Adaptations to the Cold: Tenth International Hibernation Symposium.
Edited by Geiser F., Hulbert A.J. & Nicol S.C. University of New England Press, Armidale, 1996

Effect of photoperiod on torpor and activity of *Sminthopsis crassicaudata* (Marsupialia)

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Introduction

Many small mammalian species show strong seasonal changes in thermal physiology (Morrison 1964). Generally, these species remain normothermic in the warm summer season, but become torpid during the colder winter months. These seasonal changes are most pronounced in hibernators, many of which show strong annual cycles of activity in summer followed by prolonged bouts of torpor (Pengelley 1967). Species which display daily torpor may be more opportunistic in their use of heterothermy; however, there is also a tendency towards a reduction in torpor during the summer months (Geiser & Baudinette 1987).

The regular seasonal occurrence of torpor has resulted in many investigations into the factors that govern this process. Perhaps the most common stimulus for the seasonal change is photoperiod (Heldmaier & Steinlechner 1981; Hall & Goldman 1982; Goldman *et al.* 1986; Steinlechner *et al.* 1986; Kirsch *et al.* 1991). While other factors such as ambient temperature (T_a) and food supply may also change with season, the extent of these changes can vary from year to year. Photoperiod, however, maintains a constant annual cycle and can, therefore, be used as a precise and reliable cue for seasonal changes in physiology.

While the effects of photoperiod on torpor have been studied in numerous eutherian mammals, the majority involving rodents, little is known about its effect on marsupials. Therefore, we investigated the effects of photoperiod on torpor patterns and activity in the small (16g), nocturnal dasyurid marsupial *Sminthopsis crassicaudata*, which inhabits the mesic to arid regions of southern and central mainland Australia (Morton 1978a). It is known that *S. crassicaudata* enters daily torpor, both in the wild (Morton 1978b; Frey 1991) and in the laboratory (Godfrey 1968; Geiser *et al.* 1986; Geiser & Baudinette 1987). This species has also been observed to show a seasonal change in the occurrence of torpor, with it being most prevalent in winter (Morton 1978d).

Materials and Methods

Fourteen adult male *S. crassicaudata* were obtained from a laboratory colony maintained by the Genetics Department of the University of Adelaide and transported to the University of New England (UNE), Armidale, NSW. Upon arrival at UNE in April 1992, the animals were divided into two groups of matched body mass, and kept in environmental chambers at a T_a of 18±1°C. The animals were fed *ad libitum* a mixture of dried and commercial pet food and water. Vitamin (Pentavite) and calcium supplements were given twice a week and several *Tenebrio* larvae were provided weekly.

The lighting conditions within the Adelaide colony, which were designed to optimise the breeding potential of the animals (Smith *et al.* 1978), consisted of 16 hours of daylight and 8 hours of darkness (LD 16:8) for 6 months, followed by a period of 3 weeks of LD 8:16 and then a return to the LD 16:8 photoperiod (Bennett *et al.* 1982). After their arrival at UNE all the animals were initially subjected to a photoperiod of LD 12:12 (lights on 0600h) for a period of 8 weeks before the photoperiod was changed. One group (N=7) was then exposed to a photoperiod of LD 16:8 (lights on 0400 h) and the other (N=7) to LD 8:16 (lights on 0800h). After 8 weeks under long or short photoperiods, the photoregimes of the two groups were exchanged. Light in each chamber was provided by two 8 watt fluorescent tubes which emitted a light intensity of approximately 100 lux (Gossen Panlux electronic light meter) throughout each chamber.

For determination of locomotor activity, passive infrared sensors (Jaycar Electronics, LA-5017) were placed on top of the cages. These measurements were made over a period of 4 months at T_a 18°C. Activity events were recorded continuously and summed to a maximum of 255 movements over 30 minute intervals with an eight channel Electronic Services Unit datalogger. Except where noted, food and water were available during these measurements.

Body temperatures (T_b) were determined at T_as 18, 15, 12 and 10°C by inserting a 42swg copper-constantan thermocouple probe rectally for 20mm and reading from an Omega HH-71T Electronic Thermometer. The thermocouple was calibrated to the nearest 0.1°C against a Dobros precision mercury thermometer traceable to a National Standard. For this study, any animal with a T_b below 30°C was defined as torpid (Wallis 1976).

