The heat is on: Thermoregulatory and evaporative cooling patterns of desert-dwelling bats

Ruvinda K. de Mel [a,*], Katherine E. Moseby [b], Kathleen A. Stewart [a], Kate E. Rankin [a], Zenon J. Czenze [a]

[a] Centre for Behavioural and Physiological Ecology, University of New England, Armidale, NSW, 2351, Australia
[b] School of Biological, Earth and Environment Sciences, University of New South Wales, Kensington, NSW, Australia

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ABSTRACT

For small endotherms inhabiting desert ecosystems, defending body temperatures (T_b) is challenging as they contend with extremely high ambient temperatures (T_a) and limited standing water. In the arid zone, bats may thermoconform whereby T_b varies with T_a, or may evaporatively cool themselves to maintain $T_b < T_a$. We used an integrative approach that combined both temperature telemetry and flow through respirometry to investigate the ecological and physiological strategies of lesser long-eared bats (*Nyctophilus geoffroyi*) in Australia's arid zone. We predicted individuals would exhibit desert-adapted thermoregulatory patterns (i.e., thermoconform to prioritise water conservation), and that females would be more conservative with their water reserves for evaporative cooling compared to males. Temperature telemetry data indicated that free-ranging *N. geoffroyi* were heterothermic ($T_{skin} = 18.9$–44.9 °C) during summer and thermoconformed over a wide range of temperatures, likely to conserve water and energy during the day. Experimentally, at high T_as, females maintained significantly lower T_b and resting metabolic rates, despite lower evaporative water loss (EWL) rates compared to males. Females only increased EWL at experimental $T_a = 42.5$ °C, significantly higher than males (40.7 °C), and higher than any bat species yet recorded. During the hottest day of this study, our estimates suggest the water required for evaporative cooling ranged from 18.3% (females) and 25.5% (males) of body mass. However, if we extrapolate these results to a recent heatwave these values increase to 36.5% and 47.3%, which are likely beyond lethal limits. It appears this population is under selective pressures to conserve water reserves and that these pressures are more pronounced in females than males. Bats in arid ecosystems are threatened by both current and future heatwaves and we recommend future conservation efforts focus on protecting current roost trees and creating artificial standing water sites near vulnerable populations.

1. Introduction

For small endotherms, defending body temperatures (T_b) in high ambient temperatures (T_a) is costly due to the high energy and water expenditure necessitated by their high surface area to volume ratios (McNab, 2002; Tattersall et al., 2012). At high T_as, small endotherms rely on a variety of behavioural adaptations to defend T_b (Licht and Leitner, 1967a; McKechnie and Wolf, 2019; Ochoa-Acuña and Kunz, 1999), but when $T_a > T_b$, the only physiological means of dissipating heat is via evaporative cooling (Tattersall et al., 2012). However, the effectiveness of evaporative cooling strategies appear to correlate with, among other things, sex (van Jaarsveld et al., 2021a), and roost microclimates (Czenze et al., 2021b).

Bats rest during the hottest part of the day and can tolerate high roost temperatures (T_{roost}) (>40 °C) without access to water (Bondarenco et al., 2014; Bronner et al., 1999; Maloney et al., 1999). When T_a is high, some species will allow their T_b to conform with T_{roost} (Bondarenco et al., 2014; Geiser and Brigham, 2012; Reher et al., 2018; Reher and Dausmann, 2021) and/or use evaporative cooling to defend $T_b < T_{roost}$ (Czenze et al., 2022; Czenze et al., 2021a). However, both of these strategies are associated with physiological costs, as thermoconforming at high T_as may expose animals to lethal hyperthermia (Bondarenco

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et al., 2014), while evaporative cooling increases the risk of lethal dehydration (McKechnie and Wolf, 2010; Smit and Mckechnie, 2015). Inter- and intraspecific comparrisons of small endotherms suggest that some individuals can tolerate higher T_b, or cool themselves more effectively than others, thus allowing them to contend with higher T_a(i. e, heat tolerance limit; Czenze et al., 2020a,b; Czenze et al., 2021a; Czenze et al., 2022; Noakes et al., 2021; van Jaarsveld et al., 2021a).

Bats face varying natural selection pressures on thermoregulatory strategy based on their roost choice and microclimates (Czenze et al., 2021b; Klüg-Baerwald and Brigham, 2017; Noakes et al., 2021; Reher et al., 2022). For example, species-specific differences in roost preferences are reflected in evaporative cooling capacities with species inhabiting warmer roosts exhibiting higher capacity to cool than those inhabiting cooler roosts (Czenze et al., 2021a). Indeed, some species of bats can tolerate very high T_a and Angolan free-tailed bats (*Mops condylurus*) use roosts that reach 60 °C (Bronner et al., 1999). In Australia, the inland free-tailed bat (*Ozimops petersi*) and little broad-nosed bat (*Scotorepens greyii*) can tolerate T_a of 48.1 °C (Bondarenco et al., 2014). However, temperatures during heatwaves may exceed these tolerable limits and individuals could face lethal hyperthermia and/or dehydration (Bondarenco et al., 2014; McKechnie et al., 2021; Pruvot et al., 2019).

