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RESEARCH ARTICLE

Energetics of migratory bats during stopover: a test of the torpor-assisted migration hypothesis

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ABSTRACT

The torpor-assisted migration hypothesis posits that migration is facilitated in bats by the use of torpor during stopover roosting periods, and predicts that at stopover, bats regulate time in torpor facultatively so that daily energy expenditure is independent of ambient roosting temperature. Energy savings can thus be directed to migratory movement. However, direct measurements of total roosting energy expenditure in relation to ambient and body temperature are lacking. We captured migratory silver-haired bats (*Lasionycteris noctivagans*; ~11 g) at Long Point, ON, Canada, in spring and autumn 2016. We used quantitative magnetic resonance analysis to measure body composition change and energy expenditure over a 12 h roosting period in a ventilated incubator at 10, 17 and 25°C. We assessed the effects of season, body mass, sex and age on energy expenditure. We found that daily energy expenditure was independent of roosting temperature, and that this was achieved by flexible use of torpor. Variation in body mass at capture was driven mainly by differences in fat, and the amount of body fat was negatively related to torpor use, particularly in spring. Season, sex and age also affected torpor use and energy expenditure, notably with pregnant females being generally fatter and using less torpor than males in spring. We estimate that stopover contributes only 15–20% to the total energy costs of migration in bats compared with 70% or more in typical birds. This study provides support for the torpor-assisted migration hypothesis, and furthers our understanding of the energy budgets of migratory bats.

KEY WORDS: Chiroptera, Heterothermy, Energy budget, Thermoregulation

INTRODUCTION

Behavioural and physiological differences among species can lead to divergent migration patterns and processes, even when the migratory origins and destinations are similar. For example, the combination of obligate nocturnality and heterothermy creates unusual circumstances for temperate migratory bats compared with typical migratory birds that share similar geographic distributions. Obligate nocturnality in bats causes an inherent time conflict between foraging to refuel and travelling rapidly and directly towards the destination. There is evidence that bats can, at least in some cases, forage as they travel at night (McGuire et al., 2012; Voigt et al., 2012), yet they must roost at stopover sites during daylight. Unlike most similarly sized migratory songbirds, which use the day to refuel, the daytime phase of stopover results in an energy loss for bats (Hedenström and Alerstam, 1997; Wikelski et al., 2003; McGuire and Guglielmo, 2009; McGuire et al., 2012, 2014). Heterothermy can provide a partial solution by allowing migrating bats and in rare cases birds (e.g. hummingbirds) to use torpor during roosting to save energy that would otherwise be spent maintaining a high and stable body temperature (euthermia) (bats: Cryan and Wolf, 2003; Willis et al., 2006; McGuire et al., 2012, 2014; Jonasson, 2017; hummingbirds: Carpenter, 1974; Carpenter and Hixon, 1988; Hiebert, 1993). Torpor is the active suppression of metabolic rate, which can result in body temperature (T_b) conforming closely to ambient temperature (T_a) (Geiser, 2004; Staples, 2016). Carpenter and Hixon (1988) observed a rufous hummingbird (*Selasphorus rufus*) using torpor while roosting at night during migratory stopover, and departing the following morning. They noted that the hummingbird would have incurred a negative energy balance for the day if it had not used torpor. While rare in birds, torpor during roosting appears to be a defining characteristic of bat migration.

McGuire et al. (2012, 2014) proposed the torpor-assisted migration hypothesis to explain the very brief stopovers with little evidence of refuelling of autumn-migrating silver-haired bats (*Lasionycteris noctivagans*), and to describe how a migration journey can be facilitated by the use of torpor during autumn stopovers to save energy. Based on metabolic rate measurements from respirometry and T_b data collected from free-ranging bats, McGuire et al. (2014) estimated that torpor use allows an energy saving of as much as 91% during a daytime stopover. The authors predicted that because of the facultative use of torpor, daily energy expenditure was independent of roosting T_a; however, empirical data to support this prediction are lacking.

Using similar methods to those of McGuire et al. (2014) to observe free-ranging silver-haired bats during spring migration, Jonasson and Guglielmo (2016) found that females migrate earlier and are fatter than males. Jonasson (2017) found that bats spent more time in torpor in the spring than in the autumn, which was attributed to the cooler roosting T_a in spring. However, female bats used less torpor than males during spring migration, and there was qualitative evidence suggesting that mass differences between the sexes were driving torpor use and energy expenditure during daytime stopovers. Sex differences observed in the spring may relate to the delayed fertilization and pregnancy strategy of many bats, including the migratory silver-haired and other so-called tree bats of North America (Hood and Smith, 1989; Cryan and Wolf, 2003; Solick and Barclay, 2006; Turbill and Geiser, 2006; Stawski, 2010; Dzal and Brigham, 2013). Several studies have shown that pregnant female bats reduce torpor use, hypothetically to promote fetal development (Geiser, 1996; Cryan and Wolf, 2003; Lausen and Barclay, 2003; Willis et al., 2006; Dzal and Brigham, 2013;

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Jonasson, 2017). Other studies have shown that torpor delays parturition as well as lactation post-parturition for various bat species (Racey, 1973; Racey and Swift, 1981; Wilde et al., 1999; Turbill and Geiser, 2006), which suggests that bats balance a trade-off between saving energy and maintaining fetal development.

While the evidence for migrating bats using torpor to save energy during stopovers is robust, no previous study has directly quantified the total energy expenditure of migratory bats during daytime roosting. Our objectives in this study were (1) to directly measure the total energy expended during full daytime stopover for migrating bats, and (2) to test a key prediction of the torpor-assisted migration hypothesis: that energy use during daytime stopovers is independent of roosting T_a. We hypothesized that reproductive life-history differences affect the use of torpor, and consequently energy expenditure, during daytime stopovers of migrating bats. We predicted that energy expenditure would be independent of roosting T_a in both spring and autumn, but that because of additional costs of fetal development, female bats would use less torpor and expend more energy than males in the spring.

