

Chapter Six: Relationships between Torpor and Behavioural Thermoregulation

6.1 Introduction

Energy expenditure during both normothermia and torpor varies with T_a to which an animal is exposed. For a hibernator, since the costs for normothermia are reduced in a warm environment below the T_{LC} , but the costs during an entire bout of hibernation are the lowest at the T_{TC} (chapter 5), it seems that there are two T_a s at which a hibernator can achieve a minimum energy expenditure. Either an animal displays torpor at a low T_a close to the T_{TC} , or it remains normothermic at a high T_a close to the TNZ. However, the results from a limited number of investigations on thermal preference of hibernators did not always agree with this prediction. While many insectivorous bats prefer a lower T_a range during torpor than that during normothermia as predicted (Tweney 1955; Davis 1970, Nagel and Nagel 1991; Brown and Bernard 1994), some rodents such as *Mesocricetus auratus* and *Perognathus longimembris* show preference for the relatively high T_a s during both normothermia and hibernation (Gumma and South 1970, French 1976).

Knowledge about temperature selection in daily heterotherms is even more limited than in hibernators. Because torpor depth, torpor bout duration, tendency to enter torpor, and other physiological variables of daily torpor differ significantly from hibernation, the effect of T_a on thermal relations of energetics also differ markedly between the two torpor patterns (Hudson 1973, chapter 5). Hence the preference for becoming torpid and the temperature selection for torpor of daily heterotherms cannot be predicted from behaviour of hibernators, especially if one considers the large variation among species of hibernators.

In the present study, T_a selection in relation to the use of torpor in marsupials, the present study, T_a selection, activity pattern, T_b fluctuation, and the use of torpor of *S. macroura* and *C. nanus* were investigated in a temperature gradient under *ad libitum* and restricted food conditions. By providing a choice of T_a for selection, the study was designed to answer the following questions:

1. Is temperature selection evident during normothermia, and if so, do the animals prefer a warmer rather than a cooler environment?
2. Do the animals prefer to remain normothermic rather than to display torpor?
3. If the animals become torpid, do they select a T_a for that is suitable for torpor, and what are the differences between a hibernator and a daily heterotherm in T_a selection?
4. Are the T_a selections altered when animals are energetically stressed?
5. Do torpor frequency and torpor depth differ under different food regimes?
6. What other behaviours and physiological processes do the animals use to cope with insufficient food availability?

6.2 Materials and Methods

6.2.1 Animals and Experimental Procedure

Six adult male *S. macroura* and six adult *C. nanus* (three individuals of each sex) were used in the experiment. *S. macroura* weighed 27.5 ± 2.7 g and *C. nanus* weighed 40.2 ± 5.7 g at the beginning of the experiment. All individuals were subjected alternatively to either a high T_a gradient (Table 5. 1 for *S. macroura*, Table 6. 2 for *C. nanus*) or a low T_a gradient (Table 6. 1 for *S. macroura*, Table 6. 2 for *C. nanus*). While the high T_a gradient allowed selection of a presumably energetically favourable thermal condition during normothermia (close to the TNZ), the low T_a gradient allowed

selection of a presumably optimal T_a during torpor (close to the T_{TC}). Details of the set-up of the T_a gradient are provided in chapter 2.3.

All *S. macroura* were subjected twice for 5 days to both temperature regimes. Their location, T_b , and activity were monitored continuously. During the first three days, food was freely available in all of the four compartments. During the last two days only 5 grams of fresh food (about 1/3 of the amount that the animal normally consumed when maintained in cages at 22°C) was provided. New food was introduced at 1200 hours, compartments were cleaned and animals were weighed. The mean BM loss over a 5 d measurement was 2.7 ± 3.1 g in the high T_a gradient, and 3.4 ± 2.0 g in the low T_a gradient.

Each *C. nanus* was kept in the T_a gradient for 8 days to continuously monitor their location, T_b , and activity. During the first two days in the gradient, food was provided *ad libitum* at 1200 hours in each of the four compartments. During the remained 6 days no food was provided. Animals were weighed before and after an 8-d measurement. The mean BM loss of the animals over an 8-day measurement was 2.3 ± 1.5 g in the high T_a gradient, and 1.9 ± 0.7 g in the low T_a gradient.

Water was provided in each compartment throughout the experiment for both species. Because the animals were unaccustomed to the gradient and therefore extremely active when initially introduced to the gradient, data collected during the first day in the gradient were excluded from the statistical analyses. Data recorded during the first hour after daily feeding were also excluded.

6.2.2 Data Acquisition and Analysis

Locomotor activity was monitored by PIRs (see chapter 2.3 for details). Activity of animals in each compartment was scanned and summed up over 6-min intervals. Activity counts from all four compartments were summed to determine overall daily

activity. To calculate the overall time spent active, all 6-min intervals in which activity was recorded were summed.

T_b signals were collected via the antennae placed in each compartment (see chapter 2.3 for details). T_b was recorded in 6-min intervals. The maximum, the lowest, and the average T_b s were determined for *S. macroura* on daily basis, and for *C. nanus* they were determined over a torpor bout.

Since T_b and activity signals were received separately in each compartment, they were also used to locate the animals. If an animal sat between two compartments (i.e. at the compartment gate, Fig. 2.5), or if it moved between compartments within the 6-min measuring interval, it was recorded as being in both compartments. To calculate the percentage of location for an animal, the number of measuring intervals in which the animal was present in a certain compartment was divided by the total number of location records (i.e. over 12 h or 24 h).

Torpor was defined as a T_b below 32.0°C, and torpor bout length was calculated accordingly. Torpor frequency presents the average of the percentage of torpid animals per day for each experimental condition. The percentage of location of a torpid animal was determined as the number of torpor bouts in a certain compartment divided by the total number of torpor bouts of each individual.

Differences in torpor frequency was examined by log linear modelling carried out using the statistical package Glim (Crawley 1993) which tests the χ^2 of effects of multifactors. Individual means for activity, T_b , torpor bout duration, and location at each experimental condition were calculated before these variables were statistically examined.

Table 6.1 Air temperature (°C) of the temperature gradient for measurements on *S. macroura*high gradient

compartment	1		2		3		4	
warm/cold	W	C	W	C	W	C	W	C
mean	36.8±1.9	32.0±1.3	28.2±1.0	25.2±0.8	22.3±0.9	20.5±0.7	18.7±1.0	16.0±2.1
maximum	40.0	35.0	31.3	27.5	24.5	22.3	20.8	18.5
minimum	30.5	27.8	26.0	23.5	20.0	18.8	16.0	12.8

low gradient

compartment	1		2		3		4	
warm/cold	W	C	W	C	W	C	W	C
mean	23.9±1.4	20.6±0.7	16.8±0.6	15.5±0.7	12.4±0.8	10.9±0.8	8.2±0.9	5.2±1.0
maximum	27.3	22.5	18.0	17.5	14.5	13.0	10.0	8.0
minimum	20.0	19.0	14.8	13.8	10.5	9.0	6.0	3.5

Note: "W" and "C" represent the warm est and the coldest ends of a compartment of the T_a gradient, respectively. The "mean", "Maximum" and "minimum" represent the average, the maximum and the minimum T_a s measured in 1-h intervals over 5 days.

Table 6.2 Air temperature (°C) of the temperature gradient for measurements on *C. nanus*high gradient

compartment	1		2		3		4	
warm/cold	W	C	W	C	W	C	W	C
mean	37.1±2.7	32.4±2.0	28.3±1.3	27.0±0.9	24.8±1.3	23.1±0.7	20.9±0.7	20.3±1.1
maximum	39.5	36.6	32.8	29.5	27.3	25.6	23.4	21.3
minimum	31.5	28.2	25.3	23.1	22.8	21.2	20.1	17.8

low gradient

compartment	1		2		3		4	
warm/cold	W	C	W	C	W	C	W	C
mean	22.0±1.1	20.3±0.5	17.2±0.4	15.9±0.6	12.1±0.3	10.0±0.9	7.4±0.9	3.1±1.0
maximum	23.3	21.5	18.3	17.0	13.0	12.8	8.5	6.8
minimum	18.8	18.5	15.8	13.3	11.5	8.3	5.1	1.5

Note: "W" and "C" represent the warm est and the coldest ends of a compartment of the T_a gradient, respectively. The "mean", "Maximum" and "minimum" represent the average, the maximum and the minimum T_a s measured in 1-h intervals over 8 days.