Current climate trajectories predict the intensity, frequency and spatial extent of heatwaves to increase (Dosio et al., 2018; IPCC, 2021; Viceto et al., 2019). When compared to more mesic ecosystems, the deleterious effects of heatwaves are particularly pronounced on endotherms in the arid zone. Desert-adapted bats require significantly less water for evaporative cooling (Muñoz-Garcia et al., 2022), and at high T_as, the evaporative water loss (EWL) rates of desert long-eared bats (*Otonycteris hemprichii*) and European free-tailed bats (*Tadarida teniotis*) were <50% that of bats from mesic habitats (Marom et al., 2006). Desert populations of Kuhl's pipistrelles (*Pipistrellus kuhlii*) have significantly lower EWL rates compared to mesic populations (Gearhart et al., 2020), illustrating that desert bats are adapted to conserve water and maintain a shallow gradient in the increase of T_b at high T_a using efficient evaporative cooling. In the Australian arid zone, desert-specialist inland free-tailed bats (*O. petersi*), which roosts in tree cavities, uses bouts of energy and water saving torpor and drop their T_b to <15 °C during cold T_a (Bondarenco et al., 2013, 2016). In addition, during high T_a, *S. greyii* and *O. petersi* appear to exhibit facultative hyperthermia whereby T_b may exceed 44 °C, further saving energy and water (Bondarenco et al., 2013, 2014, 2016). However, no research has been conducted to specifically investigate the evaporative cooling capacities of Australian desert bats.

The lesser long-eared bat (*Nyctophilus geoffroyi*) has a cosmopolitan distribution across Australia, which includes the arid zone. This wide geographic distribution appears to be reflected in the species' physiological variables. For example, *N. geoffroyi* from Tasmania exhibits a lower basal metabolic rate (1.12 ± 0.03 mL O$_2$ g^{-1} h^{-1}) and a lower T_a at which they enter torpor (15 °C; Dixon and Rose, 2003) than populations from New South Wales (1.36 ± 0.17 mL O$_2$ g^{-1} h^{-1}, 25 °C; Geiser and Brigham, 2000) and Western Australia (1.42 ± 0.1 mL O$_2$ g^{-1} h^{-1}, 24.2 °C; Hosken and Withers, 1999). Data on within population differences in thermoregulatory variables are scant, but Turbill and Geiser (2006) found that at 15 °C, although not statistically significant, pregnant female *N. geoffroyi*maintained a minimum torpid metabolic rate that was 62.5% that of males and a minimum resting metabolic rate that was 82.5% that of males. During summer, these reproductive females form maternity colonies while males roost solitarily (Churchill, 2008; Turbill et al., 2020). There is limited information on evaporative water loss (EWL) differences with only one study from a population in Western Australia that increased EWL significantly when T_a > 34 °C (Hosken and Withers 1999). Although it appears there are differences in thermoregulatory capacities between and within geographically distinct populations of *N. geoffroyi*, EWL at high temperatures remains relatively understudied.

Our aim was to use an integrative approach and investigate the free-ranging and experimental thermoregulatory patterns of *N. geoffroyi* from a desert population that contends with the harshest summer conditions in the species' range. Our goal was to test the hypothesis that natural selection pressures for water conservation would be reflected in the physiological traits associated with heat tolerance limits of *N. geoffroyi*. We predicted that, like other desert adapted species, *N. geoffroyi* would tolerate high experimental T_a, prioritise water conservation and high T_b over evaporative cooling and that this would be more pronounced in females due to their summer roosting habit of forming maternity colonies. To understand this population's vulnerability to heat waves in a rapidly warming world, we used these data and roost temperature recordingsto estimate the evaporative water requirements needed for bats to avoid lethal hyperthermia during summer.

2. Methods

2.1. Study site

The study was conducted during 5–16 February 2023 in Wild Deserts within Sturt National Park (−29.068373, 141.073151), NSW, Australia. Daily mean T_a for the month of February (1998–2023) ranged from 21.8 to 35.2 °C with a mean monthly rainfall of 22.5 mm. The highest temperature recorded in the month of February is 46.5 °C while the lowest is 12.7 °C (Bureau of Meteorology, 2023). Sturt National Park is comprised of grassland and sparse woodlands dominated by mulga (*Acacia aneura*), beefwood (*Grevillea striata*) and coolabah (*Eucalyptus coolabah*) trees.

2.2. Bat capture and temperature telemetry

Bats were captured using mist nets (Ecotone, Poland) and harp traps (Faunatech Austbat). Captured bats were immediately placed in cloth bags and transported to the Wild Deserts field station where they were sexed, aged, weighed to the nearest 0.1 g, and forearm length was measured to the nearest 0.1 mm. Only non-reproductive adults were used in the study.

To record free-ranging thermoregulatory patterns, we attached temperature-sensitive radio-transmitters (~0.3 g, BD-2X, Holohil Systems Inc, Carp, Ontario, Canada) using an adhesive (ADOS F2 contact adhesive) to two adult male and six adult female *N. geoffroyi* (6.6 ± 0.4 g) after clipping a small amount of fur between the scapulae. In small bats, skin temperature provides an accurate approximation of T_b (Barclay et al., 1996; Czenze et al., 2017; Willis and Brigham, 2003). All transmitters were calibrated in a water bath against a precision mercury thermometer (traceable to NIST) over a temperature range of 5–50 °C prior to attachment. Body temperature was calculated from the pulse rate (BPM) of the transmitters using a third-order polynomial regression calibration curve. Ambient temperature was recorded for the duration of the study by an iButton (Thermochron iButton, Maxim Integrated Products Inc., Sunnyvale, CA, USA) deployed in the shade at 2 m height (*sensu* Cory Toussaint and McKechnie, 2012; Czenze et al., 2022; Stawski and Currie, 2016).