MATERIALS AND METHODS

Study site and species

All methods were approved by the University of Western Ontario Animal Care Committee (Protocol 2010-020) and permitted by the Ontario Ministry of Natural Resources and Forests (Wildlife Scientific Collector's Authorization Permit: 1082592). We caught silver-haired bats, *Lasionycteris noctivagans* (Le Conte 1831), at Long Point Bird Observatory (Long Point, ON, Canada), a well-known stopover site for migratory birds and bats (Dzal et al., 2009). Bats were caught using mist nets from 1 April to 3 June 2016 (spring migration; n=60, female/male: 48/12) and 15 August to 30 September 2016 (autumn migration; n=80, female/male: 35/45) to sample an entire migratory year at the site. All bat handling and experimentation were performed in a temperature-controlled mobile laboratory (Field Laboratory for Integrative Ecological Research – FLIER; Guglielmo et al., 2011). Sex was determined by genital inspection, age (juvenile or adult in autumn only) was determined by observing the degree of epiphyseal ossification of the second metacarpal joint in the fourth digit (Kunz and Anthony, 1982), and mass was measured with a digital balance (±0.01 g; CS200; OHAUS, Parsippany, NJ, USA). We palpated the torso of female bats upon capture to assess whether any bats were noticeably pregnant (Racey, 1969; Jonasson, 2017). Up to 10 bats per night were held in light cotton bags from the time of capture until the next morning to be used in torpor experiments (see below). All bats being used for the experiment were held at ∼21°C in the FLIER from the time of capture (spring mean 02:40 h ±2 h 56 min, range 21:20–06:05 h, n=60; autumn mean 04:13 h ±2 h 14 min, range 20:08–08:11 h, n=100) until the start of the treatment (see below). The pre-treatment holding time was: spring mean 268.0±172.8 min, range 59–564 min, n=60; autumn mean 214.3±153.0 min, range: 47–682 min, n=100). There was no effect of pre-treatment holding time on energy expenditure or torpor use (all P>0.05). To prevent pathogen transfer between bats, bags were used once and machine washed in hot water using scent-free detergent (Tide Free and Gentle, Proctor and Gamble).

Temperature treatment

During a temperature treatment, individual thin cloth bags containing one bat each were hung in a ventilated, fan-circulated, temperature-controlled cabinet (±0.2°C; model PTC-1 with PELT-5 temperature controller; Sable Systems International, Las Vegas, NV, USA; dimensions: 44×30×34 cm) for approximately 12 h, from 07:00 h to 19:00 h to mimic a daytime stopover (spring mean t_{start}=07:08 h ±19.4 min; mean t_{stop}=19:40 h ±20.8 min; mean trial duration=12.2 h ±8.6 min; autumn mean t_{start}=07:45 h ±45.3 min; mean t_{stop}=19:35 h ±176.4 min; mean trial duration=12.3 h ±7.9 min). The cloth bags were thin enough to minimize any added insulatory effects to roosting bats, as well as to maintain a constant flow of fresh air. It should be noted that having bats roost in cloth bags was more natural than placing them in the cabinet because free-living silver-haired bats typically roost in small confined spaces such as crevices in tree bark, in wood piles and behind building structures where they are not directly exposed (Barclay et al., 1988; McGuire et al., 2012). While holding the bats in cotton bags and handling them at 07:00 h may have delayed the initial entry into torpor relative to what has been observed in the field (McGuire et al., 2014), all bats were treated the same and so the experimental design was sufficient to reveal the temperature and other effects we sought to test. We used three temperature treatments: cold (9.4±0.6°C), intermediate (17.0±0.2°C) and warm (25.1±0.1°C), which were selected to span a naturally occurring range of temperatures in both seasons that were within the capability of the equipment used. As we had only one temperature-controlled cabinet, each night of capture (and therefore all bats caught during a given night) was pseudo-randomly assigned to one of the three temperature treatments. We use the term 'pseudo-random', because we caught a varying number of bats each night and, therefore, as the seasons progressed, treatment temperatures were chosen to keep sample sizes as even as possible across all three treatments. Treatment temperatures were also used to control for any potential effects of acclimatization due to naturally occurring changes in T_a across arrival dates between migratory seasons (i.e. bats being captured on 15 April: T_a≈4°C and 15 September: T_a≈14°C). Cabinet temperature was measured every 30 s using a Temperature/Relative Humidity Smart Sensor cable attachment on a HOBO Micro Station (±0.01°C; S-THB-M002, H21-002, Onset Computer Corporation, Bourne, MA, USA). To ensure that the concentration of CO_2 did not build up inside of the temperature cabinet, room air was pumped in at approximately 1.3 l min^{-1} using an aquarium pump (Tetra Whisper, United Pet Group, Blackburg, VA, USA), a rate that was sufficient to maintain low [CO_2] (CO_2 measured using a S151 Infrared CO_2 Analyzer; QUBIT Systems, Kingston, ON, Canada), but not to interfere with treatment temperatures. Bats were released after sunset at the capture site.

Energy and torpor quantification

We weighed bats immediately before entry and upon exit from the temperature cabinet and body composition was quantified using quantitative magnetic resonance (QMR; EchoMRI-B, Echo Medical Systems, Houston, TX, USA). QMR is a non-invasive technique for determining fat and lean mass that does not require anaesthesia [accuracy: ±2% wet lean mass (g) and ±10% dry fat mass; McGuire and Guglielmo, 2010]. We confirmed that the loss of body mass measured gravimetrically on the balance over the 12 h roosting trials matched the sum of the losses of wet lean and dry fat mass measured by QMR [R^2=0.70, $F_{1,131}$=310.7, P<0.0001; Δmass=0.70(Δfat+Δlean)+0.19]. Changes in dry fat and wet lean mass were converted to kJ using the conversions: dry fat mass (g)×39.6 kJ g^{-1} and wet lean mass (g)×5.3 kJ g^{-1}); these were summed to calculate total energy expenditure (Gerson and Guglielmo, 2011).