6.3 Results for *Sminthopsis macroura*

6.3.1 Activity

S. macroura showed a pronounced light-dark activity cycle. During the light phase, animals normally rested, and seldom moved around. They became active immediately after lights-off. Usually 2-3 activity peaks were observed during a dark phase (not shown). Under food restriction, a profound bimodal distribution of activity was usually observed. One of the activity peaks occurred at the beginning of the dark phase, and the other during the dark phase before entering torpor, and the two activity peaks were separated by a period of low activity.

Locomotor activity during the dark phase accounted for $90 \pm 3.6\%$ and $89 \pm 10.4\%$ of the total activity counts, and for $76 \pm 7.5\%$ and $82 \pm 7.2\%$ of the total activity time in the low and the high T_a gradient, respectively (Fig. 6.1). Total activity counts did neither differ between gradients nor between food regimes ($p > 0.05$, 2-way ANOVA; Fig. 6.1). Total activity time was longer in the high than in the low T_a gradient ($p < 0.05$, 2-way ANOVA), and was not affected by food restriction ($p > 0.05$, 2-way ANOVA; Fig. 6.1).

6.3.2 Torpor

Spontaneous torpor with a substantially lowered T_b occurred in both T_a gradients. Torpor usually occurred around the time of lights on, shortly after the termination of activity. Arousal was characterised by a rapid increase of T_b and it usually occurred around midday. Although torpor frequency appeared higher in the low T_a gradient, and tended to be elevated after food restriction, these differences were not significant ($p > 0.05$, χ^2 -test; Fig. 6.2).

In the low T_a gradient animals often entered torpor earlier, or they remained torpid longer than in the high T_a gradient. However, there were no significant differences in torpor bout duration between the two T_a gradients ($p > 0.05$, 2-way

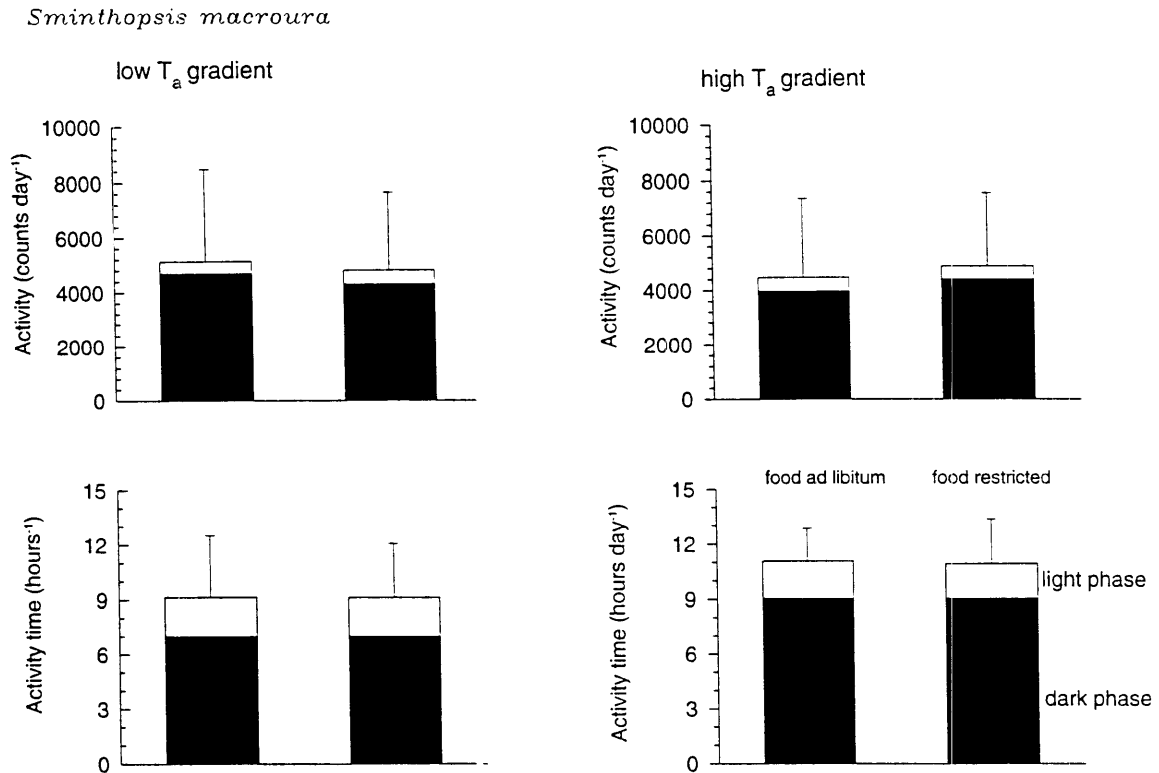


Fig. 6.1. Locomotor activity of *Sminthopsis macroura* in a low (left) and a high (right) temperature gradient with food provided *ad libitum* and restricted food supply. The top graphs show number of activity counts and the bottom graphs show the time spent active during a day. Filled bars represent the values for the dark phase, open bars those for the light phase. The error bars represent the standard deviations of the daily total values. $N = 6$, $n = 24$ for each experimental condition.

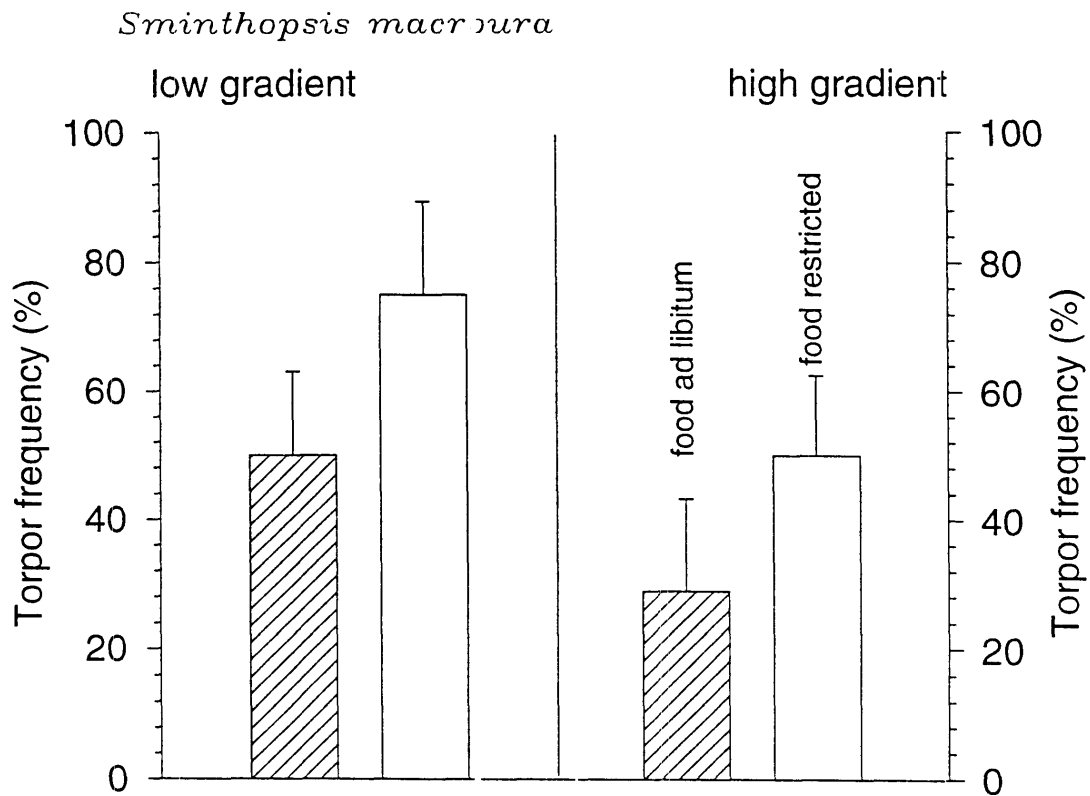


Fig. 6.2. Torpor frequency of *Sminthopsis macroura* in a low (left) and a high (right) temperature gradient under food *ad libitum* and restricted food conditions. Error bars represent the standard deviations. N = 6, n = 24.

