Seasonal energetics and torpor use in North American flying squirrels

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ABSTRACT

Seasonal cold temperatures require mammals to use morphological, behavioural, or physiological traits to survive periods of extreme cold and food shortage. Torpor is a physiological state that minimizes energy requirements by decreasing resting metabolic rate (MR) and body temperature (T_b). Many rodent species are capable of torpor, however, evidence in northern and southern flying squirrels (*Glaucomys sabrinus* and *Glaucomys volans*, respectively) has remained anecdotal. We experimentally attempted to induce torpor in wild-caught flying squirrels by lowering ambient temperature (T_a) and measuring MR using open-flow respirometry. We also studied seasonal differences in MR and T_b at various T_a. Both MR and T_b provided evidence for torpor in flying squirrels, but only infrequent, shallow torpor. MR decreased infrequently and any decreases were rarely sustained for longer than one hour. We found a significant positive relationship between T_a and T_b only in *G. volans*, which suggests that *G. volans* is more susceptible to low T_a compared with *G. sabrinus*, possibly due to their small body size. We observed no substantive seasonal or interspecific differences in the relation between MR and T_a, with the exception that northern flying squirrels expended more energy at cold T_a during warm season trials than other species-season combinations. The infrequency of torpor use in our experiments suggests that other energy-saving strategies, such as social thermoregulation, may limit the reliance on torpor in this lineage.

1. Introduction

Many birds and mammals maintain a relatively constant, high body temperature (T_b) while active that allows for optimal physiological function, even at ambient temperatures (T_a) outside of an individual's range of thermoneutrality. However, the maintenance of a high T_b comes with the costs of high resting energy expenditure and water loss, particularly at low T_a (Ruf and Geiser, 2015). Further, under limited food availability, which commonly occurs during winter at high latitudes, energetic costs associated with thermoregulation can result in a negative energy balance and increased probability of death (e.g., Körtner and Geiser, 2000).

Animals that do not migrate in winter have behavioural or physiological traits that enhance survival in extreme cold, including social thermoregulation and torpor (Hwang et al., 2007; Jefimow et al., 2011). Social thermoregulation is a crucial mechanism for winter survival in many mammals (e.g., striped skunk (*Mephitis mephitis*; Mustonen et al., 2013), Djungarian hamster (*Phodopus sungorus*; Jefimow et al., 2011), and Australian sugar gliders (*Petaurus breviceps*, Nowack and Geiser, 2016)), and is most effective for conserving energy within insulated shelters such as caves, burrows, or tree cavities (Wilson et al., 2010), and through huddling with multiple nest mates (Gilbert et al., 2010).

When faced with adverse conditions, many species forgo euthermia (the maintenance of a high T_b) and enter torpor; a temporary, controlled reduction in metabolic rate (MR), T_b, and other aspects of an individual's physiology (Ruf and Geiser, 2015). In mammals and birds, torpor is typically divided into hibernation and daily torpor; the distinction centering largely on the duration and magnitude of metabolic depression and resultant T_b reduction (Geiser and Ruf, 1995). Hibernators maintain torpor over consecutive days or weeks, and typically at a lower minimum T_b and MR. In contrast, individuals that undergo daily torpor tend to be smaller in body size, with depressions in MR and T_b being less dramatic, and lasting < 24 h (Geiser and Ruf, 1995; Ruf and Geiser, 2015). Torpor results in significant energetic savings (Barclay et al., 2001; Geiser, 2004; Humphries et al., 2003); however, it can entail substantial costs including oxidative stress, neuronal tissue damage, and reduced immunocompetence (Humphries et al., 2003; Lovegrove et al., 1999). Additionally, torpor typically requires periodic arousals to restore normal T_b and maintain necessary physiological

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processes (Lovegrove et al., 1999; Prendergast et al., 2002, but see Lovegrove et al., 2014).

Torpor has been reported in many rodent species in both hot and cold climates (Boyles et al., 2013). The rodent family Sciuridae has featured prominently in studies of torpor and thermoregulation, in part due to the diversity of thermoregulatory phenotypes (Lovegrove, 2012). For example, while ground squirrels (*Spermophilus* spp.) and chipmunks (*Tamias* spp.) are true hibernators (Barnes, 1989; Levesque and Tattersall, 2010), North American tree squirrels (e.g., *Sciuris carolinensis*) can only drop their body temperature by a few degrees (Pereira et al., 2002). In this respect, the North American flying squirrels (*Glaucomys* spp.) represent an interesting lineage for studies of comparative thermoregulation. Flying squirrels and tree squirrels are closely related, representing separate tribes within the subfamily Sciurinae (Steppan et al., 2004). However, in contrast with tree squirrels, flying squirrels are nocturnal. As a result, flying squirrels are presumably subject to lower predation risk, which may favour a different thermoregulatory phenotype (e.g., Lovegrove, 2012).

Northern (*Glaucomys sabrinus*) and southern (*G. volans*) flying squirrels are sister species endemic to North America, with largely parapatric ranges. The northern flying squirrel is a conifer specialist that inhabits the boreal forest and high elevation coniferous forests of the Appalachian and Rocky Mountains. The southern flying squirrel is a specialist of eastern deciduous forests (Weigl, 1978). The use of daily torpor in the northern and southern flying squirrel has been anecdotally reported, but, if occurring, remains poorly understood (Stapp and Mautz, 1991). For example, based on body temperature recordings, Muul (1968) reported limited torpor use in *G. volans* in the wild and in captive colonies, however, he was unable to induce torpor in the laboratory. Similarly, Neumann (1967) was unable to induce torpor in commercially bred *G. volans*. Multiple studies have since reported a lack of evidence for torpor in one or both *Glaucomys* species, however the primary objective of these earlier studies was never to induce torpor, and the absence of torpor was always a Supplementary observation (Merritt et al., 2001; Stapp et al., 1991).

