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The effect of temperature on the pattern of torpor in a marsupial hibernator

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Abstract. Physiological variables of torpor are strongly temperature dependent in placental hibernators. This study investigated how changes in air temperature affect the duration of torpor bouts, metabolic rate, body temperature and weight loss of the marsupial hibernator *Burramys parvus* (50 g) in comparison to a control group held at a constant air temperature of 2 °C. The duration of torpor bouts was longest (14.0 ± 1.0 days) and metabolic rate was lowest (0.033 ± 0.001 ml $O_2 \cdot g^{-1} \cdot h^{-1}$) at 2 °C. At higher air temperatures torpor bouts were significantly shorter and the metabolic rate was higher. When air temperature was reduced to 0 °C, torpor bouts also shortened to 6.4 ± 2.9 days, metabolic rate increased to about eight-fold the values at 2 °C, and body temperature was maintained at the regulated minimum of 2.1 ± 0.2 °C. Because air temperature had such a strong effect on hibernation, and in particular energy expenditure, a change in climate would most likely increase winter mortality of this endangered species.

Key words: Thermoregulation – Hibernation – Torpor bouts – Metabolic rate – Marsupial, *Burramys parvus*

Introduction

Environmental temperature affects the pattern of torpor in hibernating placental mammals. The duration of torpor bouts, which are interrupted by energetically costly periodic arousals, have been shown to be inversely related to T_a (Twente and Twente 1965). Other studies have demonstrated that the duration of torpor bouts, and thus energetic demands, are not always inversely related to T_a. It appears that exposure to T_as below the minimum T_b, which is metabolically defended during torpor (Heller and Hammel 1972), results in shorter bouts of torpor and increased energetic demands similar to exposure to T_as above the minimum T_b (Soivio et al. 1968; Geiser and Kenagy 1988). Thus, hibernation at T_as close to the minimum T_b should require the least energy because T_b is not metabolically defended and the metabolic rates of torpid animals will be low and the duration of torpor bouts will be long (Wolf and Hainsworth 1972; Hudson 1973; Geiser and Kenagy 1988).

Because the temperature dependence of the duration of torpor bouts in hibernators is still subject to discussion, we investigated the effect of T_a above and below the minimum T_b on the pattern of torpor and energy expenditure in the hibernator *Burramys parvus* (Marsupialia: Burramyidae). This endangered species is restricted to high altitudes of the Australian alps where it hibernates under snow-covered boulder fields (Broome and Mansergh 1990). In the laboratory *B. parvus* can survive without food for at least six months when hibernating at a T_a of 2 °C, which is close to the T_a experienced in the field during the central part of the hibernation season (Geiser and Broome 1991).

Materials and methods

Ten adult *Burramys parvus* were caught in March 1990 on Mt. Kosciusko at an altitude of about 2160 m. As this species is vulnerable the number of experimental animals that was allowed for collection was limited. Animals were transferred to the University of New England, Armidale, New South Wales, at an altitude of about 1000 m. They were maintained in temperature-controlled cabinets ($T_a \pm 0.5$ °C) and a photoperiod of LD 9.5:14.5 light from 07:30–17:00 hours AEST, which is close to the shortest photoperiod experienced by wild populations. Water was freely available throughout the experiment. Food was provided ad libitum during the time of fattening, but was removed during most of the hibernation season (Fig. 1). Food was exchanged daily and consisted of high-protein baby cereal with honey and water, canned baby food, apples, carrots, walnuts and sunflower seeds. Calcium and vitamins were mixed into the food. Animals were weighed at regular intervals and were released in November at their sites of capture.

Abbreviations: STP, standard temperature and pressure; T_a, air temperature; T_b, body temperature; $\dot{V}O_2$, rate of oxygen consumption

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All animals were exposed to a declining T_a from 12 to 2 °C in April (shown in Fig. 1), which is similar to that in their natural habitat in autumn. Environmental temperatures in a boulder field fluctuated from 7.0 to 18.0 °C in March, from −2.0 to 8.5 °C in November when little or no snow cover was provided, and was stable (0.5–3.5 °C) under snow cover between May and July. Controls (n = 5) were kept at $T_a = 2$ °C from 16 April until the end of the experiments in mid-October. Experimental animals (n = 5) were also exposed to $T_a = 2$ °C from 16 April; however, from 23 July, by which time the duration of torpor bouts in the controls had stabilized (Fig. 1), this group was exposed to different T_as ($T_a = 8$ °C, 23 July to 21 August; $T_a = 2$ °C, 21 August to 8 September; $T_a = 12$ °C, 8 September to 16 September; and $T_a = 0$ °C, 16 September to 4 October).

