

# Changes in underground roosting patterns to optimize energy conservation in hibernating bats

Caleb C. Ryan, Lynne E. Burns, and Hugh G. Broders

**Abstract:** Non-migratory bats in colder climates use hibernation to survive winter. By reducing metabolic rate (i.e., using torpor), bats can survive winter on stored fat reserves. During hibernation, bats arouse from torpor and may move within the hibernaculum, a process called “internal migration”. We hypothesized that internal migration occurs to optimize hibernation energetics in that bats move to select a microclimate to minimize energy expenditure both by seeking cooler areas of the hibernacula and avoiding those with large temperature fluctuations. Early in the winter, we observed that 62% of bats were roosting in the warmer, less energy efficient, deepest 50% of an abandoned mine hibernaculum. Late in the season, there was a shift towards the cooler entrance area, thereby decreasing energy demands during the torpid period, with 78% of bats in the mine roosting within 50 m of the entrance. Although there was no significant effect of hibernation period (i.e., early vs. late winter) on the number of bats in huddles, the largest huddles occurred close to the entrance in late winter. To fully understand and manage bat populations, it is important to understand that hibernation is a dynamic process with bats moving and interacting with one another throughout the season.

**Key words:** hibernation, torpor, energy conservation, little brown bat, *Myotis lucifugus*, northern long-eared bat, *Myotis septentrionalis*.

**Résumé :** Les chauves-souris non migratrices vivant en climat froid ont recours à l'hibernation pour survivre à l'hiver. En abaissant leur métabolisme (c.-à-d. par la torpeur), elles peuvent survivre pendant l'hiver sur leurs réserves de graisse. Durant l'hibernation, les chauves-souris émergent de leur torpeur et peuvent se déplacer dans l'hibernaculum, un processus appelé « migration interne ». Nous avons postulé que la migration interne a lieu afin d'optimiser l'énergétique de la l'hibernation, les chauves-souris se déplaçant pour choisir un microclimat qui minimise la dépense énergétique en cherchant des secteurs plus frais de l'hibernaculum et en évitant ceux où les fluctuations de température sont grandes. Nous avons observé que, au début de l'hiver, 62 % des chauves-souris étaient perchées dans la moitié plus chaude, plus profonde et la moins efficace sur le plan énergétique d'un hibernaculum dans une mine abandonnée. Vers la fin de la saison, il y avait un déplacement vers la zone de l'entrée, plus fraîche, réduisant ainsi les demandes énergétiques durant la période torpide, alors que 78 % des chauves-souris dans la mine étaient perchées dans un rayon de 50 m de l'entrée. Si la période d'hibernation (c.-à-d. début ou fin de l'hiver) n'avait aucun effet significatif sur le nombre de chauves-souris dans les entassements, les plus grands entassements se trouvaient près de l'entrée vers la fin de l'hiver. Pour bien comprendre et gérer les populations de chauves-souris, il importe de comprendre que l'hibernation est un processus dynamique dans lequel les chauves-souris se déplacent et interagissent tout au long de la saison. [Traduit par la Rédaction]

**Mots-clés :** hibernation, torpeur, conservation d'énergie, vespertilion brun, *Myotis lucifugus*, vespertilion nordique, *Myotis septentrionalis*.

## Introduction

Strategies for survival of winter in temperate regions may involve a variety of adaptations ranging from avoidance (e.g., migration) to hibernation that allow animals to cope with conditions of low temperature and food availability (Neuweiler 2000). Adaptations typically involve ways to optimize energy balance including morphology, physiology, and behaviour. Selection pressures on small animals may be particularly high because they have the potential to lose heat much more rapidly than large animals (Kleiber 1947). For year-round residents that do not have an available external energy source during the winter months (e.g., bats and ground squirrels), hibernation allows for pre-winter energy stores to last until food becomes available in the spring (Geiser 2004). During hibernation, heterothermic mammals such as bats and ground squirrels will lower their body temperature to 1–2 °C

above ambient, decreasing their metabolism and therefore the rate in which they use their stored energy reserves (Geiser and Kenagy 1988; Boyles et al. 2008; Halsall et al. 2012), and may remain in this state for up to 8 months in some areas (Buck and Barnes 1999; Norquay and Willis 2014).

Hibernation is dynamic, with heterotherms periodically arousing for short periods (hours–days) from torpor bouts that are typically days to weeks in length (Stones and Wiebers 1965; Thomas et al. 1990; Geiser 2004, 2013; van Breukelen and Martin 2015). There are a few non-mutually exclusive hypotheses to explain why heterotherms undergo these energetically expensive torpor-arousal patterns. Most are thought to occur to mitigate the physiological costs of torpor including the need to rehydrate (Thomas and Geiser 1997), voiding of metabolic waste products (urinate), fighting disease, and repaying sleep deprivation (Daan et al. 1991; Park et al. 2000). The re-warming of individuals to euthermia is

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energetically expensive and can account for 90% of an animal's total hibernation energy expenditure during hibernation (Thomas et al. 1990). Bats, for example, take advantage of this euthermic period and may engage in active behaviours such as drinking, grooming, copulating, and changing roosting location (Boyles et al. 2006).

