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Bat thermoregulation in the heat: seasonal variation in evaporative cooling capacities in four species of European bats

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A B S T R A C T

Phenotypic flexibility is an important source of physiological variation in endotherms and plays an integral role in species' response to rapid environmental changes. Studies of phenotypic flexibility have focused on winter acclimatization and cold endurance, and there are fewer data on summer acclimatization and adjustments in heat dissipation capacity, especially in Temperate-Zone species. We used indirect calorimetry and thermometry to test if thermoregulation at high air temperatures (T_a) varies between spring and summer in four species of European vespertilionid bats: *Nyctalus noctula*, *Pipistrellus nathusii*, *P. pygmaeus*, and *P. pipistrellus*. We measured subcutaneous body temperature (T_{sub}), evaporative water loss, and resting metabolic rate while exposing bats to a stepped profile of increasing T_a, from 28 °C–48 °C. We predicted that during summer, bats increase heat tolerance and evaporative cooling capacity, to better tolerate hotter T_as. In contrast, we found lower maximum ratios of evaporative heat loss (EHL) to metabolic heat production (MHP) during summer, but no seasonal differences in maximum T_a tolerated or T_{sub}. The main cause of this seasonal difference in maximum EHL/MHP seems to be from bats increasing EWL more gradually with increasing T_a in summer than spring, particularly in the smaller *Pipistrellus* species. Therefore, this seasonal variation in heat-dissipation strategies may reflect enhanced water conservation during summer to avoid dehydration, as bats are confined to roosts for longer and hotter days compared to spring.

1. Introduction

Among endothermic animals (birds and mammals), physiological traits associated with thermoregulation can vary with climate at both inter- and intraspecific levels (Chown and Gaston, 2016; Dunbar and Brigham, 2010; Fristoe et al., 2015; Jetz et al., 2012; Naya et al., 2012; Wikelski et al., 2003), and phenotypic flexibility is a major contributor to this variation (Piersma and Gils, 2011). In response to seasonal changes in the environment, many endotherms flexibly adjust thermoregulatory traits such as normothermic body temperature (Alagaili et al., 2017; Boyles et al., 2017; Glanville and Seebacher, 2010) and metabolism (Downs et al., 2012; Genoud and Christe, 2011; Smit and McKechnie, 2010; Swanson, 2010). Studies on phenotypic flexibility have historically focused on endotherms' adjustments to cold winters and specifically on physiological traits related to thermoregulation at low air temperatures (T_a) (Dawson and Marsh, 1989; Heldmaier, 1989; Lyman, 1982; Swanson and Vézina, 2015). Comparatively fewer studies have investigated changes in thermoregulatory traits during acclimatization to hotter summers.

For many endotherms, summer comes with costs associated with dissipating heat via evaporative water loss (EWL) at high T_a (Bartholomew, 1972; Marder and Gavrieli-Levin, 1986; McKechnie and Wolf, 2019; Schmidt-Nielsen, 1997). The ratio of evaporative heat loss (EHL) to metabolic heat production (MHP) correlates with heat tolerance in arid-zone passerine birds and its limits appear more constrained by increasing MHP at high T_a than by an individual's maximum capacity to increase EWL (i.e., evaporative scope (McKechnie et al., 2021)). To the best of our knowledge, published data on seasonal variation in heat dissipation traits at high T_a (T_a > body temperature) among endotherms is limited to emus (*Dromiceius novaehollandiae;* (Maloney and Dawson, 1994a; Maloney and Dawson, 1994b) and three southern African bird species (*Plocepasser mahali* (Noakes et al., 2016), *Caprimulgus tristigma* (O'Connor et al., 2017), and *Chaetops frenatus* (Oswald et al., 2018)). As far as we are aware, no studies have quantified whether seasonal changes in abiotic conditions are associated with changes in evaporative cooling capacity at high T_a in mammals, except for a summer increase in maximum sweating rates in exercising humans (Torii, 1995).

Spring temperatures can be highly variable and many European bat species rely on torpor (a physiological state characterized by reduced metabolic rate and body temperature) to conserve energy during cold days, and roost temperature can influence bat occupancy (Bartonička and Řehák, 2007; Fjelldal et al., 2023; Ruczyński, 2006; Wojciechowski

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et al., 2007). However, the artificial roost boxes that many species rely on due to anthropogenic land-use changes (Kunz and Parsons, 2009) can become hotter than natural roost sites during hot days (Bideguren et al., 2019; Crawford and O'Keefe, 2021; Czenze et al., 2022b; Lourenço and Palmeirim, 2004), which may place individuals at risk of heat-related mortality (Alcalde et al., 2017; Flaquer et al., 2014). Bats inhabiting sun-exposed artificial roosts may already be at risk of lethal dehydration during present-day heat waves (Czenze et al., 2022b), and anthropogenic climate change is driving an increase in the frequency and intensity of extreme heat events (IPCC, 2021). Heatwaves are also predicted to occur earlier in the year in the upcoming decades (IPCC, 2021) and thus bats may be at increased risk during spring if they are not acclimatized to cope with hotter T_a. However, no studies have measured seasonal acclimatization in heat tolerance and evaporative cooling capacity of bats to quantify this risk.

