

Interspecific variation in heat tolerance and evaporative cooling capacity among sympatric temperate-latitude bats

Matthew J. Noakes, Andrew E. McKechnie, and R. Mark Brigham

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Abstract: We tested the hypothesis that interspecific variation in chiropteran heat tolerance and evaporative cooling capacity is correlated with day-roost microclimates, using three vespertilionid bats that occur sympatrically during summer in Saskatchewan, Canada. We predicted that hoary bats (*Lasiurus cinereus* (Palisot de Beauvois, 1796); ~22 g) would have higher heat tolerance than little brown bats (*Myotis lucifugus* (Le Conte, 1831); ~7 g) and silver-haired bats (*Lasionycteris noctivagans* (Le Conte, 1831); ~13 g), as the latter two species roost in tree crevices or cavities that are more thermally buffered than the foliage roosts of hoary bats. We measured core body temperature (T_b ; passive integrated transponder tags), evaporative water loss, and resting metabolic rate (flow-through respirometry) while exposing individuals to a stepped profile of increasing air temperature (T_a) from ~30 °C in ~2 °C increments. Experiments were terminated when individuals became hyperthermic ($T_b \approx 42.5$ °C), with maximum T_a ($T_{a,max}$) ranging from 42.0 to 49.7 °C. As predicted, hoary bats had the highest heat tolerance and evaporative cooling capacity, reaching $T_{a,max}$ ~2.4 and 1.2 °C higher than little brown and silver-haired bats, respectively. Our results are consistent with the hypothesis that heat tolerance of bats is correlated with roost microclimates, although interspecific variation in body mass and phylogeny may confound these conclusions.

Key words: heat tolerance, evaporative cooling capacity, hyperthermia, roost temperature, *Myotis lucifugus*, little brown bat, *Lasionycteris noctivagans*, silver-haired bat, *Lasiurus cinereus*, hoary bat, Yinpterochiroptera.

Résumé : Nous avons vérifié l'hypothèse voulant que les variations interspécifiques de la tolérance à la chaleur et de la capacité de refroidissement par évaporation chez les chiroptères soient corrélées aux microclimats des gîtes diurnes, en utilisant trois chauves-souris de la famille des vespertilionidés présentes en sympatrie en été en Saskatchewan (Canada). Nous avions prédit que la tolérance à la chaleur des chauves-souris cendrées (*Lasiurus cinereus* (Palisot de Beauvois, 1796); ~22 g) serait plus grande que celles des vespertiliens bruns (*Myotis lucifugus* (Le Conte, 1831); ~7 g) et des chauves-souris argentées (*Lasionycteris noctivagans* (Le Conte, 1831); ~13 g), puisque les gîtes diurnes de ces deux dernières espèces sont des fentes ou cavités dans les arbres caractérisées par des températures moins variables que les gîtes dans le feuillage des chauves-souris cendrées. Nous avons mesuré la température corporelle centrale (T_b ; radioétiquettes passives intégrées), la perte d'eau par évaporation et le métabolisme au repos (respirométrie dynamique), alors que les spécimens étaient exposés à des températures de l'air (T_a) commençant à ~30 °C et augmentant par incréments de ~2 °C. Les manipulations cessaient que les spécimens devenaient hyperthermiques ($T_b \approx 42,5$ °C), les T_a maximums ($T_{a,max}$) allant de 42,0 à 49,7 °C. Comme prévu, les chauves-souris cendrées présentent la plus forte tolérance à la chaleur et la plus grande capacité de refroidissement par évaporation, atteignant des $T_{a,max}$ de ~2,4 et 1,2 °C supérieures à celles des vespertiliens bruns et des chauves-souris argentées, respectivement. Nos résultats concordent avec l'hypothèse selon laquelle la tolérance à la chaleur des chauves-souris est corrélée aux microclimats des gîtes, bien que des variations interspécifiques de la masse corporelle et la phylogénie puissent nécessiter de nuancer ces conclusions. [Traduit par la Rédaction]

Mots-clés : tolérance à la chaleur, capacité de refroidissement par évaporation, hyperthermie, température du gîte, *Myotis lucifugus*, vespertilion brun, *Lasionycteris noctivagans*, chauve-souris argentée, *Lasiurus cinereus*, chauve-souris cendrée, yinptérochiroptères.

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Introduction

Free-ranging endotherms frequently encounter environmental temperatures above their normothermic body temperature (T_b), for example, while foraging in sunlit microsites or occupying hot roosts during the day (e.g., Henshaw and Folk 1966; Wolf and Walsberg 1996). High heat dissipation requirements can have negative fitness consequences for endotherms, even for species residing in high-latitude regions with relatively cool summers (e.g., Oswald et al. 2008; Oswald and Arnold 2012). When environmental temperature exceeds T_b , endotherms can dissipate heat only by increasing evaporative water loss (EWL) from respiratory and cutaneous surfaces, giving rise to a trade-off between conserving water and avoiding lethal hyperthermia (reviewed by McKechnie and Wolf 2019).

As small flying mammals, bats appear particularly at risk of succumbing to extreme heat events, with tens of thousands of flying foxes (genus *Pteropus* Brisson, 1762) dying during recent heat waves in Australia (Welbergen et al. 2008; Ratnayake et al. 2019). Bats have experienced unique selection pressures among mammals associated with the evolution of flight, leading to relatively high normothermic T_b , EWL, and surface area to volume ratios (including large, uninsulated wing membranes) compared with nonvolant mammals of similar size (Studier et al. 1970; Hartman 1963; Neuweiler 2000; McKechnie and Wolf 2019). As nocturnal animals that roost during the day, many bats have no access to drinking water when experiencing high air temperatures (T_a) during summer (Licht and Leitner 1967a; Minnaar et al. 2014). Day roost selection is likely an important factor in determining bat survival during severe heat stress, for example, a free-ranging little broad-nosed bat (*Scotorepens greyii* (Gray, 1843)) abandoned its poorly insulated roost when outside T_a exceeded 48 °C during a heat wave in Australia, whereas inland freetail bats (*Mormopterus petersi* (Leche, 1884)) remained in their better-insulated roosts (Bondarenco et al. 2014). Records of roost T_a ($T_{a,roost}$) > 50 °C exist for bats roosting in anthropogenic structures such as attics and lofts (Henshaw and Folk 1966; Licht and Leitner 1967a; Maloney et al. 1999). Foliage-roosting bats likely also experience hot microclimates during summer as a result of little buffering from external T_a and high solar heat loads (Willis and Brigham 2005; Klüg et al. 2012), which can co-occur with high humidity levels that impede evaporative heat dissipation.