Torpid metabolic rate (TMR), the amount of energy the animal expends during torpor, is directly affected by the ambient temperature ( $T_a$ ) (Geiser 2004; Boyles et al. 2007). For animals that hibernate in large underground openings such as caves and mines, like little brown bats (*Myotis lucifugus* (Le Conte, 1831)) and northern long-eared bats (*Myotis septentrionalis* (Trouessart, 1897)), the microclimate conditions can vary both temporally and spatially throughout the hibernaculum. Because of this, bats select microclimates that balance the need to conserve fat stores (Kunz et al. 1998) and minimize the aforementioned risks of metabolic suppression that occur during torpor. A hibernator's ability to survive the winter hibernation period is dependent on the individual having sufficient fat stores accrued at the beginning of hibernation to offset the energy expended during the hibernation season (Boyles and Brack 2009). A study in Vermont, USA, found that adult male *M. lucifugus* gained, on average, 32.9% of their pre-hibernation body mass in the 2 months leading up to hibernation (Kunz et al. 1998), whereas male Arctic ground squirrels (*Spermophilus parryi* (Richardson, 1825)) gained 42% of their total body mass between the end of reproduction and the end of the active season (Sheriff et al. 2013), representing the energy reserves available to the animal to survive the winter.

The hibernation optimization model (Humphries et al. 2003) hypothesizes that hibernators balance trade-offs to optimize torpor conditions and conserve energy while battling with the costs of metabolic suppression. In general, individuals with greater fat reserves will select warmer microclimates than individuals with smaller reserves (Boyles et al. 2007) because hibernating at a warmer  $T_a$  requires more energy (Dunbar and Tomasi 2006). This leads to the question of why all bats would not choose to hibernate at the coldest part of the hibernaculum for the entirety of the winter. Warmer microclimates may have advantages for some individuals such as reducing the risk of bats freezing or shivering while torpid, a problem that is observed when bats hibernate in colder microclimates (Stones and Wiebers 1965). Warmer hibernation temperatures are also associated with a shallower depth of torpor, which is less physiologically damaging, but also less energetically efficient, than hibernating at cooler temperatures (Humphries et al. 2003). Lastly, thermal stability of a roost location may also play a role because roost thermal stability is known to be inversely proportional to metabolic expenditure (Kurta 1985) such that individuals may optimize their hibernation by selecting warmer, more stable locations in response to temporal thermal variation (Boyles and McKechnie 2010).

More recently, variation in hibernaculum microclimate selection among individuals within a species was demonstrated in Indiana bats (*Myotis sodalis* Miller and G.M. Allen, 1928) where individual bats used microsites at a range of temperatures within a single hibernaculum (Boyles et al. 2017). This is consistent with earlier work in the Netherlands that discusses hibernation activity and microclimate selection of nine species of European bats (Daan and Wichers 1967; Daan 1972). These studies observed a pattern of bats moving from warmer areas to cooler areas within the hibernaculum as winter progressed, a process that they termed "internal migration". Collectively, this suggests that temperature optimization by changing roosting location within a hibernaculum could reduce the energy expenditure during the winter and decrease the amount of fat required to fuel hibernation.

Another hibernation behaviour that bats employ to optimize energy conservation is huddling (sometimes referred to as "clustering"), which is defined as an active and close aggregation of animals usually for the purpose of social thermoregulation and energy conservation (Gilbert et al. 2010). In bats, huddling is used

to limit heat and evaporative water loss (Boyles et al. 2008; Boratyński et al. 2015), minimize energy required for spring arousal through synchronous re-warming (Boyles and Brack 2009; Czenze et al. 2013), and buffer bats from fluctuating ambient temperatures (Clawson et al. 1980).

The goal of this study was to characterize winter hibernation patterns to better understand behaviours that optimize energy conservation of bats during hibernation. We hypothesized that bats would change both their roost location within the hibernaculum and huddling patterns to optimize energy conservation during winter. More specifically, we predicted that bats would move from warmer areas of a hibernaculum to colder areas as winter progresses, trading off the stable temperatures of the deeper parts of the mine with the energy efficiency of the cooler front. We also predicted that a larger proportion of bats would be found huddling in colder areas of the hibernaculum, with most bats being found huddling at the entrance of the site at the end of hibernation to conserve energy during arousal through passive re-warming thereby setting the stage for spring emergence.