Metabolic rates (MR), measured as rate of oxygen consumption (VO_2), were determined over a 23 hour (±30min) period, commencing in the late afternoon, at T_a 12±1°C using an open flow system. Food and water were not available to the animals for the duration of these measurements. Animals were placed within a 0.5L respirometry vessel and VO_2 was continuously monitored, after the removal of water from the air stream, with an Applied Electrochemistry S-3A oxygen analyser connected to a Lloyd Instruments Graphic 2002 recorder. The flow rate was 350-450mL min^{-1} and was measured with calibrated rotameters. With this chamber size and these flow rates, 99% equilibrium was obtained between 5-6.5min. T_a was measured by a calibrated thermocouple placed within the respirometry vessel. Photoperiods during measurements always matched those of the environmental chambers.

The MR of normothermic resting animals (RMR) was determined during the photophase when a variation of less than 5% over 15min occurred after an inactive period of at least 30min; the MR of active animals (AMR) was derived from the maximum rate observed during the scotophase taken over a 30min interval; the arousal peak was derived from the maximum rate, measured over at least 5min, after a torpor bout; and the minimum MR of torpid animals (TMR) was determined when VO_2 was constant over at least 30min. Animals were considered torpid when MR fell below 75% of the RMR at the same T_a (Hudson & Scott 1979). Duration of torpor bouts (75% RMR during entry to 75% RMR during arousal) was derived from the measurements of VO_2. For measurements of average daily metabolic rate (ADMR), VO_2 was integrated over the entire 23 hour period using intervals of 10min for calculations of the means.

All gas values were corrected to STP and VO_2 was calculated using equation 3a of Withers (1977). For mass specific VO_2 calculations, the animals were weighed before and after the experiments and body mass interpolated assuming a constant rate of loss.

Mean values in the text and figures are shown ± standard error (SE). Paired observations were compared using a F_{max} test to see if the variances were significantly different prior to being compared by a Student's t-test for equal or unequal variances (Zar 1984). Differences were assumed significant at the 95% level ($p<0.05$). In the text and figures n=number of individuals.

Results

Photoperiod influenced both duration and intensity of activity (Fig. 1a,b). The shorter photoperiod, LD 8:16 (lights on 0800h), produced a longer duration of activity (LD 8:16 16.5h cf 12h LD 16:8), from approximately 1600h to 0830h, and this was basically confined to the period of darkness. In contrast, animals in LD 16:8 (lights on 0400h) did not usually begin activity until 2000h, but were regularly active up to 4hours after lights on. However, while the duration of the activity period was shorter in LD 16:8, the level of activity, measured as mean movements per hour, was 33% greater ($p<0.04$, t-test).

Daily activity patterns were also affected by food and water availability (Fig. 2). The level of activity increased from 150-200 movements/30min with food (Day 1) to the maximum measurement of 255 movements/30min without food (Day 2). When the food was returned on the following nights the level of activity was reduced by at least 75% to 50-60 movements/30min (Day 3), subsequently returning to normal pre-starvation levels on Day 4. Once food and water were returned, the animals were able to regain all the weight lost during the previous night in one to two days.

Photoperiod did not appear to affect T_b of *S. crassicaudata* during normothermia or torpor (Fig. 3). There was no significant difference between the mean T_bs of normothermic animals, measured at T_as ≤15°C, at LD 16:8 (mean T_b 33.55±0.44°C, n=7) and LD 8:16 (mean T_b 33.76±0.45 °C, n=8) ($p>0.05$, t-test). Similarly, mean T_bs during torpor, 20.25±1.75°C (n=6) at LD 16:8 and 22.57±2.29°C (n=6) at LD 8:16, were not significantly different ($p>0.05$, t-test). As no T_b was recorded below 15°C and at T_as below 15°C the differential between T_b and T_a increased, it is assumed that the set point for T_b during torpor is about 15°C at both photoperiods. The high mean T_bs during torpor were due to the large variation observed (LD 16:8 T_b range 15.2-28.3°C, LD 8:16 T_b range 15.6-29.5°C).

As with T_b, photoperiod appeared to have no effect on MR and there were no significant differences between the MRs in any of the metabolic states at long and short photoperiods ($p>0.05$, t-test) (Fig. 4). TMR, 0.43±0.12mL g^{-1} h^{-1} (n=6) at LD 16:8 and 0.36±0.07mL g^{-1} h^{-1} (n=5) at LD 8:16, was significantly lower ($p<0.001$, t-test) than RMR, 5.08±0.06mL g^{-1} h^{-1} (n=8) and 4.93±0.08mL g^{-1} h^{-1} (n=8) at LD 16:8 and LD 8:16, respectively, representing a 92-93% energy saving at each photoperiod. Torpor durations, 6.79±2.32h (n=6) at LD 16:8 and 3.55±1.24h (n=5) at LD 8:16, were highly variable and were also not affected by photoperiod.