We investigated thermoregulation at high T_a in four species of north temperate-zone vespertilionid bats that inhabit artificial roost boxes: *Nyctalus noctula*, *Pipistrellus nathusii*, *P. pygmaeus*, and *P. pipistrellus*. We measured resting metabolic rate (RMR), EWL, and subcutaneous body temperature (T_{sub}) over progressively higher T_as. We tested whether bats adjust their physiology in response to increasing environmental temperatures during the active season, by comparing heat tolerance, evaporative scope (minimum/maximum EHL), and EHL/MHP of male bats during spring and summer. The summer data from males were previously published in a study comparing the responses of bats between sexes in mid-summer (Czenze et al., 2022b). In contrast to previous studies on seasonal acclimatization in endotherms that compare responses between winter and summer (e.g., Noakes et al., 2016; O'Connor et al., 2017; Oswald et al., 2018), we compared responses between spring and summer, as these bats hibernate during the winter (Murariu and Gheorghiu, 2011; Petersons, 2004; Voigt et al., 2014). We predicted that males captured in mid-summer will tolerate higher T_a and have greater maximum evaporative cooling capacities compared to males captured in mid-to late spring, which would allow them to conserve water while enhancing heat loss during hot summer days.

2. Methods

2.1. Study site and species

All bats were collected from sawdust concrete roost boxes near Toruń (53.02 °N, 18.57 °E), Solec Kujawski (53.06 °N, 18.23 °E), and at the field station of Nicolaus Copernicus University in Sobiny, Poland (53.66 °N, 18.40 °E). In spring (May 3, 2021–June 7, 2021), we captured males of four species: *Nyctalus noctula* (body mass, $m_b = 24.1 \pm 2.3$ g, n = 10), *Pipistrellus nathusii* ($m_b = 6.2 \pm 0.5$ g, n = 10), *Pipistrellus pygmaeus* ($m_b = 4.1 \pm 0.3$ g, n = 9), and *Pipistrellus pipistrellus* ($m_b = 4.4 \pm 0.3$ g, n = 4). We only collected data from male bats as females are typically reproductively active during spring. We compare the data from spring to previously published data collected from males during the following summer (July 26, 2021–August 22, 2021), which was part of a study on sex-differences in heat tolerance and evaporative cooling capacity in the same species: *N. noctula* ($m_b = 29.4 \pm 1.9$ g, n = 10), *P. nathusii* ($m_b = 7.2 \pm 0.5$ g, n = 12), *P. pygmaeus* ($m_b = 4.5 \pm 0.4$ g, n = 10) and *P. pipistrellus* ($m_b = 5.2 \pm 0.4$ g, n = 7; Czenze et al., 2022b). Males in both seasons were found roosting solitarily, or in groups of two to four individuals. After capture, bats were transported to Nicolaus Copernicus University in Toruń, where they were housed as described by Czenze et al. (2022b).

2.2. Thermometry and respirometry

We used flow-through respirometry to measure the rate of CO_2 production (V_{co_2}) and EWL of bats, using the same experimental setup and protocol described by Czenze et al. (2022b). The subcutaneous body temperature (T_{sub}) of each individual was measured every 10 s using temperature-sensitive passive integrated transponder (PIT) tags (Biotherm, Biomark, Boise ID, USA). Each PIT tag was calibrated before implantation in a circulating water bath (FBH 600/FBH 612, Lauda, Germany) between 25 °C and 45 °C, against a mercury-in-glass thermometer with accuracy traceable to NIST standards. On the day of capture, we injected PIT tags subcutaneously between the shoulder blades of each individual. PIT-tagged bats were placed in individual respirometry chambers, built from 0.5-L airtight glass pickle jars for all species except *N. noctula*, which were measured in a 0.85-L glass pickle jar. Each chamber was outfitted with a cylindrical metal cage that raised individuals ~1.5 cm above a ~1 cm layer of mineral oil and allowed them to move inside the chamber and hang in a natural posture. The chambers were placed in a temperature-controlled incubator (MIR-153, Sanyo Electric, Japan or ST-1200; Pol-Eko-Aparatura, Wodzisław Śląski, Poland), in the center of a ring antenna that connected to a PIT tag reader to record T_{sub} (HPR+, Biomark, Biose ID, USA). We inserted a thermistor probe (PS104R2, Littelfuse, Chicago, IL, USA) sealed with epoxy in the top of each respirometry chamber to measure T_a.

We measured each individual's CO_2 production (V_{co_2}) and EWL using a positive-pressure flow-through respirometry system. Atmospheric air was provided into the system by a membrane pump (DOA-P501-BN, Gast Manufacturing INC., Michigan, USA), and was scrubbed of water vapour using two columns of silica gel (Silikażel, EG System Sp. Z o.o., Warsaw, Poland). After scrubbing, the air was split into baseline and experimental animal channels. A needle valve (RFO 383-1/8, Camozzi Automation S.p.A., Brescia, Italy) regulated air flow through the baseline channel and a mass flow controller (GFC 17, Aalborg Instruments & Controls, Inc., Orangeburg, NY, USA) regulated the flow rate of air entering the experimental animal chamber. All mass flow controllers were calibrated over a flow rate range of 0.2–8 L min^{-1} using a volumetric calibrator (Defender 530+, Mesa Laboratories, Inc., Butler, NJ, USA).