## Materials and methods

### Study species and site description

This study was part of a multi-year (2007–2012) hibernaculum survey at an abandoned underground gold mine at Rawdon (45°02'N, 63°49'W) in Hants County, Nova Scotia (Burns and Broders 2015a). The mine is a 325 m long horizontal adit and is primarily made up of metasedimentary slate (Day 1987). The mean height of the adit along its entire length was ≈1.9 m (range 1.75–2.5) and the mean width was ≈2.5 m, making it possible to reliably count and access most individuals along its length because few deep crevices or drill holes were present. During the winter, the mine is used as a hibernaculum by *M. lucifugus*, *M. septentrionalis*, and tricoloured bats (*Perimyotis subflavus* (F. Cuvier, 1832)) (Moseley 2007).

### Data collection

All bats observed in this study were originally captured and tagged using 0.1 g passive integrated transponder (PIT) tags (Trovan ID 100, EIDAP Inc., Sherwood Park, Alberta, Canada) during autumn swarming or upon spring emergence as part of another study (Burns and Broders 2015a). Briefly, 240 *M. lucifugus* and 170 *M. septentrionalis* were captured using harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia) set at the entrance to the mine over 26 sampling nights and tagged using PIT tags (Burns and Broders 2015a). The most up-to-date white-nose syndrome (WNS) decontamination protocols provided by the U.S. Fish and Wildlife Service were followed to minimize any potential spread of the fungus (WNS Decontamination Team 2012) from 2009 onwards. In addition to these procedures, the amount of trapping and tagging was reduced in 2011 with no tagging taking place in the spring to further prevent the spread of WNS (Burns and Broders 2015b). Methods for the capture and handling of bats were approved by the Saint Mary's Animal Care Committee (protocols 09-24, 10-11, 11-18) under yearly issued permits from the Nova Scotia Department of Natural Resources.

Hibernaculum surveys were done twice each winter, with early surveys from late November to early December and late winter surveys from late March to early April (2008–2012). Surveys were conducted by three to four people who moved as a unit through the mine to minimize disturbance. Multiple observers independently counted and scanned bats for PIT tags on ingress through the site. Counts of bats at the site were averaged from all observer counts per visit. All accessible bats (estimated >95%) were individually scanned with handheld recorders for PIT tags. Distance from the entrance was determined using a hip chain and was recorded for all tagged bats. Huddle size was determined by counting the number of bats associated with each individually tagged bat. Huddles were defined as any group of two or more bats for which each

**Table 1.** Variables and equations used to estimate hibernation energy expenditure of bats hibernating at specific measured (ambient temperature,  $T_a$ ) microclimates within the hibernaculum within the Rawdon Gold Mine, Nova Scotia, Canada.

Variable	Value	Unit	Reference
Resting metabolic rate, RMR	2.6	mL O <sub>2</sub> ·g <sup>-1</sup> ·h <sup>-1</sup>	Humphries et al. 2002
Minimum torpid metabolic rate, TMR <sub>min</sub>	0.03	mL O <sub>2</sub> ·g <sup>-1</sup> ·h <sup>-1</sup>	Humphries et al. 2002
Body temperature while euthermic, $T_{eu}$	35	°C	Humphries et al. 2002
Minimum body temperature while torpid, $T_{tor-min}$	2	°C	Humphries et al. 2002
Lowest critical body temperature, $T_{lc}$	32	°C	Humphries et al. 2002
Change in torpor metabolism owing to a 10 °C change in temperature, $Q_{10}$	$1.6 + 0.26T_a - 0.006T_a^2$		Humphries et al. 2002
Time spent torpid, $t_{tor}$	$-8.11T_a + 48.57$	days	Jonasson and Willis 2012

individual was in direct contact with at least one other within the huddle.

Hibernaculum temperature was recorded at 1-h intervals using data loggers (iButton DS1922L, Maxim Integrated, San Jose, California, USA) with a resolution of 0.5 °C, fixed to the walls of the hibernaculum 1–2 m from the ground. Data loggers were placed at the entrance and 50, 104, 227, and 275 m from the entrance from 18 December 2009 to 9 December 2010 and again from 5 December 2011 to 3 December 2012. A separate logger was placed outside the mine to record ambient environmental temperature. The data logger was mounted on a spruce tree 0.5 m above ground level between two major branch whorls to shelter from wind and solar radiation.

### Analysis

We characterized intra-hibernaculum movements by characterizing the change in the number of bats roosting at 20 m intervals inside the mine using histograms. Both sex and species were pooled for analysis. General linear mixed models (GLMM) were used to independently test both the effect of the roost distance from the hibernaculum entrance and the time of year (early or late hibernation) on huddle size with year (i.e., winter season) as the random variable in both of the tests using  $\alpha = 0.05$  following a Poisson distribution.

