

## Differences in the Physiological Response to Cold in Wild and Laboratory-bred Mountain Pygmy Possums, *Burramys parvus* (Marsupialia)

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### Abstract

The seasonal pattern of thermoregulation was investigated in wild-caught and laboratory-bred *Burramys parvus*. Wild-caught animals fattened extensively during the pre-hibernation season which was followed by about 7 months hibernation. Torpor bouts lasted 1-6 days during early hibernation and up to 19 days during the central part of the hibernation season. The metabolic rate of hibernating animals was reduced to about 2% of the basal metabolic rate. In contrast, laboratory-bred animals never fattened nor entered torpor in two consecutive winters. It is likely that the artificial conditions in the laboratory do not provide the appropriate environmental cues for seasonal physiological alterations in the species.

### Introduction

*Burramys parvus*, the mountain pygmy possum (family Burramyidae), is confined to altitudes between 1400-2200 m of the Snowy Mountains of south-eastern Australia (Calaby 1983; Broome and Mansergh 1990; Mansergh and Scotts 1990). The species had a wider distribution in the late Pleistocene (Caughley 1986). Remaining populations (about 2000 individuals) are restricted to boulder fields that are covered by snow for up to 5 months during the austral winter. Because they disappear from their habitat from May to October (Mansergh 1984) it is assumed that they hibernate. This physiological adaptation to cold temperature and food shortages may be one of the reasons why they survive competition from *Antechinus* spp. and native rats, which are unable to hibernate.

We studied the pattern of thermoregulation in wild-caught and laboratory-bred adult *B. parvus*. Wild-caught animals were studied because the pattern of torpor observed previously in this species (Fleming 1985) differed somewhat from the characteristic pattern of placental hibernators which, throughout the hibernation season, show torpor bouts of several days to weeks that are interrupted by short (about 1 day) normothermic periods (Wang 1978; French 1982; Barnes *et al.* 1986; Geiser *et al.* 1990). The relatively long normothermic periods and short torpor bouts observed in *B. parvus* (Fleming 1985) raised the question of whether this pattern is an idiosyncrasy of the species or whether it was induced by the laboratory conditions. Laboratory-bred animals were investigated because release programmes for captive-bred animals have been contemplated to stock unoccupied habitats in the wild. It is likely that these animals would only survive the harsh environment experienced by wild populations if they were equipped with the ability to hibernate.

## Material and Methods

Eight adult wild-caught animals and six adult laboratory-bred animals were studied between June 1988 and November 1989. Wild-caught animals were obtained in late March 1989 from Kosciusko National Park, New South Wales, at an altitude of about 1800 m. Animals were transported to the University of New England, Armidale, New South Wales, where they were maintained in quiet, temperature-controlled cabinets (air temperature,  $T_a \pm 0.5^\circ\text{C}$ ).  $T_a$  was  $12^\circ\text{C}$  from 28 March to 7 April,  $8^\circ\text{C}$  from 8 April to 6 May,  $2^\circ\text{C}$  from 7 May to 15 November, and  $8^\circ\text{C}$  thereafter. Environmental temperatures in a boulder field ranged from  $7.0$  to  $18.0^\circ\text{C}$  in March,  $0.5$  to  $3.5^\circ\text{C}$  between May and July, and from  $-2.0$  to  $8.5^\circ\text{C}$  in November. The artificial photoperiod was LD 9.5:14.5, which is close to the shortest photoperiod experienced by wild populations. All wild-caught animals survived hibernation and were released in November at their site of capture.

Laboratory-bred animals were obtained from the Healesville Sanctuary, Victoria, in June 1988 from stock derived from Mt Higginbotham, Victoria. The animals were the offspring of wild-caught individuals or the offspring of a first generation captivity-bred female and a wild-caught male. At Healesville Sanctuary these animals had been kept at a  $T_a$  that ranged from  $10$ – $15^\circ\text{C}$  in winter to  $15$ – $25^\circ\text{C}$  in summer. The artificial photoperiod was adjusted seasonally between extremes of LD 8:16 in winter and LD 16:8 in summer. Some of these individuals were studied for two seasons. In the first winter these animals were maintained at a photoperiod of LD 9.5:14.5 and a  $T_a$  of  $18^\circ\text{C}$  from 20 June to 10 July and at  $10^\circ\text{C}$  from 11 July to 31 September. Three of these individuals were kept during summer at a  $T_a$  of  $22 \pm 2^\circ\text{C}$  and the natural photoperiod of Armidale ( $30^\circ 31'\text{S}$ ,  $151^\circ 40'\text{E}$ ; altitude 1000 m). In the second season the laboratory-bred animals were combined with the wild-caught animals and kept under the same environmental conditions from 28 March to 12 May 1989 (i.e.  $T_a$ s of  $8$  and  $2^\circ\text{C}$ ). Because the laboratory-bred animals did not fatten and did not hibernate by that time, they were maintained at  $8^\circ\text{C}$  until 31 July after which they were removed from the temperature-controlled cabinet and the observations discontinued.

