Canadian Journal of Zoology
Revue canadienne de zoologie

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Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2016-0110.R1
Manuscript Type:	Article
Date Submitted by the Author:	24-Jul-2016
Complete List of Authors:	McGuire, Liam; Texas Tech University, Department of Biological Sciences Muisse, Kristina; University of Winnipeg, Department of Biology Shrivastav, Anuraag; University of Winnipeg, Department of Biology Willis, Craig; University of Winnipeg,
Keyword:	environmental constraints, little brown bat, <i>Myotis lucifugus</i> , plasma triglyceride analysis, TEMPERATURE < Discipline, energetics

No evidence of hyperphagia during pre-hibernation in a northern population of little brown bats (*Myotis lucifugus*)

L.P. McGuire^{1a,*}, K.A. Muise¹, A. Shrivastav¹ and C.K.R. Willis¹

¹ Department of Biology, University of Winnipeg, 515 Portage Ave, R3B 2E9, Winnipeg, Canada

Co-author E-mails: McGuire, L.P. liam.mcguire@ttu.edu

Muise, K.A. k_muise@hotmail.com

Shrivastav, A. a.shrivastav@uwinnipeg.ca

Willis, C. c.willis@uwinnipeg.ca

*Corresponding Author:

Liam P. McGuire
Department of Biological Sciences
Texas Tech University
Box 43131
Lubbock, Texas 79409
p: (806) 834-5129
e: liam.mcguire@ttu.edu

^aCurrent address: liam.mcguire@ttu.edu, Department of Biological Sciences, Texas Tech University, Lubbock, Texas, United States 79409

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Abstract

During autumn, temperate bats must deposit fat stores for hibernation. Populations at high latitudes face four challenges: a shorter active season and shorter nights during the active season (less time to accumulate fat), a longer hibernation period (larger fat store needed), and colder nights (reduced prey availability). Mating also occurs during the pre-hibernation period, placing time constraints for mating and fattening on northern populations. We tested the hypothesis that these factors constrain pre-hibernation foraging in northern populations of little brown bats (*Myotis lucifugus* LeConte, 1831). We measured plasma triglyceride concentration to study pre-hibernation fueling rates of a population near the northern range limit of the species, and compared our results with previously published results from a more southern population. In contrast to bats at lower latitude, we found consistently low concentrations of plasma triglycerides, indicating a low fuelling rate throughout the pre-hibernation period. However, despite an apparently low fuelling rate, bats achieved a substantially greater body mass. The discrepancy between populations suggests that environmental constraints lead northern populations to employ different behavioural or physiological strategies to prepare for hibernation.

Key words: environmental constraints, little brown bat, *Myotis lucifugus*, plasma triglyceride analysis, temperature, torpor, energetics

Introduction

Hibernation is an energy-saving strategy characterized by reduced body temperature and metabolic rate (Lyman et al. 1982; Heldmaier and Ruf 1992; Geiser 2004) that allows temperate mammals to survive the winter period of low ambient temperature (T_a) and reduced food availability (Humphries et al. 2004; Dunbar and Tomasi 2006). To prepare for hibernation animals must either store a food cache (Humphries et al. 2002) or accumulate a substantial fat store to survive a prolonged period when food resources may not be available (Young 1976; Humphries et al. 2002; Humphries et al. 2003). Like many hibernators, little brown bats (*Myotis lucifugus* LeConte, 1831) accumulate large fat stores to meet their winter energy requirements (Kunz et al. 1998; Humphries et al. 2002, 2003). Rapid fat deposition is achieved by becoming hyperphagic in autumn and dramatically increasing feeding rate (Kronfeld-Schor et al. 2000; McGuire et al. 2009b).

The period of fat accumulation prior to hibernation coincides with a behaviour known as swarming (Fenton 1969; Thomas et al. 1979a,b). During swarming, bats aggregate at hibernacula, mate promiscuously and females store sperm until spring (Davis and Hitchcock 1965; Fenton 1969; Anthony and Kunz 1977; Thomas et al. 1979b; Fenton and Barclay 1980; Schowalter 1980). The need to store fat for winter while also engaging in mating behaviour creates a potential trade-off between investment in autumn reproduction and investment in overwinter survival. Little is known however, about the relative allocation of energy and time to pre-hibernation fattening compared to mating behaviour during this critical period of the annual cycle.