To enhance air mixing within the chambers, we made the air inlet a metal tube that ended ~5 cm from the bottom of the chamber, and the air outlet was placed at the top of the chamber. We adjusted flow rates depending on m_b and the behavioural and physiological responses of individuals during measurements, to maintain chamber water vapour pressures consistently <0.41 kPa (dewpoint < −5.4 °C). As higher flow rates lower the CO_2 and water vapour signal deflections, we ensured that we could still accurately measure the differences in CO_2 and water vapour between baseline and experimental animal air. The flow rates during measurements ranged from: 0.6–4.4 L min^{-1} for *P. pygmaeus*, 0.5–4.2 L min^{-1} for *P. pipistrellus*, 0.5–4.9 L min^{-1} for *P. nathusii*, and 1.0–8.0 L min^{-1} for *N. noctula*.

We used a three-way solenoid valve controlled manually to switch between baseline and experimental channels, and an SS-4 Subsampler (Sable Systems Int., Las Vegas, NV, USA) to pull air through the gas analysers at a flow rate of ~200 ml min^{-1}. Subsampled air was pulled through a CO_2/H_2O analyzer (LI-850, LI-COR, Lincoln, NE, USA), which was calibrated prior to measurements. The H_2O sensor was zeroed using nitrogen and spanned using air saturated with H_2O at two dew point temperatures as described by Marom et al. (2006). The CO_2 sensor was also zeroed with nitrogen and spanned using a known concentration of CO_2 (10 000 ppm, Air Products, Brussels, Belgium). Tubing upstream of the respirometry chamber was polyurethane (Polyurethane 98 MB-Longlife, Mebra Plastik Italia S.p.A., Sacconago, Italy) and downstream tubing was Bev-A-Line IV (Thermoplastic Processes Inc., Warren, NJ, USA). Air was pushed through a 2 m copper tube coil upstream of the chamber to ensure it entered the respirometry chamber at the same temperature as the incubator. All voltage outputs of the instruments were digitized by an analog-digital converter (model UI-2, Sable Systems, Las Vegas NV, USA) and recorded at 0.5 Hz using Expedata software v. 1.7.31 (Sable Systems Int., Las Vegas, NV, USA).

We used Expedata software to correct the data for drift and lag in analyser readings, and used equations 9.5 and 9.6 from Lighton (2008)

to calculate V_{co_2} and EWL. For each target T_a, we used the lowest stable 3-min periods of CO2 and water vapour readings to calculate the values of V_{co_2} and EWL. We assumed 0.803 mg mL^{-1} H_2O (Lighton, 2008), calculated RMR from V_{co_2} assuming respiratory exchange ratio of 0.7 as bats were post-absorptive (Walsberg and Wolf, 1995), and converted V_{co_2} to metabolic rate (W) assuming 27.8 J ml^{-1} CO_2 (Withers, 1992). We converted EWL rates to evaporative heat loss (EHL, W) assuming a latent heat of vaporization of water of 2.406 J mg^{-1} H_2O at 40 °C (Tracy et al., 2010). We then calculated EHL/MHP, using EHL and MHP (i.e., RMR) at the same T_a. To examine seasonal changes in the dry heat transfer though the integument we also calculated thermal conductance (C; W °C^{-1} cm^{-2}) in each species at $T_a = 36$ °C, the highest T_a for which we have data and were confident that bats were normothermic. We used the equation from Dawson and Schmidt-Nielsen (1966): $C\left(W\,^{\circ}C^{-1}cm^{-2}\right) = \frac{MHP-EHL}{(T_b-T_a)\bullet A_s}$ to calculate thermal conductance using Dawson and Hulbert (1970) to estimate body surface area (A_S; cm^2) = 10 * m_b $(g)^{0.67}$.

2.3. Experimental protocol

Measurements occurred during the bats' rest phase. Changes in T_{sub}, EWL, and RMR were measured while exposing individuals to increasingly higher T_a, in 4 °C increments between 28 and 40 °C and then in 2 °C increments from 40 to 48 °C. It took approximately 20 min to increase from one T_a to the next. Bats were placed in the respirometry chamber at 28 °C for at least 1 h to habituate to the experimental protocol before the trial began. Bats were continuously monitored using an infrared camera and measurements were terminated when bats showed behavioural and physiological indications that they were approaching their upper thermoregulatory limit (i.e., heat tolerance limit; indicated by loss of coordination or balance, prolonged escape behaviour, a sudden and rapid increase in T_{sub}, rapid decrease in water vapour pressure or V_{co_2}). Individuals spent a minimum of 20 min at each setpoint T_a (excluding the time taken to change between T_as), until gas exchange values stabilized, after which data were extracted from periods when V_{co_2} and water vapour pressure traces remained stable for at least 3 min. The entire measurement period lasted for approximately 3–5 h, including the ~1 h habituation time. Our methods follow protocols from previous studies of the upper limits of heat tolerance and evaporative cooling of rodents (Ramirez et al., 2022; van Jaarsveld et al., 2021), bats (Czenze et al., 2020a; Noakes et al., 2021), and birds (Czenze et al., 2020b; Smit et al., 2018; Whitfield et al., 2015). Recently, it was shown that this stepped-state T_a protocol yields similar results as a steady-state protocol in birds (Short et al., 2022).