Several laboratory studies have measured thermoregulatory responses of bats at T_a up to 40 °C (Bartholomew et al. 1964, 1970; Herreid and Schmidt-Nielsen 1966; Herreid 1967; Studier and O'Farrell 1972; Cryan and Wolf 2003; Marom et al. 2006; Muñoz-García et al. 2012; Minnaar et al. 2014), and some studies have examined thermoregulation at T_a > 40 °C (highest T_a ≈ 41–45 °C; Hock 1951; Henshaw and Folk 1966; Carpenter and Graham 1967; Licht and Leitner 1967b; Laburn and Mitchell 1975; Maloney et al. 1999; Cory Toussaint and McKechnie 2012; Czenze et al. 2020). However, the lack of standardized methods among these studies likely confounds interspecific comparisons. For example, the humidity level in chambers can affect the ability of bats to cope with high T_a (Licht and Leitner 1967b) and humidity has a major effect on evaporative cooling efficiency at high T_a among birds (Gerson et al. 2014; van Dyk et al. 2019). To facilitate comparisons among studies, there is a need to quantify thermoregulation of bats at T_a above normothermic T_b using a standardized protocol (e.g., Whitfield et al. 2015).

Considering the variety of day-roost types used by bats, it is possible that roost microclimate is a predictor of interspecific variation in heat tolerance and evaporative cooling capacity. Some support for this idea includes the results of two studies on interspecific thermoregulatory variation at high T_a in the Limpopo River valley of southern Africa, where environmental T_a regularly exceeds 40 °C during summer (Cory Toussaint and McKechnie 2012; Czenze et al. 2020). Variation among three sympatric bats revealed the lowest evaporative cooling capacity in Cape long-eared

bats (*Nycteris thebaica* E. Geoffroy, 1818; measured up to T_a ≈ 42 °C), which roost in thermally buffered baobab tree cavities where $T_{a,roost}$ remains <30 °C despite outside T_a > 35 °C (Cory Toussaint and McKechnie 2012). In contrast, Mauritian tomb bats (*Taphozous mauritanus* E. Geoffroy, 1818) and Robert's flat-headed bats (*Sauromys petrophilus* (Roberts, 1917)) had higher evaporative cooling capacity and roost in rock crevices where $T_{a,roost}$ is routinely >2 °C above outside T_a during hot days (Cory Toussaint and McKechnie 2012). Czenze et al. (2020) reported that a cave-dwelling bat (the Egyptian rousette, *Rousettus aegyptiacus* (E. Geoffroy, 1810)) had relatively low heat tolerance and evaporative cooling capacity, reaching maximum T_a ($T_{a,max}$) ~ 6 °C lower than two bats that roost exposed in trees (Mauritian tomb bat and long-tailed house bat, *Eptesicus hottentotus* (A. Smith, 1833)). However, small sample sizes ($n = 3$ –6 individuals per species), large phylogenetic differences (different suborders), and 10-fold variation in body mass (M_b) of the study species may confound evaluating the hypothesis of variation associated with roost type.

We investigated whether upper thermoregulatory limits vary with roost type among three insectivorous bats at a site where they occur sympatrically during summer, the Cypress Hills Interprovincial Park in Saskatchewan, Canada (hereinafter Cypress Hills). All three species are vespertilionids and vary about 3-fold in M_b : little brown bats (*Myotis lucifugus* (Le Conte, 1831); ~7 g), silver-haired bats (*Lasiurus noctivagus* (Le Conte, 1831); ~12 g), and hoary bats (*Lasiurus cinereus* (Palisot de Beauvois, 1796); ~22 g). Male and non-reproductive female little brown bats (Anthony et al. 1981; Broders and Forbes 2004) and silver-haired bats (Barclay et al. 1988; Campbell et al. 1996; Vonhof and Gwilliam 2007) typically roost solitarily in well-concealed crevices under loose bark. Reproductive females of both species form maternity colonies that roost in tree cavities during summer (Barclay and Cash 1985; Mattson et al. 1996; Betts 1998; Crampton and Barclay 1996; Vonhof and Barclay 1996; Psyllakis and Brigham 2006). Little brown bats may also roost in anthropogenic structures where available (e.g., barns and attics; Anthony et al. 1981; Burnett and August 1981; Menaker 1962). In contrast, hoary bats are one of the few temperate-zone bats that roost solitarily on the open foliage of trees regardless of reproductive state (Shump and Shump 1980; Koehler and Barclay 2000; Klüg et al. 2012), typically choosing sites where there is less forest canopy and density, and thus higher solar heat loads (Willis and Brigham 2003, 2005).

The more exposed roost sites of hoary bats suggest they experience hotter roost microclimates during summer compared with individual little brown and silver-haired bats, as a result of less protection from outside T_a and exposure to direct sunlight during the day (Willis and Brigham 2005; Willis et al. 2006; Klüg et al. 2012). Despite summer maximum T_a being substantially lower than bats experience in more arid regions at lower latitudes, T_a in the Cypress Hills can nevertheless exceed normothermic T_b (highest recorded $T_a = 40.5$ °C, 29 May 1988; <https://climate.weather.gc.ca>). We predicted that heat tolerance and evaporative cooling capacity (ratio of evaporative heat loss to metabolic heat production; EHL/MHP) would be greater in the species using more exposed roost sites, with the foliage-roosting hoary bats having higher maximum EHL/MHP and $T_{a,max}$ compared with cavity- or crevice-roosting little brown and silver-haired bats.

Materials and methods

Study site and species

We quantified heat tolerance and evaporative cooling capacity in little brown, silver-haired, and hoary bats ($n = 12$ individual per species) caught during summer (29 June – 10 August 2018) in the Cypress Hills (49°34'N, 109°53'W). Daily maximum T_a at this site regularly exceeds 30 °C during summer, with the highest recorded T_a during our study period being 36.1 °C (mean (±SD) daily maximum $T_a = 24.8 ± 5.0$ °C; climate data obtained from

the nearest Government of Canada weather station, ~28 km northeast of our study site; <https://climate.weather.gc.ca>).

We captured bats at night in mist nets set across the main water body in the park, Battle Creek. Individuals were housed in cloth bags at room temperature, with roughly equal numbers from each sex (little brown bats: $n = 7$ males and 5 females; silver-haired bats: $n = 5$ males and 7 females; hoary bats: $n = 6$ males and 6 females). All bats were inspected to ensure that they were adults and that females were non-reproductive (i.e., checked for signs of lactation and abdomens palpated to confirm that they were not pregnant). Gas exchange measurements were conducted the day following capture, and bats were released that evening (<24 h in captivity), except for two silver-haired bats for which measurements occurred the second day after capture. Bats were handfed mealworms and given water from a pipette until satiated regularly while in captivity (two to three times per day).

Ethics approval

We received a permit to conduct research on Saskatchewan Provincial Park Land from the Ministry of Parks, Culture and Sport. We undertook our research following animal use protocols approved by the University of Regina, the University of Pretoria (protocol NAS342/2019), and the South African National Biodiversity Institute (protocol P19/26).