Water was freely available throughout the experiments. Food was provided *ad libitum* during the period of fattening and when animals were normothermic. Food was exchanged daily and consisted of high protein cereal with honey and water, canned baby food, apples, carrots, sunflower seeds and walnuts. Calcium and vitamins were mixed into the food. During each winter, food was withheld from the laboratory animals for 1 day to determine whether starvation induces torpor. Hibernating animals were not fed throughout most of the hibernation season (20 May–22 November).

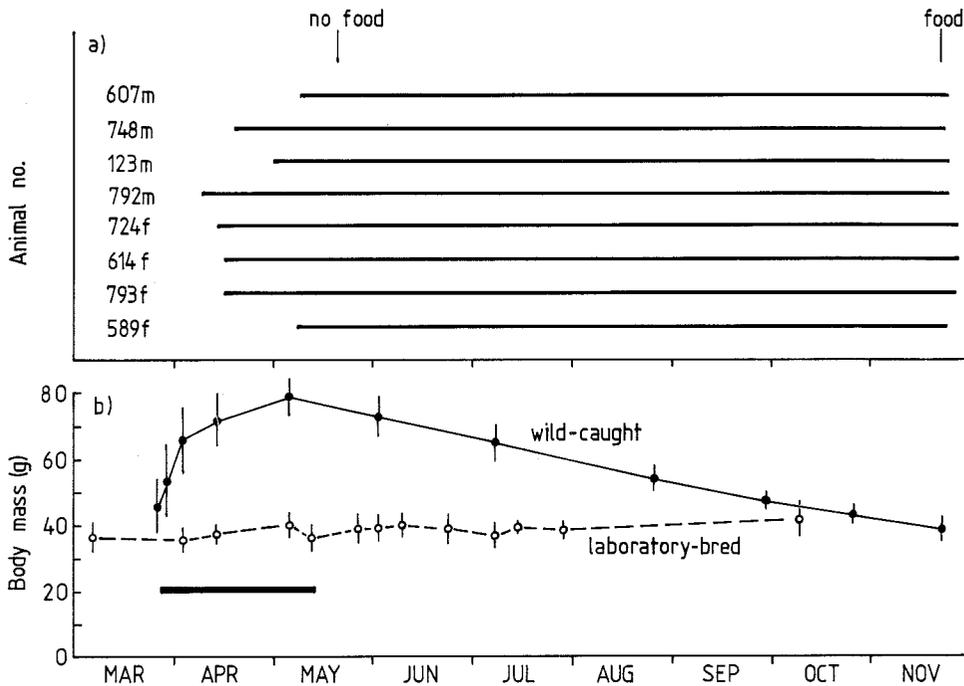
The duration of the hibernation season and the duration of torpor bouts in hibernating individuals were determined by observing daily, at 0900–1000 h, the displacement of sawdust from the back of the animals that occurs when they arouse. The metabolic rate, measured as oxygen consumption ( $\dot{V}\text{O}_2$ ), of torpid animals were also determined. These measurements were made after animals had been hibernating for at least 2 months because  $\dot{V}\text{O}_2$  is lowest during the central part of the hibernation season (Geiser *et al.* 1990). For the  $\dot{V}\text{O}_2$  measurements, hibernating animals were transferred from their holding chambers at  $T_a 2.0 \pm 0.5^\circ\text{C}$  to a respirometry vessel at the same  $T_a$ .  $\dot{V}\text{O}_2$  was measured, after removal of water from the air stream, with an Applied Electrochemistry S-3A oxygen analyser. The flowrate of dry air through the  $0.75$  L respirometer vessel was  $80$  mL  $\text{min}^{-1}$ . The  $\dot{V}\text{O}_2$  at  $T_a 2.0 \pm 0.5^\circ\text{C}$  was integrated over 1 hour, corrected to STP and calculated according to Withers (1977). The basal metabolic rate (BMR) of three post-absorptive individuals was also determined at a  $T_a$  of  $30^\circ\text{C}$ .

Numeric values in the text are expressed as mean  $\pm$  1 standard deviation.

## Results

Wild-caught animals showed a prolonged hibernation season in the laboratory (Fig. 1). Most individuals began to hibernate in April at  $T_a 8^\circ\text{C}$ ; however, two individuals commenced hibernation only after  $T_a$  was reduced to  $2^\circ\text{C}$ . The hibernation season, which lasted about 7 months, began with short torpor bouts of 1–6 days, which increased to a maximum of 19 days. All wild-caught animals began to hibernate when food and water were freely available and when body mass of individuals was between 71 and 83 g, after intensive fattening during the prehibernation period (Fig. 1). When all individuals were hibernating, food was withdrawn. Normothermic periods during the main part of the hibernation season were shorter than 1 day. The metabolic rate of torpid animals during the central part of the hibernation season was  $0.025 \pm 0.007$  mL  $\text{O}_2$   $\text{g}^{-1}$   $\text{h}^{-1}$  at  $T_a 2^\circ\text{C}$  ( $n=8$ ). All animals ended the hibernation season after the first arousal after food was reintroduced. The BMR of three individuals measured in spring was  $1.12 \pm 0.08$  mL  $\text{g}^{-1}$   $\text{h}^{-1}$  (body mass  $33.9 \pm 2.9$  g) and the  $Q_{10}$  for the change of  $\dot{V}\text{O}_2$  between normothermia (BMR) and torpor was about 3.2.

