variables, explaining 66% of the variation in rewarming rate, while the other top model included BMR and latitude, explaining 47% of the variation in rewarming rate (Table 2).

In our full models, latitude appeared in all of the top PGLS models (vw = 0.88) and the top GAM model (vw = 0.99) (Table 2). Roost score was moderately important as a predictor variable as it appeared in almost all PGLS (vw = 0.44) and GAM models (vw = 0.79) (Table 2). Colony size did not appear in any of the top global models, indicating it was not a strong predictor of rewarming rate. Mass appeared in three of the reported models (vw = 0.36 for PGLS, vw = 0.30 for GAM) (Table 2).

In our partial PGLS models, BMR and latitude appeared in both of the top models (vw = 0.55, 0.71 respectively) and were positively correlated with rewarming rate (Figs. 2, 3). Colony size (vw = 0.53) and roost score (vw = 0.53) were moderately important predictors, appearing in the top model (Table 2).

4. Discussion

Our results suggest that physiology, behavior and climate have influenced the evolution of rewarming rates in bats. In contrast to an earlier, smaller analysis on bats [9], but consistent with trends across mammals in general [7], species with high BMR (i.e., potentially greater capacity for metabolic heat production) had relatively high rewarming rates (as seen in [7]). Species at higher latitudes (i.e., in temperate regions) also tended to exhibit higher rewarming rates, possibly as a result of climate-mediated selection. Species roosting in thermally unstable roost sites had higher rewarming rates than those roosting in stable microclimates. Finally, we observed a weak correlation between colony size and rewarming rate, with species roosting in smaller colonies exhibiting higher rewarming rates, suggesting a potential co-evolutionary relationship between social behavior and thermoregulatory energetics of torpor. Taken together these results suggest that, for heterothermic endotherms, the ability to maintain positive energy balance has been
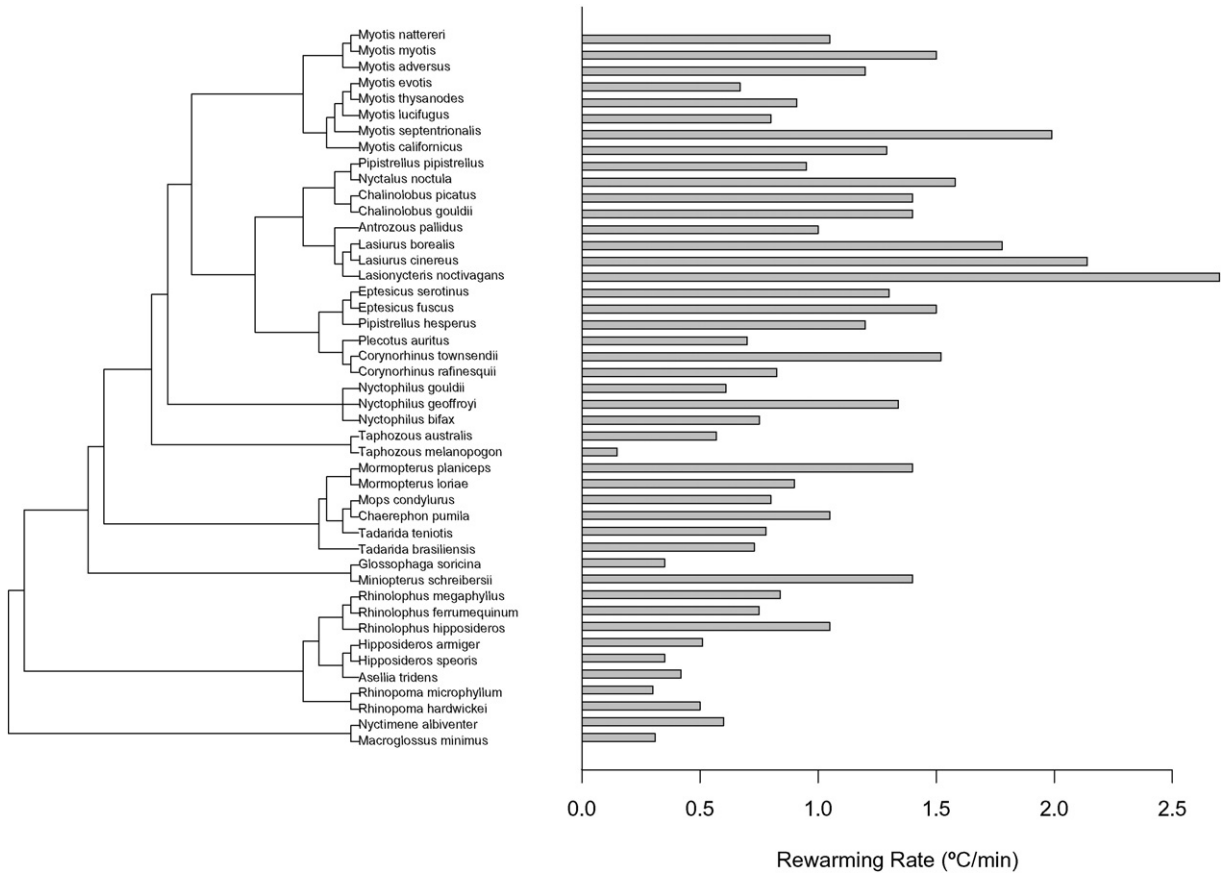
Fig. 1. Phylogenetic tree (left; adapted from [34]) used in the Phylogenetic Least Squares (PGLS) analysis alongside the distribution of rewarming rate values (right) for the 45 bat species used in our analyses.