Gas exchange measurements

We used open flow-through respirometry to estimate EWL and RMR using a similar experimental setup to that described by Whitfield et al. (2015), with modifications described below. We placed bats individually into airtight respirometry chambers constructed from clear plastic containers with volumes of either 1.8 L (General Mills, Minneapolis Minnesota, USA) or 1.2 L (Lock & Lock, Seoul, South Korea). A plastic mesh platform elevated bats above a layer of mineral oil and a frame positioned on top of the platform covered the sides and roof of the chamber, allowing the bat to climb and hang in a natural roosting posture.

We constructed a temperature-controlled cabinet from a 50 L refrigerator (FR100, Igloo, Katy, Texas, USA) by mounting an infrared ceramic heat emitter bulb inside (100 W, 90–120 V, non-light, black, CRLight, People's Republic of China). We connected the lead from the heating lamp and the refrigerator to a digital thermostat (ITC-308, Inkbird, Shenzhen, People's Republic of China) so that both heating and cooling functions could regulate the T_a inside the cabinet. We placed the respirometry chambers containing bats inside the temperature cabinet during measurements, shielded from the heating lamp by a cardboard partition.

Air was supplied to the system by a pump (DOA-P701-AA, Gast Manufacturing, Benton Harbor, Michigan, USA). Flow rates into the respirometry chamber were regulated by a mass flow controller (MC-10SLPM-D/5m, Alicat Scientific Inc., Tucson, Arizona, USA) and were frequently adjusted during measurements to maintain low humidity levels inside the chamber (range of flow rates: 0.9–7.6 L/min; chamber water vapour partial pressure: <0.44 kPa). The range of 99% equilibrium times for the above flow rates and chamber volumes was 0.7–9.2 min (Lasiewski et al. 1966), and we consistently allowed sufficient time at each T_a to ensure equilibrium within chambers. Air from the different channels were alternately subsampled using a V3 Multiplexer and TR-SS3 subsampler (Sable Systems, Las Vegas, Nevada, USA) and passed through a $\text{CO}_2/\text{H}_2\text{O}$ analyser (LI-840 A, LI-COR, Lincoln, Nebraska, USA) at ~130 mL/min. Voltage outputs from the $\text{CO}_2/\text{H}_2\text{O}$ analyser were digitized using an analog–digital converter (UI3, Sable Systems, Las Vegas, Nevada, USA) and recorded with a sampling interval of 5 s using Expedata software (Sable Systems, Las Vegas, Nevada, USA). We calibrated mass flow controller and $\text{CO}_2/\text{H}_2\text{O}$ analyser using the same protocol as Whitfield et al. (2015).

Air and body temperature measurements

We continuously recorded T_a in the respirometry chambers during measurements using a thermistor probe (TC-100, Sable Systems, Las Vegas, Nevada, USA) inserted through a hole in the metabolic chamber sealed with a rubber grommet. Core T_b was measured using passive integrated transponder (PIT) tags (BioThermo13, Biomark, Boise, Idaho, USA; tag mass = 0.109 ± 0.030 g, resolution = 0.1 °C) calibrated following Whitfield et al. (2015). We injected PIT tags subcutaneously between the scapulae of bats using a sterile syringe and secured the injection site with a drop of cyanoacrylate glue. PIT-tag injection typically occurred on the night of capture of an individual (i.e., ≥ 8 h before measurements), although some individuals were injected the morning prior to measurements (≥ 2 h before measurements). We recorded T_b during measurements using a racket antenna and PIT-tag reader (HPR Plus, Biomark, Boise, Idaho, USA).

Experimental protocol

We estimated the heat tolerance and evaporative cooling capacity of bats during rest-phase (daytime) using the standardized protocol described by Whitfield et al. (2015), with modifications described below. Prior to the onset of gas exchange measurements, we sexed individuals and measured M_b using a portable balance (PT600, Sartorius AG, Göttingen, Germany). Individuals were exposed to a ramped T_a profile, starting at a relatively moderate T_a (~30 or 32 °C) and increasing by ~2 or 4 °C increments until $T_a \approx 40$ °C, and thereafter in ~2 °C increments until bats approached their upper thermoregulatory limits (max $T_a \approx 48$ °C). We maintained bats at the initial moderate T_a for ≥ 30 min (47.5 ± 14.2 min, mean \pm SD), and then at subsequent T_a set points for ≥ 10 min (17.7 ± 6.5 min, mean \pm SD). The behaviour of individuals was continuously monitored using a camcorder with a built-in infrared light (HDR-XR200, Sony, Tokyo, Japan). Experimental measurements typically lasted about 3 h (196.1 ± 34.5 min, mean \pm SD).

We determined when individuals were approaching their upper thermoregulatory limit by considering all available variables: T_b , EWL, RMR, and behavioural responses. Experiments were immediately terminated if bats became hyperthermic (considered $T_b > 42.5$ °C) or exhibited clear signs of behavioural or physiological distress (e.g., increased activity levels, escape behaviours, or sudden spikes in EWL, RMR, or T_b). We chose a T_b of ~42.5 °C because preliminary trials indicated bats became distressed at higher T_b and exhibited sudden and seemingly uncontrolled elevations in EWL and RMR. We considered the maximum T_a an individual reached as an indication of their heat tolerance ($T_{a,max}$). We provided individuals with water until satiated immediately before and after measurements; all bats recovered before release. We released bats at dusk on the evening following measurements.

Data analysis

We calculated whole-animal RMR, EWL, and EHL/MHP following Noakes et al. (2016), assuming a respiratory exchange ratio of 0.85 (i.e., a metabolic substrate consisting of 50% carbohydrates and 50% lipids) and using thermal equivalence data from Withers (1992). We present mean values in the format: mean \pm SD. We used R version 3.5.2 (R Core Team 2018) to fit linear models and linear mixed-effects models (“nlme” package; Pinheiro et al. 2018) to the data. We tested model assumptions (normality, homogeneity of variance and, multicollinearity) and fit (residuals, leverage, and Cook's D values) using model residual plots. We excluded data from the first (lowest) T_a measured for each individual from statistical analyses, due to greater among-individual variation in thermoregulatory data at these T_a . This variation suggests that bats might have still been habituating to the experimental setup, although behavioural records indicated bats were calm.

We fitted general linear models to investigate M_b variation between sexes, as well as variation in $T_{a,max}$ between sexes and

Table 1. Statistical results from the top linear mixed-effects models fitted to body temperature (T_b), evaporative water loss (EWL), resting metabolic rate (RMR), and the ratio of evaporative heat loss to metabolic heat production (EHL/MHP) of little brown (*Myotis lucifugus*), silver-haired (*Lasionycteris noctivagans*), and hoary (*Lasiurus cinereus*) bats exposed to high air temperatures ($\sim 30^\circ\text{C} \leq T_a \leq 48^\circ\text{C}$).