2.4. Data analysis

All statistical analyses were done in R 4.0.5 (R Core Team, 2021). We compared average and maximum daily environmental temperatures between field sites and seasons during the period of thermoregulatory data collection. The temperature data were obtained from a public meteorological data repository (https://danepubliczne.imgw.pl/) for Toruń and Chojnice (~55 km W of Sobiny, same latitude: 53.7°N). Average and maximum temperatures were compared using a two-way ANOVA, including season, location, and their interaction as factors. Mean values of physiological variables per species per season are presented with standard deviations (Table S1). In cases where the maximum T_a reached for a species was represented by a single individual, we also report the mean values at the next highest T_a.

We determined the relationship between each thermoregulatory variable (EWL, RMR, EHL/MHP, and T_{sub}) and T_a in each species during spring and summer separately, using linear mixed effects models from the package *nlme* version 3.1–149 (Pinheiro et al., 2009). In all models, we accounted for multiple measurements from individuals by including individual identity as a random factor. We identified inflection points in the relationships with T_a using the package *segmented.lme* (Muggeo, 2016). We calculated 95% confidence intervals (CI) for each inflection point, and considered inflection points to differ between seasons if their CIs did not overlap. We compared segmented models to linear models (i.e., without breakpoints) using an ANOVA based on residual sum of squares. If the segmented fit outcompeted the simple linear fit, we analysed data above and below inflection points separately using linear mixed-effect models.

To determine if thermoregulatory responses at high T_a differ between seasons within species, we pooled spring and summer EWL, RMR, EHL/MHP, and T_{sub} data over the T_a ranges where variables were increasing most steeply (i.e., typically above the season-specific inflection points calculated above). We analysed these data using linear mixed-effects models, including T_a, season, and the T_a × season interaction term as predictor variables in the global models. We also compared the m_b and thermal conductance of bats between spring and summer using a Welch's *t*-test. We found that individuals of all species were heavier in summer compared to spring (*p* value = <0.001–0.2; Supplementary Table 1), and therefore included m_b in all global models. As we were testing a hypothesis and thus modelling for inference, we selected models based on null hypothesis testing (Tredennick et al., 2021). R^2 represents the total proportion of variance described by explanatory variables and random effects (*sensu* Nakagawa and Schielzeth, 2013).

After model selection, we used the *emmean* package version 1.4.6 (Lenth, 2021) to calculate estimated marginal means (EMM, reported with ±standard error) for mean maximum fractional increase in EWL (evaporative scope = maximum EWL/minimum EWL), mean maximum EHL/MHP, heat tolerance limit (maximum T_a tolerated), and T_{sub} at the heat tolerance limit per season in each species. These values were then compared between seasons using a Tukey's HSD test adjusting for multiple comparisons.

3. Results

Daily average air temperatures during the study period did not differ between Toruń and Chojnice ($p > 0.05$), but maximum temperatures were ~1.5 °C higher in Toruń than in Chojnice ($F_{1,124} = 4.304$, $p < 0.05$; Fig. S1). Regardless of location, both daily average temperatures ($F_{1,124} = 73.372$, $p < 0.001$) and maximum temperatures ($F_{124} = 53.59$, $p < 0.001$) were ~5 °C higher in summer than in spring (Fig. S1). The m_b of bats was higher in summer compared to spring ($0.001 < p < 0.2$; Supplementary Table 1).

During thermoregulatory measurements, individuals of all species reached their heat tolerance limit at T_a between 44 and 46 °C regardless of season (one individual *N. noctula* reached 48 °C). The T_{sub}, RMR, EWL, and EHL/MHP of bats generally increased linearly with increasing T_a, either across the entire range of T_a or above respective inflection points (Figs. 1–4; Table S1). In all species during both seasons, we recorded a linear increase in EWL above inflection T_as that ranged from 34.2 to 40.6 °C (Fig. 2; Table S1). The RMR of *P. pygmaeus* and *P. pipistrellus* during spring increased linearly over the entire T_a range, whereas in all other species-season groups RMR increased above an inflection T_a that ranged from 34.1 to 38.8 °C (Fig. 3). The EHL/MHP of most species in both seasons increased linearly with increasing T_a from inflection T_as ranging from 34.2 to 39.0 °C (Fig. 4; Table S1). There was one exception where the EHL/MHP of *N. noctula* during summer increased linearly with T_a until 43 °C and then plateaued.