The duration of torpor bouts in hibernating individuals was determined by observing at 09:00–10:00 hours daily the displacement of sawdust from the back of the animals that occurs when they arouse. The sawdust had been placed on the animals when they first were observed in torpor and was replaced after they had removed it during arousal and had re-entered torpor. The average off all undisturbed torpor bouts of each individual was used to calculate the mean bout length of control and experimental groups over a month or a particular time interval as shown by horizontal bars in Fig. 1. When a torpor bout extended over 2 months it was included in the month in which the longer part of the bout occurred. For investigation of temperature-dependence of the duration of torpor only bouts of the experimental group measured between 23 July and 4 October were included, when torpor bouts of control animals were stable.

The metabolic rate (measured as $\dot{V}O_2$) and the T_b of experimental individuals hibernating at different T_as was also determined. For these measurements hibernating experimental animals were transferred from their holding chamber to a respirometer vessel at the same $T_a \pm 0.5$ °C. These measurements were conducted at T_as of 8, 2, and 0 °C; animals were not measured at $T_a = 12$ °C because handling induced premature arousals. T_b was measured, at the end of the $\dot{V}O_2$ measurements, by a 4-cm rectal insertion of a 38-Gauge thermocouple probe calibrated to the nearest 0.1 °C and read with a Omega Model HH-71 T Microcomputer thermometer. To ensure that T_b and $\dot{V}O_2$ were at steady-state levels, and because the sensitivity to disturbance increases towards the end of a torpor bout, measurements were performed between days 2 and 4 of a torpor bout. $\dot{V}O_2$ was measured, after removal of water from the air stream, with an Applied Electrochemistry S-3A/II oxygen analyser. The flow rate of dry air through the 0.75-l respirometer vessel was 80 ml · min^{-1}. $\dot{V}O_2$ was measured for several hours and the minimum $\dot{V}O_2$ observed over 1 h was integrated, corrected to STP and calculated according to Withers (1977). Measurements of $\dot{V}O_2$ and T_b in torpid animals were made between 23 July and 4 October. Numerical values in the text are expressed as means ± 1 standard deviation (SD).

Results

The occurrence of torpor and the duration of torpor bouts in *B. parvus* changed with food availability, season and temperature (Fig. 1). In March/April, when all animals were maintained at $T_a = 12$ °C, only four of the ten individuals entered torpor and the torpor bouts lasted between 1 and 4 days. Lowering T_a increased the incidence of torpor to five individuals ($T_a = 8$ °C) and nine individuals ($T_a = 2$ °C). In May, when food was withheld, all individuals hibernated and the duration of bouts increased to 9.1 ± 1.9 days ($n = 10$). In the five control animals the duration of torpor bouts reached a plateau that remained stable from July to mid-October with mean torpor bouts ranging between 13.6 ± 3.1 days (July) and 11.9 ± 2.9 days (October). The torpor bouts of the five experimental animals lasted 14.9 ± 0.9 days at $T_a = 2$ °C in June/July, which was indistinguishable from the controls during the same time period ($P > 0.05$; t-test). However, these individuals subsequently shortened torpor bouts to 9.6 ± 1.8 days at $T_a = 8$ °C (23 July–21 August), lengthened torpor bouts to 12.0 ± 2.0 days at $T_a = 2$ °C (21 August–8 September), shortened torpor