To characterize what extent changes in  $T_a$  have on total hibernation energy expenditure, we modified an energetics model to estimate energy expenditure at different locations in the hibernaculum. The model is based on that of Humphries et al. (2002) and Twente et al. (1985), but it is purposefully generalized to isolate the effect that changes in  $T_a$  have on the amount of fat required for hibernation in *M. lucifugus*, which is the most abundant species at our study site. The resting metabolic rate (RMR), minimum torpid metabolic rate (TMR<sub>min</sub>), body temperature while euthermic ( $T_{eu}$ ), minimum body temperature while torpid ( $T_{tor-min}$ ), and lowest critical body temperature ( $T_{lc}$ ) were standardized using values from the literature for this purpose of this model, whereas the  $Q_{10}$  (change in torpor metabolism owing to a 10 °C change in temperature) and the time spent torpid ( $t_{tor}$ ) were estimated as a function of  $T_a$  (Humphries et al. 2002; Jonasson and Willis 2012) (Table 1):

$$E_{winter} = [t_{winter}/(T_a + 3.75)] \times \{ [2.6 + (32 - T_a)0.2638]3 + [0.03(1.6 + 2.6T_a - 0.006T_a^2)^{(T_a-2)/10}]^{(0.04224T_a+2.4227)} \} 10 \times [0.03(1.6 + 2.6T_a - 0.006T_a^2)^{(T_a-2)/10} + (35 - T_a)0.131]$$

where  $E_{winter}$  is the grams of fat (g fat) required for hibernation,  $t_{winter}$  is the duration of the hibernation period, and  $T_a$  is the temperature at the given location. The temperature data used in this study was taken at five different locations within the mine, not at the exact roost sites of the bats. Because of this, we are only able to determine the  $E_{winter}$  at the locations where temperature was recorded.

## Results

### Intra-hibernaculum movement

Temperature and temperature stability were positively related with distance from the entrance, with temperatures recorded in the front 50 m being cooler and more variable (mean = 6.698 °C, SD = 0.5845 °C), fluctuating 3.0 °C over the winter (Fig. 1). However, during the second half of the hibernation season, the front 50 m became more stable (mean = 7.908 °C, SD = 0.3240 °C), fluctuating by just 1.0 °C. Temperatures at the back of the mine fluctuated by only 1.0 °C during the entire winter. Using this temperature information, the estimated energy expenditure (g fat) required to survive the winter was determined to be greater in the back (1.370 g) than in the front (1.169 g) of the hibernaculum (Table 2).

Based on the 368 early-winter and 280 late-winter PIT-tagged bat records within the hibernaculum, species composition of the study sample was 85% *M. lucifugus* (510 males, 41 females) and 15% *M. septentrionalis* (91 males, 6 females).

The distribution of bats within the hibernaculum during each of the winters from 2008 to 2011 changed from early to late in the season. All bats were recorded hibernating between 4 and 300 m from the entrance. Early in the hibernation season (December), ~62% of bats were found hibernating in the deepest 50% of the mine (between 150 and 300 m from the entrance; Fig. 2A), which changed at the end of hibernation (April) where ~78% of the hibernating bats were found within the front 50% of the mine (Fig. 2B).