For all thermoregulatory variables, the final reduced model explaining variation between seasons within species (spring and summer data pooled from the respective T_a ranges where T_{sub} was increasing most steeply) explained a large portion of variance in the data (range of r^2 of models: 0.78–0.96, Table S2). For all species, the final models for T_{sub} only included T_a, indicating no T_{sub} difference between spring and summer (Fig. 1; Table S2). In all species except *N. noctula* there was no difference in the inflection point of EWL between seasons. The confidence intervals for the breakpoint in EWL did not overlap for *N. noctula*

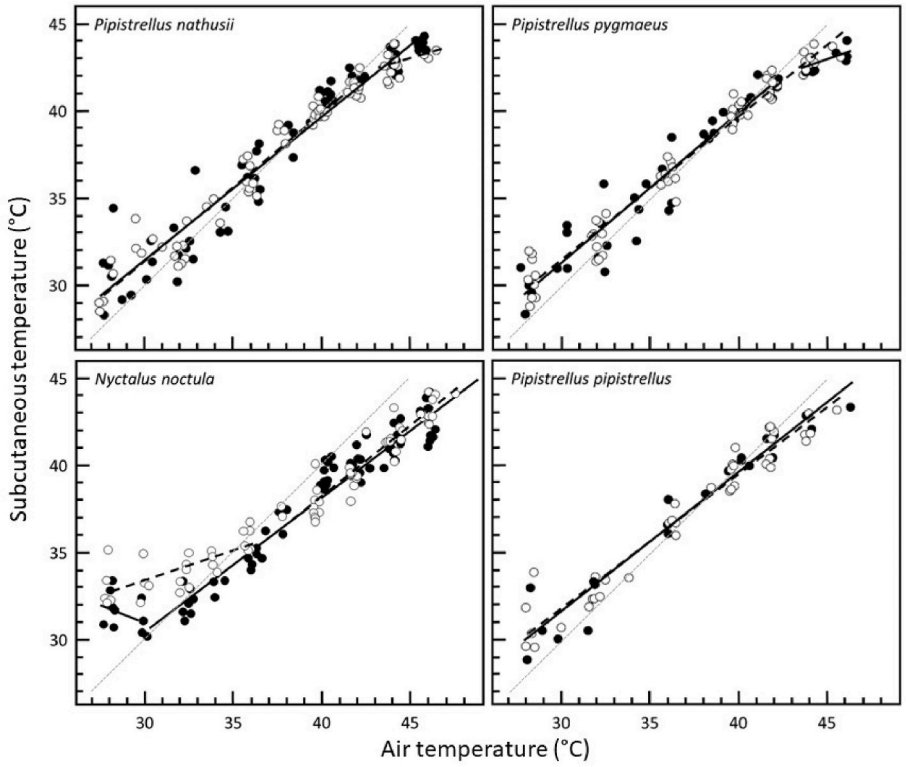


Fig. 1. Subcutaneous temperature (T_{sub}) as a function of air temperature (T_a) in the males of four European bat species during spring and summer. Black circles represent data from spring and white circles represent summer. The grey dotted line indicates the line of equality between T_{sub} and T_a. The solid (spring) and dashed (summer) lines indicate linear regressions between T_a and T_{sub}. In *Pipistrellus nathusii* during summer and *P. pygmaeus* during spring, an inflection occurred at T_a = 42.0 and 42.7 °C respectively, above which the slope of increasing T_{sub} was shallower.

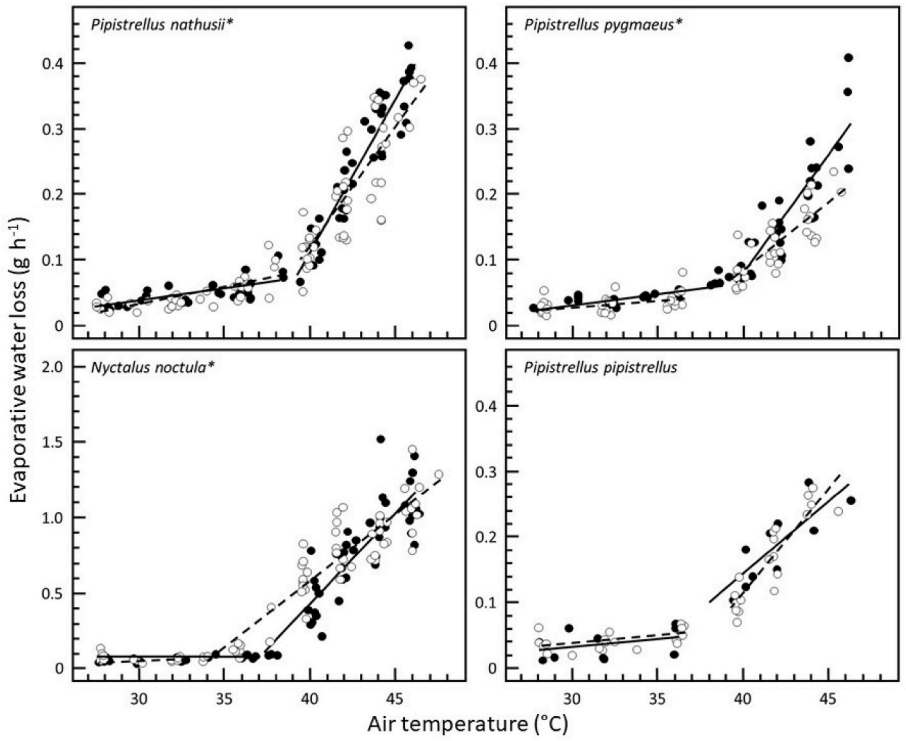


Fig. 2. Evaporative water loss (EWL) as a function of air temperature (T_a) in the males of four European bat species during spring and summer. Black circles represent data from spring and white circles represent summer. The solid (spring) and dashed (summer) lines indicate linear regressions between T_a and EWL. The asterisk in the panel of some species indicates a significant interaction term between season and T_a in linear mixed effects models fitted to EWL data above inflection points. Note the difference in y-axis scale for *Nyctalus noctula*.