Response variables	Bat species	Top global models: predictor variables						Models investigating relationship with air temperature							
		Air temperature			Sex			Inflection point		Equations					
		<i>F</i>	df	<i>p</i>	<i>F</i>	df	<i>p</i>	<i>p</i>	T_a ($^\circ\text{C}$)	<i>F</i>	df	<i>p</i>	<i>m</i>	y-int	
T_b	Little brown	138.036	1, 33	<0.001				0.581		138.036	1, 33	<0.001	0.572	16.521	
	Silver-haired	234.311	1, 42	<0.001				0.366		234.311	1, 42	<0.001	0.553	16.911	
	Hoary	304.161	1, 46	<0.001				0.003	<42.273 >42.273	253.334 39.780	1, 26 1, 8	<0.001	0.732 0.184	9.775 32.959	
EWL	Little brown	65.176	1, 34	<0.001				<0.001	<40.963 >40.963	51.507 29.189	1, 19 1, 5	<0.001	0.135 0.693	-3.280 -25.739	
	Silver-haired	61.033	1, 40	<0.001	20.87	1, 10	0.001	0.004	<42.884 >42.884	37.400 3.003	1, 30 1, 1	<0.001	0.217	-5.920	
	Hoary	91.658	1, 51	<0.001				0.002	<38.914 >38.914	4.973 61.283	1, 12 1, 26	0.046 <0.001	0.096 2.121	-0.835 -80.353	
RMR	Little brown	73.61	1, 34	<0.001				0.333		73.610	1, 34	<0.001	0.006	-0.137	
	Silver-haired	<i>Top model supported null hypothesis</i>													
	Hoary	45.4	1, 51	<0.001				0.237		45.400	1, 51	<0.001	0.017	-0.454	
EHL/MHP	Little brown	27.501	1, 34	<0.001				0.002	<41.294 >41.294	2.058 8.701	1, 19 1, 5	0.168 0.032	0.265	-10.060	
	Silver-haired	17.02	1, 40	<0.001	11.336	1, 10	0.007	0.123		19.013	1, 40	<0.001	0.057	-1.346	
	Hoary	81.835	1, 51	<0.001				<0.001	<39.238 >39.238	0.000 45.397	1, 13 1, 25	0.999 <0.001	0.206	-7.463	

Note: Potential predictor variables included air temperature (T_a), sex, body mass (M_b), and all possible interaction terms. We only included predictor variables in final models if model selection indicated that the predictor variable improved model fit (neither M_b nor any interaction terms were included in any top models). If T_a was a significant predictor for a response variable (all except RMR of silver-haired bats), then we used a Davies test to determine whether there was a significant inflection point in this relationship. Where appropriate, we fitted broken-stick linear regressions to identify inflection T_a and a linear mixed-effects model to obtain the slope (m) and y intercept (y -int) of the equations of significant relationships. The p values in boldface type are significant at $p < 0.05$.

with M_b , for each species separately. We fitted linear mixed-effects models to investigate variation in thermoregulatory variables (RMR, EWL, T_b , and EHL/MHP) with predictor variables (T_a , M_b , and sex) for each species, with individual included as a random factor in all models. We used the “dredge” function (“MuMIn” package; Bartoń 2018) to determine the combination of predictor variables that produced models best explaining the variation in thermoregulatory variables by comparing Akaike’s information criterion values. As T_a was included in the top models for all thermoregulatory variables from all species (except RMR of silver-haired bats), we used Davies test to determine whether there were significant inflection points in the relationship between each variable and T_a . If a significant inflection point was identified, then we fitted broken stick linear regressions to determine the T_a value of this point (“segmented” package; Muggeo 2003) and used an ANOVA to confirm that the broken stick regression provided significantly better fit than a simple linear regression. We then fitted a linear mixed-effects model separately to data either side of each inflection T_a . The equations plotted in figures are from these final models. We repeated statistical analyses for each species with CO_2 production as the response variable to confirm that converting CO_2 production into RMR in watt (W) was not a source of error.

Results

Body mass

The null model provided the best fit for M_b of little brown and hoary bats (i.e., no significant difference between sexes), with mean (\pm SD) values of 7.1 ± 0.6 and 22.1 ± 5.0 g for each species respectively. In contrast, the M_b of silver-haired bats was significantly higher in females (12.3 ± 1.2 g) compared with males (9.5 ± 0.6 g; $F_{1,10} = 21.498$, $p < 0.001$). M_b was not included in the top models for any thermoregulatory variables of all three species.

Maximum air temperature reached

The top models explaining variation in $T_{a,\text{max}}$ was the null model in all three species (i.e., including neither M_b nor sex; Table 1). Individual $T_{a,\text{max}}$ ranged from 42.0°C in a little brown bat to 49.7°C in a hoary bat. Hoary bats reached the highest mean $T_{a,\text{max}}$, ~ 2.4 and 1.2°C higher than little brown and silver-haired bats, respectively (Fig. 1).

Body temperature

The top model explaining variation in T_b of all three species included only T_a (Table 1). The T_b of little brown and silver-haired bats increased significantly with increasing T_a at a consistent rate across all experimental T_a (Figs. 2a and 2b; Table 1). The T_b of hoary bats also increased significantly with increasing T_a , but there was an inflection point in this relationship (Table 1). At $T_a > 42.3^\circ\text{C}$, T_b increased at $\sim 4\times$ slower rate with increasing T_a , remaining at a near-constant $T_b \approx 41^\circ\text{C}$ (Fig. 2c).

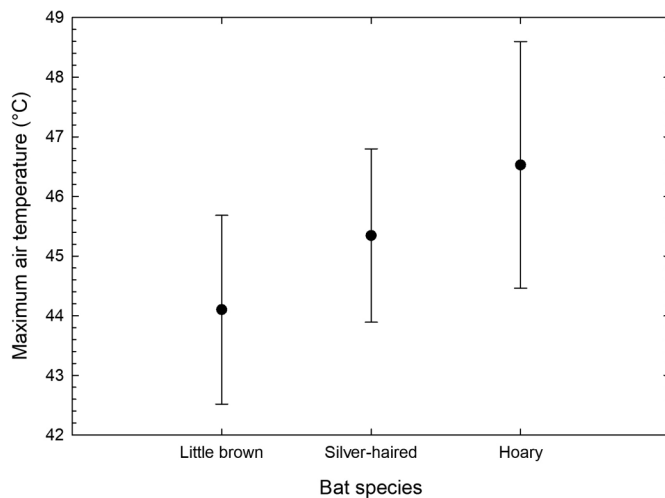
Evaporative water loss

The top model explaining variation in EWL of all species included T_a , with inflection points in these relationships (Table 1). EWL increased significantly with increasing T_a across all experimental T_a in little brown and hoary bats, but at a $\sim 5\times$ and $4\times$ faster rate at $T_a > 41.0$ and 38.9°C , respectively (Table 1; Figs. 3a and 3c). In contrast, EWL of silver-haired bats increased significantly with increasing $T_a < 42.9^\circ\text{C}$, although a visual inspection of the data suggests a sharp EWL increase at $T_a > 42.9^\circ\text{C}$ (Table 1; Fig. 3b). The top model for silver-haired bats also included sex (but not the $T_a \times$ sex interaction term; Table 1), with EWL generally higher in males compared with females regardless of T_a (Fig. 3b).