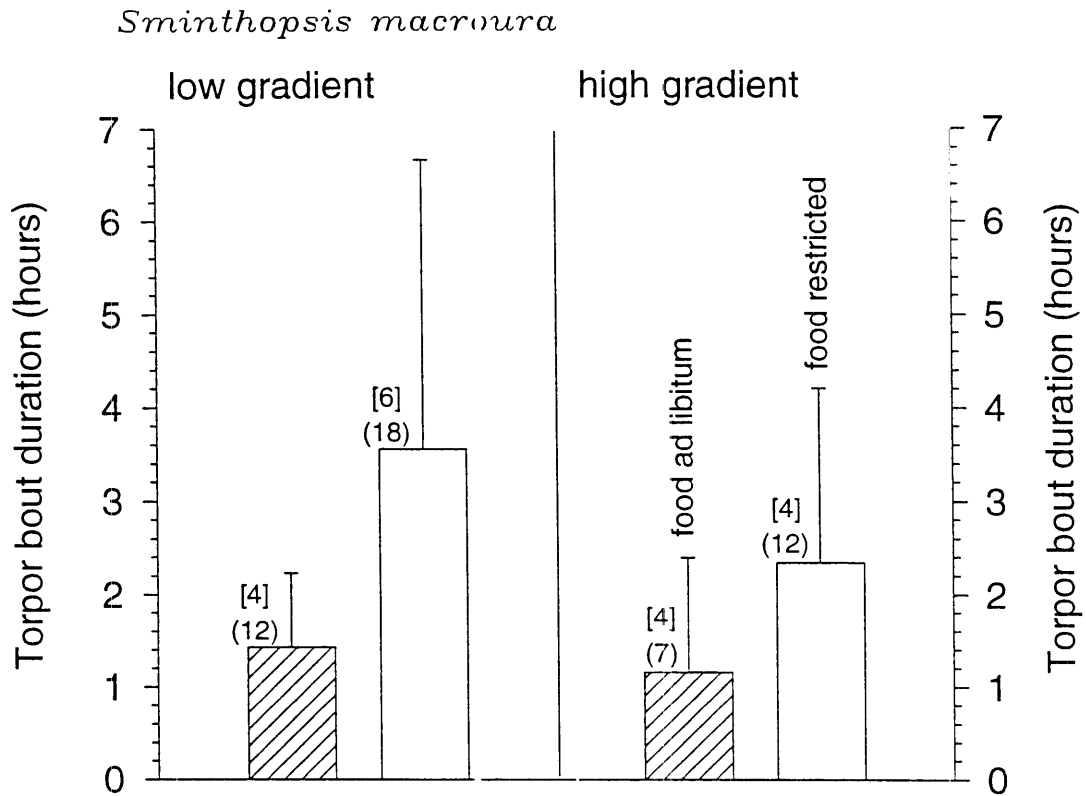


Fig. 6.3. Torpor bout duration of *Sminthopsis macroura* in the low (left) and high (right) temperature gradient under food *ad libitum* and restricted food conditions. The error bars represent the standard deviations. Numbers of animals are given in the square brackets, and numbers of observations are shown in parentheses.

ANOVA). Insufficient food supply usually delayed arousals. Torpor bout duration increased significantly under food restriction ($p < 0.05$, 2-way ANOVA; Fig. 6.3).

6.3.3 Body Temperature Fluctuation

T_b fluctuated profoundly in accordance with the activity patterns and torpor cycles. The lowest daily T_b (T_{b1}) of torpid animals under both food regimes was 3.3°C and 3.2°C lower than normothermic individuals in the low and the high T_a gradient, respectively ($p < 0.001$, $p < 0.05$, 2-way ANOVA; Fig. 6.4). For these torpid animals, food restriction resulted in a significant decrease in T_{b1} ($30.9 \pm 1.0^\circ\text{C}$ to $29.3 \pm 2.4^\circ\text{C}$ in the low T_a gradient; $30.9 \pm 0.3^\circ\text{C}$ to $29.1 \pm 2.1^\circ\text{C}$ in the high T_a gradient; $p < 0.001$, $p < 0.05$, 3-way ANOVA). However, no difference in T_{b1} after food restriction was found in individuals that remained normothermic ($33.0 \pm 0.5^\circ\text{C}$ to $33.5 \pm 0.7^\circ\text{C}$ in the low T_a gradient; $33.4 \pm 0.4^\circ\text{C}$ to $33.2 \pm 0.4^\circ\text{C}$ in the high T_a gradient; Fig. 6.4).

The daily maximum T_b (T_{bmax}) was about 38°C and was indistinguishable under all experimental conditions ($p > 0.05$, 3-way ANOVA; Fig. 6.4). In animals that displayed torpor, the average T_b (T_{bmean}) over one day was reduced by an average of $0.7 \pm 0.5^\circ\text{C}$ ($p < 0.001$, 3-way ANOVA), and this reduction was independent of food regime and T_a gradient ($p > 0.05$, 2-way ANOVA; Fig. 6.4).

6.3.4 Selection of Ambient Temperature

S. macroura favoured a relatively warm environment. In the low T_a gradient, when food was freely available, they stayed for $86.4 \pm 6.7\%$ of the day in the compartment with the highest available T_a of $20.6 \pm 0.7^\circ\text{C}$ to $23.9 \pm 1.4^\circ\text{C}$. However, this compartment was more frequently occupied during the light phase than during the dark phase ($p < 0.001$, 2-way ANOVA). Food reduction did not effect the distribution of the animal in the gradient ($p > 0.05$, 2-way ANOVA), but interacted significantly with the light-dark phase ($p < 0.05$, 2-way ANOVA), for which animals spent more

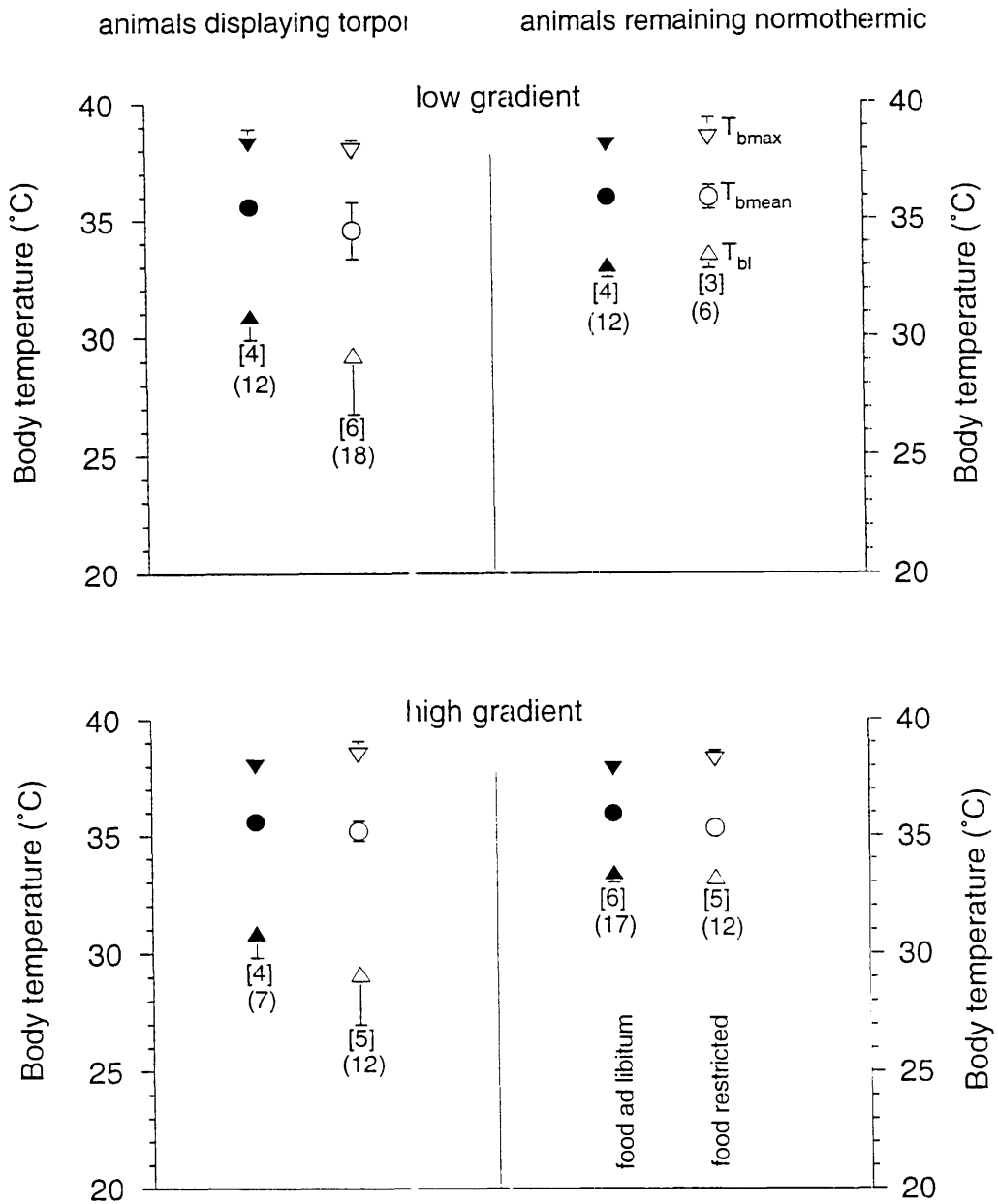


Fig. 6.4. Average daily body temperatures (\pm SD) of *Sminthopsis macroura* under food *ad libitum* (filled symbols) and restricted food (open symbols) conditions of individuals that displayed torpor (left) and those that remained normothermic (right). Top graph shows the values in the low gradient and bottom graph the values in the high temperature gradient. Inverted triangles represent daily maxima (T_{bmax}), upright triangles the lowest daily (T_{bl}), and circles the daily mean (T_{bmean}). Numbers of individuals are given in square brackets and numbers of observations are given in parentheses.

time in the warmest compartments during the light phase, but less time during the dark phase (Fig. 6.5). Under both food regimes all torpid individuals were located in the warmest compartment.