In contrast, the laboratory animals, which were kept under identical environmental conditions as the wild-caught individuals from 28 March–12 May, did not fatten and never entered torpor (Fig. 1). When food was withheld from laboratory-bred animals for 1 day (27 May;  $T_a$  8°C), all animals remained normothermic. Exposure to 10°C during the previous winter (1988) also produced no periods of torpor. Mean body mass of the six individuals ranged from  $32.8 \pm 1.9$  g (21 June) to  $34.4 \pm 2.1$  g (3 October) and the highest body mass,  $37.8 \pm 2.4$  g, was observed on 8 September. Food withdrawal for 1 day in July resulted in a 5.6 g loss of mean body mass and all the individuals were sluggish or hypothermic and required external heat for rewarming. None of these individuals showed the characteristic curled posture that was observed in hibernating animals.



**Fig. 1.** (a) The duration of the hibernation season shown as horizontal bars of wild-caught *Burramys parvus*. The hibernation season was interrupted by periodic arousals. Food was withheld from hibernating animals from 20 May to 22 November 1989. Animal numbers and sex (m male; f female) are shown. (b) The body mass (mean  $\pm$  standard deviation) of wild-caught (closed circles) and laboratory-bred (open circles) adult *B. parvus*. The time during which wild-caught and laboratory-bred animals were kept under identical environmental conditions is indicated by a solid bar below the body masses.

## Discussion

Our study shows distinct differences in temperature regulation of wild and laboratory-bred *Burramys parvus*. While the wild animals fattened extensively during the prehibernation season and hibernated for up to 7 months, laboratory-bred animals never fattened, nor entered torpor during 2 consecutive winters.

The pattern of hibernation in the wild *B. parvus* was similar to that observed in placental hibernators (Lyman *et al.* 1982). Initial short torpor bouts increased to long bouts during the main part of the hibernation season and normothermic periods were shorter than 1 day (Wang 1978; French 1982; Barnes *et al.* 1986; Geiser *et al.* 1990). This is in contrast to earlier observations of relatively short bouts of torpor and long normothermic periods (Fleming 1985). However, our observations support Fleming's (1985) suggestion that fattening is very important for hibernation in *B. parvus*. All adult animals that hibernated with access to food and water had a body mass exceeding 70 g. Laboratory-bred animals, which did not hibernate, always had a body mass of less than 45 g.

The very low metabolic rate of torpid animals is in agreement with previous measurements (Fleming 1985). The  $Q_{10}$  of 3.2 for the change in metabolism between normothermia (BMR) and torpor at  $T_a$  2°C was considerably greater than the predicted  $Q_{10}$  of 2.5 for biological reactions. This observation suggests that *B. parvus*, like other small hibernators, employs metabolic inhibition to enhance energetic savings during the prolonged hibernation season (Geiser 1988).

The lack of hibernation in laboratory-bred animals raises the question of whether such animals could survive the harsh winters if reintroduced to the wild. During winter, the main food source of *B. parvus* (Bogong moths, *Agrotis infusa*) is absent (Mansergh 1988) and numbers of other subnivean invertebrates are low (Green 1989). While non-perishable food items, e.g. seeds of *Podocarpus lawrencei*, are available and may at times be cached, high densities of *B. parvus* can occur in boulder screens with little vegetation cover (Broome, unpublished). Results from our study suggest that wild animals can survive without food for at least 185 days because of their ability to hibernate. Enormous food caches would be required in normothermic animals to survive this prolonged period. It is therefore unlikely that individuals could survive without hibernation if food is not provided from external sources.

Laboratory-bred *B. parvus* lack the seasonal changes of physiology exhibited by wild animals. This could be due either to genetic effects or to maintenance in captivity that does not provide the appropriate environmental cues for seasonal adjustments. Breeding and raising animals in captivity removes the selective pressure from the harsh winters. Lack of ability to hibernate would have no consequences for captive animals that have free access to food and are maintained at a relatively high  $T_a$ . However, it is unlikely that the first generation in captivity would completely lose the ability to hibernate. Therefore, the second possibility—that laboratory conditions changed aspects of the animals' behaviour and physiology—is more likely. In studies to date on captive animals, the artificial photoperiod, the high environmental temperatures that show little seasonal variation, or the constant supply of artificial food may have blunted the correct seasonal response of the species. Many hibernators require a combination of exposure to cold temperatures, short photoperiod and food shortage for the hibernation response to be elicited (Lyman *et al.* 1982). Further studies, which will investigate factors that control seasonal physiological changes in *Burramys parvus*, are planned.

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