Bats were tracked to their roosts each subsequent morning and a data logger (SRX-1200, SRX-800, Lotek, Canada, custom logger *sensu* Körtner and Geiser, 1998)) was deployed and pulse rate recorded at 10 min intervals. Bats were considered to be torpid when their T_{skin} was <28 °C for more than 30 min (Bondarenco et al., 2016). Passive arousals from torpor were distinguished by T_{skin} closely following T_a while active arousals were characterised by a sharp increase in T_{skin} above the torpor threshold (Bondarenco et al., 2016; Turbill and Geiser, 2008). When accessible tree roosts ($n = 7$) were located, a temperature data logger (Thermochron iButton, Maxim Integrated Products Inc., Sunnyvale, CA, USA; logging interval 60 min) was deployed in the roost to record T_{roost} later when the bat was not occupying the roost.

2.3. Gas exchange measurements

The bats used in the respirometry experiments (11 adult females, 6 adult males) were transported in cloth bags to the research station after capture and were held in a quiet, dark room for <48 h. Every morning and evening bats were offered water using a pipette and fed mealworms (*Tenebrio molitor*) until satiated or until they gained ≥10% body mass. After feeding individuals on the first night, a temperature sensitive PIT-tag (Biotherm, Biomark, Biose ID, USA, accuracy ± 0.5 °C) was implanted subcutaneously between the scapulae of each individual. Gas exchange measurements began the morning after initial capture.

An open flow-through respirometry system was used to measure carbon dioxide ($\dot{V}_{CO2}$), oxygen ($\dot{V}_{O2}$), and evaporative water loss (EWL) during measurements. Airtight respirometry chambers were constructed from 0.5 L glass pickle jars and included a wire mesh platform elevated ∼2 cm above ∼1 cm of mineral oil on the bottom, intended to trap excreta. The walls and top of the chamber were covered in mesh to allow the bat to crawl and hang in natural roosting postures. The chamber was placed in a modified warming/cooling chest (WAECO mobile refrigeration unit: 14 L), the temperature of which could be controlled manually. The T_b readings from the PIT-tags were recorded every 10 s throughout the procedure using a racket antenna placed inside the ice box next to the chamber (Biotherm, Biomark, Biose ID, USA).

Air was supplied using an air pump with a maximum capacity of 30 L min⁻¹. Atmospheric air was passed through a column of silica to scrub water and this dried air was then split into baseline and chamber channels. A needle valve was used to regulate the baseline air flow while a mass flow controller (MFC; model MC-10SLPM-D/5m, Alicat Scientific Inc., Tuscon AZ, USA) was used to regulate the chamber airflow. The air inlet in the chamber was placed near the top of the chamber while the air outlet was placed below the mesh platform to maximise air mixing. The flow rate was adjusted as necessary depending on the behaviour of the bat at each T_a. Flow rates ranged from 0.47 to 3.56 L min⁻¹.

Excurrent air from the baseline and chamber was split using a multiplexor (MUX3-1101-18M, Sable systems) in manual mode and then subsampled using a SS4 (Sable Systems). Subsampled air was pulled through a CO_2/H_2O analyser (LI-840A, LI-COR, Lincoln, NE, USA) and an oxygen analyser (Sable Systems, FC-10). The CO_2/H_2O analyser was regularly zeroed using nitrogen and spanned for CO_2 using a known CO_2 concentration (2000 ppm). The H_2O sensor of the LI-840A was also regularly zeroed using nitrogen and spanned using air saturated with H_2O at two dew point temperatures as described by Marom et al. (2006). Bev-A-Line IV tubing (Thermoplastic Processes Inc.) was used within the system. An analog-digital converter (UI-2, Sable Systems, Las Vegas, NV, USA) was used to digitise the voltage outputs from the analysers and was recorded using Expedata software (Sable Systems) with a sampling interval of 5s.

2.4. Experimental protocol

Experimental runs, were conducted during the diurnal rest phase of the bats (07:00–17:00) following established methods (Czenze et al., 2022; Whitfield et al., 2015) where animals were exposed to incrementally higher T_as in a stepped profile; a method that is analogous to the sliding cold exposure protocol used for summit metabolism measurements (Swanson et al., 1996). This method was used as it produces results indistinguishable from steady-state measurements without prolonged exposure to deleteriously high temperature (Short et al., 2022).

Individuals spent 1 h at $T_a = 28$ °C to acclimatise to the respirometry chamber before we recorded physiological values. Data collection was initiated at $T_a = 28$ °C and T_a was increased in 4 °C increments until 40 °C. Thereafter, the T_a was increased in 2 °C increments. Temperature inside the respirometry chamber was measured with a thermocouple probe (Type T thermocouple; 0.1 °C accuracy) inserted into the lid and connected to a digital thermometer (Omega Handheld Digital

Thermometer HH81A). At each subsequent increase in T_a, individuals spent a minimum of 20–30 min at this experimental T_a before we recorded values. Once an individual had been exposed to the experimental T_a for 20–30 min, we waited for T_b, $\dot{V}_{CO2}$, and EWL to stabilise at constant values before we recorded a 5-min sample. Individuals were continuously monitored using an infra-red video camera and runs were immediately terminated when the individual displayed signs of having reached its heat tolerance limit. These included loss of coordination, rapid and uncontrolled increase in T_b, prolonged ceaseless or escape behaviour. Immediately after removal from chamber, bats were weighed and offered water. Bats were then placed in cloth bags and allowed to rest before being offered mealworms and water 1 h later, and then again before being released at the site of capture the same night. Each experiment lasted 4.5–5.5 h in total. This protocol has been used to measure heat tolerance limit and evaporative cooling capacities of bats (Czenze et al., 2022; Czenze et al., 2021a; Noakes et al., 2021), birds (Czenze et al., 2021a; Czenze et al., 2020b) and rodents (van Jaarsveld et al., 2021b) and individuals have been recaptured without losing mass (Czenze et al., 2021b, 2022).