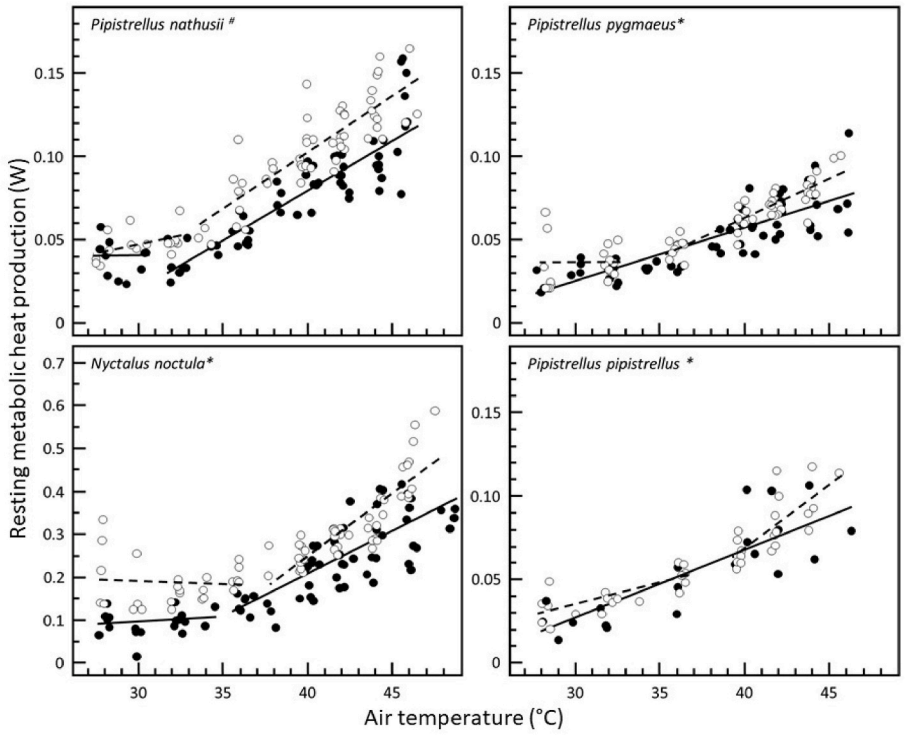


Fig. 3. Resting metabolic heat production as a function of air temperature (T_a) in the males of four European bat species during spring and summer. Black circles represent data from spring and white circles represent summer. The solid (spring) and dashed (summer) lines indicate linear regressions between T_a and RMR. The hash mark in the panel for *Pipistrellus nathusii* signifies a significant difference between seasons in linear mixed effects models fitted to data above inflection points. The asterisks in all other panels indicates a significant interaction term between season and T_a. Note the difference in y-axis scale for *Nyctalus noctula*.