In the high T_a gradient, animals spent a similar amount of time in the two warmest compartments with $T_a > 25.2 \pm 0.8^\circ\text{C}$ together for $87 \pm 8.9\%$ of a day (Fig. 6.5). Animals resided in the two warmest compartments more frequently during the light phase than during the dark phase ($p < 0.001$, 2-way ANOVA), and the distribution was not affected by food availability ($p > 0.05$, 2-way ANOVA, Fig. 6.5).

In the high T_a gradient, torpor was observed in one individual on four occasions in the second warmest compartment and in another two individuals in the warmest compartment on 3 occasions. Thus the location frequency of torpid individuals was 66.7% and 33.3% in the warmest and the second warmest compartment, respectively. When food was restricted, torpid individuals were evenly distributed between the two warmest compartments, with frequency of 50% each.

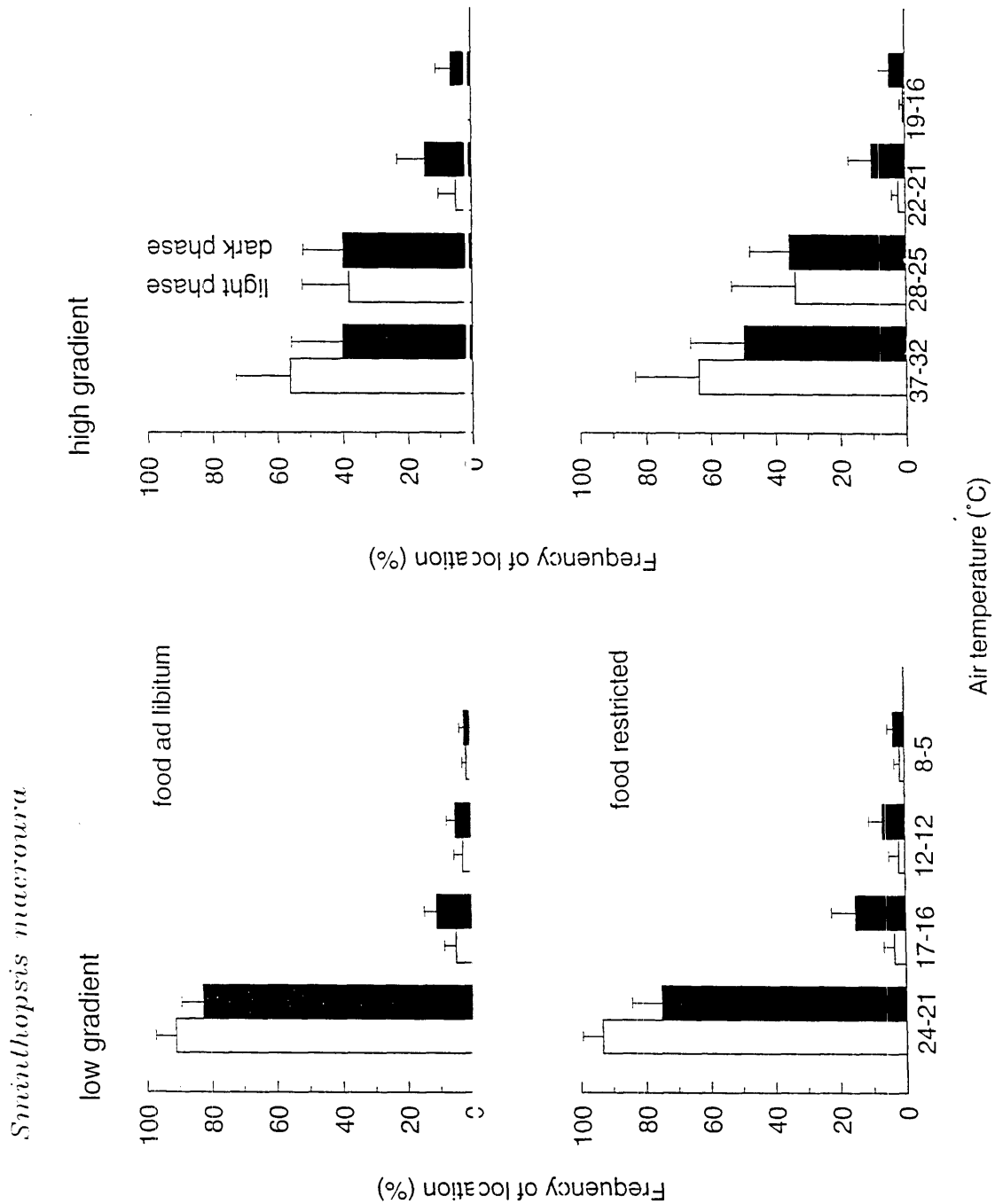


Fig. 6.5. location of *Sminthopsis macroura* in a low (left) and a high (right) temperature gradient under food provided *ad libitum* (top) and with restricted food supply (bottom). Filled bars represent values for the dark phase and open bars that for the light phase. The error bars represent the standard deviations of 6 individuals.

6.4 Results for *Cercartetus nanus*

6.4.1 Activity

Due to the differences in torpor employment and torpor patterns, the timing, counts, and duration of activity of *C. nanus* all varied markedly among individuals and among different days for the same individual. The number of activity counts per day of normothermic animals was neither affected by food regimes nor by differences in the T_a range of the gradient ($p > 0.05$, 2-way ANOVA; Fig 6.6). However, daily activity time was significantly longer in the low T_a gradient, and animals spent more time active when food was available *ad libitum* than when food was withheld ($p < 0.05$, 2-way ANOVA; Fig 6.6), apparently due to differences of time that they spent in torpor.

6.4.2 Torpor

Torpor was widely employed by *C. nanus* in both T_a gradients under both food regimes. In the low gradient, torpor frequency was 100% under both food regimes. In the high gradient, four of a total of six individuals displayed torpor on every day. However, one animal remained normothermic constantly throughout an 8-day measurement, and another individual entered torpor only after food was withheld for 4 days. Thus the torpor frequency in the high T_a gradient was 66.7% and 77.1% under food *ad libitum* and food restricted conditions, respectively.

In the T_a gradient, *C. nanus* often displayed torpor on a daily basis. Animals usually entered torpor several hours after lights-off, and aroused simultaneously shortly before the onset of the dark phase. However, they sometimes also displayed long bouts of torpor lasting for more than one day. These were more frequently observed when food was not available and in the low T_a gradient (Fig. 6.7). When food was withheld, the average torpor bout duration was 29.7 ± 21.6 h in the low T_a gradient and 21.6 ± 21.2 h in the high T_a gradient. This was 8.5 ± 3.5 h and 8.2 ± 3.9 h longer than under food *ad libitum* condition in the low and high T_a gradient,