Little brown bats are among the most widespread of North American mammals, occurring from Alaska to Newfoundland and Labrador at the northern limit, to Southern

California and Northern Arizona at the southern limit (Fenton and Barclay 1980). Over such a large range of latitudes, populations face varying environmental conditions that could influence the trade-off between autumn mating and pre-hibernation fattening (Vernberg 1962; Garland and Adolph 1991). Little brown bats living further north face four energetic challenges that could influence this trade-off and affect the distribution and northern limit of the species (Humphries et al. 2002, 2004; Lausen and Barclay 2006). First, the active season is reduced at northern latitudes which leaves less time available to recover from hibernation, rear pups, and deposit fat stores in preparation for the coming winter. Second, bats face a longer hibernation period and presumably need to deposit a larger fat store to endure the longer winter. Third, late summer nights are shorter at higher latitudes which leave less time for nocturnal foraging and mating. Fourth, bats face colder nighttime temperatures in the pre-hibernation fattening phase which could reduce the availability of insects. The abundance of flying insects tends to decline below about 10°C (Racey et al. 1987; Hickey and Fenton 1996), resulting in lower prey availability. Thus bats from northern populations need to deposit a larger fat store but face lower prey availability, a shorter active season, and less time available each night. This suggests that northern bats may be energetically constrained and differ from southern populations in terms of their allocation of resources to foraging versus mating.

Previous research has documented foraging rates during pre-hibernation fattening at a site in eastern Ontario, Canada using plasma metabolite analysis (McGuire et al. 2009b). Plasma metabolite analysis can be used to indicate feeding behaviour (i.e., fasting or feeding) of individual animals and has been validated for many taxa (e.g., Guglielmo et al. 2005; Price et al. 2013), including insectivorous bats (McGuire et al. 2009a). Specifically, circulating triglycerides indicate fat deposition as they are transported in the circulation regardless of the combination of

proteins, carbohydrates and lipids that an animal has ingested (Robinson 1970; Jenni-Eiermann and Jenni 1994). McGuire et al. (2009b) described a bi-phasic temporal pattern of plasma triglyceride concentrations during autumn with high levels during the first phase of swarming (indicative of hyperphagia) and low levels during the second phase. The abrupt decrease in triglyceride concentrations coincided with the first observed copulations, and thus the authors hypothesized that bats face a trade-off between time spent foraging outside the hibernaculum and time spent mating inside the hibernaculum.

Our objective was to examine the influence of energetic constraints at higher latitudes on allocation of time and energy to pre-hibernation fat accumulation. We tested the hypothesis that the combination of the shorter active season, shorter nights, longer hibernation period and cooler temperatures (i.e., lower insect abundance) would constrain the potential for pre-hibernation fattening. This hypothesis predicts that the temporal pattern of plasma triglyceride concentrations measured for bats captured throughout autumn swarming should differ from the biphasic pattern observed by McGuire et al. (2009b). We predicted that this energetic constraint would lead to one of three possible scenarios consistent with energy limitation: 1) Consistently high levels of plasma triglyceride throughout pre-hibernation would suggest that prey availability is sufficient to support prolonged hyperphagia but bats invest less time on pre-hibernation mating at the end of the swarming period; 2) Consistently low levels of plasma triglycerides would suggest that prey availability is low and that northern bats rely more heavily on alternative energy-saving mechanisms (e.g., torpor; Krzanowski 1961, Speakman and Rowland 1999); 3) Plasma triglyceride levels that fluctuate on a short-term (i.e., day-to-day) basis would suggest that northern bats adjust their time investment in foraging versus mating opportunistically, perhaps influenced by ambient conditions and insect availability.