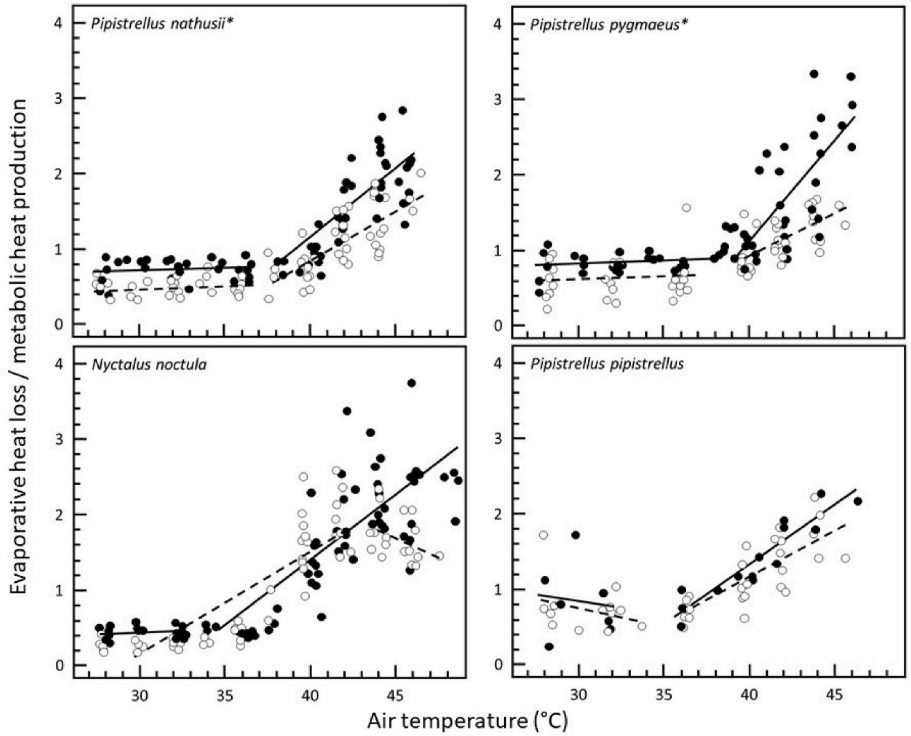


Fig. 4. The ratio of evaporative heat loss to metabolic heat production (EHL/MHP) as a function of air temperature (T_a) in the males of four European bat species during spring and summer. Black circles represent data from spring and white circles represent summer. The solid (spring) and dashed (summer) lines indicate linear regressions between T_a and EHL/MHP. The asterisk in the panel of some species indicates a significant interaction term between season and T_a in linear mixed effects models fitted to EHL/MHP data above inflection points. In *N. noctula* during summer, an inflection occurred at $T_a = 43.1$, above which the slope of increasing EHL/MHP was shallower.

with summer individuals increasing EWL at lower T_a than spring individuals. In *N. noctula, P. nathusii,* and *P. pygmaeus*, the final model describing changes in EWL included the interaction between season and T_a, with bats exhibiting a more gradual increase in EWL with increasing T_a in summer compared to spring (Fig. 2; Table S2). In *P. pipistrellus,* the final model explaining variation in EWL included T_a and season, but not their interaction. In *P. nathusii*, *P. pygmaeus*, and *P. pipistrellus,* the final model also included m_b, with EWL increasing significantly with increasing mass (Table S2).

In *N. noctula, P. pygmaeus,* and *P. pipistrellus*, the change of RMR with T_a above inflection points differed between seasons (season × T_a interaction; Fig. 3; Table S2), with bats exhibiting higher and steeper increases in RMR in summer compared to spring. For *P. nathusii*, the interaction term approached significance ($p = 0.07$) but was not retained in the final model, which included T_a and season. The RMR of *P. nathusii* was consistently higher in summer compared to spring at T_a above the inflection points (Fig. 3). Body mass was only included in the final RMR model of *N. noctula,* with RMR increasing significantly with increasing m_b (Table S2).

Seasonal patterns in the relationship between EHL/MHP and T_a varied between species. The rate that EHL/MHP increased with T_a did not differ between seasons in *N. noctula* or *P. pipistrellus* However, *P. nathusii* and *P. pygmaeus* increased EHL/MHP more gradually with increasing T_a during summer compared to spring. In all species except *P. pipistrellus,* the maximum EHL/MHP of individuals was significantly lower in summer than in spring (Table S1). The greatest seasonal difference in the EMMs of maximum EHL/MHP was in *P. pygmaeus* (spring: 2.2 ± 0.21, summer: 1.4 ± 0.20), followed by *P. nathusii* (spring: 2.1 ± 0.13, summer: 1.4 ± 0.12), *N. noctula* (spring: 2.5 ± 0.14, summer: 2.1 ± 0.14), and finally *P. pipistrellus* (spring: 1.8 ± 0.22, summer: 1.5 ± 0.26). In all species, the mean of individual evaporative scope, maximum T_{sub}, and heat tolerance limit did not differ between spring and summer. At $T_a = 36$ °C, in all species there was no difference in thermal conductance between seasons (*N. noctula*: df = 22.9, $t = -0.5$, $p = 0.6$; *P. nathusii*: df = 24.7, $t = 0.3$, $p = 0.7$; *P. pygmaeus*: df = 20.5, $t = -0.04$, $p = 0.9$; *P. pipistrellus*: df = 9.1, $t = 1.3$, $p = 0.2$).

4. Discussion

We report the first recorded seasonal change in thermoregulatory responses at high T_a within bat populations. However, in contrast to our predictions, we did not find higher maximum evaporative cooling capacities and heat tolerance limits (maximum T_a tolerated) during summer compared to spring. Instead, we found that all species showed no seasonal variation in heat tolerance limits, and all species except *P. pipistrellus* had significantly lower maximum EHL/MHP in summer compared to spring. In *P. nathusii* and *P. pygmaeus*, lower summer EHL/MHP is driven by lower EHL during summer compared to spring, and less from seasonal changes in MHP. Moreover, EWL increased more gradually with increasing T_a in summer than spring in *P. nathusii*, *P. pygmaeus*, and *N. noctula*, and these patterns may reflect a water conservation strategy to avoid dehydration during hot summer days. This is similar to thermoregulatory differences between sexes in the same four species during summer (Czenze et al., 2022b). Despite no sex difference in heat tolerance limits, females had patterns of evaporative cooling that may be related to greater water conservation, perhaps due to the hotter roost microclimates of maternity colonies relative to males that roost solitarily or in smaller groups (Czenze et al., 2022b).