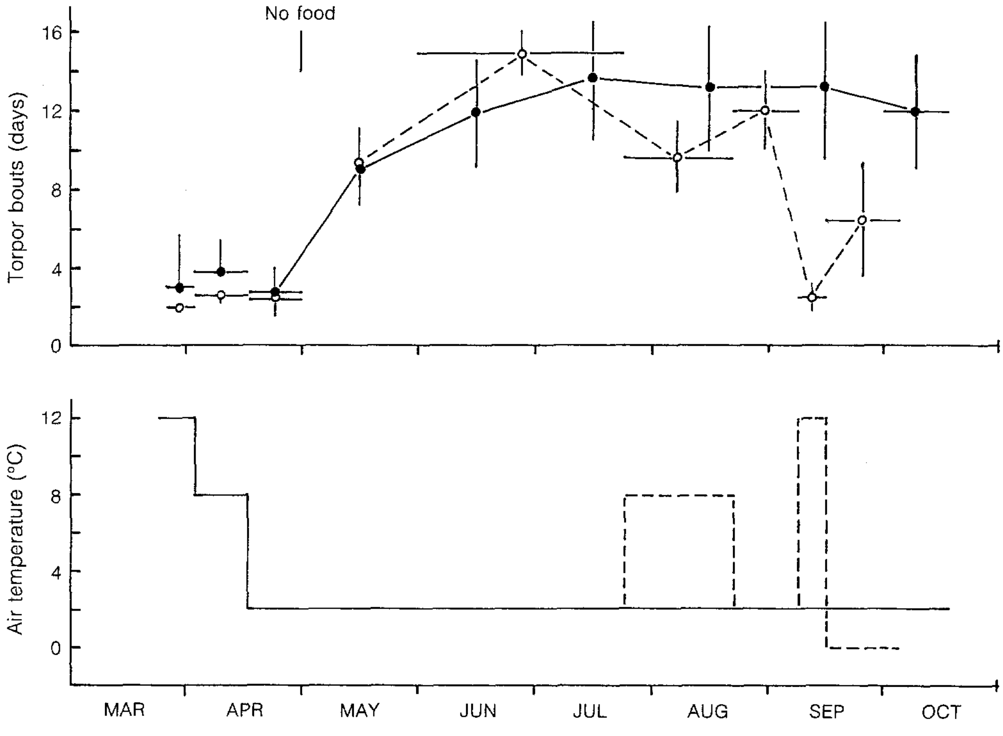

Fig. 1. The duration of torpor bouts (means ± 1 SD) of *Burramys parvus (upper)* and the air temperature T_a *(lower)*. The time intervals over which mean torpor bouts were determined are indicated by *horizontal bars;* if no bars are shown the means represent values for 1 month. Controls (*solid lines and symbols; n* = 5) were kept at constant T_a of 2 °C from 16 April to October. The time intervals when experimental animals (*broken lines* and *open symbols; n* = 5) were exposed to different T_as than controls are indicated by *broken lines*

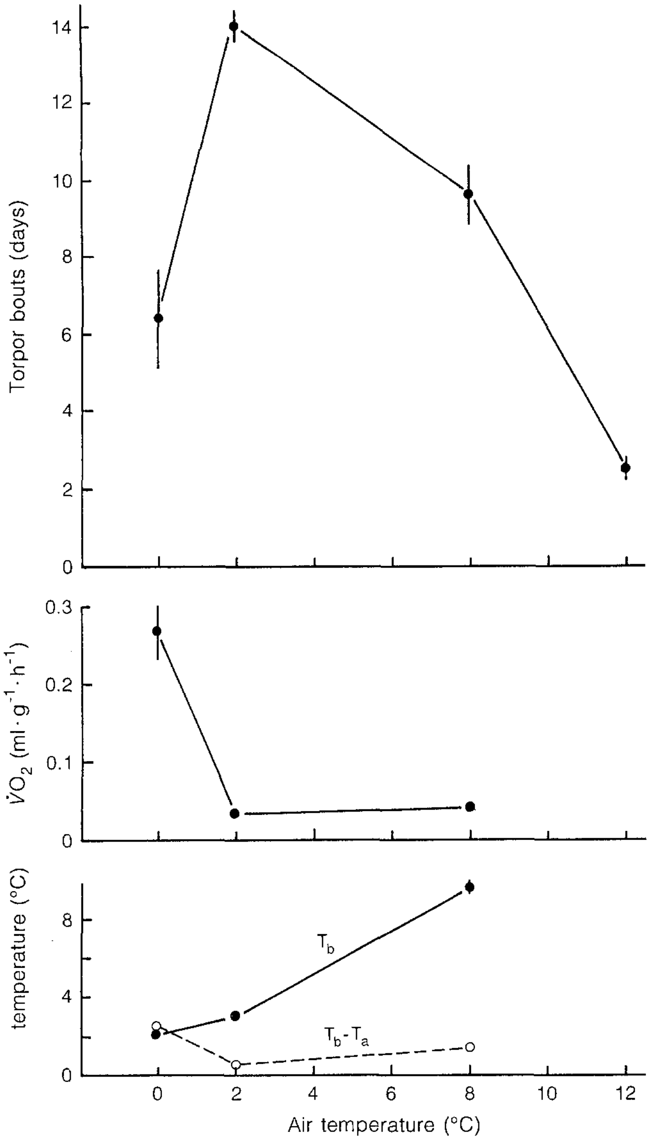

Fig. 2. The duration of torpor bouts *(upper)*, the metabolic rate measured as oxygen consumption, $\dot{V}O_2$ *(middle)*, and the body temperature, T_b and $T_b - T_a$ *(lower)* of hibernating *Burramys parvus*. Values are means ± 1 SE for the five experimental animals. Body masses of animals at the end of the $\dot{V}O_2$ measurements were: 54.9 ± 4.5 g (SD) at T_a = 8 °C, 50.2 ± 3.6 g at T_a = 2 °C, and 40.0 ± 0.9 g at T_a = 0 °C