Skin temperature (T_{sk}) is considered an accurate approximation of core T_b of small bats (Audet and Thomas, 1996; Barclay et al., 1996;

Willis et al., 2005; Willis et al., 2006; McKechnie et al., 2007), including silver-haired bats (McGuire et al., 2014; Jonasson, 2017). McGuire et al. (2014) found no significant difference between skin and rectal temperature measured by a thermocouple and so we consider variation in T_{sk} measured with radio-transmitters to be a reliable measure of variation in T_b. To record T_{sk} and measure torpor use, bats were outfitted with temperature sensitive radio-transmitters (0.3 g; ±0.1°C; BD-2XT, Holohil Systems Ltd, Carp, ON, Canada) immediately after the QMR scan, prior to being placed in the temperature-controlled cabinet. A small amount of hair was trimmed from the interscapular region and the transmitter was attached using a non-toxic latex glue (Osto-Bond, M.O.C., Montreal, QC, Canada). Each transmitter produced radio pulses that were shorter than 23 ms, on a different VHF radio frequency within 0.048 MHz of the tag frequency, 3 dB above background noise, and the pulse frequency varied with temperature. Raw transmitter signals (indicating T_{sk}) were received by a custom-built Sensorgnome receiver (www.sensorgnome.org), which sampled each active transmitter for repeated ∼4–8 s intervals throughout the day. Transmitters were removed after a treatment using a small amount of an adhesive solvent (Remove, Smith & Nephew, USA) and reused on other bats. The fur that surrounded the transmitter was then washed of all solvent with a dilute biodegradable soap solution and dried thoroughly before the final QMR scan.

Filtering and conversion of raw transmitter pulse frequency data to T_{sk} was done using methods outlined by Jonasson (2017). The raw transmitter signals were filtered for background and harmonic noise, using the *hampel* function in R software (package: pracma; v 3.3.2), then converted from pulse frequency to T_{sk} (°C) by applying the pulse frequency data to a calibration curve from the respective transmitter provided by the manufacturer. Prior to use on bats, the calibration curves provided by the manufacturer were verified for accuracy by placing each transmitter in a precision water bath (±0.1°C; Lauda Eco, LAUDA-Brinkmann, Delran, NJ, USA) at 5°C increments from 5 to 45°C for 5 min (or until pulse frequency readings were stable). After the conclusion of all experiments, a second water bath calibration was completed using the same method as above, with all transmitter pulse frequencies drifting <0.02% from the initial calibration (mean drift: 0.02±0.03%).

To quantify torpor use, we used R software to produce plots of T_{sk} against time (s) as time course images (.png files) and processed them using ImageJ (version 1.49v, National Institutes of Health, USA; Fig. 1). Following the methods of previous studies, we set a conservative torpor threshold for each bat using an equation that takes the lower limit of a 99% confidence interval of a reported normothermic mean for silver-haired bats (34°C; McGuire et al., 2014; Jonasson, 2017), subtracts 3°C from the normothermic mean (to take into account differences in transmitter attachment) and corrects for each bat's body mass (equation 4 of Willis, 2007; McGuire et al., 2014; Jonasson, 2017). We used the 'area' tool of ImageJ to calculate the area between the torpor threshold and T_{sk} curve (hereafter: A_{torpor}), for as long as T_{sk} was below the torpor threshold for a minimum of 15 min (McGuire et al., 2014; Jonasson, 2017). We validated the use of ImageJ in the calculation of A_{torpor} using a linear model to compare A_{torpor} of a subset of bats from ImageJ (from all treatments) and the integrated area under the curve calculated using Expedata software (v.0.2045, Sable Systems International) [area ImageJ=0.80(area Expedata)+1.15; $F_{1,14}$=8910, P<0.0001, R^2=0.99]. By quantifying the area under the torpor threshold, we were able to take into account some of the variability in T_{sk} in response to T_a as well as the duration of each torpor bout. Individuals that used a longer (duration), deeper (a greater difference between euthermic and torpid T_{sk}) torpor therefore would have a larger A_{torpor} value. We acknowledge the limits of using A_{torpor} values to describe torpor behaviour, especially for individuals that may have entered shallow torpor for a long duration, and individuals that may have entered deep torpor for a short duration, but achieving this level of differentiation is not within the scope of the experiment or the questions we aimed to address. However, by using A_{torpor} values instead of 'time spent in torpor' in combination with an 'average depth of torpor', we were able to simultaneously take into account differences in torpor use within treatments and energy used for any partial arousals (i.e. increases in T_{sk} that did not cross the torpor threshold). To allow our data to be comparable to previous studies of our study system (McGuire et al., 2014; Jonasson, 2017), we chose not to use techniques recently proposed for classifying torpor use (heterothermy index: Boyles et al., 2011; non-stationary waveform analysis: Levesque et al., 2017).