2.5. Statistical analysis

Analyser drift and lag were corrected using Expedata software. Equations 9.5 and 9.6 (Lighton, 2008) were used to calculate $\dot{V}_{CO2}$ and EWL from the lowest stable 5-min periods of CO_2 and water vapour at each T_a, assuming 0.803 mg H_2O ml⁻¹ vapour (Lighton, 2008). Resting metabolic rate (RMR) was calculated from $\dot{V}_{CO2}$ assuming a respiratory exchange ratio = 0.71 (Walsberg and Wolf, 1995) and $\dot{V}_{CO2}$ was converted to metabolic rate (W) assuming 27.8 J CO_2 ml⁻¹ (Withers, 1992) since individuals were post-absorptive. Whole animal RMR (waRMR) and whole animal EWL (waEWL) was calculated for each individual and subsequently sex. Rates of waEWL were converted to evaporative heat loss (EHL, W) assuming a latent heat of vapourisation of water of 2.406 J/mg at 40 °C (Tracy et al., 2010). All statistical analyses were performed in R 4.2.3 (R Core Team, 2021). The *SEGMENTED. LME* package (Muggeo, 2016) was used to identify inflection points of the physiological responses in relation to T_a. ANOVA conducted between the segmented regression and the linear regression was statistically different in favour of the segmented regression for all physiological responses to T_a. When comparing the inflection points between males and females, we considered a lack of overlapping confidence intervals to be a significant difference. Linear mixed effect models were then fitted using the NLME package (Pinheiro et al., 2009) above the sex-specific inflection points. Individual identity was included as a random factor in the models to account for repeated measurements from individuals. Chamber temperature, body mass (M_b), sex and their interaction terms were used as predictor variables in the global models. We compared the global models to reduced models without the interaction term and, based on null hypothesis testing, we selected which predictor variables to retain in the final model (Beilke and O'Keefe, 2023; Tredennick et al., 2021). In all cases M_b was non-significant and was not included in the final models. Estimated marginal means for T_b, waEWL, waRMR and EHL/metabolic heat production (MHP) were calculated using the emmeans package (Lenth, 2021). In all analyses, significance was assigned at $\alpha < 0.05$ or in cases where confidence intervals did not overlap.

2.6. Estimated evaporative water requirements

Daily evaporative water requirement was defined as the mass of H_2O required for bats to maintain thermal balance while resting (Czenze et al., 2022). We estimated water requirements for the hottest day of our study period (11 February, maximum $T_a = 42.7$ °C), and the hottest day on record for the locality (January 12, 2013, maximum $T_a = 48.1$ °C;

Bondarenco et al., 2014; Bureaue of Meteorology, 2023). We used the NicheMapR micro-climate hindcaster to generate T_a values for January 12, 2013 (Kearney and Porter, 2017). Using the T_{roost} that was best correlated with T_a ($R^2 = 0.96$) we calculated the operative T_{roost} for the 2013 heatwave. We then used these T_{roost} values to estimate the evaporative water requirement using the sex-specific relationships between sex and EWL at high T_as.

3. Results

3.1. Temperature telemetry

We collected 35 bat-days of temperature-telemetry data. During 5–16 February, the shaded iButton recorded a mean daily maximum T_a of 37.0 ± 2.9 °C (absolute minimum 17.2 °C, absolute maximum = 42.7 °C, daily range = 14.2 ± 3.5 °C). All bats roosted under exfoliating bark of both live and dead trees ($n = 18$). Roost temperatures were higher than T_a (mean daily maximum $T_{roost} = 39.2 ± 4.0$ °C; absolute maximum 48.7 °C) and maximum T_{skin} of resting bats exceeded 40 °C on 16 bat days (mean maximum daily $T_{skin} = 39.9 ± 2.9$ °C), with an absolute maximum of 44.9 °C (Table 1).

We recorded torpor bouts on 10 bat-days ($n = 5$ individuals). Torpor bouts occurred within the first 3 h of sunrise ($T_a = 20.5$–31.6 °C; Fig. 1), with a mean duration of 99 ± 52 min. The longest torpor bout we recorded was 190 min, and individuals appear to rewarm both passively ($n = 6$) and actively ($n = 4$) with increasing T_a (Fig. S1).

3.2. Respirometry

The mean maximum T_a tolerated by female *N. geoffroyi* ($n = 11$; 45.0 ± 1.4 °C; one female tolerated 48.1 °C before reaching its heat tolerance limit) was approximately 2 °C higher than males ($n = 6$; 43.38 ± 1.6 °C; Table 2). Although both sexes defended similar T_b at their heat tolerance limit (female = 42.9 ± 0.4 °C; male = 43.1 ± 0.5 °C) and reached an identical absolute maximum T_b of 43.9 °C, the estimated marginal mean of T_b for females was significantly lower than males at every experimental T_a (40–46 °C; Table S1).

All variables increased linearly with T_a for both sexes (Table S1). Both males and females showed distinct inflection points in the relationship between increasing T_a and T_b, waEWL, waRMR, and EHL/MHP (Fig. 2). There were no significant differences in the inflection points of T_b, waRMR or EHL/MHP between males and females. However, females tolerated a significantly higher T_a (42.5 °C, CI = 42.0–42.9 °C) than males (40.7 °C, CI = 40.22–41.53 °C) before increasing the rate of waEWL (Fig. 2).

In the final models for both T_b and waEWL, sex and T_a were included as fixed effects with females exhibiting lower values than males at high temperatures (Table 3). For the relationship between waRMR and T_a, the final model included the interaction term T_a x sex, which indicated that females increased waRMR more gradually at higher T_as than males (Fig. 2b). Although the values for EHL/MHP were higher for females at most high temperatures, there was no statistical sex difference in the relationship between increasing T_a and EHL/MHP (Fig. 2d).