bouts to 2.5 ± 0.7 days at T_a = 12 °C (8 September–16 September), and again lengthened torpor bouts to 6.4 ± 2.9 days at T_a = 0 °C (16 September–4 October). The duration of torpor bouts at T_a = 2 °C in August/September, as in June/July, did not differ between control and experimental groups ($P > 0.05$; *t*-test). Torpor bouts of the experimental animals were, except at T_a = 2 °C, shorter than the corresponding values at the same time of the year of the control groups ($P < 0.05$; *t*-test).

The duration of torpor bouts, metabolic rate and T_b of the five experimental animals as a function of T_a is shown in Fig. 2. Torpor bouts lengthened from T_a = 12 °C to T_a = 8 °C and reached a maximum of 14.0 ± 1.0 days at T_a = 2 °C (mean value of June/July and August/September). The lengthening of torpor bouts from T_a of 8 to 2 °C was accompanied by a significant decrease in $\dot{V}O_2$ (0.043 ± 0.005 ml · g^{-1} · h^{-1} at T_a = 8 °C, body mass 54.9 ± 4.5 g; 0.033 ± 0.001 ml · g^{-1} · h^{-1} at T_a = 2 °C, body mass 50.2 ± 3.6 g; $P < 0.01$, *t*-test; Fig. 2). A further reduction of T_a to 0 °C resulted in torpor bouts that were reduced by more than 50% in comparison to values at T_a = 2 °C and in an eightfold increase of $\dot{V}O_2$ to 0.27 ± 0.07 ml · g^{-1} · h^{-1} (body mass 40.0 ± 0.9 g) in comparison to the $\dot{V}O_2$ at T_a = 2 °C. T_b fell with T_a from T_a = 8 °C (T_b–T_a = 1.3 ± 0.2 °C) to T_a = 2 °C (T_b–T_a = 0.5 ± 0.1 °C); between T_a = 2 °C and 0 °C, T_b fell only slightly to the regulated minimum T_b of 2.1 ± 0.2 °C (T_b–T_a = 2.5 ± 0.2 °C; Fig. 2).

The loss of body mass during undisturbed hibernation of experimental animals was also strongly affected by T_a. Mass loss was lowest at T_a = 2 °C (0.158 ± 0.0275 g · day^{-1}), slightly higher at T_a = 8 °C (0.168 ± 0.057 g · day^{-1}), and increased by more than twofold at T_a = 0 and 12 °C (combined value 0.416 ± 0.122 g · day^{-1}; $P < 0.01$; *t*-test in comparison to mass loss at T_a = 2 and 8 °C). The mass loss of control animals at T_a = 2 °C was 0.127 ± 0.013 g · day^{-1} which was indistinguishable from that of the experimental animals at that T_a ($P > 0.05$; *t*-test).

Discussion

As has been found in placental hibernators, the duration of torpor bouts and other physiological variables were affected by T_a in the marsupial *Burramys parvus*. Torpor bouts were longest, metabolic rate and mass loss lowest at a T_a close to the minimum T_b of the species. At T_as above and below the minimum T_b torpor bouts shortened and energy expenditure increased.

Thermal response of torpor bouts

The temperature dependence of torpor bouts observed here is not generally accepted nor are the mechanisms that control the duration of torpor bouts understood. The inverse relationship between torpor bout length and T_a, which seems to hold at T_as above the minimum T_b (Twente and Twente 1965), is often seen as the general pattern and the reduction of torpor bouts at T_as below the minimum T_b is ignored or portrayed as an exception. However, it is likely that lack of observations on shortening of torpor bouts at T_as below the minimum T_b in some studies (e.g. Twente and Twente 1965; French 1982) are due to lack of measurements in this temperature range. A number of hibernators and species exhibiting daily torpor show the shortening of torpor bouts at very low T_as as described here (Pengelley and Kelley 1966; Soivio et al. 1968; Geiser 1986; Geiser and Kenagy 1988). Because costs of metabolic defence of T_b during torpor are inversely related to body mass (Geiser and Baudinette 1987), the shortening of torpor bouts at T_as below the minimum T_b should be more pronounced in small than in large species.