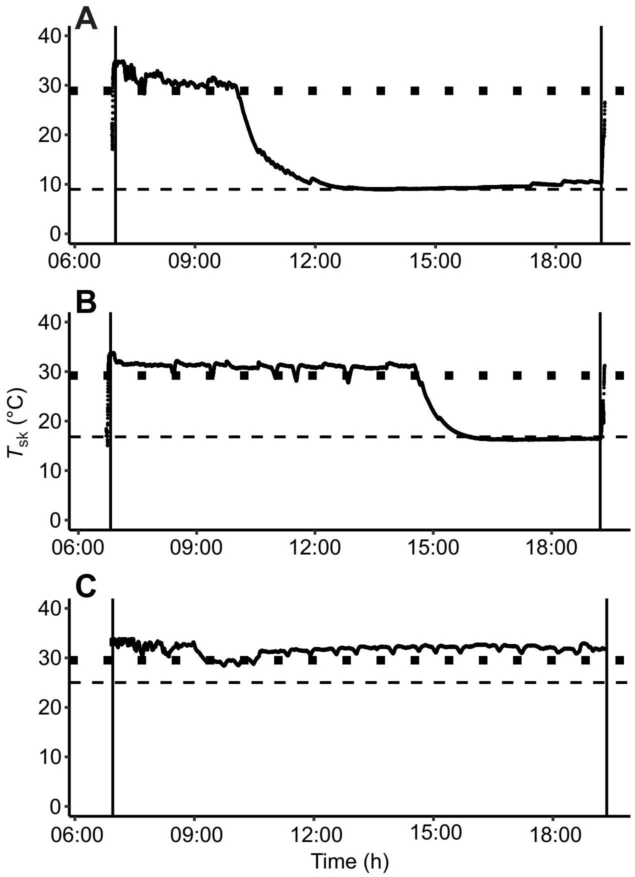

Fig. 1. Skin temperature (T_{sk}) of silver-haired bats (*Lasionycteris noctivagans*) during a 12 h daytime stopover at one of three treatment temperatures. Bats roosted at (A) 9.39±0.64°C, (B) 16.95±0.2°C and (C) 25.09±0.1°C inside a temperature-controlled cabinet. Dotted lines represent the torpor threshold of each respective individual. Dashed lines represent the treatment temperature of each respective trial.

Statistical analyses

All statistical analyses were run in R (version 3.3.2, http://www.R-project.org/). Linear models (analysis of variance: ANOVA and analysis of co-variance: ANCOVA) were used to analyse the effects of season, sex, body mass and age on A_{torpor} and total energy expenditure (E_{total}) (Zuur et al., 2009). We used backwards step elimination to remove non-significantly contributing terms from

Table 1. Body mass, dry fat mass and wet lean mass of adult and juvenile silver-haired bats (*Lasionycteris noctivagans*) during stopover at Long Point, ON, Canada, in spring and autumn 2016 before entering a daytime stopover temperature treatment

Sex	Season	Age	*n*	Body mass (g)	Fat mass (g)	Lean mass (g)
Female	Spring	Adult	48	11.59±1.29^{a}	1.58±0.64^{a}	8.54±0.65^{a,b}
	Autumn	Adult	23	11.03±1.39^{a}	1.88±1.22^{a}	8.72±0.75^{a}
		Juvenile	12	10.71±0.70^{a,b}	1.09±0.24^{a,b}	8.36±0.61^{a,b,c}
Male	Spring	Adult	12	9.24±1.00^{c}	0.67±0.31^{b}	7.34±0.75^{d}
	Autumn	Adult	31	11.58±1.68^{a,b}	1.88±1.16^{a}	8.12±0.57^{b}
		Juvenile	14	10.02±0.86^{b,c}	1.04±0.28^{a,b}	7.92±0.57^{c,d}

Shared letters indicate no significant difference at α=0.05 by ANOVA followed by a Tukey's multiple comparison test. *n*, sample size.

models (α=0.05) until only significant terms remained (Zuur et al., 2009). In the case of significant interaction terms (between two or more predictor variables), we reanalysed the data by creating new linear models (ANOVA or ANCOVA) by sub-setting the dataset according to the type of interaction (Zuur et al., 2009). After sub-setting, we included all predictor variables from the original linear model, including all possible interactions between the remaining predictor variables. For comparing means of two groups independently of other factors, we used Student's *t*-tests (Zuur et al., 2009). General additive models (GAMs) were used to take into account non-linear relationships, specifically in models that included temperature and torpor use (*mgcv* function: R; Zuur et al., 2009). Akaike's information criterion (AIC) was used to compare linear and non-linear models that included the same variables to determine the model of best fit for the given data in question. A model is 'preferred', or better fits a dataset, when it has the lowest AIC value, by at least 2 units, between the models in question (Welham et al., 2015). All results were reported using Type III sum of squares using the *drop1* function in R to report values taking into account a randomized order of terms included in each linear model. The distribution of all variables was assessed using Shapiro–Wilks test (*shapiro.test* function: R) and *qqplot* when assessing normality of residuals from linear models. Variables that were not normally distributed were transformed (i.e. natural log or square root) to satisfy the assumption of normality within linear models. Mass was transformed using a natural log [hereafter ln(mass)] and A_{torpor} was square-root transformed (hereafter A_{torpor}) to meet the assumption of normality. Sample sizes for each group (i.e. season, sex and age) can be found in Table 1.

To determine whether energy expenditure during the daytime migratory stopover was independent of roosting T_{a} across both autumn and spring migratory seasons, we created linear models to test for effects of predictor variables on energy use and to address significant effects of two- and three-way interactions among predictor variables on E_{total}. We carried out a similar analysis but replaced E_{total} with A_{torpor} to assess the effects of the same predictor variables on torpor use. To address the three-way interaction of season, sex and ln(mass), we divided the full E_{total} model into two female-only, season-controlling models (one for spring and one for autumn females) and two male-only, season-controlling models (one for spring and one for autumn males), where we included E_{total} as the response variable, ln(mass), temperature and the two-way