Discussion

The present study shows that the pattern of daily torpor in *S. crassicaudata* is unaffected by photoperiod and that this species should be able to utilise torpor at all times of the year. This is in contrast to many species, particularly rodents, in which photoperiod has been found to be the instigating factor in the timing of torpor (Heldmaier & Steinlechner 1981; Goldman *et al.* 1986; Steinlechner *et al.* 1986). Nevertheless, photoperiod did influence both the duration and intensity of activity in this species.

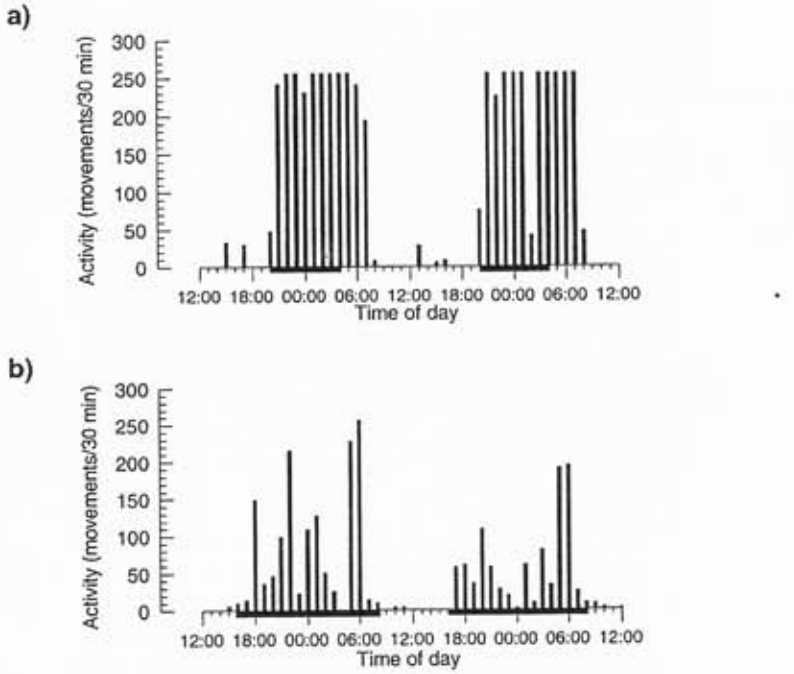

Fig. 1. Activity measurements, determined over two consecutive days, for an individual *S. crassicaudata* at **a)** LD 16:8 and **b)** LD 8:16. All other animals showed a similar behaviour. Horizontal bars indicate period of darkness. Photoperiod affected both duration and intensity of activity.

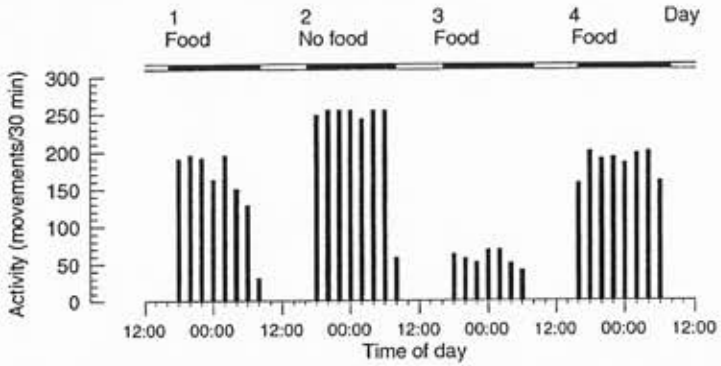

Fig. 2. Activity measurements for an individual *S. crassicaudata* at LD 8:16 over 4 consecutive days. All animals showed a similar pattern. Food and water were removed on Day 2 and returned on Day 3. Dark bars indicate night.

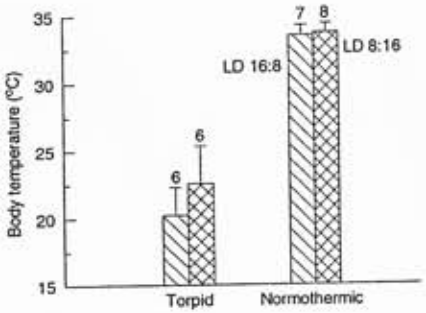

Fig. 3. Effect of photoperiods LD 16:8 and LD 8:16 on the mean body temperatures (T_b), ±SE, of torpid and normothermic *S. crassicaudata* at ambient temperatures ≤15°C. The numbers above the columns indicate the number of individuals. Photoperiod had no signficant effect on the T_bs of either torpid or normothermic individuals ($p>0.05$, t-test).