shaped by thermogenic capacity, adaptation to local climate and various behavioral mechanisms.

Consistent with our first hypothesis, we identified a correlation between rewarming rate and BMR for 23 species, suggesting that heat production during rewarming from torpor is related to metabolic rate [7,8] (Fig. 2). Although BMR is a metric of "maintenance" costs and not necessarily representative of thermogenic capacity, rewarming from torpor requires intense metabolic output and BMR does correlate with overall metabolic performance [7,40]. Rewarming rates could also be strongly influenced by other metrics of metabolism (e.g., summit metabolism)

Table 2

Model selection parameters (AICc = Akaike's Information Criterion corrected for small sample size; w_i = Aikaike weight) of (A) global and (B) partial Phylogenetic Least Squares models (PGLS) and (C) global General Additive Models (GAM),testing the effects of body mass, absolute latitude, colony size, roost stability and mass-corrected basal metabolic rate (BMR) on rewarming rate. Models listed are those considered the most parsimonious (ΔAICc values <2). In PGLS models, λ is an indicator of the phylogenetic correlation in our data, and can range from 0 (no phylogenetic signal) to 1 (very strong phylogenetic signal).

(A) Global PGLS Models	λ	R^2	AICc	ΔAICc	w_i
Latitude	0.40	0.08	56.1	0.0	0.32
Latitude + Roost Score	0.00	0.31	57.1	1.0	0.20
Latitude + Roost Score + Mass	0.00	0.32	58.1	2.0	0.12
Latitude + Mass	0.39	0.07	58.1	2.0	0.12
(B) Partial PGLS Models					
BMR + Latitude + Roost Score + Colony	0.00	0.66	10.94	0.0	0.34
BMR + Latitude	0.00	0.47	11.27	0.3	0.29
(C) Global GAM Models					
Latitude + Roost Score	–	0.31	63.3	0.0	0.45
Latitude + Roost Score + Mass	–	0.30	64.9	1.6	0.20

or thermoregulatory parameters (e.g., thermal conductance, which is also strongly correlated to BMR [41]). However, due to a lack of published data on these parameters for bats we were unable to include them in our analysis. As more data become available, incorporating thermal conductance and other measures of thermogenic capacity (e.g., summit metabolism) could be useful for further resolving the relationship between heat generation capacity and rewarming.