Methods

We captured little brown bats between 6 August and 28 September 2013 at Abyss Cave, located north of the town of Grand Rapids, Manitoba (53° 10' N 99° 16' W), using a harp trap positioned directly in front of the opening of the cave. Traps were continuously monitored and bats were removed from the trap immediately to ensure that blood samples could be collected within 10 min of capture, a time window beyond which the stress of capture and handling can affect plasma triglyceride concentrations (Jenni-Eiermann and Jenni 1996, 1997). Upon removal from the trap, the interfemoral vein was punctured with a 27 gauge syringe needle (Becton, Dickinson and Company, Mississauga, ON), and approximately 70 µl of blood was collected in a heparinized capillary tube (Fisher Scientific, Pittsburgh, Pennsylvania), which was then sealed at one end with Critoseal (McCormick Scientific, St. Louis, Missouri). Sex, age, body mass (± 0.1 g), and forearm length (± 0.05 mm; a measure of body size) were recorded for each bat, as well as the time of capture (minutes after sunset) and the total bleed time (time from capture to the end of blood collection). Age (adult or sub-adult) was determined by the degree of ossification of the third metacarpal-phalanges joint (Anthony 1988).

Blood samples were centrifuged for 5 min at 10000 rpm with a hematocrit centrifuge (ZipocritTM, LW Scientific Inc, Lawrenceville, Georgia), plasma was transferred to a 1 mL O-ring sealed cryotube (Fisherbrand: Thermo Fisher Scientific, Pittsburgh, Pennsylvania), and stored in a liquid-nitrogen cooled dry shipper (Taylor-Wharton, Theodore, Alabama).

Approximately every 12 days the samples were transferred to a -80°C freezer at the University of Winnipeg where they were stored for up to 13 weeks prior to analysis.

Climate data were obtained from a meteorological station located approximately 60 km from the cave (Grand Rapids, Manitoba; Station 5031A10; Environment Canada). Ambient temperature recorded at the meteorological station at 23:00 (time chosen arbitrarily) on all sampling nights (11.37 ± 5.11 °C, $n=27$ nights) was strongly correlated with T_a measured using a digital thermometer (AccuTemp Products Inc., Fort Wayne, Indiana) at the field site (10.56 ± 5.45 °C) (linear regression, $F_{1,26} = 3563$, $p < 0.0001$, $R^2 = 0.99$, zero intercept and 95% CI for slope includes 1). Therefore, we used data from the meteorological station for statistical analysis because it was a more complete dataset. We averaged hourly T_a between 20:00 and 05:00, the approximate times of sunset and sunrise, as a nightly measurement of T_a at the site. Change in barometric pressure (ΔBP) can reflect changes in prey availability and influence activity of little brown bats as a drop in BP predicts advancing weather fronts (Paige 1995; Baerwald and Barclay 2011; Czenze and Willis 2015). Therefore, we recorded ΔBP (difference between BP at noon and at sunset) from the meteorological station following Baerwald and Barclay (2011). We estimated relative nightly insect abundance using a modified version of spotlight sampling (e.g., Taylor and O'Neill 1988, O'Donnell 2002). We counted the number of medium to large (approximately 10-40 mm in length) flying insects that flew in the beam of a handheld spotlight over a 30 second interval. Spotlight sampling was conducted at 23:00 every night in the same location (approximately 20 m away from cave). This approach is biased toward photophilic and biting insects attracted to either the spotlight or the observer. However, because we conducted it consistently each night (i.e., one observer, at the same time, in the same location using the same spotlight) it provides a reasonable approximation of night-to-night variation in insect abundance that could be useful for understanding foraging behaviour of bats.

Triglyceride concentration was quantified in plasma samples using a serum triglyceride determination kit (Sigma-Aldrich) modified for small sample volumes in 96-well microplates as described by Guglielmo et al. (2002, 2005). As for previous studies using this technique with insectivorous bats, plasma was analyzed undiluted (McGuire et al. 2009*a,b*). When sample volume was sufficient analyses were conducted in duplicate and the coefficient of variation was calculated between measurements (CV). Volume permitting, any samples with a $CV > 15\%$ were re-analysed and only samples with $CV \leq 15\%$ were included in our analyses.