Table 1

Summary of skin temperature (T_{skin}) of adult *N. geoffroyi* ($n = 8$), ambient temperature (T_a) and roost temperature (T_{roost}) ($n = 7$), from 5–15 Feb in Wild Deserts within Sturt National Park. All values are in °C and mean ± SD are reported.

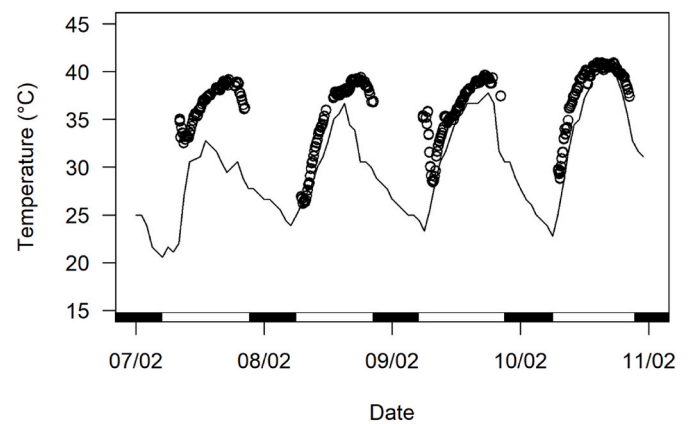|	T_{skin}	T_a	T_{roost}
Mean daily minimum	29.6 ± 4.7	22.8 ± 2.8	22.7 ± 2.9
Absolute minimum	18.9	17.2	16.7
Mean daily maximum	39.9 ± 2.9	37.0 ± 2.9	39.2 ± 4.0
Absolute maximum	44.9	42.7	48.7
Absolute maximum daily range	10.3 ± 4.7	14.2 ± 3.5	16.4 ± 4.8

Fig. 1. Skin temperature fluctuation (open circles) of a female *Nyctophilus geoffroyi* at Wild Deserts within Sturt National Park, NSW, Australia during February 2023. Shaded air temperature indicated by the unbroken line and black and white bars denote night and day respectively.

Table 2

Summary of thermoregulatory performance as a function of air temperature (T_a) in *Nyctophilus geoffroyi* from Wild Deserts within Sturt National Park (5–16 February 2023). Thermoregulatory variables include subcutaneous temperature (T_b), whole animal evaporative water loss (waEWL), whole animal resting metabolic rate (waRMR) and evaporative heat loss/metabolic heat production (EHL/MHP). 'Max' and 'Min' indicate maximum and minimum values for each variable respectively. Mean values are reported ± SD with sample sizes in parentheses. Inflection points reported with 95% confidence intervals.

Variable	Male	Female
Body mass (g)	6.8 ± 0.7 (6)	6.9 ± 0.7 (11)
Subcutaneous temperature (T_b)		
Min. T_b (°C)	34.8 ± 0.3 (4)	35.5 ± 1.5 (9)
Inflection T_a (°C)	33.2	34.1
	32.5–36.1	33.0–36.1
Max. T_b (°C)	43.6 (1)	42.9 (1)
	42.9 ± 0.5 (4)	42.8 ± 0.6 (6)
Max. Experimental T_a (°C)	46 (1)	48 (1)
	44 (3)	46 (5)
Evaporative water loss		
Min. waEWL (gh^{-1})	0.02 ± 0.01 (4)	0.02 ± 0.02 (9)
Inflection T_a (°C)	40.7	42.5
	40.2–41.5	42.1–42.9
Max. waEWL (gh^{-1})	0.35 (1)	0.40 (1)
	0.33 ± 0.09 (4)	0.35 ± 0.08 (6)
Max. waEWL/Min. waEWL	17.5	20
Min. EHL/MHP	0.26 ± 0.16 (4)	0.21 ± 0.06 (10)
Max. EHL/MHP	1.79 (1)	2.18 (1)
	1.23 ± 0.08 (4)	1.94 ± 0.81 (6)
Resting metabolic rate		
Min. waRMR (W)	0.05 ± 0.01 (5)	0.06 ± 0.02 (10)
Inflection T_a (°C)	38.5	39.6
	37.1–40.30	37.9–41.6
Max. waRMR (W)	0.18 ± 0.04 (4)	0.13 ± 0.04 (6)
Max. waRMR/Min. waRMR	3.60	2.16