Resting metabolic rate

The top model explaining variation in RMR of little brown and hoary bats only included T_a , with RMR increasing consistently with increasing T_a (Table 1; Figs. 4a and 4c). The model supporting

Fig. 1. Hoary bats (*Lasiurus cinereus*) reached higher maximum air temperatures ($T_{a,max}$) than silver-haired (*Lasionycteris noctivagans*) and little brown (*Myotis lucifugus*) bats. We placed individuals in metabolic chambers at $T_a \approx 30^\circ\text{C}$, increasing T_a in $\sim 2^\circ\text{C}$ increments until bats approached their upper thermoregulatory limits (≥ 10 min per T_a ; $n = 12$ per species). We terminated experiments if bats became hyperthermic (body temperature $> 42.5^\circ\text{C}$) or exhibited clear signs of thermoregulatory or behavioural distress. The $T_{a,max}$ is the T_a recorded when bats were removed from the chambers. $T_{a,max}$ did not differ significantly between sexes in any species, and thus mean \pm SD are presented including all individuals.



the null model provided the best fit for RMR of silver-haired bats (Fig. 4b). Repeating analyses using CO_2 production instead of metabolic rate (W) as the response variable revealed the same general patterns of variation in each species.

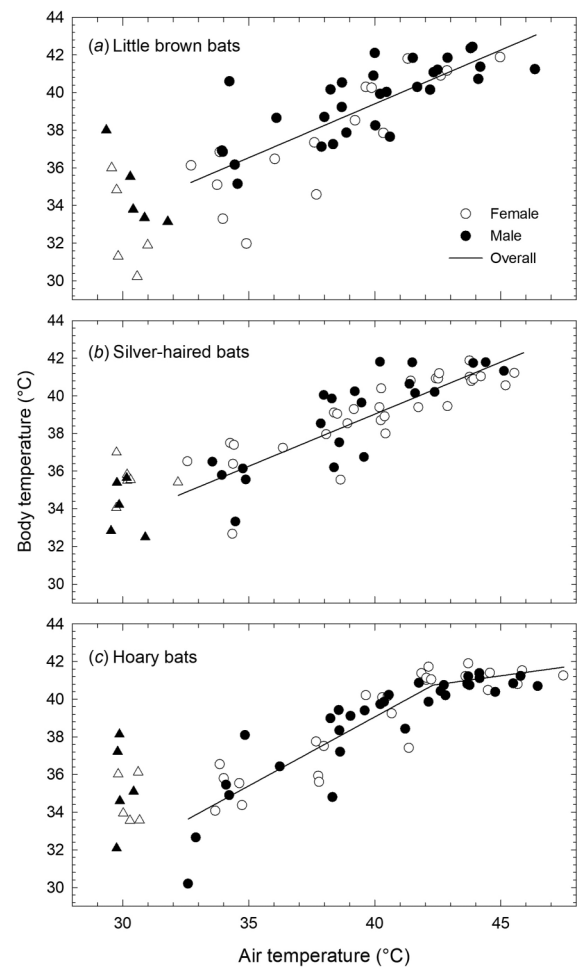
Ratio of evaporative heat loss to metabolic heat production

The top model explaining variation in EHL/MHP in all three species included T_a (Table 1). There was an inflection point in this relationship for little brown and hoary bats, with EHL/MHP only increasing significantly with increasing $T_a > 41.3$ and 39.2°C , respectively (Table 1; Figs. 5a and 5c). In contrast, EHL/MHP of silver-haired bats increased at a consistent rate across all experimental T_a (Fig. 5b; Table 1). The top model for silver-haired bats also included sex (but not the $T_a \times$ sex interaction term; Table 1), with EHL/MHP generally higher in males compared with females regardless of T_a (Fig. 5b). The mean maximum EHL/MHP of silver-haired bats was also higher for males (1.7 ± 0.4 at $T_a = 43.1 \pm 1.8^\circ\text{C}$) compared with females (1.3 ± 0.4 at $T_a = 42.5 \pm 2.6^\circ\text{C}$). There was no significant difference between sexes in little brown and hoary bats, with mean maximum EHL/MHP of 1.3 ± 0.6 (at $T_a = 41.1 \pm 3.9^\circ\text{C}$) and 2.0 ± 0.6 (at $T_a = 44.5 \pm 1.7^\circ\text{C}$) for each species, respectively. The highest EHL/MHP ratios recorded for an individual little brown bat, silver-haired bat, and hoary bat were 2.6, 2.2, and 3.1, respectively (all male individuals at $T_a = 44.1$, 45.1 , and 45.8°C , respectively).

Discussion

The thermoregulatory responses of the temperate-zone bats that we tested were typical of endotherms experiencing high T_a (McKechnie and Wolf 2019), with individuals generally increasing T_b , EWL, RMR, and EHL/MHP with increasing T_a above normothermic T_b . As predicted, foliage-roosting hoary bats were better at tolerating high T_a than the other two species, both of which use less exposed roost sites (tree crevice or cavity roosts). Hoary bats had higher $T_{a,max}$ values than little brown and silver-haired