Statistical Analyses

Sub-adult bats are relatively uncommon at our study site, and are known to follow different patterns of body mass accumulation than adults (Kunz et al. 1998; McGuire et al. 2009*b*). Therefore, we restricted our analysis of blood samples and body mass data to adult bats. All statistical analyses were conducted in R (v. 3.0.1., R Development Core Team, 2013). In all models, date was converted to Julian date. Plasma triglyceride concentration was highly right skewed and required $\log_{10}$ transformation to achieve normality. We examined variation in plasma triglyceride concentration with general linear models to test for effects of sex and date, including capture time, body mass, forearm length, bleed time, T_a , relative insect abundance, and ΔBP as covariates. We included two-way interactions between sex and each of the covariates. We followed a backwards stepwise procedure to identify the best model, sequentially removing the least significant term in the model. Initial visual inspection of the body mass data suggested a non-linear relationship between body mass and date. Therefore, we tested for the influence of date on body mass using a general additive model (GAM). We included date as a non-linear term in the model and included linear terms to test for effects of sex, forearm length, capture time, T_a ,

relative insect abundance and ΔBP . We began our model building procedure by comparing a model with a common date smoothing fit for all bats, and a model with separate smoothing curves fit by sex. Optimal smoothing functions were determined using penalized cubic regression splines, selecting the optimal degree of smoothing by cross-validation (Zuur et al. 2009). All further model selection followed a backwards stepwise procedure as described above.

We used GAM to compare body mass between our study population and the more southern population studied by McGuire et al. (2009b). General additive models allowed us to model non-linear patterns of body mass change over time while including effects of sex and forearm length (body size). The methods in both studies were identical (same study species, time period, sampling and storage methods, and same analytical kits) so we make direct comparisons of plasma triglyceride concentrations between the two studies.

All procedures were approved by the University of Winnipeg Animal Care Committee (Protocol #155) and conducted under Manitoba Conservation Wildlife Scientific Permit WB0612.

Results

We captured 401 adult bats in 27 nights of capture over a 54 night period, and collected sufficient blood for analysis from 123 bats. Mean time ($\pm$ s.e.) from capture to blood sampling was 8.2 ± 0.25 min (range 2 – 10 min). Following analysis we excluded 21 samples where it was not possible to determine triglyceride concentration with $CV \leq 15\%$, resulting in a final sample size of 102 bats. There was no effect of sex or date, nor any of the covariates (i.e., capture time, body mass, forearm length, total bleed time, T_a , ΔBP , relative insect abundance), or any two-way interactions on plasma triglyceride concentration (all $p > 0.05$). There was no change in plasma

triglyceride concentration throughout the swarming period, nor was there a difference between males and females (Fig. 1). Mean triglyceride concentration was 0.30 ± 0.03 mmol/L, and the maximum observed triglyceride concentration was 1.22 mmol/L.

The pattern of change in body mass was the same for males and females ($F_{3,2,149.6} = 1.7$, $p = 0.16$) and there was no effect of capture time, T_a , relative insect abundance, or ΔBP on body mass (all $p > 0.05$). Controlling for body size (forearm length: $F_{1,393} = 33.4$, $p < 0.0001$) there was a nonlinear pattern of body mass increase with date ($F_{6,8,393} = 66.3$, $edf = 5.725$, $p < 0.0001$). Body mass was relatively low in early August then increased sharply from approximately 28 August until 7 September, and remained high for the remainder of the study (Fig. 2).

Although both our Manitoba population and the Ontario population studied by McGuire et al. (2009b) gained mass throughout swarming, the pattern of mass change differed between populations. A model including separate smoothing curves was better supported than a common smoothing curve model ($F_{5,9,556} = 101.98$, $p < 0.0001$). In other words, the timing of abrupt changes in body mass differed for the two populations and it would have been inappropriate to directly compare body masses throughout the swarming period. Therefore, to minimize temporal effects we limited our analysis to an early period (first 10 days at each site) and late period (final 10 days at each site) and used a two-way ANOVA to compare body mass between populations and periods. There was a significant population*period interaction ($F_{1,242} = 15.4$, $p = 0.0001$). Controlling for body size, body mass was lower in Ontario bats during the early ($F_{1,151} = 114.0$, $p < 0.0001$) and late period of swarming ($F_{1,90} = 127.2$, $p < 0.0001$). Bats in Manitoba weighed more than bats from Ontario during the early period, but also increased body mass more than the Ontario population (24% vs 8%; Fig. 3).