G. sabrinus and *G. volans* exhibit some overlap in the periphery of their geographic ranges (Weigl, 1978) and this degree of overlap appears to be increasing due to contemporary climate change (Bowman et al., 2005; Garroway et al., 2011). The southern flying squirrel appears to be limited at its northern range edge by cold winter temperatures and has undergone a poleward range expansion (Bowman et al., 2005; Myers et al., 2009). Recently, these two species were reported to exhibit novel hybridization as a result of the climate-change induced range overlap (Garroway et al., 2010). One proposed mechanism underlying hybridization is heterospecific social nesting, possibly to minimize thermoregulatory costs for the invading southern flying squirrel at the range frontier (Gilbert et al., 2010; Walpole and Bowman, 2011). The ability to enter torpor in the face of suboptimal thermal conditions may reduce the necessity of such heterospecific nesting. Therefore, establishing whether flying squirrels use torpor is an important step in developing a mechanistic understanding of the processes leading to their interspecific hybridization.

We evaluated the metabolic performance of wild-caught *G. sabrinus* and *G. volans* at different T_a, and attempted to quantify whether and under what conditions flying squirrels entered torpor. We asked whether there were seasonal differences in metabolic rate, within and between *Glaucomys* species. We also tested for the presence of torpor, and asked whether there were seasonal or daily torpor patterns in *Glaucomys* species. We hypothesized that metabolic requirements will change between seasons for each species, and that both *G. sabrinus* and *G. volans* will use daily torpor as an energy-saving strategy during extreme T_a, as suggested by Muul (1968). We predicted that we would observe synchronous decreases in T_b and metabolic rate coinciding with cold T_a, indicating torpor.

2. Methods

2.1. Animal capture and husbandry

Field work took place from February 2014 to March 2015 in Harvey Township near Buckhorn, Ontario, Canada. *Glaucomys* individuals were live-trapped in mixed-wood forest at the southwest end of Mississagua Lake, west of Kawartha Highlands Provincial Park (44.688°N, 78.335°W and 44.684°N, 78.361°W). We used Tomahawk model 102 live traps (Tomahawk, Wisconsin) with sunflower hearts and apple slices as bait, and the addition of peanut butter and/or bacon grease in winter. Traps were strapped to platforms made of 2.54 × 10.16 cm lumber mounted on a shelf bracket approximately 2 m up a tree. All trapped individuals were subcutaneously injected with passive integrated transponder (PIT) tags (model TX1411SST, 12.50 mm × 2.07 mm, 134.2 kHz ISO, 0.1020 g, Biomark Inc. Boise, Idaho) for identification. Trapping protocols were executed under an Ontario Ministry of Natural Resources and Forestry Wildlife Scientific Collector's Authorization Permit.

A total of 49 squirrels (*G. sabrinus*: 8 males and 11 females; *G. volans*: 17 males and 13 females) were brought to the Trent University Animal Care Facility (44.360°N, 78.285°W). Ambient temperature at the facility varied from a maximum average daily temperature of 16.5 °C in July 2014 to a minimum of −3.7 °C in January 2015 (Appendix 1). Squirrels were kept at the facility for no longer than one month before being released at the site of capture. Squirrels were kept in gerbil cages in an outdoor aviary exposed to T_a and natural photoperiod. Food (Topcrop Squirrel Mix, LabDiet Mouse Diet 5015 [Land O' Lakes, Inc.], cuttlefish bone, cubed cooked chicken, praline nut mix) and water were provided ad libitum, except when squirrels were fasted for 12 h prior to respirometry trials. All experiments were approved by the Trent University Animal Care Committee.

2.2. Experiment I: identification of lower critical temperature and BMR

To characterize the thermal strategy of flying squirrels, we first estimated the lower critical temperature (T_{lc}) of the thermal neutral zone (TNZ) and BMR. Because T_{lc} and BMR can vary with season, we first compared measurements between two temperature periods: warmseason (from August to November 2014) and cold-season (December 2014 to April 2015). We chose these periods based on a retrospective assessment of temperatures at the university's climate station from 1981 to 2010 (Environment Canada, 2015).

As an index of energy expenditure, we measured each individual's oxygen consumption rate (VO_2) at various T_a using flow-through respirometry. To ensure a post-absorptive state, we fasted squirrels for 12 h overnight prior to trials, but we provided water ad libitum. Food and water were not provided during respirometry. In warm season trials, we measured MR of each squirrel over an 8 h period at a range of incubator temperatures (T_{inc}). We started with a 2 h acclimation period at 30 °C, followed by 6 h of recording, for 1 h each at 30 °C, 20 °C, 10 °C, 0 °C, −5 °C, −10 °C. Each 1 h measurement period included the length of time taken for the incubator to reach the desired T_{inc}. To reduce temperature shock during cold-season measurements arising from the wide temperature range being tested, we started trials at 10 °C. Rather than conduct a single trial with decreasing temperature as we did during the warm season trials, it was necessary to perform two separate trials: the first trial started at 10 °C for a 2 h acclimation period, followed by recording for 1.5 h at each of 4 decreasing T_{inc}: 10 °C, 0 °C, −5 °C, −10 °C. The second metabolism trial started at 10 °C for a 2 h acclimation period and recorded for 1.5 h at each of 4 increasing T_{inc}: 10 °C, 20 °C, 30 °C, 35 °C. In the split cold-season trials, the first 30 min between T_{inc} changes were not used to record VO_2 to keep the measurement time at each temperature identical between warm-season and cold-season trials. The split cold-season trials were performed on separate days and trial order was randomized for each squirrel.