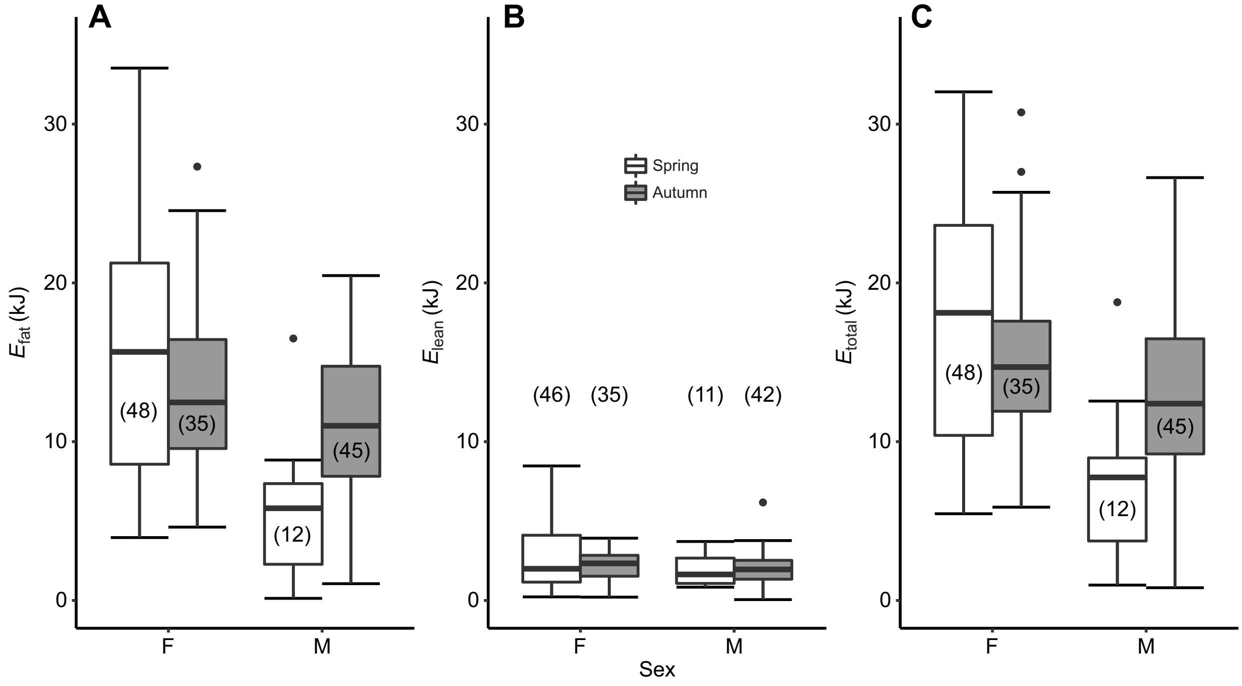

Fig. 2. Body composition and total energy expenditure of silver-haired bats during a 12 h daytime stopover within their respective migratory season. (A,B) Change in body composition, shown as (A) fat and (B) lean energy expenditure (E_{fat} and E_{lean}, respectively) based on substrate conversions of dry fat mass (39.6 kJ g^{-1}) and wet lean mass (5.3 kJ g^{-1}). (C) Total energy expended (E_{total}), calculated as the summation of E_{fat} and E_{lean}. Data are for female and male bats regardless of age and roost temperature treatment. Numbers in parentheses represent the sample size of each respective group. Box plots represent the 25th and 75th percentiles, while bold black lines represent median values and whiskers represent 5th and 95th percentiles; dots are outliers.

interaction between mass and temperature as predictor variables. Age and all interactions with the other predictor variables (stated above) were also included in the autumn models. Total body mass was used in these models instead of a body condition index, as it has been shown to be a more appropriate metric of body condition (McGuire et al., 2018), and because it includes the potential effects of both fat and lean mass in one variable.

RESULTS

Body mass ($F_{5,143}$=10.46; P<0.0001), fat ($F_{5,134}$=8.83; P<0.0001) and lean mass ($F_{5,134}$=9.78; P<0.0001) of silver-haired bats varied among season, sex and age class upon capture (Table 1). In general, females had greater body mass, fat mass and lean mass than males in the spring. In spring, males were lighter and leaner compared with both spring females and autumn bats of all sex and age classes. In spring, females did not differ greatly in body composition and mass from autumn bats. There were minimal differences observed in body composition and body mass among sexes and ages of bats in the autumn (Table 1).

Energy expenditure during roosting

Total energy expenditure during roosting was dominated by the catabolism of fat (Fig. 2). Combining seasons and temperature