Discussion

Our results suggest that little brown bats from a northern population in central Manitoba experience constraints in foraging compared to bats from a more southern population in eastern Ontario. However, despite this constraint on energy intake, northern bats are still able to accumulate substantially larger fat stores than southern bats. We found consistently low concentrations of plasma triglycerides throughout the swarming period (mean 0.30 ± 0.03 mmol/L, max 1.22 mmol/L). We can consider these values in context by comparing to previously published studies of little brown bats. McGuire et al. (2009b) described two phases of swarming in eastern Ontario- an early phase of hyperphagia during which plasma triglyceride concentrations exceeded 3.0 mmol/L in some cases, and a later phase where plasma triglyceride concentrations were nearly an order of magnitude lower (mean 0.42 mmol/L, max 1.1 mmol/L excluding three outliers). Even during the latter phase of reduced foraging observed by McGuire et al. (2009b) plasma triglyceride concentrations were approximately twice as high as we observed in Manitoba (0.42 mmol/L vs 0.24 mmol/L). In a study of little brown bats at a summer maternity colony in southern Ontario, Canada McGuire et al. (2009a) reported post-foraging concentrations of 0.84 ± 0.10 mmol/L during good foraging weather but, on nights with rain when bats were unable to forage, triglyceride concentrations were, again, much lower (0.22 ± 0.05 mmol/L). Thus, the triglyceride concentrations we observed consistently throughout the swarming period (0.30 ± 0.03 , max 1.22 mmol/L) were similar to published values for little brown bats on rainy nights with low foraging success (McGuire et al. (2009a). The low fuelling rates we observed are particularly notable when compared to the pre-hibernation hyperphagia or even the relatively lower fuelling rates observed in the latter phase of swarming by McGuire et al. (2009b). The low fuelling rates in our study are consistent with the hypothesis that northern

populations are energetically constrained during autumn swarming relative to bats from lower latitudes.

Despite the fact that plasma triglyceride concentration remained very low, body mass increased substantially over the course of the sampling period (Figs. 2, 3) which is not consistent with energetic limitation. Over a two week period in late August – early September, there was an abrupt increase in body mass (~3 g or ~30%). Bats achieved this substantial increase despite low plasma triglyceride concentrations, indicating low fuelling rates. Thus the question arises: How were bats able to substantially increase fat stores over the swarming period, seemingly without increasing food intake? There are several possible explanations for these apparently contradictory results.

One explanation for increased body mass in the absence of increased foraging is that bats may have concentrated foraging away from swarming sites on some nights. Little brown bats commonly feed on emergent aquatic insects (e.g., Coleoptera and Diptera) (Belwood and Fenton 1976; Moosman et al. 2012; Clare et al. 2013). On warmer nights with greater prey availability bats may have foraged at a nearby body of water prior to being captured at the swarming site. From our study site, the closest body of water is approximately 3.5 km from the cave. With an estimated flight speed of 5 m/s (Hayward and Davis 1964) a little brown bat could commute between the lake and hibernaculum in approximately 12 min. Thus bats could travel to the body of water on warmer nights to increase their foraging success. In passerine birds, plasma triglyceride concentration begins to increase ~ 10 min after feeding, peaks around 30 – 40 min (Zajac et al. 2006) and remains elevated for several hours following feeding (Jenni-Eiermann and Jenni 1994). With a commute time of < 30 min, even if bats foraged away from the swarming site, the foraging signal should remain detectable as triglyceride concentrations remain elevated

for several hours (McGuire et al. 2009a). Therefore we suggest that foraging away from the swarming site is not the most likely explanation for rapid mass gain in the absence of evidence for increased fuelling rate based on plasma triglyceride concentration.