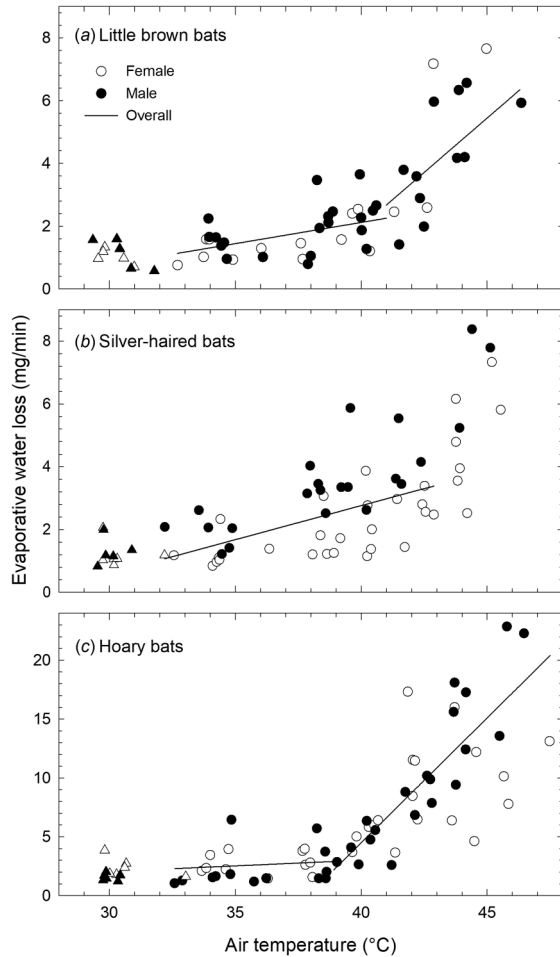
Fig. 2. The body temperature (T_b) of little brown (*Myotis lucifugus*; a), silver-haired (*Lasionycteris noctivagans*; b), and hoary (*Lasiurus cinereus*; c) bats increased significantly with increasing experimental air temperatures (T_a ; $\sim 30^\circ\text{C} \leq T_a \leq 48^\circ\text{C}$). There was a significant inflection point in this relationship only for hoary bats, with T_b increasing at a slower rate with increasing $T_a > 42.3^\circ\text{C}$ (Table 1). There were no significant T_b differences between sexes in any species. We measured T_b of individuals using temperature-sensitive transponder tags ($n = 12$ per species;). Data measured at the first (lowest) T_a for each individual were excluded from statistical analyses (indicated as triangles; statistical values and equations reported in Table 1).



bats (~ 2.4 and 1.2°C higher, respectively; Fig. 1), as well as higher maximum evaporative cooling capacities (EHL/MHP; Figs. 4a–4c). These results are consistent with the hypothesis that the abilities of bats to cope with high T_a vary among species in a manner correlated to roost microclimate (Cory Toussaint and McKechnie 2012; Czenze et al. 2020).

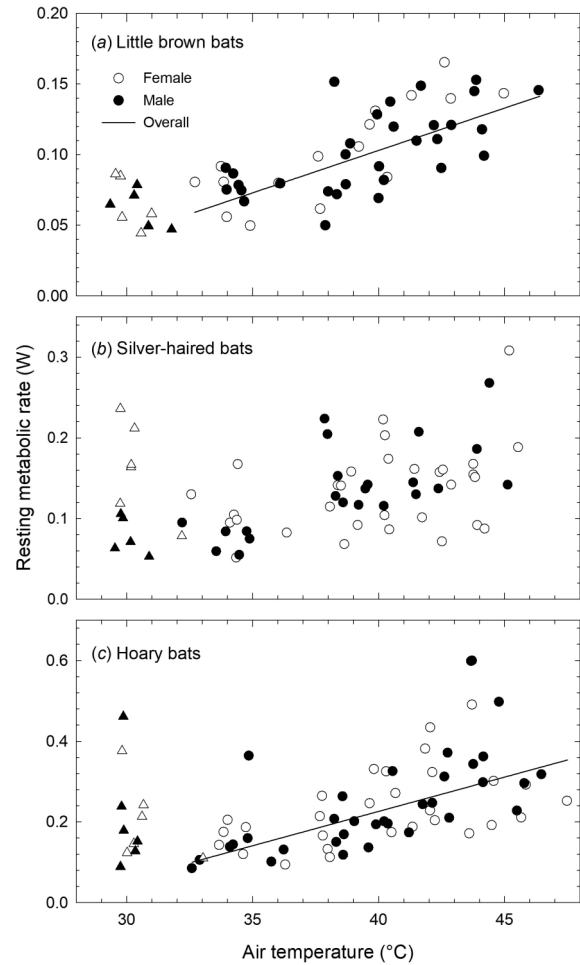
A limitation of our study is that we could not directly measure $T_{a,roost}$ used by bats in Cypress Hills; however, numerous studies have investigated the roosting habits of these species. Male and non-reproductive female little brown and silver-haired bats roost solitarily during summer, protected from direct sunlight and buffered from external T_a in crevices beneath tree bark (Anthony et al. 1981; Barclay et al. 1988; Campbell et al. 1996; Mattson et al. 1996; Broders and Forbes 2004; Vonnhof and Gwilliam 2007). Reproductive female bats of both species form maternity colonies in natural tree cavities during summer (Barclay and Cash 1985; Betts 1998; Crampton and Barclay 1996; Mattson et al. 1996; Vonnhof and Barclay 1996; Psyllakis and Brigham 2006), although

Fig. 3. The evaporative water loss (EWL) of little brown (*Myotis lucifugus*; a) and hoary (*Lasiurus cinereus*; c) bats increased with increasing experimental air temperatures (T_a ; $\sim 30^\circ\text{C} \leq T_a \leq 48^\circ\text{C}$), but at a faster rate above an inflection point at $T_a > 41.0$ and 38.9°C , respectively. The EWL of silver-haired bats (*Lasionycteris noctivagans*; b) only increased with increasing $T_a < 42.9^\circ\text{C}$. There were no significant differences between sexes in little brown and hoary bats, but EWL of silver-haired bats was significantly higher in males relative to females across all T_a . We measured EWL of individuals using open flow-through respirometry ($n = 12$ per species). Data measured at the first (lowest) T_a for each individual were excluded from statistical analyses (indicated as triangles; statistical values and equations reported in Table 1).



little brown bats frequently use anthropogenic structures when available (Menaker 1962; Burnett and August 1981; Kurta and Kunz 1988). Maternity colonies commonly use cavities of trembling aspen (*Populus tremuloides* Michx.) trees in this region (e.g., Crampton and Barclay 1996; Bohn 2017), and a study on the microclimate of these trembling aspen cavities occupied by big brown bats (*Eptesicus fuscus* (Palisot de Beauvois, 1796)) in the Cypress Hills indicates that they are considerably buffered from outside maximum T_a ($T_{a,roost}$ rarely $> 30^\circ\text{C}$ during summer; Willis and Brigham 2007). In contrast, hoary bats roost solitarily in the open foliage of coniferous trees regardless of reproductive state, typically choosing sites in the Cypress Hills where there is less forest canopy and density, and thus higher solar heat loads (Willis and Brigham 2005, 2003). Willis et al. (2006) recorded $T_{a,roost} > 30^\circ\text{C}$ during June for hoary bats in the Cypress Hills, but there are no similar data for the hottest parts of summer in July and August.