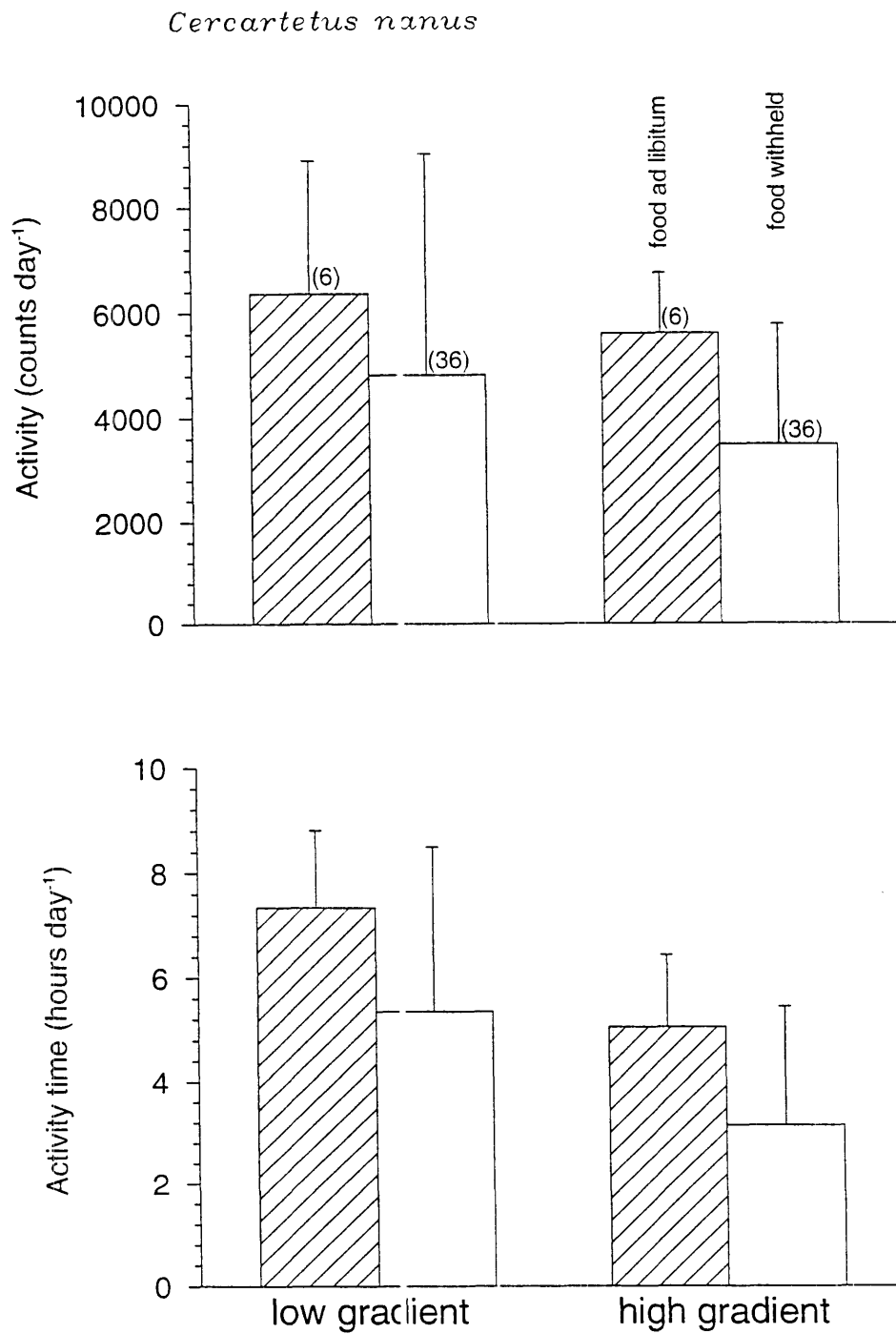


Fig. 6.6. Locomotor activity (mean \pm SD) of *Cercartetus nanus* in a low and a high T_a gradient with food provided *ad libitum* and restricted food supply. The top graph shows number of activity counts and the bottom graph shows time spent active during a day. Numbers of measurements are given in parentheses, and 6 individuals were used at each experimental condition.

respectively ($p < 0.05$, 2-way ANOVA; Fig. 6.7). However, mean torpor bout duration did not differ significantly between the animals exposed to high and low T_a gradients ($p > 0.5$, 2-way ANOVA; Fig. 6.7).

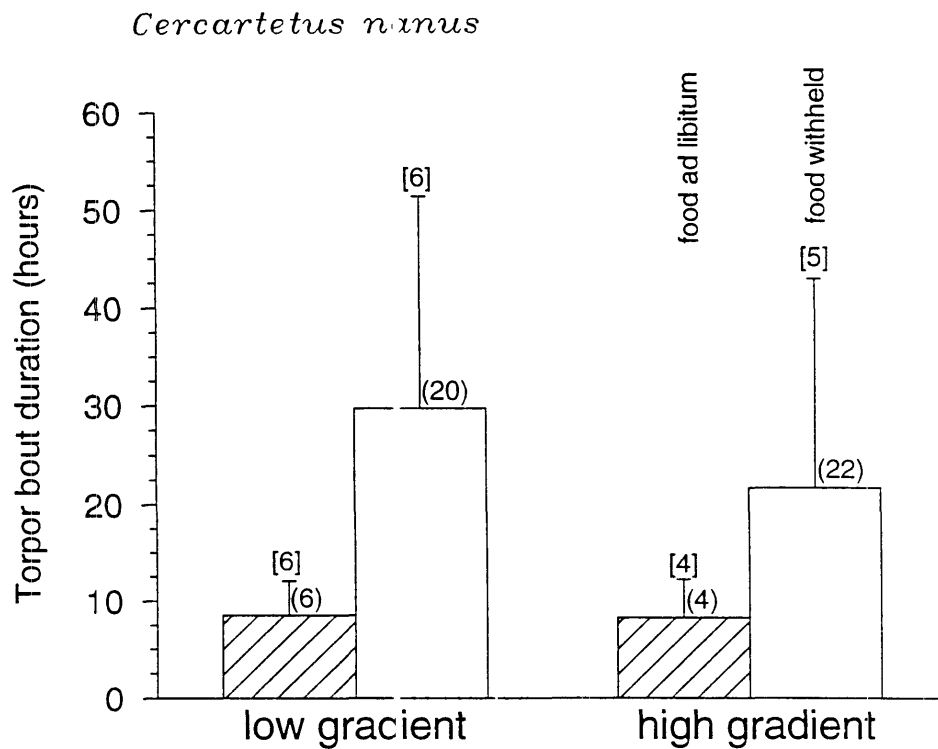


Fig. 6.7. Torpor bout duration (Mean \pm SD) of *Cercartetus nanus* in the low and high T_a gradient under different food regimes. Numbers of individuals are given in square brackets and numbers of measurements are given in parentheses.

6.4.3 Body Temperature Fluctuation

During the days on which two individuals remained normothermic in the high T_a gradient, neither the daily T_{bmean} nor T_{bmax} differed by more than 0.3°C between food *ad libitum* and food restriction, but the daily T_{bl} was 1.1°C lower when food was withheld.

For the animals that employed torpor, the T_{bl} during a bout of torpor in the low T_a gradient ($16.5 \pm 1.0^\circ\text{C}$) was significantly lower than that in the high T_a gradients ($26.2 \pm 1.2^\circ\text{C}$) ($p < 0.001$, 2-way ANOVA; Fig. 6.8), suggesting the effect of T_a on T_b during torpor. Nevertheless, food regimes did not significantly affect T_{bl} during torpor ($p > 0.1$, 2-way ANOVA; Fig. 6.8).

Exposure to low T_a gradient and withdrawal of food both significantly decreased the daily T_{bmean} ($p < 0.05$, 2-way ANOVA; Fig. 6.8), apparently due to deeper and prolonged torpor bouts.

The T_{bmax} during inter-bout normothermia was not affected by different food regimes or different T_a gradients ($p > 0.05$, 2-way ANOVA; Fig. 6.8).