3.3. Evaporative water requirements

On 11 February we recorded $T_{roost} > 40$ °C for 7 h. During this time T_{roost} exceeded the waEWL inflection point of males (40.7 °C) and females (42.5 °C) for 7 and 4 h, respectively. Males required 1.74 g (25.5% body mass) while females required 1.24 g (18.3% body mass) of water to maintain thermal balance. During the 2013 heatwave, our estimates suggest that T_{roost} may have remained >45 °C for over 5 h and >48 °C for over 3 h. These T_{roost} would have exceeded the male and female inflection point of waEWL for 6 and 4 h, respectively. During this time, males would have required 3.2 g (47.3% body mass) of water while
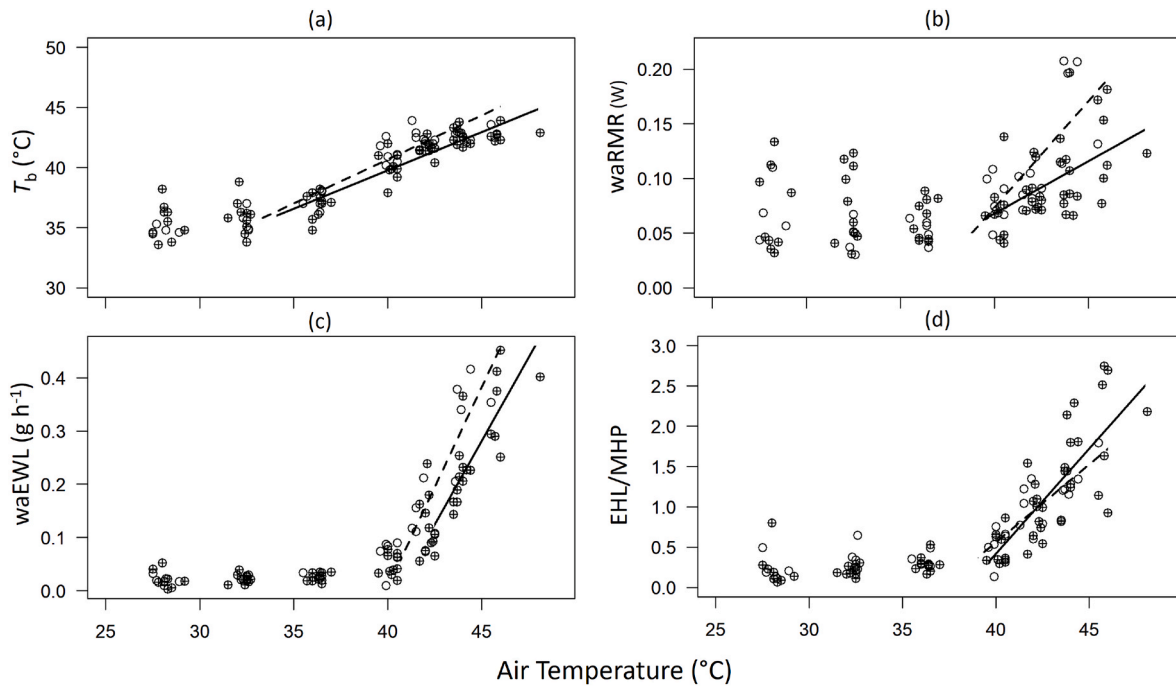
Fig. 2. (a) Subcutaneous body temperature (T_b), (b) whole animal resting metabolic rate (waRMR), (c) whole animal evaporative water loss (waEWL) and (d) evaporative heat loss (EHL)/metabolic heat production (MHP) at high air temperatures of male ($n = 6$) and female ($n = 11$) lesser long-eared bats (*Nyctophilus geoffroyi*). Dashed and solid lines represent regressions for males and females respectively above inflection points (Table 3). Open circles indicate males and filled circles indicate females.

Table 3
Final reduced linear mixed effect models of thermoregulatory performance as a function of chamber air temperature (T_a) in *Nyctophilus geoffroyi* from Sturt National Park (5–16 February 2023). Thermoregulatory variables include subcutaneous temperature (T_b), whole animal evaporative water loss (waEWL), whole animal resting metabolic rate (waRMR) and evaporative heat loss/metabolic heat production (EHL/MHP). Numbers in parenthesis = (no. of observations, no. of individuals). Interactions between fixed effects denoted with 'x'.

Dependant variable	R^2	Fixed effect	d.f.	t	p
T_b (71, 17)	0.83	T_a	59.48	17.97	<0.001
		Sex	14.51	−3.36	<0.001
waRMR (55, 17)	0.51	T_a	45.38	4.78	<0.001
		T_a x Sex	44.82	−2.06	0.045
waEWL (31, 17)	0.67	T_a	28	7.80	<0.001
		Sex	28	−2.95	0.006
EHL/MHP (55, 17)	0.64	T_a	49.29	9.42	<0.001

females would have required 2.5 g (36.5% body mass) of water to avoid lethal dehydration.

4. Discussion

In the hottest part of their range, *N. geoffroyi*'s patterns of thermoregulation during hot weather mirror those of desert specialists. Our results indicate that the *N. geoffroyi* from this population are heterothermic and may use torpor and possibly facultative hyperthermia depending on the conditions. Female *N. geoffroyi* appear to be more tolerant of high temperatures and are substantially more conservative with their water reserves than males. In fact, females only began active evaporative cooling at $T_a = 42.5$ °C which is, to our knowledge, the highest waEWL inflection point ever recorded for a bat. This reflects

adaptations likely driven by selective pressures for dehydration risk avoidance and water conservation in arid desert ecosystems where water is scarce or absent.

4.1. Temperature telemetry

All bats we tracked roosted under exfoliated bark that was relatively unbuffered from ambient conditions. During this period, one roost ranged from 20.4 to 48.4 °C in a single day and we recorded T_{skin} that fluctuated in a similar manner (21.0–44.2 °C). This degree of thermal flexibility is critical as individuals can reduce energy and water expenditure during torpor in the early mornings when temperatures are low and drastically reduce their evaporative water loss when T_a is high (Bondarenco et al., 2016; Cryan and Wolf, 2003; Maloney et al., 1999; Marom et al., 2006; Muñoz-Garcia et al., 2022). The bats from our study used relatively short torpor bouts (maximum duration = 190 min) when the T_a was at a minimum (06:21–09:51; $T_a = 21.6$–31.6 °C, Fig. 1). Further, although we recorded both passive and active arousals from torpor that occurred at $T_{skin} = 25.6$–27.9 °C when $T_a = 24.4$–31.6 °C (Fig. 1), passive arousals were more common. The energetic costs of rewarming from torpor can be reduced by >50% if an individual can passively rewarm (Currie et al., 2015; Turbill et al., 2008). By using a single torpor bout to reduce energy demands when temperatures are low and then passively rewarming when temperatures increase at midday while within an unbuffered tree roosts allows individuals to minimize energy and water use during lower temperatures. This is particularly important as the saved water can then be used in evaporative cooling during the hottest part of the day (Czenze et al., 2022; Licht and Leitner, 1967b; Maloney et al., 1999).