Consistent with our second hypothesis, we identified a positive relationship between latitude and rewarming rate (Fig. 3). Our results suggest that variation in rewarming rates may reflect an adaptation to regional climate, highlighting the importance of seasonality and temperature as selective forces on both thermogenesis and behavior in small mammals [14–17]. A handful of physiological traits associated with thermogenesis are known to vary along latitudinal gradients. For example, species that evolved in cooler, more seasonal climates (i.e., higher absolute latitude) exhibit higher MR (both BMR and summit MR; [15,42,43]) and greater capacity for non-shivering thermogenesis [16]. To our knowledge, ours is the first study to identify a similar latitudinal gradient for maximum rewarming rate in endotherms. To cope with unpredictable climate and extreme variation in T_a associated with higher latitudes, individuals of temperate species should benefit from the capacity to rewarm rapidly. In contrast, maximum rewarming rates of tropical species were relatively low (Table 1, Fig. 3) even though all the tropical species in our analysis would regularly experience temperatures well below thermoneutrality and all readily express torpor. Despite their use of torpor, however, lower rewarming rates for tropical species likely reflect a relatively low thermoregulatory scope (i.e., lower range of T_b variation; [17]) compared to temperate species. The costs of slower rewarming in the tropics may be lower because of a smaller T_b differential between torpor and normothermia. It is clear that latitude is an important predictor of various physiological parameters [14,15,
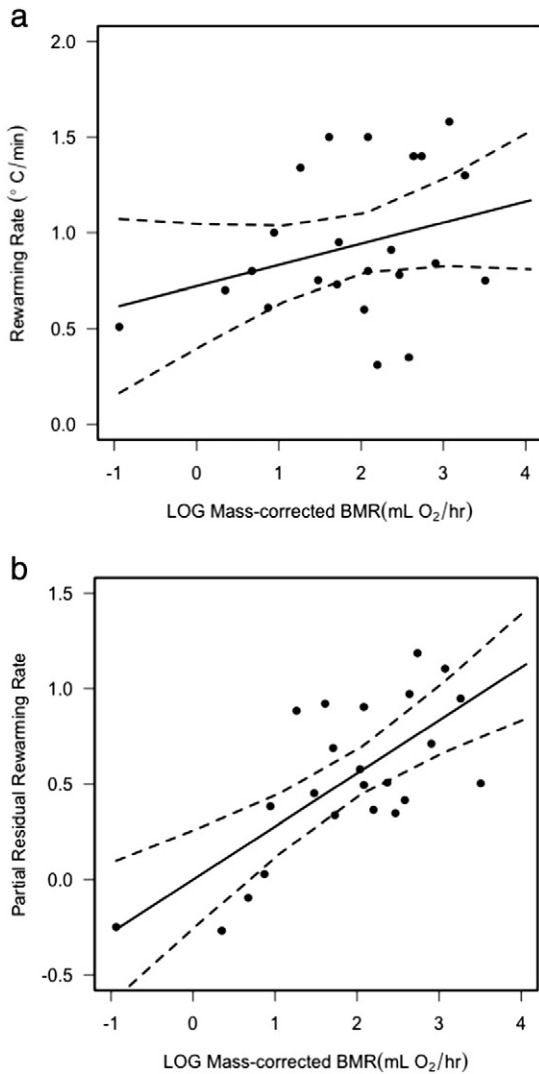
Fig. 3. Positive correlation between absolute latitude and rewarming rates (°C/min) for 45 bat species. Absolute latitude values for each species were taken to provide a measure of distance from the equator without differentiating between the two hemispheres. Dashed lines represent 95% confidence interval around the regression line.

Fig. 2. (a) Positive relationship between mass-corrected BMR residuals (mL O_2/h; log_{10} transformed) and rewarming rate (°C/min) for 23 bat species. (b) Partial residual plot demonstrating the effect of mass-corrected BMR (mL O_2/h; log_{10} transformed) on partial residual rewarming rate, taking the effect of absolute latitude into account. Dashed lines represent 95% confidence interval around the regression line

microclimate preferences of bats would benefit from more precise T_a measurements for a broad range of roost types and better data on spatial and temporal variation in roost temperature.

Contrary to our behavioral predictions, colony size was not an important predictor of rewarming rate. It is known that huddling with a large number of conspecifics can reduce heat loss to the environment, increase local T_a, and facilitate passive rewarming for individuals via heat generated by huddle-mates, thereby minimizing the energetic cost of arousal [44,45]. One explanation for only a weak effect of colony-size in our analysis is that thermoregulatory capacity, local climate and thermal stability of roosts outweigh the effects of social thermoregulation. Perhaps only bat species that reside in temperate, thermally unstable roost sites or that lack sufficient thermogenic capacity are obligated to overcome thermoregulatory demands by clustering with

17] and the relationship between rewarming rate and latitude we observed suggests another example of adaptation to local climate and the importance of temperature as a selective force on energy expenditure and behavior of small mammals.