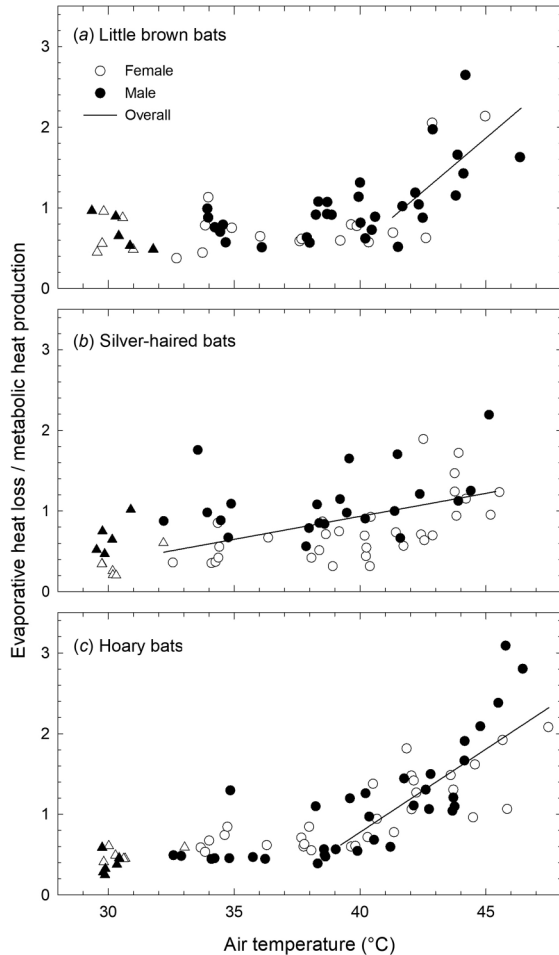
Fig. 4. The resting metabolic rate (RMR) of little brown (*Myotis lucifugus*; a) and hoary (*Lasiurus cinereus*; c) bats increased consistently with increasing experimental air temperatures (T_a ; $\sim 30^\circ\text{C} \leq T_a \leq 48^\circ\text{C}$). The RMR of silver-haired bats (*Lasionycteris noctivagans*; b) did not vary significantly with T_a . There were no significant RMR differences between sexes in any species. We measured RMR of individuals using open flow-through respirometry ($n = 12$ per species). Data measured at the first (lowest) T_a for each individual were excluded from statistical analyses (indicated as triangles; statistical values and equations reported in Table 1).



Collectively, these studies support our assumption that hoary bats experience hotter summer roost microclimates than little brown and silver-haired bats in the Cypress Hills.

At experimental T_a of $30\text{--}34^\circ\text{C}$, the range in T_b of all bats was similar to the normothermic range reported for bats in general (~ 31 to 38°C ; Figs. 2a–2c; McKechnie and Wolf 2019). These findings are also similar to previous studies (both in New Mexico, United States) on female little brown bats caught in attics ($T_b \approx 33\text{--}34^\circ\text{C}$ at $T_a \approx 32^\circ\text{C}$; Studier and O'Farrell 1972) and hoary bats of both sexes during their spring migration ($T_b \approx 30\text{--}39^\circ\text{C}$ at $T_a \approx 30\text{--}34^\circ\text{C}$; Cryan and Wolf 2003). We terminated experiments when $T_b \approx 42.5^\circ\text{C}$ because individuals showed signs of behavioural or physiological distress at higher T_b , which is within the range of maximum T_b reported for small bats in general (~ 42 to 45°C ; McKechnie and Wolf 2019). Little brown bats reached cut-off T_b at $T_{a,max} = 44.1 \pm 1.6^\circ\text{C}$, which is similar to cave-dwelling conspecifics from Kentucky (USA) that tolerated $T_b \approx 42^\circ\text{C}$ when exposed to T_a of up to 45°C (Henshaw and Folk 1966). The $T_{a,max}$ of hoary bats was $46.5 \pm 2.1^\circ\text{C}$, whereas previous studies have

Fig. 5. The ratio of evaporative heat loss to metabolic heat production (EHL/MHP) of silver-haired bats (*Lasiurus noctivagans*; *b*) increased consistently with increasing experimental air temperatures (T_a ; $\sim 30^\circ\text{C} \leq T_a \leq 48^\circ\text{C}$). The EHL/MHP of little brown (*Myotis lucifugus*; *a*) and hoary (*Lasiurus cinereus*; *c*) bats only increased with increasing $T_a > 41.3$ and 39.2°C , respectively. There were no significant differences between sexes in little brown and hoary bats, but EHL/MHP of silver-haired bats was significantly higher in males relative to females across all T_a . We measured EHL/MHP of individuals using open flow-through respirometry ($n = 12$ per species). Data measured at the first (lowest) T_a for each individual were excluded from statistical analyses (indicated as triangles; statistical values and equations reported in Table 1).



only measured thermoregulatory responses up to $T_a \approx 40^\circ\text{C}$ in this species (maximum $T_b \approx 42^\circ\text{C}$; Cryan and Wolf 2003). We are not aware of any similar data on the thermal physiology of silver-haired bats, which reached the cut-off T_b at $T_{a,\text{max}}$ intermediate between the other two species ($45.3 \pm 1.5^\circ\text{C}$; Fig. 1).

Hoary bats increased T_b at a faster rate with increasing $T_a > 32^\circ\text{C}$ compared with little brown and silver-haired bats ($\sim 0.2^\circ\text{C}(T_b)/^\circ\text{C}(T_a)$ faster; Table 1 and Fig. 2c). Moreover, hoary bats were the only species to reduce the rate of increasing T_b at higher T_a , maintaining a near-constant $T_b \approx 41^\circ\text{C}$ at $T_a > 42.3^\circ\text{C}$. Generalized models of heat stroke include initial rapid increases in T_b followed by the regulation of hyperthermic T_b at an approximately constant level (Leon 2006). A plateau in hyperthermic T_b at $T_a > \text{normothermic } T_b$ could arise from active metabolic suppression, a phenomenon broadly analogous to torpor that is receiving increased attention among bats and other endotherms (Lovegrove et al.

2014; Reher et al. 2018). For example, Commerson's leaf-nosed bat (*Macronycteris commersonii* (E. Geoffroy, 1813)) from Madagascar reduced RMR at high T_a (up to 40°C) to values representative of torpid individuals, despite maintaining hyperthermic T_b of up to 41.5°C (Reher et al. 2018). However, EWL and RMR of hoary bats continued to increase at the same rate with increasing $T_a > 42.3^\circ\text{C}$ (Figs. 3c and 4c), and thus we are not certain how they maintained a near-constant T_b . We cannot rule out that this is an artefact of our experimental protocol (e.g., T_b endpoints), although there was no plateau at high T_a in any thermoregulatory variables of the little brown or hoary bats, nor in a previous study on three subtropical bat species using a similar experimental protocol (Czenze et al. 2020).

We know of no studies that have measured EWL at $T_a > 30^\circ\text{C}$ in little brown or silver-haired bats, although we report the expected increase to values about 6- or 7-fold higher at $T_a \approx 46^\circ\text{C}$ compared with EWL at $T_a \approx 32^\circ\text{C}$ (Figs. 3a and 3b). Cryan and Wolf (2003) measured EWL of hoary bats, reporting about a 2-fold increase in mass-specific values from $T_a \approx 32\text{--}40^\circ\text{C}$. We similarly found a 2-fold increase in whole-animal EWL of hoary bats over the same T_a range, although we found an overall 8-fold increase from $T_a \approx 32\text{--}46^\circ\text{C}$ (Fig. 3c).