To increase body mass, animals must be in a state of positive energy balance which may be achieved either by increasing energy intake or reducing energy output. In our study we observed no indication of increased energy intake suggesting that the dramatic increase in body mass we observed is best explained by reduced energy output. To decrease energy expenditure, it is possible that on nights with lower T_a , and thus reduced prey availability, little brown bats used torpor to reduce energy output. Torpor is an energy-saving strategy widely used by insectivorous bats in which body temperature and metabolic rate are reduced (Stawski et al. 2014) and has been suggested as a strategy for increasing mass gain during pre-hibernation fattening (Speakman and Rowland 1999). McGuire et al. (2009b) observed an increase in the number of torpid bats inside the hibernaculum later in the swarming period, before the start of hibernation which would have allowed bats to save substantial amounts of energy, and thus continue to build fat stores despite reduced foraging. Similarly bats in our study may have used torpor more extensively on nights with lower T_a , and thus lower prey availability, allowing for conservation of energy stores. Further study of thermoregulatory patterns and differences among populations across latitudes is required to fully understand the interplay of fuelling rates, torpor use, mass gain, and reproductive investment. In the same way that increased time spent foraging reduces time available for reproductive activities, torpid bats are not reproductively active. Therefore, regardless of whether the solution for northern bats involves increased foraging activity (increased energy intake) increased torpor use (decreased energy output), or some combination of the two, our results are consistent with is a trade-off between pre-hibernation fattening to

ensure overwinter survival and mating activity to increase reproductive success. This possible life-history trade-off requires further research attention.

Our study emphasizes the importance of considering environmental variation across the range of widely distributed species. Body mass, controlling for body size, was substantially greater for bats in central Manitoba, with a maximum of 16.0 g (enormous for a little brown bat) as compared to the bats in eastern Ontario (maximum 11.1 g). Little brown bats in central Manitoba are ultimately able to deposit the fat stores required to survive the longest hibernation periods yet recorded for any species of bat (Norquay and Willis 2014) and thus do not appear to lack foraging resources. However, the triglyceride signal of foraging of the bats in our study was much lower than the triglyceride signal previously observed for a more southern population. Therefore, while resources are adequate to permit fat accumulation, the behavioural differences that must occur to allow for fat accumulation by the more northern bats indicate that living at a higher latitude poses some constraints. Future studies should further examine the ecological and population level consequences of these constraints. For example, if bats forego mating opportunities at swarming sites to forage, constraints associated with high latitude could have consequences for the mating system, reproductive fitness, and potentially genetic diversity of the population.

Acknowledgments

We thank A. Uilhoorn for her help collecting blood samples, J. Lawler, B. Bendure, G. Luzwick and T. Townsend for their assistance in the field, and H. Mayberry for logistical assistance throughout the study. We are grateful to two anonymous reviewers for their helpful comments on the manuscript. We also acknowledge the Misipawistik Cree Nation for the

opportunity to conduct research on bats on their traditional territory. Funding was provided to CKRW from the National Science and Engineering Research Council (NSERC) and the Kenneth M Molson Foundation. Two anonymous reviewers provided helpful feedback on the manuscript.

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Figure Captions

Figure 1. Plasma triglyceride concentrations of little brown bats (*Myotis lucifugus*) captured during swarming at a hibernaculum in central Manitoba ($n = 102$, 6 August – 28 September 2013). Triglyceride concentration was not affected by either sex or date, nor any other factor we considered. Refuelling behavior of our study population during swarming is characterized by a consistently low concentration of triglycerides.

Figure 2. Body mass ($n=401$) of adult little brown bats (*Myotis lucifugus*) captured at a cave throughout the swarming period (6 August – 28 September 2013). The solid line represents a GAM smoothing curve and dashed lines represent ± 1 SE. There was a dramatic increase in body mass between approximately 28 August and 7 September despite no indication of increased nutrient intake during this time period (Fig. 1). Note the large body masses, with individual bats weighing as much as 16.0 g compared with maximum body mass of 11.1 g observed from a more southerly population (McGuire et al. 2009b).

Figure 3: Comparison of body mass in populations from Manitoba and Ontario (mean $\pm$ SE). Sample sizes indicated in parentheses below each bar. The population in Ontario (black bars) had lower body mass than the more northern population in Manitoba (white bars) in both early and late periods of swarming. A population*period interaction indicated that the change in body mass from early to late swarming was greater for the Manitoba population, despite a lack of hyperphagia in the Manitoba population (Fig. 1).

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