Environmental temperatures and the minimum T_b

The shortening of torpor bouts at very low T_as has important implications for the evolution of the species-specific minimum T_b which varies among hibernators. It is widely believed that the low T_b during hibernation primarily functions to reduce metabolic rate during torpor (Kayser 1961). While this may be important, a low minimum T_b would also ensure that metabolically defended T_bs with the resulting shorter torpor bouts and relatively high metabolic rates during torpor are rarely reached (Wolf and Hainsworth 1972). When exposed to low T_a, individuals with a relatively high minimum T_b and thus frequent arousals would have a reduced chance of survival in comparison to individuals with a low minimum T_b, and the latter individuals would therefore be selected. At high environmental temperatures this selective pressure toward low minimum T_bs does not exist and individuals that regulate at a high T_b would have a chance of survival similar to those with a low set point. Within possible physiological limits the minimum T_b of hibernators therefore should be close to the environmental temperature experienced during hibernation. Examples supporting this hypothesis are the rodents *Eutamias amoenus* and *Spermophilus saturatus*. Both species have a minimum T_b which changes seasonally but remains slightly below the soil temperature throughout most of the hibernation season (Geiser et al. 1990). Hibernators from warmer environments, *Spermophilus beecheyi* and *S. variegatus,* have a relatively high minimum T_b (Strumwasser 1960; Pengelley 1964).

It is not implied that the species-specific set point for the minimum T_b has evolved in response to a single adaptive pressure. Since thermoregulatory costs during torpor when T_b is metabolically defended are inversely proportional to body mass (Geiser and Baudinette 1987) and seem to be linked to the duration of torpor bouts, temperature-dependent changes in torpor bouts and energy expenditure during hibernation should be less extreme in large than in small species. Thus, the selective pressure of T_a on the minimum T_b should be reduced with increasing body mass. Relatively high minimum T_bs have, for example, been observed in the large marmots *Marmota flaviventris* (Florant and Heller 1977) and *Marmota marmota* (Arnold et al. 1991) and very high T_bs in the overwintering badger, *Meles meles* (Fowler and Racey 1988). Furthermore, other factors than T_a, or in addition to T_a, seem to influence the minimum T_b in daily heterotherms, many of which regulate T_b well above the T_a experienced in the wild (Wolf and Hainsworth 1972; Hudson 1973; Ruf et al. 1991). Nevertheless, some hummingbirds appear to show a reasonable relationship between the mean minimum T_a of their habitat and the minimum T_b during daily torpor (Wolf and Hainsworth 1972). It therefore appears that the minimum T_b of heterothermic mammals and birds is influenced by a multitude of factors out of which environmental temperature and body mass are only the most conspicuous.

Temperature, energetics and survival

The strong effect of T_a on the physiological variables of torpor has implications for the use of energy throughout the hibernation season of *B. parvus.* As indicated by the measurements of $\dot{V}O_2$ and loss of body mass, hibernation at T_as above and below the minimum T_b is relatively costly. Arousals consume most of the energy expended during the hibernation season (Wang 1978) and therefore more frequent arousals accelerate the loss of stored body fat. Since the metabolic rate of torpid animals also increases below and above the minimum T_b additional energy is consumed. If one assumes that the 30 g of fat, which is about the amount accumulated by *B. parvus* during the pre-hibernation season in the laboratory (Geiser and Broome 1991), is the only energy available throughout the hibernation season, then the longest possible period of hibernation, derived from the loss of body mass at the various T_as, would be about 236 days at $T_a = 2$ °C, about 179 days at $T_a = 8$ °C and only about 72 days at T_as of 0 and 12 °C. If one assumes that the stored fat of *B. parvus* suffices for 20 arousals, which is approximately the number of arousals in a hibernation season (Geiser and Broome 1991), similar figures are deduced from the duration of torpor bouts at the various T_as (about 240 days at $T_a = 2$ °C, 190 days at $T_a = 8$ °C, 120 days at $T_a = 0$ °C and 50 days at $T_a = 12$ °C).

In the wild the animals appear to accumulate only about 20 g of fat during the pre-hibernation season (Broome 1992) which would shorten the possible hibernation season by about one-third. From these calculations it appears that successful hibernation in *B. parvus,* which in subadults and adults lasts for about 180–214 days in the wild (Broome 1992), is possible only at $T_a = 2$ °C (when the fat storage in the wild is considered) or between 2 and 8 °C (when fat storage and torpor bouts in the laboratory are considered). Especially lower T_as would require too much energy and would reduce the likelihood of survival. As the hibernation season is a time of high mortality in this species with mortality rates of between 30% in adult females and 60% in juvenile females (Broome 1992), a shift in environmental temperature would probably further worsen the already precarious situation of this species. For example, lack of snow cover as a temperature buffer, which could be caused by climatic change, would expose animals to greater temperature fluctuations and probably increase winter mortality.

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