Consistent with our third, behavioral hypothesis, roost preference was correlated with rewarming rate (Table 2, Fig. 4). Although we predicted less thermally stable roost sites might allow for greater passive rewarming opportunities, we found that species roosting in less stable environments have slightly higher rewarming rates than those roosting in the most thermally stable environments. Well insulated roost sites that maintain relatively stable microclimates could be better suited to social thermoregulation if they retain more heat generated by the bats themselves. In some circumstances, this could provide greater energy savings than the passive rewarming opportunities provided by fluctuating T_a in less thermally stable roost sites. However, this result should be treated cautiously because the need to treat roost stability as a categorical variable in our analysis likely reduced our ability to quantify its relationship to rewarming rate. For example, bats could also exploit spatial variation in microclimate within the roost structure to maximize rewarming opportunities but our analysis would not be able to detect this pattern. We suggest that future analyses of roost and/or
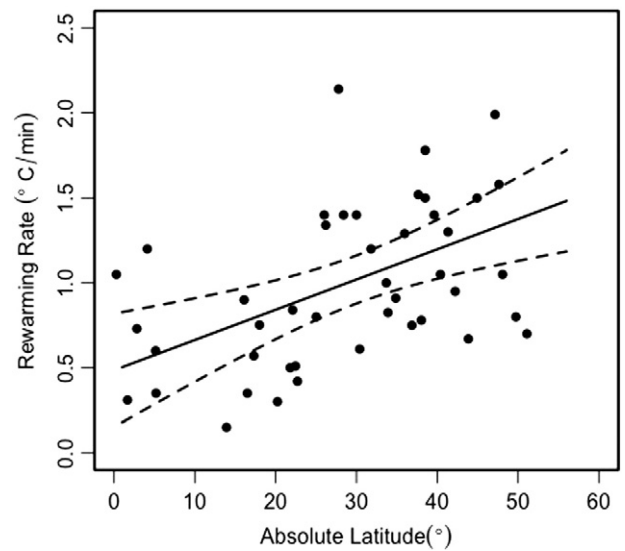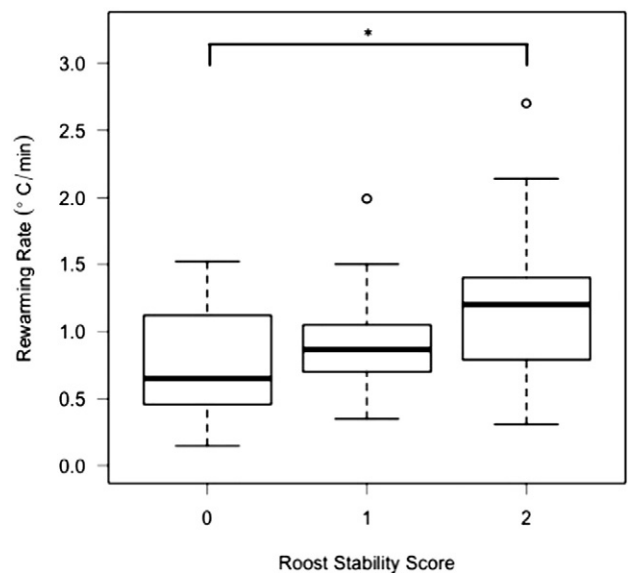

Fig. 4. Relationship between roost stability (i.e., roost score) and rewarming rate (°C/min) for 45 bat species. Roost score represents the temperature stability of roosts on a three-point scale ranging from 0 (highly stable roosts; e.g., caves) to 2 (highly unstable roosts; e.g., foliage). The * represents a significant difference (p < 0.05) between roost stability scores.

other individuals. We were restricted to the use of maximum colony size, rather than the average, because estimating mean colony size would require an accurate estimate of a large sample of different colony sizes of a given species. For many species this would be next to impossible and, therefore, so few species accounts report an "average" colony size and, instead, report minimum or maximum colony sizes. It is also possible that a measurement of maximum colony size is not ideal for capturing the thermoregulatory consequences of group size. This measure may not describe the most common colony size of a species, but may represent a "best-case scenario".