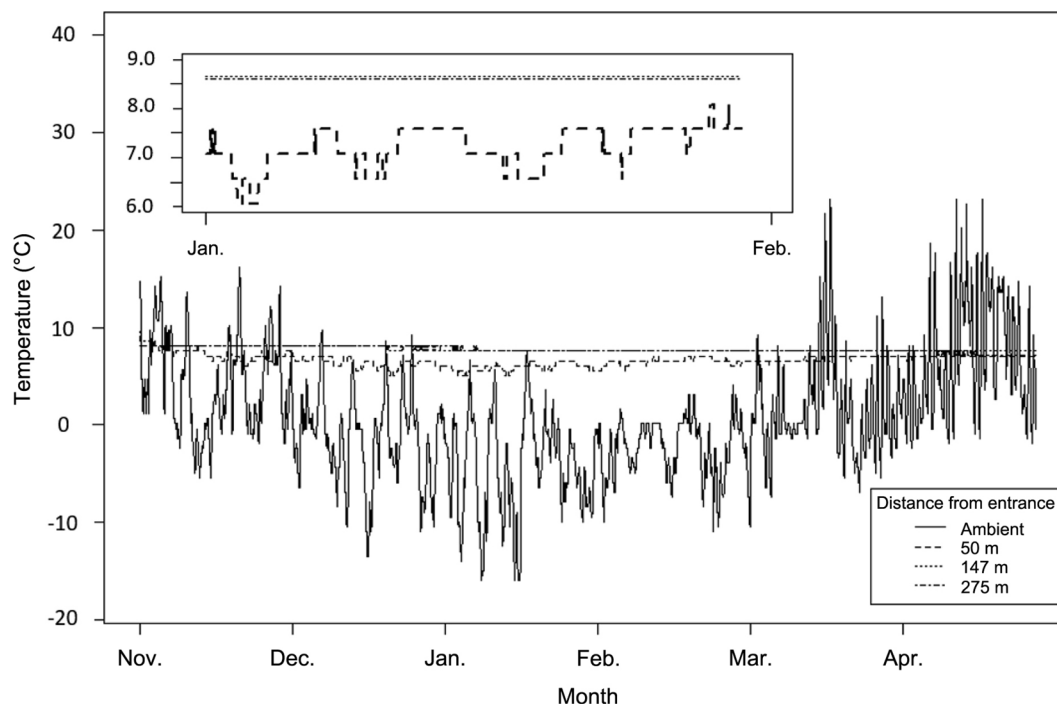
Of the 188 marked bats for which we had both early and late winter records for the same year, 137 (73%) moved from the back of the hibernaculum to the front (Fig. 3B), 44 (23%) were found within the same 5 m each time, and 7 (4%) moved away from the entrance. The mean roosting distance for bats that moved towards the front of the hibernaculum was 50.7 m (SD = 39.8 m) from the entrance at the end of hibernation. The 44 bats that had no net movement were distributed around the middle of the mine adit at both the beginning and the end of the hibernation period (Fig. 3A).

### Huddling patterns

At the beginning of hibernation, a mean of 55.7% (SD = 9.3%) of the bats in the hibernaculum were found in huddles compared with 48.8% (SD = 3.0%) at the end of hibernation regardless of roosting location inside the mine. There was no significant effect of hibernation period (i.e., early vs. late winter) on the total number of bats in huddles ( $\beta = 0.02$ , SE = 0.11,  $p = 0.84$ ). Little variation in roosting distance was observed between huddling and non-huddling bats in the early winter; however, in the late winter, huddling bats were found, on average, 63.3 m (SD = 9.7 m) from the entrance compared with 115.2 m (SD = 13.8 m) for non-huddling individuals (Table 3). A larger proportion of bats were observed to be huddling at the front of the hibernaculum in the late winter compared with the early winter, when there was a larger proportion of huddling bats deeper inside the mine (Fig. 4). The number of bats in a huddle decreased with distance from the entrance ( $\beta = -0.002$ , SE = <0.001,  $p = 0.02$ ), with the largest huddles being found close the entrance of the mine.



**Fig. 1.** Temperature data recorded hourly from 1 November 2011 to 1 May 2012 at 50, 147, and 275 m inside Rawdon Gold Mine, Nova Scotia, Canada, and the ambient temperature recorded outside the mine. Inset: Temperature data from 1 January to 1 February 2012 at 50, 147, and 275 m inside the mine.



**Table 2.** Predicted winter energy expenditure for winter hibernation, 14 November to 8 May, within Rawdon Gold Mine, Nova Scotia, Canada, based on the mean ambient temperature ( $T_a$ ) at each distance from the entrance.

Distance from entrance (m)	$T_a$ (°C)	Range (°C)	Energy expenditure (g fat)
50	6.698	3.015	1.169
108	7.419	1.005	1.282
150	7.888	0.502	1.366
227	7.615	1.002	1.316
275	7.908	1.004	1.370

## Discussion

As hypothesized, changes in both roost location and huddling patterns were observed between the start and end of the hibernation period with bats being observed moving towards the front of the hibernaculum. Although no increase in the total number of bats huddling was observed, an increase in total proportion of huddling bats was found at the front of hibernaculum at the end of the winter.