6.4.4 Selection of Ambient Temperature

When food was provided *ad libitum*, the location of animals over the four compartments (from warm to cold) was $49.7 \pm 17.0\%$, $25.3 \pm 12.2\%$, $17.9 \pm 8.4\%$, and $7.2 \pm 4.0\%$, and $50.3 \pm 20.6\%$, $34.0 \pm 17.4\%$, $9.8 \pm 6.0\%$, and $5.9 \pm 7.2\%$ in the low and high T_a gradient (Fig. 6.9). This distribution was not affected by the light-dark cycle ($p > 0.05$, ANOVA for each compartment). When food was withheld, animals show marked individual differences in T_a selection. Some animals tended to spend more time at the warmest compartments, others were located more frequently in the middle of the gradient, and the rest seemed to prefer different compartments on different days, relating largely to whether torpor was displayed and what torpor

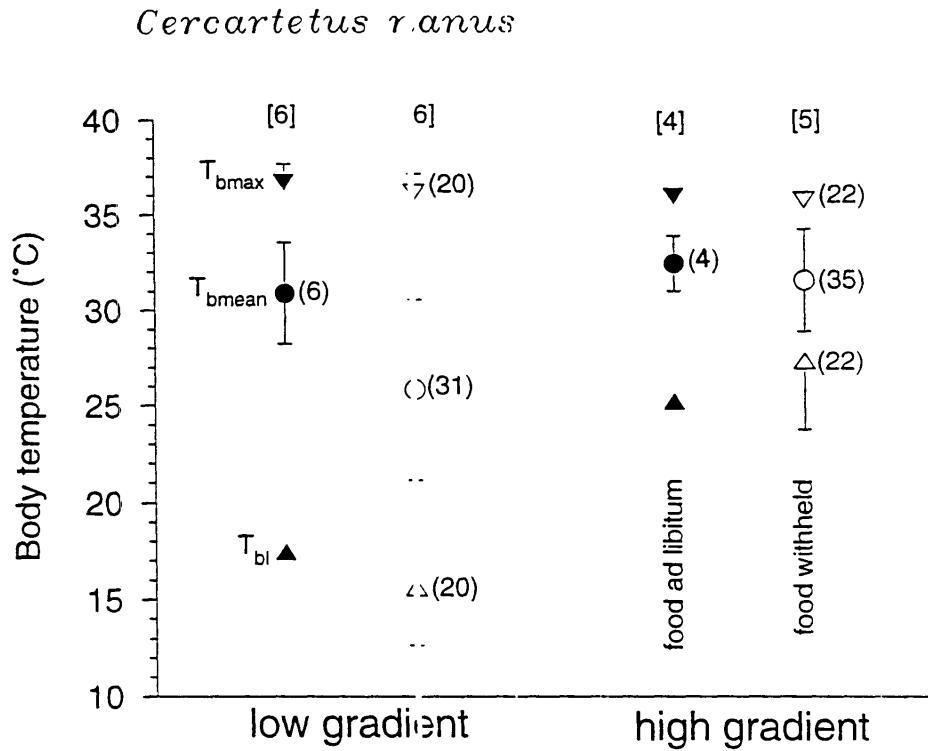


Fig. 6.8. Body temperatures of *Cercartetus nanus* that displayed torpor in the high and low T_a gradient. filled symbols represent for values under food *ad libitum* condition and open symbols for values under restricted food condition. The values show maximum T_b (T_{bmax} , down facing triangles) during inter-torpor normothermia, the mean body temperature over the measuring days (T_{bmean} , circles), and the lowest T_b during a bout of torpor ($T_{b|}$, up facing triangles). Numbers of animals are given in square brackets and numbers of measurements are given in parentheses.

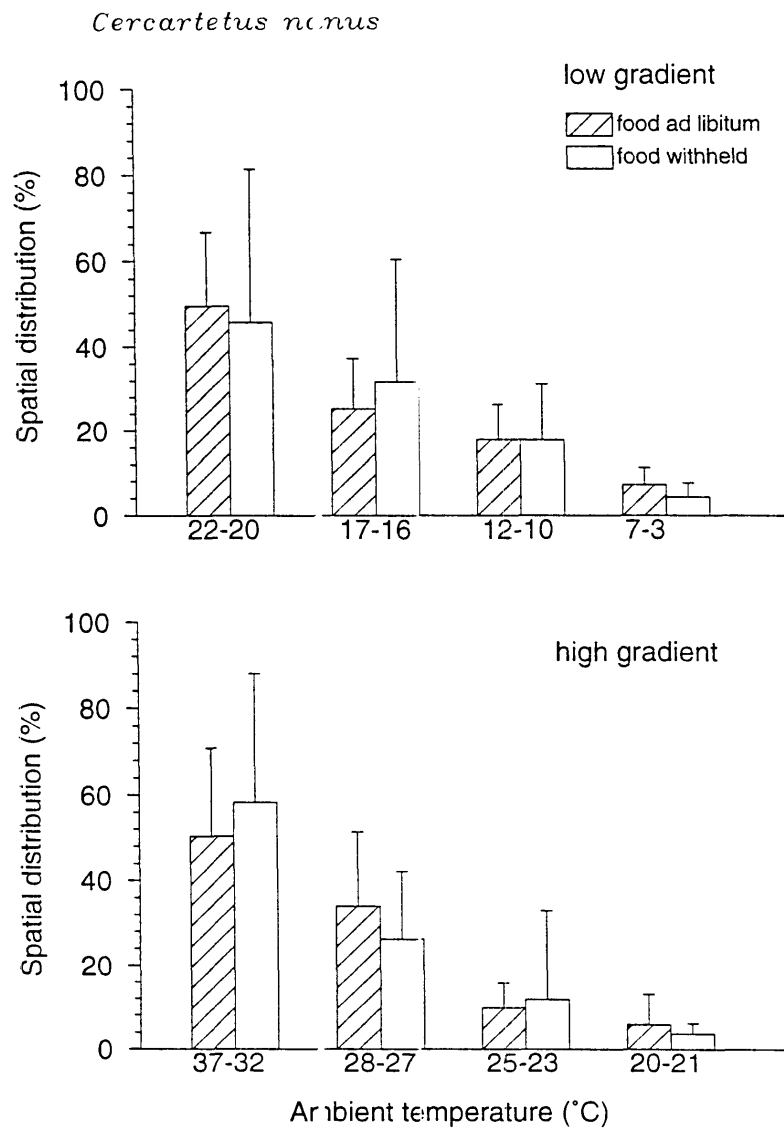


Fig. 6.9. Location (mean \pm SD) of *Cercartetus nanus* in the four compartments of the low (top) and the high (bottom) T_a gradients with food provided *ad libitum* (hatched bars) and restricted food supply (open bars). Six individuals were used at each experimental condition.

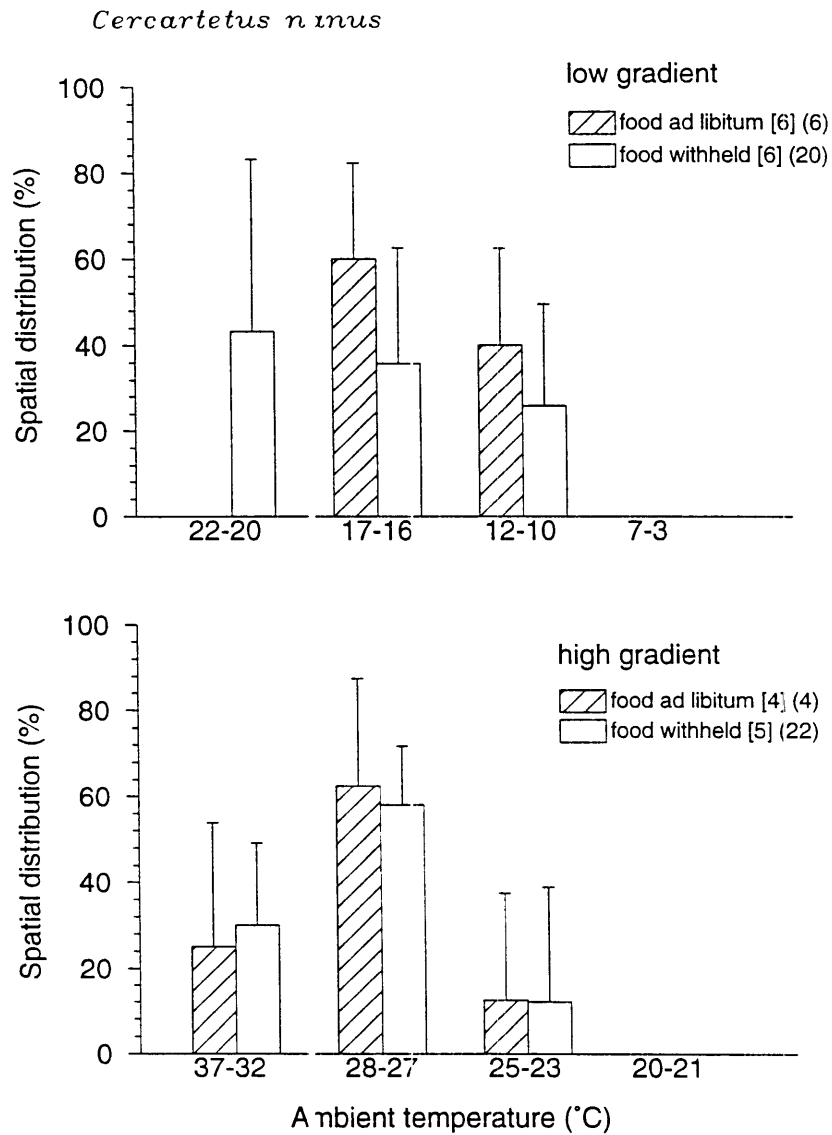


Fig. 6.10. Location (mean \pm 3D) of torpid *Cercartetus nanus* during a torpor bout in the low (top) and high (bottom) T_a gradient. Six animals were used for each experimental condition, but not all entered torpor in the high T_a gradient. Numbers of torpor bouts are given in parentheses and individuals displayed torpor in square brackets.

patterns (i.e. short bout or long bout torpor) were employed on that particular day. The location of the animals in the gradient was not affected by food regimes ($p > 0.05$, ANOVA for each compartment).