Past studies of rewarming rate have either not controlled for phylogeny [7] or have corrected for phylogeny using independent contrasts which assume a significant impact of phylogeny on the trait of interest [8,9]. Ours is the first analysis to quantify the effect of phylogeny on rates of arousal from torpor across a range of bat species and our data suggest the effect of phylogeny is relatively small (Table 2; λ ranging from 0 to 0.40 [35]). However, intermediate values of λ, such as those reported here, are difficult to interpret because the "strength" of λ is context-dependent and can be influenced by both the scale of the dataset and/or convergent evolution [38,46]. Therefore, although we found values of λ approaching 0, we cautiously interpret phylogeny as having at least some limited effects on rewarming rates in bats (Table 2), a result which is qualitatively supported through visual inspection of Fig. 1 (i.e. rewarming rates range within clades). However, the relatively weak phylogenetic signal we observed, the large agreement between phylogenetic and non-phylogenetic analyses, and the strong influence of BMR, latitude, and roost preference supports the hypothesis that mammalian heterothermy has undergone extensive adaptive modification in response to climate and local environment, resulting in a range of thermoregulatory strategies spread throughout the phylogeny [17,44,47]. While phylogeny may determine the historic origin and evolution of heterothermy, the contemporary expression of torpor-related traits, like rewarming rate, appear to have been more strongly shaped by local climate, physiology, and behavior [e.g., [17]].

In conclusion, our findings suggest that the costs of arousal from torpor exert selection pressure on rewarming capacity and that behavior and distribution of heterothermic endotherms influences the evolution of traits associated with thermoregulation. Some species can benefit from behavioral mechanisms and environmental conditions that favor passive rewarming [10,11] whereas others have evolved more rapid endogenous warming rates to reduce energetic costs. Our results contribute to the growing literature on the evolution of species-specific energetic traits in bats as well as other taxa and highlight that local climate, physiology, and behavior are likely mechanisms associated with the proliferation of heterothemic endotherms.

Acknowledgments

References

[1] J.R. Speakman, A. Rowland, Preparing for inactivity: how insectivorous bats deposit fat store for hibernation, Proc. Nutr. Soc. 58 (1999) 797–813.

[2] F. Geiser, Metabolic rate and body temperature reduction during hibernation and daily torpor, Annu. Rev. Physiol. **66** (2004) 239–274.

[3] F. Geiser, R.M. Brigham, The Other Functions of Torpor. Living in a Seasonal World, Springer, Berlin Heidelberg, 2012 109–121.

[4] F. Geiser, T. Ruf, Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns, Physiol. Zool. (1995) 935–966.

[5] D.W. Thomas, M. Dorais, J. Bergeron, Winter energy budgets and cost of arousals for hibernating little brown bats, *Myotis lucifugus*, J. Mammal. 71 (1990) 475–479.

[6] A.E. McKecknie, B.O. Wolf, The energetics of the rewarming phase of avian torpor. *Life in the cold:* evolution, *mechanisms, adaptation and application*, in: B.M. Barnes, H.V. Carey (Eds.), Twelfth International Hibernation Symposium, Institute of Arctic Biology, University of Alaska, Fairbanks 2004, pp. 265–273.

[7] F. Geiser, R.V. Baudinette, The relationship between body mass and rate of rewarming from hibernation and daily torpor in mammals, J. Exp. Biol. 151 (1990) 349–359.

[8] G.N. Stone, A. Purvis, Warm-up rates during arousal from torpor in heterothermic mammals: physiological correlates and a comparison with heterothermic insects, J. Comp. Physiol. B. 162 (1992) 284–295.

[9] C.K.R. Willis, Do roost type or sociality predict warming rate? A phylogenetic analysis of torpor arousal, in: B.G. Lovegrove, A.E. McKecknie (Eds.),Hypometabolism in Animals: Hibernation, Torpor, and Cryobiology 2008, pp. 373–384.

[10] F. Geiser, R.L. Drury, Radiant heat affects thermoregulation and energy expenditure during rewarming from torpor, J. Comp. Physiol. B. 173 (2003) 55–60.

[11] F. Geiser, R.L. Drury, G. Kortner, C. Turbill, C.R. Pavey, M. Brigham, Passive rewarming from torpor in mammals and birds: energetic, ecological and evolutionary implications, in: B.M. Barnes, H.V. Carey (Eds.), Life in the Cold: Evolution, Mechanisms, Adaptation and Application. Twelfth International Hibernation Symposium, Institute of Arctic Biology, University of Alaska, Fairbanks 2004, pp. 45–56.