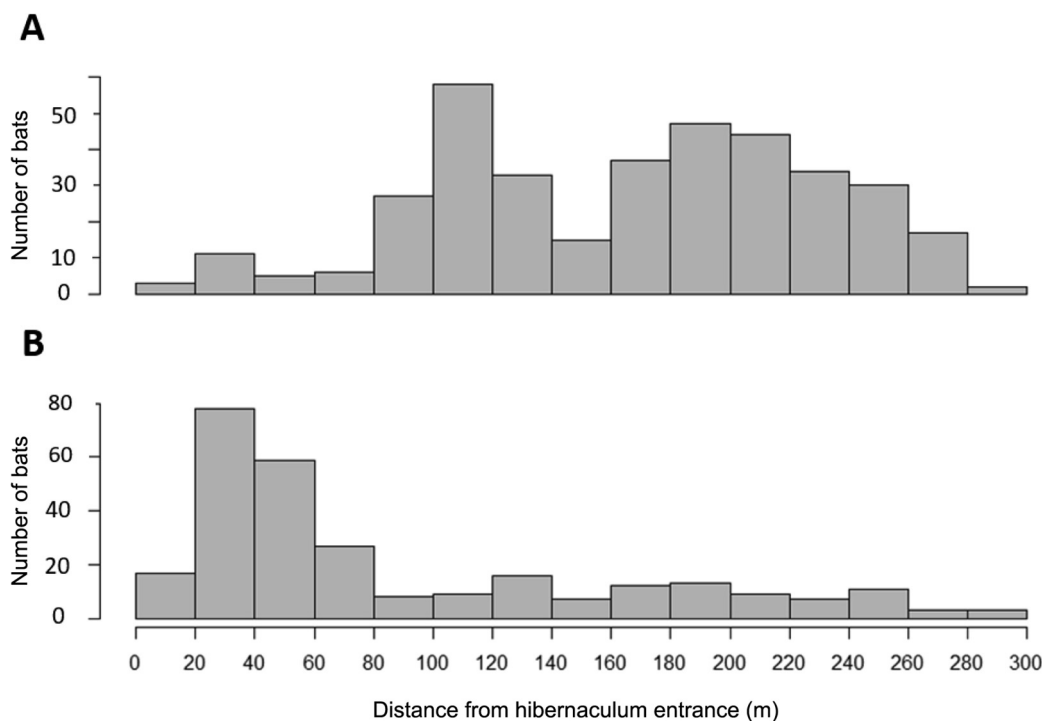
An internal migration of the bats that used the mine as a hibernaculum occurred with bats moving from the back of the hibernaculum in the early winter towards the front of the mine in late winter. This behaviour may be at least partially temperature driven, as bats could be selecting and changing microclimates to optimize their energy expenditures in relation to their decreasing fat reserves as hibernation progresses. At the beginning of the hibernation season, most bats roosted towards the back of the mine, which is warmer with more stable temperatures compared with the front. These warmer temperatures are more energetically costly hibernation sites than the cooler front of the mine. However, the more stable temperatures found in the back 50% of the hibernaculum insulate the bats from fluctuating temperatures that in turn decreases hibernation energy expenditure, because although the temperatures in the back of the mine are warmer, there is an inverse relationship between roost thermal

stability and metabolic expenditure (Humphries et al. 2002). This means that it can be more energetically efficient to roost at warmer, more stable temperatures than cooler, more variable ones (Boyles and McKechnie 2010). However, in the late winter, bats are found roosting in the cooler, less temperature stable front portion of the mine. Although the front of the mine is cooler, it is also a potentially more harmful hibernation site both from the increased risk of predation and the physiological harms of hibernation (e.g., freezing) at cooler temperatures (Stones and Wiebers 1965; Humphries et al. 2003). Therefore, the front may not be a more efficient hibernation site until the amount of temperature fluctuation decreases at the end of the winter (Boyles and McKechnie 2010).

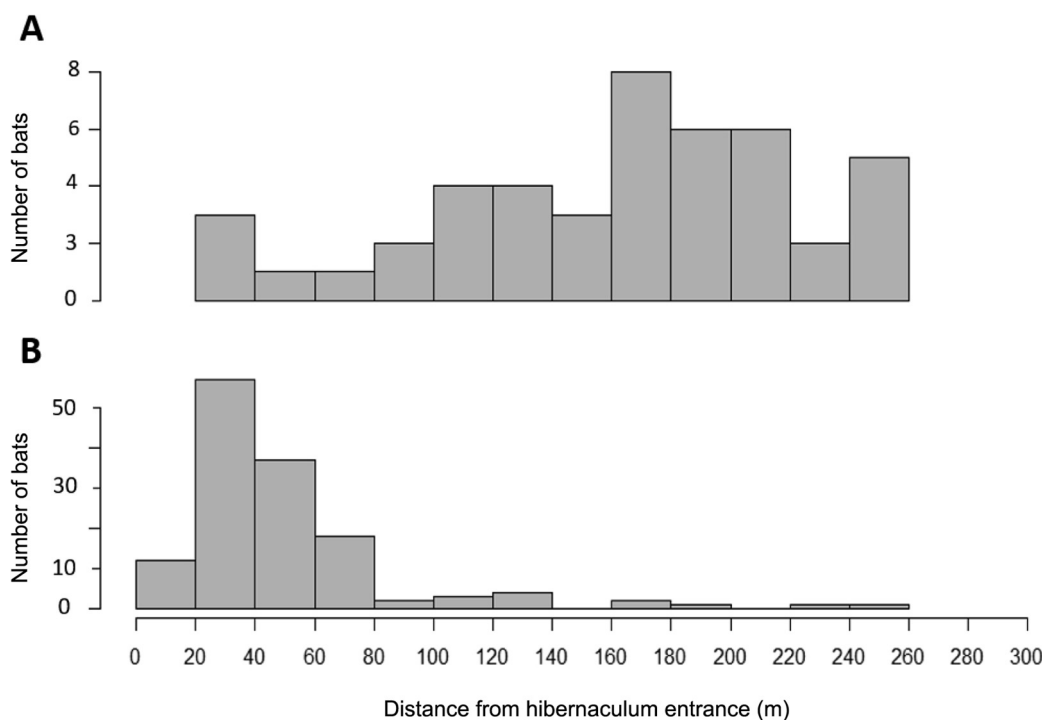
Energy expenditure may be useful to explain why some bats move roost locations during hibernation and some bats do not change locations during hibernation. Non-moving bats remained grouped at the relatively warmer centre (compared with the front) of the mine ahead through the winter, whereas the majority of moving bats made a forward movement from the back to the front of the hibernaculum. A possible reason for this difference in behaviour is that non-moving bats may have had larger fat reserves than forward-moving bats, so it was not necessary for these bats to move towards the front of the mine and risk the increased physiological harms of deeper torpor (Humphries et al. 2003). Declining fat reserves have been shown to influence arousal timing and frequency, with arousals occurring closer together towards the end of the hibernation season (Czenze and Willis 2015). These arousals then create opportunities for bats to change positions within the hibernaculum to optimize their energy use for the remaining hibernation period.