The RMR of little brown bats increased about 2.5-fold from $T_a \approx 32\text{--}46^\circ\text{C}$ (Fig. 4a); the only comparable data are from cave-dwelling conspecifics in Pennsylvania and New York (USA), which had a two-fold RMR increase from $T_a = 30\text{--}41.5^\circ\text{C}$ (Hock 1951). In contrast to our data, Hock found little brown bats subsequently decreased RMR at $T_a \approx 44^\circ\text{C}$, where death occurred rapidly; this discrepancy is likely the result of different experimental protocols (e.g., RMR was measured using a Scholander semi-microrespirometer). The RMR of hoary bats increased from the lowest experimental T_a (~ 32 to 34°C) to maximum values about 3-fold higher at the highest T_a measured (~ 44 to 47.5°C ; Fig. 4c), which is consistent with Cryan and Wolf (2003) who found that the upper critical T_a of thermoneutrality is 34°C in hoary bats. In contrast to the expected endothermic pattern of increasing RMR with increasing $T_a > \text{normothermic } T_b$ (Fig. 4b; McKechnie and Wolf 2019), there was no significant relationship between RMR and T_a in silver-haired bats. Other studies have also reported the lack of an RMR increase at high T_a in both bats and birds (e.g., Maloney et al. 1999; Noakes and McKechnie 2019; van Dyk et al. 2019).

There are no comparable EHL/MHP data for conspecifics of our study species at $T_a > 30^\circ\text{C}$, but we report maximum values higher than typically reported in bats (Figs. 5a–5c). For example, the mean maximum EHL/MHP of little brown bats (1.3 ± 0.6 at $T_a = 41.1 \pm 3.9^\circ\text{C}$), silver-haired bats (males: 1.7 ± 0.4 at $T_a = 43.1 \pm 1.8^\circ\text{C}$; females: 1.3 ± 0.4 at $T_a = 42.5 \pm 2.6^\circ\text{C}$), and hoary bats (2.0 ± 0.6 at $T_a = 41.1 \pm 3.9^\circ\text{C}$) are higher than recorded for bats using a similar experimental protocol in southern Africa. Czenze et al. (2020) reported maximum EHL/MHP of 1.2 ± 0.2 in long-tailed house bats (at $T_a = 41.8 \pm 0.06^\circ\text{C}$, $M_b = 13.6 \pm 1.6$ g) and 1.04 ± 0.56 in Mauritian tomb bats (at $T_a = 42.2 \pm 0.6^\circ\text{C}$, $M_b = 24.4 \pm 0.9$ g). As far as we are aware, the maximum EHL/MHP of hoary bats exceeds the highest values previously recorded in any bat species, EHL/MHP = 1.5 ± 0.5 at $T_a = 39.5 \pm 0.9^\circ\text{C}$ in lesser naked-backed fruit bats (*Dobsonia minor* (Dobson, 1879); $M_b = 87.0 \pm 9.3$ g) from Madang, Papua New Guinea (data extracted from fig. 4 in Bartholomew et al. 1970). Hoary bats are one of the few temperate-zone bats that roost solitarily on the open foliage of trees regardless of reproductive state (Shump and Shump 1980), and thus relatively high evaporative cooling capacities likely allow them to cope with hotter roost temperatures and high solar heat loads.

There was a significant thermoregulatory difference between sexes only among silver-haired bats, with higher EWL and EHL/MHP in males compared with females (Figs. 3b and 5b). It is possible that variation could exist between sexes due to different roosting habits in the reproductive season. However, despite the summer roosting habits of little brown bats also varying between sexes, there were no significant sex differences in any thermoreg-

ulatory responses for this species. Moreover, there were no significant differences in T_b , RMR, or $T_{a,max}$ between sexes of silver-haired bats ($T_{a,max}$), nor in the relationship of EWL and EHL/MHP with increasing T_a (i.e., $T_a \times \text{sex}$ interaction). Overall, this suggests that male and female silver-haired bats have a similar ability to cope with high T_a .

We report that foliage-roosting hoary bats have the highest summer heat tolerance and evaporative cooling of our study species (Fig. 1), which is consistent with previous studies reporting interspecific variation among sympatric bats in relation to roosting habits in the Limpopo River valley of southern Africa (Cory Toussaint and McKechnie 2012; Czenze et al. 2020). Our data are not directly comparable with those of Cory Toussaint and McKechnie (2012), as these authors did not aim to quantify upper thermoregulatory limits. However, little brown, silver-haired, and hoary bats had higher mean $T_{a,max}$ (Fig. 1) and evaporative cooling capacities than reported by Czenze et al. (2020; $T_{a,max} \approx 39\text{--}44$ °C, maximum EHL/MHP $\approx 0.75\text{--}1.3$). The apparent lower heat tolerance in southern African bats is surprising considering the Limpopo River valley is substantially hotter during summer (T_a regularly >40 °C) than the Cypress Hills, although this study had smaller sample sizes ($n = 3\text{--}6$ per species; Czenze et al. 2020). Direct comparisons among our three study species are potentially confounded by phylogeny (different genera albeit all from the same family: Vespertilionidae) and body mass (3-fold variation), but arguably less so than previous similar studies (different families, ~ 2.5 -fold M_b variation: Cory Toussaint and McKechnie 2012; different suborders, 10-fold M_b variation: Czenze et al. 2020).

Our data are consistent with the hypothesis that species using hotter summer roosts have a greater ability to cope with high T_a (Cory Toussaint and McKechnie 2012; Czenze et al. 2020). Foliage-roosting hoary bats have higher heat tolerance and evaporative cooling capacities compared with tree crevice- and cavity-roosting little brown and silver-haired bats. Ideally, future studies should investigate variation in heat tolerance and evaporative cooling capacity using multiple species per roost type while simultaneously controlling for M_b and phylogeny, although finding sympatric species meeting these criteria is challenging. However, any further studies investigating heat tolerance and evaporative cooling capacity in bats using a similar standardized protocol will add to the collective dataset to investigate interspecific thermoregulatory variation at a broader scale. Quantifying variation in chiropteran heat tolerance and evaporative cooling capacity is critical for understanding how bats will respond to changing climates.

Contributors' statement

M.J.N.: conceptualization, methodology, formal analysis, investigation, writing (original draft and editing), visualization, project administration, and funding acquisition. A.E.M.: conceptualization, methodology, resources, writing (reviewing and editing), and supervision. R.M.B.: conceptualization, methodology, resources, writing (reviewing and editing), supervision, project administration, and funding acquisition.

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Data availability

Data are available from the corresponding author (M.J.N.).

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