[12] L. Warnecke, J.M. Turner, F. Geiser, Torpor and basking in a small arid zone marsupial, Naturwissenschaften 95 (2008) 73–78.

[13] A.E. McKecknie, B.O. Wolf, B.M. Barnes, H.V. Carey, Solar Radiation and the Energetic Cost of Rewarming from Torpor, Life in the Cold: Evolution, Mechanisms, Adaptation and Application. Twelfth International Hibernation Symposium, Institute of Arctic Biology, University of Alaska, Fairbanks 2004, pp. 63–70.

[14] M.M. Humphries, D.W. Thomas, J.R. Speakman, Climate-mediated energetic constraints on the distribution of hibernating mammals, Nature 418 (2002) 313–316.

[15] B.G. Lovegrove, The influence of climate on the basal metabolic rate of small mammals: a slow-fast continuum, J. Comp. Physiol. B. 173 (2003) 87–112.

[16] F. Bozinovic, P. Calosi, J.I. Spicer, Physiological correlates of geographic range in animals, Annu. Rev. Ecol. Evol. Syst. 42 (2011) 155–179.

[17] J.G. Boyles, A.B. Thompson, A.E. McKecknie, E. Malan, M.M. Humphries, V. Careau, A global heterothermic continuum in mammals, Glob. Ecol. Biogeogr. 22 (2013) 1029–1039.

[18] J.G. Boyles, B. Smit, A.E. McKechnie, A new comparative metric for estimating heterothermy in endotherms, Physiol. Biochem. Zool. 84 (2011) 115–123.

[19] W. Arnold, Social thermoregulation during hibernation in alpine marmots (*Marmota marmota*), J. Comp. Physiol. B. 158 (1988) 151–156.

[20] C.K.R. Willis, R.M. Brigham, Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat, Behav. Ecol. Sociobiol. 62 (2007) 97–108.

[21] F. Geiser, N. Goodship, C.R. Pavey, Was basking important in the evolution of mammalian endothermy? Naturwissenschaften 2002 (2002) 412–414.

[22] C. Stawski, C.K.R. Willis, F. Geiser, The importance of heterothermy in bats, J. Zool. 292 (2014) 86–100.

[23] T.H. Kunz, L.F. Lumsden, T.H. Kunz, M.B. Fenton, Ecology of Cavity and Foliage Roosting Bats, Bat Ecology, University of Chicago Press, Chicago, 2003 3–89.

[24] L.P. McGuire, K.A. Jonasson, C.G. Guglielmo, Bats on a budget: torpor-assisted migration saves time and energy, PLoS ONE 9 (2014) e115724, http://dx.doi.org/10.1371/journal.pone.0115724.

[25] R.M.R. Barclay, M.C. Kalcounis, L.H. Crampton, C. Stefan, M.J. Vonhof, L. Wilkinson, R.M. Brigham, Can external radiotransmitters be used to assess body temperature and torpor in bats? J. Mammal. 77 (1996) 1102–1106.

[26] C.K.R. Willis, M. Brigham, Defining torpor in free-ranging bats: experimental evaluation of external temperature-sensitive radiotransmitters and the concept of active temperature, J. Comp. Physiol. B. 173 (2003) 379–389.

[27] J.R. Speakman, D.W. Thomas, T.H. Kunz, M.B. Fenton, Physiological ecology and energetics of bats, Bat Ecology, University of Chicago Press, Chicago 2003, pp. 430–490.

[28] R. Bivand, N. Lewin-Koh, Maptools: Tools for reading and handling spatial objects. R package version 0.8-29, 2014.

[29] R Development Core Team, R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2014 (URL: http://www.R-project.org).

[30] A. Grafen, The phylogenetic regression, Philos. Trans. R. Soc. B 346 (1989) 119–157.

[31] T. Garland, A.R. Ives, Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods, Am. Nat. 155 (2000) 346–364.

[32] E. Paradis, J. Claude, K. Strimmer, APE: analyses of phylogenetics and evolution in R language, Bioinformatics 20 (2004) 289–290.