Changing position to find a new more suitable microclimate for the remainder of hibernation may not be the only adaptive behaviour that bats facing decreasing fat reserves can use as they optimize their hibernation in a season. As we predicted, the size and location of huddles varies throughout the hibernation season. We observed little difference in the location of huddles during early hibernation, but we found a larger proportion of huddled bats

**Fig. 2.** Number of all tagged *Myotis* spp. found at 20 m intervals within Rawdon Gold Mine, Nova Scotia, Canada, from 2008 to 2011: (A) early in the hibernation period and (B) late in the hibernation period.



**Fig. 3.** Number of tagged *Myotis* spp. found in late winter at 20 m intervals within Rawdon Gold Mine, Nova Scotia, Canada, that (A) had no net movement through the winter and (B) moved towards the entrance of the hibernaculum over the course of the winters of 2008–2011.



close to the entrance of the mine at the end of hibernation. This pattern is consistent with the hypothesis that bats use huddling at the end of hibernation to prepare for spring arousal (Czenze and Willis 2015). Increased huddle sizes allow for synchronous arousals (Czenze et al. 2013; Czenze and Willis 2015) and reduces the energy required for arousal (Boyles et al. 2008), thus allowing bats to arouse from torpor in colder locations at the front of the hiber-

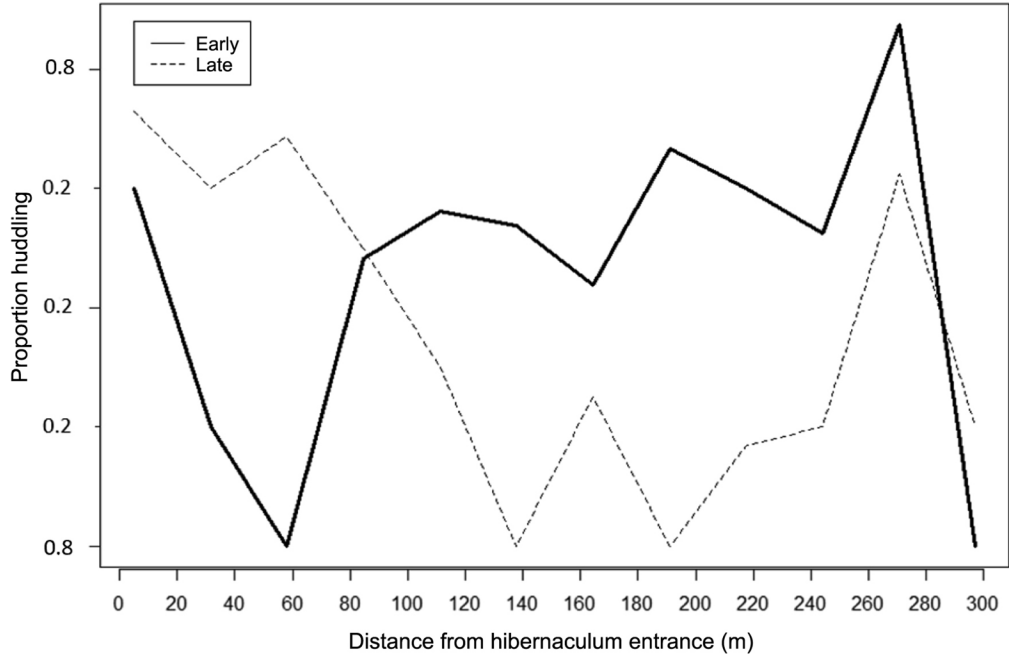
naculum more efficiently. Huddling towards the front of the mine at the end of hibernation may provide other advantages, such as protection against fluctuations in ambient temperature and allowing for more efficient arousals if bats are threatened by an external disturbance (Clawson et al. 1980).