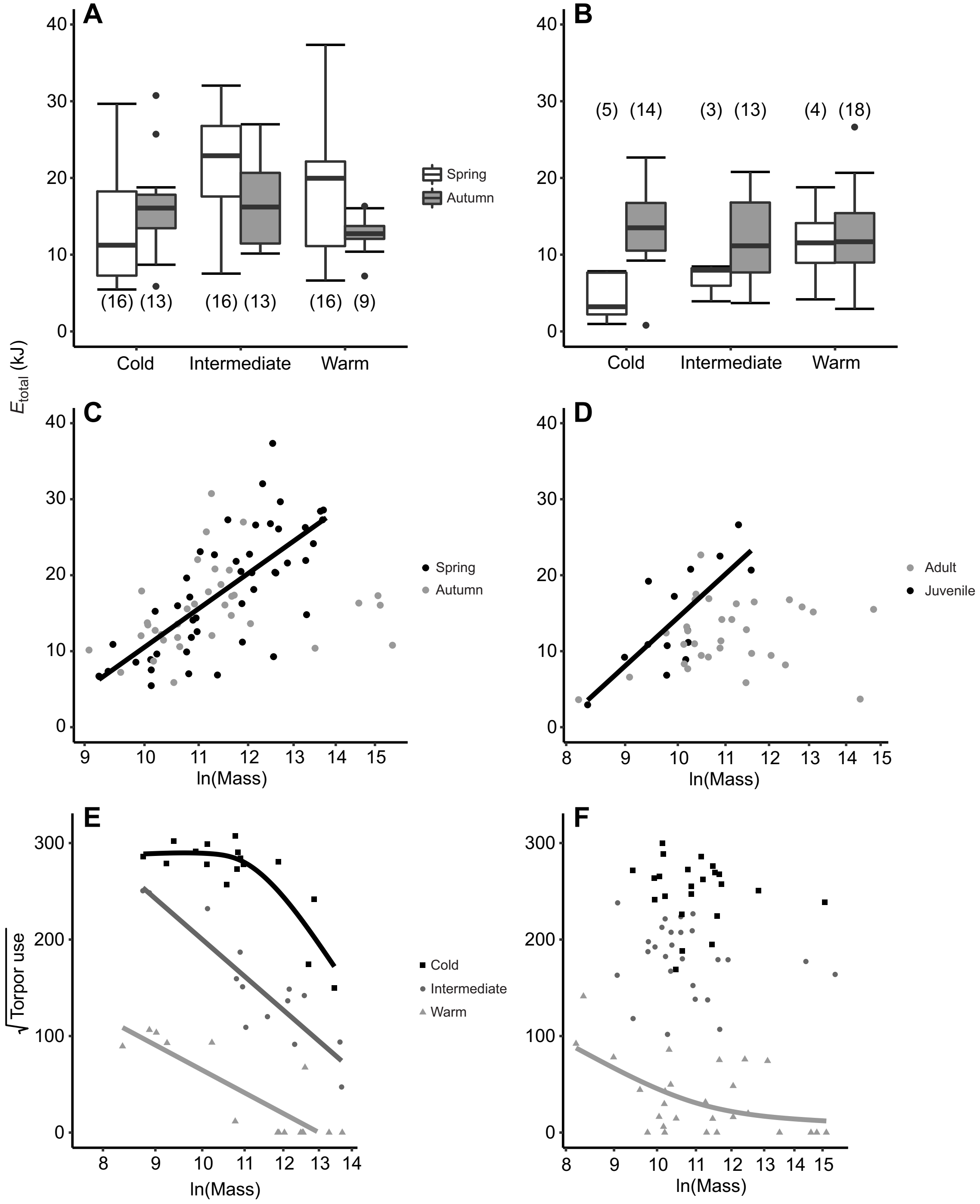

Fig. 3. See next page for legend.

Fig. 3. The effects of sex, season, treatment temperature and body mass on energy and torpor use by silver-haired bats during a 12 h daytime stopover. (A,B) Total energy (E_{total}) use by silver-haired bats during a full daytime stopover (~12 h) from either the spring or autumn migration season, at one of three temperature treatments (cold, intermediate or warm). Energy use is independent of ambient temperature (T_a) in both seasons for both female (A) and male (B) bats. Numbers in parentheses represent the sample size of each respective group. Box plots represent the 25th and 75th percentiles, while bold black lines represent median values and whiskers represent 5th and 95th percentiles; dots are outliers. (C) The effect of body mass (g; natural log transformed) on total energy use during daytime stopover for females in both seasons. The black trend line represents the positive relationship between E_{total} and ln(mass) within female bats migrating in the spring (Eqn: y=53.20x–111.96; R^2=0.54, $F_{1,46}$=55.52, P<0.0001). There was no relationship between energy use and ln(mass) within female bats in the autumn. (D) The effect of body mass (g; natural log transformed) on total energy use during daytime stopover of young-of-the-year (juvenile) and older than 1 year (adult) male silver-haired bats during autumn migration. The black trend line represents the positive relationship between energy use and ln(mass) within the juvenile male bats (Eqn: y=60.22x–124.26; $F_{1,12}$=16.24, P=0.002, R^2=0.54). There was no relationship between energy use and body mass for adult male bats. (E) Higher body mass (g; natural log transformed) results in less torpor use (calculated as A_{torpor}, square-root transformed) during daytime stopover at all temperature treatments (cold, intermediate and warm) for spring silver-haired bats (two sexes combined). The non-linear line (black) was calculated from a general additive model (GAM) that describes the relationship between A_{torpor} and ln(mass) for bats roosting at cold temperatures (edf=1.91, Ref. d.f.=1.99, F=20.77, P<0.0001, R^2=0.78). The negative linear relationships between A_{torpor} and ln(mass) for both intermediate and warm treatments are described by the dark grey (Eqn: y=1172.04–420.21x; $F_{1,9}$=16.34, P=0.003, R^2=0.61) and light grey trend lines (Eqn: y=623.40–243.12x; $F_{1,10}$=10.59, P=0.009, R^2=0.47). (F) Higher body mass (g; natural log transformed) results in less A_{torpor} (square-root transformed) during daytime stopover at only warm temperatures for autumn silver-haired bats (two sexes combined). The non-linear line (light grey) was calculated from a GAM that describes the relationship between A_{torpor} and ln(mass) for bats roosting at the warm temperature treatment (edf=2.84, Ref. d.f.=2.98, F=7.94, P<0.001, R^2=0.45).

treatments, female bats, on average, used around 31% more energy than males during a daytime stopover (females: 16.38±6.83 kJ; males: 11.27±5.73 kJ; t=4.79, d.f.=132.45, P<0.0001). During spring migration, females used approximately 59% more energy than males (females: 17.32±7.65 kJ; males: 7.11±5.04 kJ; t=5.59, d.f.=25.33, P<0.0001; Fig. 2C), whereas females in the autumn used around 17% more energy than males (females: 15.09±5.36 kJ; males: 12.54±5.17 kJ; t=2.15, d.f.=71.90, P=0.035; Fig. 2C). In general, energy expenditure was negatively related to the amount of torpor used within a given treatment temperature ($F_{1,104}$= 4.274; P=0.041). We found the relationship between E_{total}, A_{torpor} and treatment temperature to be complex because of the additional effect of body composition on both E_{total} and A_{torpor}; hence, we examine it in more detail below.

The initial full 'energy use' model included E_{total} as the response variable, season, ln(mass), sex, temperature and all two-way, three-way and four-way interactions. The three-way interaction between season, sex and ln(mass) was significant ($F_{1,131}$=6.79; P=0.01). There was also a significant effect of the temperature×season interaction ($F_{1,129}$=4.77, P=0.03), while all other two- and three-way interaction terms including temperature did not affect E_{total} (P>0.05).

In spring, there was a positive relationship between ln(mass) and E_{total} ($F_{1,46}$=55.52; P<0.0001) in female bats, with heavier females using more energy (Eqn: y=53.20x−111.96; R^2=0.54; Fig. 3C). There was no effect of the interaction between, or the main effects of, ln(mass) and temperature (P>0.05). For autumn-migrating female bats, temperature, ln(mass), age and all of the two- and three-way interactions between the predictor variables did not predict E_{total} (P>0.05). When compared with that of female bats in the spring, there was considerably more variation in E_{total} in the autumn as a result of the greater number of heavier female bats and greater maximum body mass than in the spring. The largest females captured in the autumn drove the lack of a relationship between E_{total} and body mass. Thus, body mass was a strong predictor of roosting energy expenditure in the spring, but not in the autumn, and in both seasons temperature was not important.