In comparison to normothermic animals, animals clearly favoured relatively cooler sites during torpor. Although torpor was observed in all but the coldest compartments of both T_a gradients, for both feeding regimes torpid animals were most frequently confined to the second warmest compartments of both T_a gradients under different food regimes (Fig 6.10).

6.5 Discussion

6.5.1 Temperature Selection

During normothermia, both *S. macroura* and *C. nanus* most frequently appeared in the compartments with the highest available T_a which was close to the TNZ. This preference is shown even during the dark phase, while animals foraged. This selection of a relatively high T_a during normothermia has also been reported for many bat and rodent hibernators (Twente 1955; Harmata 1969; Davis 1970; Gumma and South 1970; French 1976; Nagel and Nagel 1991; Brown and Bernard 1994). Thus it may represent a general trait for normothermic heterotherms, as for homeothermic endotherms (Schmidt 1984; Gordon 1985). Obviously, the selection of relatively high T_a results in a reduction of energy expenditure, since homeothermy can be maintained at low metabolic costs (Stinson and Fisher 1953; Morrison and Warman 1967; Schmidt; Gordon 1985).

However, during a bout of torpor, the thermal relations of energy expenditure are more complex. As indicated in chapter 5, above the T_{tc} , energy savings by the use of torpor declines with increasing T_a , while that of thermoregulating torpid animals decreases at low T_a s. Nevertheless, it does not necessarily mean that torpor at the T_{tc} should be energetically most favourable. When the cost for arousal, which increases at

low T_a s, is included in the calculation, the smallest overall energy expenditure of torpor is achieved at different T_a s in the two species measured in this study (chapter 5). For *S. macroura*, due to a relatively large proportion of arousal costs, torpor at a high T_a close to the T_{lc} is energetically the cheapest. Thus, it is not surprising to find that *S. macroura* were always located at high T_a s in the temperature gradient, since being either torpid or normothermic at a high T_a is energetically more economical than being at a low T_a . This is supported by the observation that the average daily metabolic rate of *S. macroura* that were torpid at a T_a close to the T_{tc} was significantly higher than that of individuals at a T_a close to the T_{lc} , although torpor at a T_a close to the T_{tc} reduced energy expenditure more significantly in comparison to normothermic values (Song and Geiser 1997).

Although total energy expenditure provides a good explanation for the preference of high T_a of *S. macroura*, it does not appear to explain temperature selection for torpor in *C. nanus*. As has been showed in chapter 5, the arousal costs of *C. nanus* are relatively small and the overall energy expenditure of torpor is minimal at the T_{tc} . In the T_a gradient *C. nanus* favoured lower T_a s for torpor than for normothermia, and when food was withheld, some individuals moved to even lower T_a s. Although its selection of T_a in the gradient appeared to be closely correlated with the length and depth of torpor, and thus energy savings, *C. nanus* did not select T_a s near the T_{tc} . This suggests other important factors contribute to the T_a selection for torpor.

The result on *C. nanus* might suggest that torpor represents a less favourable physiological state than normothermia, and there are potential disadvantages associated with deep torpor. Although torpor is an effective strategy for an animal confronted with environmental constraints, it is unavoidably accompanied by temporary sacrifice of the advantages of being active and mobile. This is not only important from an individual point of view which involves a potential decrease of the ability to avoid predation, but also reduces access to food resources. Moreover,

immune functions may also be impaired at lower T_b s (Lyman 1982e; Willis 1982a). Inactivity is also important from a population point of view as most reproductive processes such as spermatogenesis and fetal and neonatal growth may cease or may be slowed during torpor (Racey 1973; Barnes et al. 1986a, b). Furthermore, it has been suggested that normothermia is a very important physiological state for a hibernator especially because it is necessary for abolishing various kinds of metabolic imbalances developed during heterothermy (Willis et al. 1971; French 1986), or for restoring biological functions that may be retarded at low T_b s (Willis 1982a, b; Florant et al. 1984). Thus it is likely that the preference of a high T_a for torpor is associated with essential requirements of being normothermic (French 1986).

Nevertheless, small heterothermic mammals inhabiting high latitudes of the northern hemisphere frequently encounter food shortages during severe winters. Thus survival of periodical energy crises appears to depend on the efficiency of employment of torpor. This environmental stress may have provided another strong selection pressure for optimising selection of the most favourable T_a during torpor. Small hibernators which rely on a restricted small amount of body fat for energy metabolism, such as insectivorous bats, appear to prefer cold hibernacula close to the set-point for T_b during torpor (Twente 1955; Harmata 1969; Davis 1970; Brown and Bernard 1994). The T_a selection of these animals ensures prolonged low-cost torpor bouts and less frequent energy expensive arousals, and thus preservation of their limited energy reserves. Since a favourable T_a is critical for these hibernators to survive winter, some hibernators even change hibernacula between torpor bouts, presumably to meet their T_a requirements (Körtner and Geiser 1996).

However, animals living in mild environments may have a reasonable chance to find some food during the hibernating season. Thus the pressure to select a low T_a for minimising energy expenditure during torpor may not be strong enough. This is also true for animals that are associated with harsh winters, but have ability to store large

amounts of food for the hibernation season. It has been reported that several hibernators which store food prefer a high T_a during hibernation, which ensures that they have sufficient time for being normothermic to access and digest their cached food (Gumma and South 1967, 1970; French 1976). Supporting this point of view, the longest torpor bout duration of animals that store food are usually shorter than that of animals that rely exclusively on body fat (French 1982b, 1986). Moreover, when a large amount of stored food is available, animals remain normothermic for longer periods and torpor bout duration is reduced (French 1986; 1989).

C. nanus lives in Australian forests along the south-east coast in a relatively mild climate. Their main diet includes nectar and pollen of a range of native banksias and eucalyptus. Since these plants may alternatively blossom throughout the year, the animals have the opportunity to obtain food even during winters (Turner and Ward 1983; Turner 1984). In addition, *C. nanus* may store body fat towards the end of summer (Turner and Ward 1983). Body fat plus available food should provide the animal with sufficient energy to survive unfavourable seasons. Torpor in the field is most likely used opportunistically by the species, depending largely on its immediate energy requirements such as for handling short-term food shortage, for balancing energy depletion, or for accumulating energy in the form of body fat by consuming less food intake. These functions of torpor in facilitating rapid fattening and in balancing short-term energy deficit have generally been considered to be strategies of daily heterotherms, but not of hibernators (Carpenter and Hixon 1988; Wang 1989). The view that eastern pygmy-possums employ torpor opportunistically is supported by the observations in the field that some *C. nanus* from the same population may be active, in shallow torpor, or in deep torpor on different days during all seasons of the year (Bladon 1993). Thus the selection of a high T_a during torpor in *C. nanus* seems to favour the use of frequent, short bout torpor, which allows them to be active and forage during normothermia.

6.5.2 Torpor and Responses to Food Restriction

Both *S. macroura* and *C. nanus* frequently employed torpor spontaneously in the T_a gradient, even under seemingly ideal circumstances, including sufficient food and water supplies, and relatively high T_{as} . However, these conditions are not commonly encountered in the animals' natural environments, and energy benefits of torpor are potentially much larger in the field. For both species torpor that may occur on a cold night, even during summer when it is warm during the day. This can reduce their energy expenditure for normothermic thermoregulation. Since time of activity and the associated costs are reduced, less food needs to be collected for keeping a positive energy balance. Moreover, it has also been proposed that an animal that employs torpor during night time may be able to reduce overall arousal costs by a passive increase of T_b with T_a on the following warm day (Schmid 1996). These large energy savings by the use of torpor make it likely that torpor is frequently used in the field. Therefore, torpor may form part of a daily routine and spontaneous torpor may reflect a long-term adaptation to the environment for both *S. macroura* and *C. nanus*.