The highest T_{roost} we recorded (48.7 °C) was considerably higher than the corresponding T_a recorded in the shade (40 °C). As most roosts would be directly exposed to full sun and solar radiation, it is likely that they regularly have operative temperatures higher than T_a. The scarcity of trees in this ecosystem limits the number of suitable roosts available to *N. geoffroyi*. Although we never recorded T_{skin} from individuals in roosts we outfitted with iButtons, *N. geoffroyi* appear to contend with

high T_{roost} by allowing T_{skin} to increase to values similar to other species inhabiting hot roosts (Bronner et al., 1999; Cory Toussaint and McKechnie, 2012; Czenze et al., 2022). Hyperthermia tolerance allows individuals to avoid lethal dehydration, and the maximum T_{skin} we recorded (44.9 °C) in a roost is the highest ever recorded for a free-living *N. geoffroyi*. Further, to the best of our knowledge, this T_{skin} for a resting bat is only exceeded by two individual *O. petersi* recorded with resting T_{skin}s of 45.8 °C and 45.4 °C (Bondarenco et al., 2014). The physiological mechanisms by which these bats allow their T_b to reach these extremes are unknown, but may be a result of reduced thermogenesis through active metabolic suppression (Lovegrove et al., 2014), which has recently been recorded where bats remain torpid at very high temperatures (Reher et al., 2018; Reher and Dausmann, 2021). Although the strategy of using torpor combined with hyperthermia has only been recorded in one species of Malagasy bat (*Macronycteris commersoni*), it may be that other species that share similar natural selection pressures (i.e., thermally unbuffered roosts in dry arid ecosystems) are capable of this strategy and this is simply yet to be discovered. Regardless, tolerating very high T_b is an efficient strategy to conserve water in extremely hot and dry environments where standing water is absent or extremely scarce.

Roosting habitat is critical for bats in the Australian desert (Williams and Dickman, 2004). If roost temperatures exceed the tolerable levels, an individual may be forced to abandon the roost or face mortality (Flaquer et al., 2014). Roost abandonment comes with several risks for individuals as they may be taken by birds of prey or potentially overheat while flying during warmer temperatures (Speakman et al., 1994; Voigt and Lewanzik, 2011). Further, unlike other species that may have a range of microclimates to choose from, *N. geoffroyi* in this population is limited to small dead trees with few deeper hollows available (unpublished data). Larger, living trees with trunk and branch hollows are preferred by *O. petersii* (unpublished data) as they provide more buffered microclimates due to active water transport throughout the tree via transpiration (Briscoe et al., 2014; Kunz et al., 2003). Indeed, Bondarenco et al. (2014) found that bats roosting in small dead trees abandoned their roosts in Sturt National Park during a 2013 heatwave. Therefore it is critical that conservation managers identify and protect older living trees to aid conservation efforts for arid zone bats.

4.2. Respirometry

The heat tolerance limits, T_bs at these limits, evaporative scopes, and maximum evaporative cooling capacities we recorded fit within the results from previous work on other bat species (Czenze et al., 2020b; Czenze et al., 2021b, 2022; Licht and Leitner, 1967b; Maloney et al., 1999; Noakes et al., 2021). Interestingly, we found that most physiological variables varied between sexes, suggesting that females may possess higher overall heat tolerance capabilities.

Although both females and males tolerated similar mean maximum T_b (~42.9; Table S1), the T_a at which these T_b were reached was significantly higher in females ($T_a = 46$ °C) than males ($T_a = 41.3$ °C). Further, at all temperatures between 40 and 46 °C, females maintained significantly lower T_b than males (Estimated marginal means, Table S1, fitted lines Fig. 2). Our knowledge about sex differences in T_b at high T_a is relatively scant. A study of three insectivorous bats from Canada found no sex differences in maximum T_b (Noakes et al., 2021) and similarly Czenze et al. (2022) found no significant sex differences in maximum T_b among three of four species of insectivorous bats from Poland. However, one species (*Nyctalus noctula*) did exhibit sex differences in T_b with females increasing T_b more gradually than males at high temperatures (Czenze et al., 2022). The authors argue that, due to roosting habits, female bats from these mesic habitats face stronger selection pressures for effective thermoregulation at high T_a. Our results agree with this interpretation and suggest that these selection pressures may be more pronounced in desert ecosystems.

Femaleshad lower metabolic rates compared to males when thermoregulating at high temperatures, and the slope at which their waRMR increased with chamber temperatures was significantly lower (Fig. 2). This, coupled with lower concomitant rates of waEWL, leads to more efficient evaporative cooling by females compared to males at high temperatures. For example, at $T_a = 44$ °C females' waRMR was only 57% of males, and females evaporatively dissipated 151% of metabolic heat produced compared to 123% for males. Unlike birds, waRMR does not increase substantially to power the increased waEWL in bats as they do not pant. This low waRMR at high T_as allow them to generate less heat and thereby maintain a low T_b. Females also maintained significantly lower values of waEWL than males at high T_a and this was particularly evident at $T_a = 40$ °C and 44 °C where females' waEWL was only 70% and 65% of males. The trend of greater cooling efficiency by females is similar to southern yellow-billed hornbills (*Tockus leucomelas*; van Jaarsveld et al., 2021a) that spend the summer entombed in high temperature/humidity tree hollows without access to water and are reliant on metabolic water production from food provisioned by the male. The conservative water economy by female *N. geoffroyi* is comparable to results from similarly sized European female bats in Poland at 40 °C (*Pipistrellus nathusii*, *P. pygmaeus* and *P. pipistrellus*; Czenze et al., 2022). However, our results from desert-dwelling *N. geoffroyi* indicate an even wider disparity than the study from temperate Poland and point to a greater selection pressure for water conservation and efficient thermoregulation at high T_a on females. In the North American arid zone, non-reproductive fringed myotis (*Myotis thysanodes*) females' drinking habits fluctuated with weather, but lactating females bats required water every night and drank 13 times more often than non-reproductive females (Adams and Hayes, 2008). Many Australian arid zone bats do not have regular access to standing drinking water and although we did not study bats during periods of lactation, a conservative water economy may be a strategy by females to allocate water savings from evaporative cooling to milk production. Together, our data further emphasizes that species facing limitations in their daily water intake due to scarcity or the unpredictability of prey acquisition rates are subject to significant evolutionary pressure for water conservation.