For spring-migrating male bats, there was no effect of ln(mass), temperature or their interaction (P>0.05) on E_{total}. For males in the autumn, the age×ln(mass) interaction was significant ($F_{1,41}$=11.44, P=0.002). There was no effect, however, of all other two- and three-way interactions or main effects of temperature, ln(mass) and age (P>0.05) on E_{total} for males in autumn. The interaction between ln(mass) and age was probably produced by the difference in mean body mass between adult and juvenile males in the autumn, and in both seasons temperature was not important.

To address the interaction between age and mass for males in autumn, we separated the ages and compared the effects of mass on E_{total}. Adult males had greater body mass in the autumn than juveniles (t=−2.95, d.f.=34.41, P=0.006). However, E_{total} did not respond the same to ln(mass) between age categories. Fig. 3D shows the positive relationship between E_{total} and mass for juvenile males (Eqn: y=60.22x−124.26; $F_{1,12}$=16.24; P=0.002; R^2=0.54), and the lack of relationship between mass and E_{total} for adult male bats in the autumn (P>0.05).

Torpor use

Additional metrics of torpor use are included in Table 2 to allow readers to compare our quantification of torpor (A_{torpor}) with other torpor studies. From the full 'torpor-use' model, both the interaction between ln(mass) and season as well as temperature significantly predicted A_{torpor} [ln(mass)×season: $F_{1,117}$=17.65, P<0.0001; temperature: $F_{1,117}$=851.64, P<0.0001]. There was no effect of any of the other interaction terms between predictor variables or sex on A_{torpor} (P>0.05). Thus, the body mass upon arrival at stopover affected torpor use differently between seasons and there was a negative relationship between torpor use and temperature.

After separating seasons and combining sexes, there were negative relationships between ln(mass) and temperature on A_{torpor} in the spring at all temperatures, and for bats roosting at warm temperatures in the autumn. In the spring, there were significant negative effects of ln(mass) ($F_{1,41}$=86.95, P<0.0001) and increasing temperature ($F_{2,41}$=215.47, P<0.0001) on A_{torpor}, where heavier bats and bats roosting at warmer temperatures used less torpor (Fig. 3E). In the autumn, there were also significant negative effects of temperature ($F_{2,73}$=256.55, P<0.0001) and ln(mass) ($F_{1,73}$=8.11, P<0.006) on A_{torpor}, where bats roosting at cold temperatures used torpor more than bats roosting at warm temperatures. However, when bats from each temperature were analysed separately, there was only an effect of body mass at warm temperatures, where heavier bats used less torpor than lighter bats (Fig. 3F).

DISCUSSION

We directly quantified energy expenditure of migrating bats during full-length daytime stopovers in spring and autumn to test key predictions of the torpor-assisted migration hypothesis and to examine the effects of sex, season and age. Overall, we provide empirical evidence in support of the prediction that, regardless of sex and season, energy expenditure during a daytime stopover for migratory bats is independent of roosting T_a. Heavier spring females and autumn juvenile male bats used more energy than lighter individuals within

Table 2. Characteristics of torpor use by silver-haired bats (*Lasionycteris noctivagans*) during a 12 h daytime stopover

Season	Sex	Treatment	Mean time spent in torpor (min)	Time in torpor (%)	Average torpor depth (°C)	Max. depth of torpor (Δ°C)	A_{torpor}	*n*
Spring	Female	Cold	609.5±98.0	84.6±13.6	14.4±3.8	18.0±2.8	72,401±22,374	13
		Intermediate	415.3±186.0	57.7±25.8	23.5±3.6	10.2±3.8	19,834±14,339	11
		Warm	88.2±171.1	12.3±23.8	28.2±1.5	0.4±2.1	1833±3440	13
	Male	Cold	677.0±45.6	94.0±6.3	14.7±1.3	18.2±0.7	76,179±8820	3
		Intermediate	663.0±63.6	92.1±8.8	19.8±4.0	7.2±4.4	49,976±21,279	2
		Warm	493.6±63.9	68.6±8.9	26.4±0.4	4.1±0.3	1014±1774	3
Autumn	Female	Cold	600.6±109.8	83.4±15.2	16.4±3.6	20.1±1.2	69,157±15,693	12
		Intermediate	486.0±138.5	67.5±19.2	21.3±2.1	12.7±0.4	31,445±11,823	13
		Warm	76.4±124.3	10.6±17.3	28.5±0.9	2.0±1.5	1205±2403	9
	Male	Cold	569.5±57.8	79.1±8.0	15.5±4.4	20.3±1.2	61,965±17,455	13
		Intermediate	525.6±136.4	73.0±19.0	20.5±2.4	12.6±0.4	35,531±13,349	13
		Warm	201.2±218.0	27.9±30.3	27.9±1.4	2.6±1.7	3322±4968	18

Characteristics are separated by migratory season (spring and autumn), sex and treatment (cold: 9.4±0.6°C; intermediate: 17.0±0.2°C; warm: 25.1±0.1°C). Percentage of time in torpor was calculated based on the percentage of time bats spent in torpor during a 12 h trial. Details on the calculation of A_{torpor} can be found in Materials and Methods. *n*, sample size.

their respective sex and migratory season. We also found body mass to be an important predictor of torpor use, where heavier spring individuals, regardless of sex, and autumn individuals roosting at warm temperatures, used less torpor. We also confirmed the previous findings that during stopover, torpor use by migratory bats is dependent on roosting T_a. Our experimental procedures with short-term captive bats may have delayed the morning entry into torpor compared with free-living bats (McGuire et al., 2014). This could make our estimates of energy savings from torpor more conservative, but the effects of temperature and other factors are robust.