Despite maintaining similar T_{sub} during both seasons at high T_a, the male bats of three of the four species increased RMR more rapidly and EWL more gradually with T_a during summer compared to spring. There is limited data on patterns of seasonal variation in endothermic heat dissipation traits at high T_a (Maloney and Dawson, 1994a; Maloney and Dawson, 1994b; Noakes et al., 2016; O'Connor et al., 2017; Oswald et al., 2018). The total EWL at $T_a \approx 40$–50 °C of arid-zone white-browed sparrow-weavers (*Plocepasser mahali*) was lower during summer compared to winter, which may reflect a higher need for water conservation during the drier summer season (Noakes et al., 2016). This is similar to patterns observed at the interspecific level in bats, as species restricted to more arid areas have lower total EWL than species that occur in more mesic areas (Muñoz-Garcia et al., 2016). It is possible that individual bats acclimatize to summer by increasing their capacity to conserve water on account of longer and hotter days during which they are confined in roosts. Angolan free-tailed bat (*Mops condylurus*) can tolerate extremely high summer roost temperatures (up to 50–60 °C, and >40 °C > 6 h per day), and conserve water by producing more concentrated urine in summer compared to winter (Bronner et al., 1999; Buffenstein et al., 1999; Maloney et al., 1999). Seasonal changes in the integument or pelage could lead to greater water conservation, but we found that thermal conductance values did not differ between seasons, and thus the potential mechanism of lower summer EWL remains unclear. Regardless, it remains that although male bats have lower maximum EHL/MHP during summer, they appear more efficient at water use for thermoregulation as they require lower EWL rates to defend similar T_{sub} and tolerate similar maximum T_a as males in spring.

Interestingly, we found that the drivers of the higher EHL/MHP of male bats during spring compared to summer appear to differ among species, influenced by both higher EWL and lower RMR at high T_a during spring (Supplementary Table S2). The pattern in *N. noctula* (e.g., at $T_a =$ 46 °C, there was <1 % seasonal difference in EWL but ~ 10 % lower MHP during spring) is consistent with previous reports that higher maximum EHL/MHP are driven by lower MHP (Czenze et al., 2022a; McKechnie et al., 2021; Wojciechowski et al., 2021). In contrast, higher EHL/MHP in spring males of *P. nathusii* and *P. pygmaeus* was more the result of higher EHL (~31 % and 41 % higher EWL relative to summer, respectively, at $T_a = 46$ °C) rather than lower MHP (~7 % and 11 % lower RMR, respectively) compared to summer males. The EHL/MHP of these two species increased more rapidly with increasing temperature during spring than during summer. Interestingly, EHL/MHP in *N. noctula* during summer appeared to plateau at a value of ~2 at T_a >43 °C, whereas spring individuals continued to increase EHL/MHP to values > 3 in some cases. Some of the differences in thermoregulatory patterns we observed among species may be due to variation in m_b (*N. noctula* is ~5–6 times heavier than the other species studied). Further work should investigate how heat-dissipation strategies vary among bat species in relation to m_b, as much of the current literature focuses on species in the range of ~10–15 g.

The dehydration tolerance limits of bats during acute heat exposure remain largely unknown, but for small *Myotis* species ranged between 23 and 32 % of m_b (Studier et al., 1970). Czenze et al. (2022b) estimated that during present-day summer heatwaves, the same species studied here would require daytime evaporative water requirements equivalent to between ~ 23 and 34 % of m_b if roosting in sun-exposed roosts. Applying these estimates to bats inhabiting the same roosts during a similar heat wave in spring (see Czenze et al., 2022b for calculation details), our data suggest there would be little seasonal difference in evaporative water requirements for male *N. noctula* (spring = 24.7 % and summer = 24.2 % of m_b per day). However, the estimated daytime water requirements for all of three *Pipistrellus* species are higher in spring than in summer (*P. pipistrellus*: 33.8 % of m_b during summer and 43.1 % during spring; *P. nathusii*: summer = 26.3 %, spring = 32.9 %; *P. pygmaeus*: summer = 28.9 %, spring = 34.8 %), with spring values being consistently above the range of dehydration tolerance limits reported for small *Myotis* species (Studier et al., 1970). Considering that heatwaves are already happening progressively earlier in the year due to anthropogenic climate change (IPCC, 2021), high T_{roost} may threaten the viability of the smaller species, particularly if heatwaves occur before bats are fully summer acclimatized. This reiterates the importance of placing bat boxes in positions varying in sun exposure and orientation, thus mimicking the thermal heterogeneity of natural roost sites to allow for roost selection and switching if necessary (Czenze et al., 2022b).

In conclusion, our study is the first to examine seasonal changes in

the heat tolerance and evaporative cooling capacity of bats and revealed phenotypic changes in thermoregulatory responses at high T_a. However, without conservation actions designed to enhance roosting oppurtunities for bats in degraded ecosystesms, these adaptaions may be insufficient for species survival under predicted climate change scenarios. Understanding phenotypic flexibility in thermoregulatory strategies at high T_a is vital for predicting how endothermic animals will respond to increasing temperatures and heat waves, and such information needs to be incorporated into mechanistic models predicting the responses of species to anthropogenic climate change (Ratnayake et al., 2019; Welbergen et al., 2008).

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CRediT authorship contribution statement

Zenon J. Czenze: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Matthew J. Noakes:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Michał S. Wojciechowski:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2024.103911.

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