[33] D. Orme, R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, W. Pearse, Comparative Analyses of Phylogenetics and Evolution in R, 2013.

[34] I. Agnarsson, C.M. Zambrana-Torrelio, N.P. Flores-Saldana, L.J. May-Collado, A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia), PLoS Curr. 3 (2011).

[35] M. Pagel, Inferring the historical patterns of biological evolution, Nature 401 (1999) 877–884.

[36] J. Felsenstein, Confidence limits on phylogenies: an approach using the bootstrap, Evolution (1985) 783–791.

[37] R.P. Freckleton, P.H. Harvey, M. Pagel, Phylogenetic analysis and comparative data: a test and review of evidence, Am. Nat. 160 (2002) 712–726.

[38] M.R.E. Symonds, S.P. Blomberg, A primer on phylogenetic generalized least squares, in: L.Z. Garamszegi (Ed.), Modern Phylogenetic Comparative Models and Their Application in Evolutionary Biology, Springer-Verlag, Berlin, Heidelberg 2014, pp. 105–130.

[39] K.P. Burnham, D.R. Anderson, Model Selection and Multimodal Inference: A Practical Information-Theoretic Approach, Springer, New York, 2002.

[40] J.R. Speakman, E. Krol, M.S. Johnson, The functional significance of individual variation in basal metabolic rate, Physiol. Biochem. Zool. 77 (2004) 900–915.

[41] D.E. Naya, L. Spangenberg, H. Naya, F. Bozinovic, Thermal conductance and basal metabolic rate are part of a coordinated system for heat transfer regulation, Proc. R. Soc. B 280 (2013) 20131629.

[42] E.L. Rezende, F. Bozinovic, T. Garland Jr., Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents, Evolution 58 (2004) 1361–1374.

[43] A. Sparti, Thermogenic capacity of shrews (Mammalia, Soricidae) and its relationship with basal rate of metabolism, Physiol. Zool. 65 (1992) 77–96.

[44] J.G. Boyles, J.J. Storm, V. Brack Jr., Thermal benefits of clustering during hibernation: a field test of competing hypotheses on Myotis sodalis, Funct. Ecol. 22 (2008) 63–636.

[45] J.S. Boratynski, C.K.R. Willis, M. Jefimow, M.S. Wojciechowski, Huddling reduces evaporative water loss in torpid Natterer's bats, Myotis nattereri, Comp. Biochem. Physiol. A 179 (2015) 125–132.

[46] J.M. Kamilar, N. Cooper, Phylogenetic signal in primate behaviour, ecology and life history, Philos. Trans. R. Soc. B 368 (2013) 20120341.

[47] B.G. Lovegrove, A single origin of heterothermy in mammals, in: T. Ruf, C. Bieber, W. Arnold, E. Millesi (Eds.), Living in a seasonal world: thermoregulatory and metabolic adaptations, Springer-Verlag, Berlin, Heidelberg 2012, pp. 3–11.

[48] P. Licht, P. Leitner, Physiological responses to high environmental temperatures in three species of microchiropteran bats, Comp. Biochem. Physiol. 22 (1967) 371–387.

[49] D.J. Hosken, P.C. Withers, Temperature regulation and metabolism of an Australian bat, Chalinolobus gouldii (Chiroptera: Vespertilionidae) when euthermic and torpid, J. Comp. Physiol. B. 167 (1997) 71–80.

[50] C.K.R. Willis, J.E. Lane, E.T. Liknes, D.L. Swanson, R.M. Brigham, Thermal energetics of female big brown bats (Eptesicus fuscus), Can. J. Zool. 83 (2005) 871–879.

[51] B.K. McNab, The economics of temperature regulation in neotropical bats, Comp. Biochem. Physiol. 31 (1969) 227–268.

[52] J.-N. Liu, W.H. Karasov, Hibernation in warm hibernacula by free-ranging Formosan leaf-nosed bats, Hipposideros terasensis, in subtropical Taiwan, J. Comp. Physiol. B. 181 (2010) 125–135.

[53] W. Bartels, B.S. Law, F. Geiser, Daily torpor and energetics in a tropical mammal, the northern blossom-bat Macroglossus minimus (Megachiroptera), J. Comp. Physiol. B. 168 (1998) 233–239.