The amount of energy expended during stopover is an important, and in some cases dominant, component of the overall energy budget of migrants. In birds, stopover energy expenditure may comprise 70% or more of total migration energy (Wikelski et al., 2003), but we expect this to be much lower in migratory bats that roost during daylight and use torpor. Using respirometry calibrations of field skin temperature telemetry recordings McGuire et al. (2014; Fig. 4) estimated roosting energy expenditure of silver haired bats to range between approximately 0.003 and 0.02 W g^{-1}, or 0.13 W for an 11 g bat. This is equivalent to about 1.3–1.5 times the basal metabolic rate (BMR), based respectively on allometric prediction (0.10 W) from Speakman and Thomas (2003) and on indirect calorimetry data (0.08 W) from McGuire et al. (2014) (assuming 27.6°C and with 16% of energy from protein and 84% fat measured in our experiments). Using our data on body composition change for 131 bats, we estimate an average energy expenditure of about 14 kJ over a 12 h period, or about 0.3 W (3×BMR). The energy expenditure measured in our study could be greater than that of free-living bats because of the artificial situation of roosting in a cloth bag in a temperature-controlled cabinet (Geiser et al., 2000); however, we did not find differences between temperature traces from silver-haired bats roosting in captivity (this study) and roosting at similar T_a in a natural setting (McGuire et al., 2014). Concurrently, the estimate from McGuire et al. (2014) may represent a lower bound because it is derived from minimum oxygen consumption during 200 s of respirometry measurement at various temperatures. Using these estimates and assuming a flight energy cost of 1.5 W (McGuire et al., 2014), a typical stopover of <1 day (McGuire et al., 2012; Jonasson, 2017) and a flight duration of 8 h, we calculate that stopover may comprise as little as 15–20% of total migration energy cost in bats. Some bats stopover for multiple days, particularly in spring (Jonasson, 2017), and more detailed activity budgets of roosting, foraging and migratory flight are needed to adequately model the energetics of migration in these species.

There were differences in energy use between sexes in the spring, with females using more energy at the cold temperatures than males, and tending to do so at all temperatures. The difference in energy use between sexes agrees with findings and predictions proposed by Jonasson (2017). Using radio-telemetry of free-ranging bats, Jonasson (2017) found female bats to use less torpor than males, suggesting that this was an adaptation to maintain the rate of fetal development, which has been observed several times in bats (Geiser, 1996; Cryan and Wolf, 2003; Lausen and Barclay, 2003; Willis et al., 2006; Dzal and Brigham, 2013).

The tendency for females to carry larger fat stores than males in the spring could be solely responsible for the reduced use of torpor by females. Alternatively, a decrease in torpor use by females could be unrelated in any direct causal way to their larger fat stores if both ultimately contribute to a greater reproductive success. However, in saying this, there was no difference in the extent of torpor use (A_{torpor}) between seasons for females, despite the fact that females are pregnant in the spring, but not reproductive in the autumn. Although the lack of difference in A_{torpor} between seasons for females appears contrary to our hypothesis regarding the effect of pregnancy, we believe it is more likely that both reproduction and migration require sufficiently large fat stores, thus forcing females to remain relatively fat for the duration of the year, while males only seem to build large fat stores during autumn migration.

We found significant negative relationships between body mass and torpor use for spring migrants, regardless of sex at all three treatment temperatures, and for bats held in the warm treatment in the autumn. The variation in roosting energy expenditure of bats with intermediate body masses in the autumn could be related to variation in age. However, age did not significantly predict energy expenditure, which one might predict if juvenile bats were still maturing physiologically and therefore would not respond to changes in T_a in the same way as adults. It could be that our age classification system is too coarse to truly assess physiological capacity at different stages of development for juvenile bats. However, given that our metric of assessing age is based on the lack of ossification of metacarpal joints in juvenile bats (Kunz and Anthony, 1982), it is likely that they would be under different energetic demands than adults as juvenile bats require extra energy for growth and development.

Jonasson and Guglielmo (2016) and the present study showed that females have more fat than males upon arrival at stopover in spring, fitting with the prediction that females that are pregnant or are preparing for pregnancy will have sufficient fat stores for the costs of fetal development and lactation. Alternatively, migratory bats could benefit from a relatively low percentage body fat (compared with pre-hibernal bats) to facilitate increased manoeuvrability for foraging while at stopover or en route (Dechmann et al., 2014). Maintaining a lower body mass reduces wing loading, which increases the ability to make rapid and tight turns that may be necessary for catching insects during flight (Norberg and Rayner, 1987). The constraint on the size of fat stores due to reductions in manoeuvrability may explain why we see such thermal plasticity and variable energetic strategies (i.e. using torpor during stopover) in temperate bat species.

It is likely that migratory bats supplement their energy budgets by foraging at some point during migration. If, as our data suggest, fat requirements during stopover are greater than predicted by McGuire et al. (2012), it is also likely that supplementing energy budgets by foraging would remove the need for bats to evolve the capacity to store, in advance, all of the fuel required for an entire migratory journey. Isotopic analysis of breath CO_2, collected immediately after capture, indicates that bats we sampled in both seasons appeared to be metabolizing ^{13}C-enriched substrates from their diet rather than from endogenous fat stores (D.E.B., C.G.G. and K. A. Hobson, unpublished data), which suggests that bats forage upon arrival at stopover sites (Voigt et al., 2008, 2012). This is the first study to directly quantify energy expenditure of full daytime stopovers of migrating bats from spring and autumn migratory seasons. We found support for predictions made by the torpor-assisted migration hypothesis that energy expenditure during daytime stopovers for bats is independent of roosting T_a in both migratory seasons, and that this was achieved by flexible use of torpor. We found evidence that the size of fuel stores (fat) is the determining factor for torpor use, which in turn determines energy expenditure.

Studying the intersection between heterothermy and migration in bats provides us with information regarding the energetic adaptations of heterothermic species to migratory and reproductive life-history stages. Bats have adapted, physiologically and perhaps behaviourally, to save energy during expensive stopovers by entering torpor. By understanding the dynamics of energy use during migration, we are able to understand the energy and nutritional requirements of individual bats, giving us a starting point for quantifying the demands of migrating bats on their environment.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.E.B., C.G.G.; Methodology: D.E.B., C.G.G.; Formal analysis: D.E.B.; Investigation: D.E.B.; Resources: C.G.G.; Writing - original draft: D.E.B.; Writing - review & editing: D.E.B., C.G.G.; Visualization: D.E.B.; Supervision: C.G.G.; Funding acquisition: C.G.G.

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