Changes in roost location and huddling patterns are likely associated with the specific microclimatic conditions found within

**Table 3.** Percentage of bats found in a huddle and the mean roosting distance for huddled, non-huddled, and all bat records inside the Rawdon Gold Mine, Nova Scotia, Canada, during the winters of 2009–2012 recorded at both early and late in the hibernation season.

Winter	Early hibernation season				Late hibernation season			
	% Huddled	Mean roosting distance (m)	Mean roosting distance of huddled bats (m)	Mean roosting distance of non-huddled bats (m)	% Huddled	Mean roosting distance (m)	Mean roosting distance of huddled bats (m)	Mean roosting distance of non-huddled bats (m)
2009–2010	68.8	163.8	171.1	147.9	48.8	88.9	65.4	111.4
2010–2011	46.5	167.2	169.2	165.4	46.7	107.9	73.7	139.9
2011–2012	57.1	178.1	188.7	162.8	53.5	67.6	50.5	87.4
All records	55.7	169.7	179.2	161.3	48.8	89.6	63.3	115.2

**Fig. 4.** The proportion of bats found in clusters at 20 m intervals along Rawdon Gold Mine, Nova Scotia, Canada, both early and late in the hibernation seasons of 2009–2012.



our study site and these conditions may vary between hibernation sites. Hibernacula climates vary among sites, depending on a variety of spatial and non-spatial factors such as the size and shape of the hibernaculum, height of the ceiling, and the amount of air circulation (Perry 2013). Differences in hibernacula climates are apparent even within other known nearby hibernacula in Nova Scotia. For example, Hayes Cave (South Maitland, Nova Scotia, Canada) has a mean winter temperature of 5 °C at 75 m inside the cave (H.G. Broders and L.E. Burns, unpublished data), cooler than any mean temperature recorded at our study site. Cluster sizes of bats hibernating within Hayes cave were observed as being larger, on average, than those in Rawdon Gold Mine, which is consistent with the idea of a negative relationship between  $T_a$  and huddle size (Boyles et al. 2008). Our results support other studies that suggest there is no single optimal condition for bat hibernation (Webb et al. 1996; Wojciechowski et al. 2007; Boyles et al. 2017). Rather, variation exists in the conditions and strategies used by individuals (e.g., huddling patterns) that permits successful hibernation.

Hibernation physiology and behaviour of bats have come into focus recently due to the emergence of WNS in hibernating bat populations, with the causative fungus *Pseudogymnoascus destructans* (Bleher & Gargas) Minnis & D.L. Lindner reaching Nova Scotia in April of 2011. Changes in hibernation physiology and not in pre-hibernation body condition are the main cause of mortality in bats affected by WNS (Boyles and Willis 2010). Recent studies have

demonstrated that cryptic community connections between bats, specifically during huddling and movements within and between species during hibernation appear to play a role in the spread of WNS (Hoyt et al. 2018). Hibernaculum temperature has an effect on the growth performance of *P. destructans*, with little growth occurring between 3 and 5 °C and an increasing growth rate towards the optimal temperature of 12.5–15.8 °C (Verant et al. 2012). This means that as bats change their location within the hibernaculum, the rate at which *P. destructans* grows can change exponentially. By quantifying when and how bats are moving and huddling during hibernation, we can better understand how the fungus may be spread during hibernation. Unfortunately, the latest count of bats at this study site in December 2018 was zero.

As observed in this study, bats moved from warmer to colder locations as winter progressed, and although more bats were found in huddles at the beginning of hibernation than at the end, a greater proportion of the huddling bats at the end of hibernation were found at the front of the hibernaculum. This suggests that bats modify their behaviours during hibernation to conserve energy and to mitigate the physical and physiological risks of extended torpor. To better test our hypothesis, more accurate temperature data need to be collected. Rock and ambient temperature should be recorded at more frequent intervals to create a more accurate temperature map of the hibernaculum. To determine if larger bats (i.e., those in better body condition) are in fact more likely to be non-moving compared with smaller individuals,

masses of bats at the beginning and end of hibernation would need to be collected for tracked individuals. Lastly, work examining if huddle mates remain constant or change through time would provide valuable insight into the potential social dynamics of these bats during hibernation.

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