Insufficient food supply enhanced torpor frequency of both *S. macroura* and *C. nanus* in the T_a gradient. In addition, food withdrawal prolonged the time spent torpid and lowered the T_b of individuals displaying torpor. This suggests that the main strategy the two species applied to overcome energy crises is to remain torpid for longer periods at lower T_b s. In many other hibernators and daily heterotherms torpor is also facilitated by an energy deficit (Brown and Bartholomew 1969; Buffenstein 1985; Tannenbaum and Pivorun 1987; Ruf et al. 1991). It has been proposed that restriction of food reduces stored energy reserves and the threshold for remaining normothermic is easily approached even when little energy is used (Dawson 1989; Hiebert 1990). However, it is also possible that the energy threshold is reached due to a faster depletion rate, where the low energy availability requires more efforts and thus

costs in searching for food. If energy requirements are not met during foraging, torpor may be employed to make food reserves last longer (Buffenstein 1984a).

It seems that enhancement of torpor when food supply is restricted is related to the intense foraging activity in *S. macroura*. Under food restriction, the locomotion of the animal at low T_{aS} during the dark phase increased while during the light phase the frequency of location at high T_{aS} increased. This suggests that after animals foraged in the gradient and failed to gain enough food, they displayed longer torpor bouts, but in relatively warm areas.

Although enhancement of torpor is applied to reduce energy expenditure to meet low food availability, daily activity counts of both *S. macroura* or *C. nanus* were similar with both food *ad libitum* and restricted food supply. This suggests that despite the high energetic costs for activity animals would not reduce activity to save energy, most likely because low activity would limit their chance to obtain food. Thus, the enhancement of torpor allows the animals to maintain foraging activity even under food restriction.

In summary, if given a choice, both *S. macroura* and *C. nanus* select relatively high T_{aS} for normothermia and torpor to reduce energy expenditure and/or to ensure sufficient foraging activity during normothermia. Enhancement of torpor appears to be the main strategy for both species to handle food shortage, which enables the animals to maintain activity by reducing energy expenditure.

Chapter Seven: General Conclusions

The present study investigated thermal and metabolic strategies of the marsupials, *Sminthopsis macroura*, a daily heterotherm, and *Cercartetus nanus*, a hibernator. The metabolic reduction and energetics between the daily heterotherm and the hibernator were compared over a wide range of T_a , distinguishing phases of torpor entry, steady-state torpor, and arousal. It is the first study to provide such a detailed comparative analysis of thermal physiology and energetics of daily torpor and hibernation. The conclusions drawn from the study are as follows:

As both animals are small heterothermic marsupials, *S. macroura* and *C. nanus* share some thermophysiological aspects. During normothermia, they both showed a relatively low BMR and T_b compared with many similar-sized placentals. However, the thermogenic capacity of both species are even higher than that predicted for similar-sized placental mammals. As *C. nanus* is a hibernator and *S. macroura* is a daily heterotherm, the two animals showed differences in physiological variables such as the minimum metabolic rate and the minimum T_b . Nevertheless, the physiological variables of *C. nanus* were within the ranges predicted for mammalian hibernators, while those of *S. macroura* were comparable to other mammalian daily heterotherms. This is further support for the view that torpor in marsupials and in placentals are similar.

During steady-state torpor, two clearly different responses of physiological variables to a change of T_a were observed at T_a s below and above the critical temperature during torpor for both patterns of torpor. These two responses reflect differences in the determination of metabolic rate in the two T_a ranges. Below the critical temperature, torpid animals regulated their T_b above the set-point for T_b by proportional metabolic thermogenesis. This proportional increase of metabolic rate

with ΔT was similar to that during normothermia, but T_b during torpor was regulated at a substantially lower level, and with less precision. In contrast, above the critical temperature, animals in steady-state torpor did not regulate their T_b s, but allowed T_b and metabolic rate to drop with T_a . Therefore, in this temperature range, metabolic rate of both non-thermoregulating *C. nanus* and *S. macroura* was a function of T_b , and not the ΔT .

It appears that the effects of the lowered T_b on enzymatic reaction rates can well explain the reduction of metabolic rate in *S. macroura* during daily torpor. In contrast, temperature effects alone are not sufficient to explain the extremely low metabolic rate during hibernation in *C. nanus*. Thus the differences of metabolic rate between daily torpor and hibernation in the two species cannot be attributed only to the differences of their T_b s. It appears that during hibernation a substantial metabolic inhibition also contributes to the reduction of metabolic rate. A most important result of this study is that *C. nanus* exhibited hibernation in the thermoneutral zone. This is the first clear evidence for largely temperature-independent metabolic inhibition in endotherms.

During entry into torpor, metabolic rate always declines before T_b falls. This is partially due to the cessation of normothermic thermoregulation. However, the extent of the initial drop of MR was much greater during entry into hibernation than into daily torpor. This difference provides further evidence that metabolic inhibition is an important factor during hibernation in *C. nanus*. In contrast, the contribution of metabolic inhibition to metabolic rate reduction during torpor entry appears to be minor or nonexistent in the daily heterotherm, *S. macroura*, similar to observations during steady-state torpor.

The decline of T_b during torpor entry of both species did not follow a single Newtonian cooling curve. Cooling was affected by the changing heat production. The somewhat larger species, *C. nanus* showed a slower rate of cooling than *S. macroura*. This is contrast with a lower metabolic rate during entry into torpor in *C. nanus* than

in *S. macroura*, but is as expected from their body size and levels of their thermal conductance. Effects of decreasing thermal conductance were apparent at the end of torpor entry, especially at low T_{as} .

However, a low conductance is not a prerequisite for reduction of metabolic rate during torpor, since metabolic rate is apparently not determined directly by thermal conductance in either species. In contrast, it is likely that the low conductance during torpor is largely caused by the low metabolic rate. The better insulation, compared to that observed during normothermia should prevent T_b from reaching the set-point for thermoregulation during torpor.

Also related to levels of conductance, rewarming from torpor is most obviously dependent on T_a as it affects rate of heat loss, which increases with ΔT . However, this temperature effect is partially compensated by the increase of metabolic rate during arousal at low T_{as} . It appears that *S. macroura* reaches its maximum thermogenic capacity during arousal. In contrast, hibernating *C. nanus*, which possess a higher thermogenic capacity than *S. macroura*, make little use of their full thermogenic capacity during spontaneous arousal. Despite of the sub-maximum heat production, the rewarming rate of *C. nanus* was still higher than in *S. macroura*. A high average metabolic rate during arousal is essential to ensure a faster arousal, which is often associated with reduction of total energy expenditure for both the hibernator and the daily heterotherm.

Energy savings by employing torpor in comparison to RMR was the greatest around the critical temperature during torpor for both *S. macroura* and *C. nanus*. However, for the daily heterotherm, the relative energy cost for rewarming increased overall energy expenditure during torpor because of relatively short torpor bouts. This resulted in overall lower energy expenditure during torpor at high T_{as} , although metabolic rate in torpid state was relatively high. Consequently, if given a choice, *S.*

macroura selected high temperatures around the thermoneutral zone for both normothermia and torpor.

In contrast, arousal cost for hibernating *C. nanus* was only a small proportion of the overall energy expenditure during torpor because their torpor bouts were long. Torpor in this species was cheaper near the critical temperature than at high T_{as} . However, *C. nanus* also selected relatively high temperatures for hibernation although these were slightly lower than those selected for normothermia. These observations suggest that although *C. nanus* can hibernate for many days, prolonged normothermic activity periods combined with short torpor bouts, which are influenced both by metabolism and circadian rhythms, are favoured. This illustrates that *C. nanus* employs torpor mainly opportunistically to meet its immediate energy requirements. In this respect, *C. nanus* is similar to most daily heterotherms of both northern and southern hemispheres, but different from strictly seasonal hibernators from the northern hemisphere.

Accordingly, both species maintained energy expensive activities even when food was withheld. This is apparently because a reduced foraging activity would mean a reduced chance to obtain food. The maintenance of activity is especially important for daily heterotherms like *S. macroura*, since they have little stored fat and must feed frequently. Therefore, the enhancement of torpor during the normal resting phase when confronted with unfavourable food conditions allows them to maintain foraging activity. It seems that the employment of torpor can not only decrease overall energy expenditure, it also serves as a mechanism to reallocate precious energy resources.

In general, the present study confirms that both daily torpor and hibernation of small mammals are efficient adaptive strategies to temporarily reduce energy expenditure. However, many physiological variables differ quantitatively between the hibernator and the daily heterotherm. In addition, many interrelations between physiological variables differ between hibernation and daily torpor. This suggests that

different physiological adjustments are involved in the two species to achieve different metabolic rates during torpor. Physiological differences also influence how energetics of the animals are affected by temperature, which in turn, is reflected by their thermal behaviour.