Females from our study did not markedly increase their waEWL rates until $T_a = 42.5$ °C, which represents the highest such value yet recorded (~34–41°C; Czenze et al., 2022; Noakes et al., 2021). Consequently, females delay the onset of evaporative cooling behaviours like gaping or saliva spreading, and thus reduce EWL, until extremely high temperatures. Several physiological traits vary among populations of *N. geoffroyi* (Dixon and Rose, 2003) but, to our knowledge, the only data on EWL rates of *N. geoffroyi* comes from the temperate zone of Western Australia (Hosken and Withers, 1999). Although we caution that direct comparison to this work may not be appropriate as some of the experimental design differed, the previous study recorded an inflection point in EWL at 34 °C compared to 40.7 °C (males) and 42.5 °C (females) in this study. Further, after digitizing the data from Figure 3 of Hosken and Withers (1999), it appears that average values of mass specific EWL at $T_a = 40$ °C (~10.6 mg H_2O g^{-1} h^{-1}) are 155% of female bats from this study (6.8 mg H_2O g^{-1} h^{-1}). This indicates that the *N. geoffroyi* populations adapted to arid conditions delay the increase of EWL until higher temperatures and consequently lower their risk of dehydration. We recommend that further studies investigate the EWL of *N. geoffroyi* from other ecoregions across Australia to determine the phenotypic flexibility or geographic differences in thermoregulatory traits of a wide-ranging species.

Even minor fluctuations in summer temperatures can trigger substantial and cascading impacts on desert populations. Using our waEWL data from respirometry experiments in conjunction with T_{roost} we recorded on the hottest day of our study period (11 February), we estimate females would have required 18.3% of body mass (1.24 g) allocated to water loss for evaporative cooling while males would have required 25.5% (1.74 g). However, during the heatwave of 2013 in Sturt National Park, our data suggest that T_{roost} would have exceeded the highest T_a tolerated by a single bat in our study, and the water

requirements for evaporative cooling would have been 36.5% (2.5 g) and 47.3% (3.2 g) for females and males, respectively. The lethal dehydration limit for bats is high among mammals (Studier, 1970). A study on *Myotis lucifugus* (~6.7 g) and *Myotis yumanensis* (~5.5 g) reported that water loss greater than 31% body mass was lethal for half the study sample (Studier, 1970). If we use 31% as an estimate for mortality, heat waves like those recorded in 2013 may have led to mortality in individuals occupying poorly buffered roosts, particularly for juveniles that are at higher risk of dehydration (Griffiths, 2022). The severe effects of heatwaves on Australian pteropodid bats and European insectivorous bats has already been documented (Flaquer et al., 2014; Ratnayake et al., 2019; Welbergen et al., 2008), and as heatwaves become more frequent due to climate change this threat will only increase (Cowan et al., 2014; Dosio et al., 2018).

In Australia, water plays a critical role for bats regardless of their ecoregion, but particularly in the arid zone (Blakey et al., 2018). Some arid zone bat species may be able to survive without access to permanent water so long as food availability and roosting potential is high (Williams and Dickman, 2004). However, when artificial watering points were placed in Sturt National Park the species richness and diversity were concentrated at the points up to five times higher than sites away from water (Velez, 2002). The management strategy of artificial watering points in arid zones is contentious (Letnic et al., 2014), and in other parts of the world they are used as a conservation strategy for bats (Korine et al., 2016) but they should be considered on a case by case basis for certain populations of Australian arid zone bats.

5. Conclusion

Our data highlight sexual variation in thermoregulatory strategies in endotherms from desert environments. Females demonstrate more efficient water conservation than males that may reflect selection pressures related to reproduction. We recommend future studies with larger sample sizes further exploring the variation in EWL at high temperatures as our preliminary results lend support to intraspecific variation in EWL between arid and mesic populations (Gearhart et al., 2020; Muñoz-Garcia et al., 2016, 2022). Bat populations in hyper-arid ecosystems are at risk of hyperthermia and dehydration from current and future heatwaves, which underscores the importance of immediate conservation efforts such as creating artificial waterholes, installing bat boxes and creating artificial tree-hollows to prevent these species from being locally extirpated.

Statement on inclusion

Our study is a combined effort of scientists originating from Australia, Canada, and Sri Lanka. We consulted local experts when designing our methodology and publications by Australian scientists have been heavily cited.

Data availability statement

Data available from authors upon reasonable request.

Funding sources

CRediT authorship contribution statement

Ruvinda K. de Mel: Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Katherine E. Moseby:** Writing – review & editing, Supervision, Methodology, Investigation. **Kathleen A. Stewart:** Writing – review & editing, Methodology, Investigation. **Kate E. Rankin:** Writing – review & editing, Methodology, Investigation. **Zenon J. Czeze:** Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing Interest

Authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2024.103919.

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