[54] S.K. Maloney, G.N. Bronner, R. Buffenstein, Thermoregulation in the Angolan free-tailed bat Mops condylurus: a small mammal that uses hot roosts, Physiol. Biochem. Zool. 72 (1999) 385–396.

[55] A. Kurta, T.H. Kunz, Roosting metabolic rate and body temperature of male little brown bats (Myotis lucifugus) in summer, J. Mammal. (1988) 645–651.

[56] K. Hanu, Body temperatures and metabolism in bats at different environmental temperatures, Physiol. Bohemoslov. 8 (1959) 250–259.

[57] M.J. O'farrell, E.H. Studier, Fall metabolism in relation to ambient temperatures in three species of Myotis, Comp. Biochem. Physiol. 35 (1970) 697–703.

[58] F. Geiser, Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect of physiological inhibition? J. Comp. Physiol. B. 148 (1988) 25–37.

[59] C. Stawski, F. Geiser, Do season and distribution affect thermal energetics of a hibernating bat endemic to the tropics and subtropics? Am. J. Phys. Regul. Integr. Comp. Phys. 301 (2011) R542–R547.

[60] D.J. Hosken, P.C. Withers, Metabolic Physiology of euthermic and torpid lesser long-eared bats, Nyctophilus geoffroyi (Chiroptera: Vespertilionidae), J. Mammal. 80 (1999) 42–52.

[61] F. Geiser, R.M. Brigham, Torpor, thermal biology, and energetics in Australian long-eared bats (Nyctophilus), J. Comp. Physiol. B. 170 (2000) 153–162.

[62] J.A. McLean, J.R. Speakman, Energy budgets of lactating and non-reproductive Brown Long-Eared Bats (Plecotus auritus) suggest females use compensation in lactation, Funct. Ecol. 13 (1999) 360–372.

[63] J.R. Hirshfeld, M.J. O'Farrell, Comparisons of differential rewarming rates and tissue temperatures in some species of desert bats, Comp. Biochem. Physiol A 55 (1976) 83–87.

[64] E. Kulzer, J.E. Nelson, J.L. McKean, P.F. Andmohres, Untersuchungen iiber die Temperaturregulation australischer Flederma'use, Z. Vgl. Physiol. 69 (1970) 426–451.

[65] M. Eisentraut, Der Winterschlaf der FledermSuse mit besonderer Beriicksichtigung der Warmeregulation, Z. Morphol. Okol. Tiere 29 (1934) 231–267.

[66] D.H. Kelm, O. von Helversen, How to budget metabolic energy: torpor in a small Neotropical mammal, J. Comp. Physiol. B. 177 (2007) 667–677.

[67] B.J. Chruszcz, R.M.R. Barclay, Thermoregulatory ecology of a solitary bat, Myotis evotis, roosting in rock crevices, Funct. Ecol. 16 (2002) 18–26.

[68] R.C. Stones, J.E. Wiebers, Temperature regulation in the little brown bat, Myotis lucifugus, in: K.C. Fisher, A.R. Dawe, C.P. Lyman, E. Schonbaum, F.E. South (Eds.), Mammalian Hibernation, vol. Ill, Oliver & Boyd, Edinburgh 1967, pp. 97–109.

[69] G.A. Bartholomew, W.R. Dawson, R.C. Lasiewski, Thermoregulation and heterothermy in some of the smaller flying foxes (Megachiroptera) of New Guinea, Z. Vgl. Physiol. 70 (1970) 196–209.

[70] C. Stawski, F. Geiser, Fat and fed: frequent use of summer torpor in a subtropical bat, Naturwissenschaften 97 (2009) 29–35.

[71] C. Turbill, G. Kortner, F. Geiser, Natural use of heterothermy by a small, tree-roosting bat during summer, Physiol. Biochem. Zool. 76 (2003) 868–876.

[72] C. Turbill, Roosting and thermoregulatory behaviour of male Gould's long-eared bats, Nyctophilus gouldi: energetic benefits of thermally unstable tree roosts, Aust. J. Zool. 54 (2006) 57–60.

[73] G.A. Bartholomew, T.R. Howell, T.J. Cade, Torpidity in the white-throated swift, anna hummingbird and poorwill, Condor 59 (1957) 145–155.