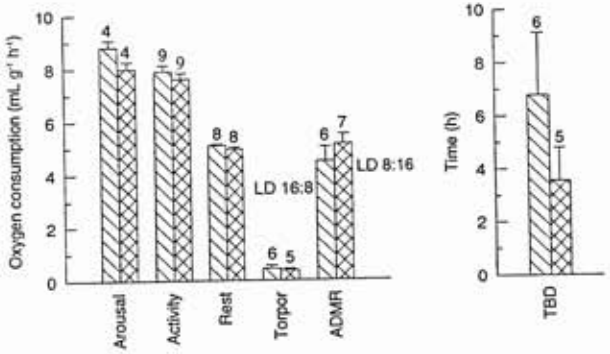

Fig. 4. Effect of photoperiods LD 16:8 and LD 8:16 on metabolic rates (mean ± SE), measured as rate of oxygen consumption, and torpor bout duration in *S. crassicaudata* at T_a 12°C. The numbers above the columns indicate the number of individuals, ADMR = average daily metabolic rate, TBD = torpor bout duration. Photoperiod had no signficant effect on any of the metabolic states or the duraton of torpor bouts ($p>0.05$, t-test).

Sminthopsis crassicaudata held in outdoor conditions show seasonal changes in metabolism and torpor (Geiser & Baudinette 1987). In winter the tendency to enter torpor was greater and TMR, T_b and set point were lower, as was RMR, compared to summer. The present study suggests that these changes are not controlled by photoperiod and that some other factor(s) must be involved.

Since in the study of Geiser and Baudinette (1987) animals were exposed to both changes in photoperiod and T_a, it appears that an important stimulus for the seasonal change in thermoregulation and torpor patterns in this species is T_a. It is also likely that food restriction acts as a major stimulus, as Morton (1978d) only observed torpid *S. crassicaudata* in the field at relatively moderate T_as of 9-17°C, despite night-time T_as often falling below 5°C, and concluded that the use of torpor in this species is a response to short-term food shortages. That food availability is an important factor is confirmed by laboratory studies where torpor can be induced through food deprivation (Godfrey 1968; Frey 1991; Holloway & Geiser 1995) and spontaneous torpor (food available) occurs only occasionally (Geiser & Baudinette 1987). Therefore, it appears that in *S. crassicaudata* a combination of both low T_a and food shortage act as a stimulus for the use of torpor.

Sminthopsis crassicaudata inhabits an arid to semi-arid environment which is subject to unpredictable and variable rainfall and, as such, large fluctuations in insect abundance (Morton 1978a, b). In addition, the activity of their insect prey is also affected by the weather, with the insects being less active in cold and wet conditions (Morton 1978b; Frey 1991). The majority of rodent species, on the other hand, which consume seeds and green plant material, are less prone to unpredictable fluctuations in abundance of their food source. Consequently, with the unpredictable fluctuations in food supply, combined with cool-cold nights, it would make sense for *S. crassicaudata* to be more opportunistic in its use of torpor, rather than relying, as do many rodent species, on a set cue such as photoperiod.

Since activity is on average 55% more energetically expensive than resting, an additional way of conserving energy during times of food shortage, instead of using torpor, is to reduce activity. As only a small percentage of *S. crassicaudata* have been observed torpid in the field (Morton 1978d; Frey 1991), there may be some disadvantages to its use, such as an increased risk of predation. In addition, Carey (1989) found that nutrient absorption in the digestive tract is slowed at low T_bs. Therefore, there may be times when it might be more suitable for the animal to reduce its activity and quickly replace the nutrients it lost while deprived of food, and still conserve some energy, than to enter torpor.

The length of photoperiod appeared to have two effects on activity, influencing both duration and intensity. By reducing the level of its activity during the short photoperiod, when the activity period is prolonged, this species appears able to maintain its ADMR at a rate similar to that during the long photoperiod. In addition, this reduction in activity, together with a prolonged period of feeding, may contribute towards the increase in body mass observed in the wild during winter (Morton 1978c). This increase in weight is thought to be due to behavioural changes and the use of energy conserving mechanisms as well as the endocrinological changes associated with breeding (Morton 1978c).

Sminthopsis crassicaudata is well adapted to its arid to semi-arid habitat, utilising a number of energy conserving mechanisms, including torpor and a reduction in activity levels, to cope with the unpredictable nature of this environment. However, the observed seasonal occurrence of torpor (Morton 1978d; Frey 1991) is most likely a reflection of an opportunistic response to low T_as and/or reduction in food availability, rather than to photoperiod.

Acknowledgements

We would like to thank Drs R. Hope and C. Chesson of the Genetics Department, University of Adelaide for providing the animals. Thanks also to Dr G. Körtner for reading the manuscript and to Dr S. Cairns for advice on the statistics. This work was supported by a University of New England Zoology Department Postgraduate Internal Research Grant to J. Holloway and a grant from the Australian Research Council to F. Geiser.

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