

Chapter Five: Energetics of Hibernation and Daily Torpor

5.1 Introduction

The significance of mammalian torpor in reducing energy expenditure is often evaluated from ratios of TMR to FMR. During a bout of deep hibernation, TMR can account for less than 1% of RMR (Hock 1951; Kayser 1964; Wang and Hudson 1971). Although the reduction of metabolic rate during daily torpor is generally less pronounced, TMR can still be as low as 10% of RMR (Wang and Hudson 1970; Fleming 1980; Thompson 1985). While torpor, especially deep, prolonged torpor, greatly reduces energy expenditure, the relative energy savings of torpor evaluated by this ratio declines as T_a increases (Buffenstein 1985), because RMR decreases and TMR increases with T_a over this T_a range (Hainsworth and Wolf 1970; chapter 3).

Nevertheless, the overall energy turnover during torpor is not only determined by the cost during the torpid state, it is also determined by the cost during arousal, the energetically most expensive phase of a bout of torpor (Wang 1979). A change of T_a may influence energy expenditure of these two energy-determining components differently. For example, during torpor at T_a s above the T_{tc} , an increase of T_a results in an increase of TMR, thus increases the energy cost during torpid state. In contrast, during an arousal at a high T_a a smaller gradient of T_b has to be bridged, thus the energy spent on arousal should be decreased (Hiebert 1990). Because of the opposite effects of a change of T_a on overall energy expenditure, responses of energy consumption during torpor to T_a changes are not always immediately obvious. A factor that makes these responses even more difficult to identify is that many variables in relation to energy consumption respond to T_a changes differently in the T_a ranges below and above the T_{tc} (Hainsworth and Wolf 1970). For example, while torpor bout is inversely related to T_a above the T_{tc} (Twente and Twente 1967; French 1985;

Geiser and Kenagy 1988), it decreases at low T_a s below the T_{tc} (Geiser and Kenagy 1988). In addition, due to the differences in torpor duration, T_{tc} , and TMR between hibernation and daily torpor, the response of energy consumption during torpor to changes of T_a may also differ between the two torpor patterns. Obviously, the ratio of TMR to RMR is not sufficient to reflect all these important energy characteristics during torpor.

Therefore, energetic characteristics during torpor and energy conservation by the use of torpor as a function of T_a can only be assessed in a meaningful way if the total energy expenditure during both torpor phase and arousal phase are included into the calculation of energy expenditure (Wang 1989; Hiebert 1990; Ellison 1993; Webb and Skinner 1996). In addition, different T_a ranges above and below the T_{tc} must be considered. Unfortunately, at present information regarding this aspect is scarce for both mammalian hibernation and daily torpor.

The present chapter attempts to investigate total energy expenditures and energy savings of daily torpor in *S. macroura* and hibernation in *C. nanus* over a wide range of T_a s. It also provides a detailed analysis of the relationships between the cost and the duration of torpor and arousal. To accomplish these, the following questions were addressed for both species:

1. What are the energy costs of torpor and arousal at different T_a s?
2. How does the overall energy expenditure during a bout of torpor change with T_a ?
3. Does torpor result in net energy savings in comparison to the normothermic resting state at different T_a s?
4. Are energy expenditure during a bout of torpor or arousal and the duration of torpor or arousal correlated?
5. What is the minimum torpor duration that results in a saving of energy at different T_a s?

6. How does thermoregulation during torpor affect the length of torpor and arousal, and related energy expenditure?"

5.2 Materials and Methods

5.2.1 Animals

Seven adult male *S. macroura* were used in this study. Their mean BM throughout the experiments was 24.6 ± 3.3 g. Oxygen consumption of the animals over one day were recorded at five T_{as} ranging from 8 to 28°C. Both system A and system B of the respirometry equipment were used and the flowrate through the respirometry chamber was adjusted to 200 mL h^{-1} (chapter 2.2). Data from complete steady-state torpor bouts were used for analysis.

Seven adult *C. nanus*, four males and three females (mean BM 36.3 ± 6.2 g) were used. The measuring routine for *C. nanus* was similar to that for *S. macroura*, but only system B was used. Records were taken at seven T_{as} ranging from 1 to 30°C. Each measurement included at least one complete torpor bout and lasted between 1 - 18 days, depending on T_a .

Photoperiod was set to 12L : 12D (lights on 0600 - 1800 hours) for both species. Food and water were not available during measurements.

5.2.2 Data Analysis

The entire torpor bout duration (TBD) was determined as the interval between the time when $\dot{V}O_2$ fell below 75% RMR during torpor entry and when $\dot{V}O_2$ returned to RMR after a MR overshoot during arousal (see also chapter 2.4.3).

Duration of torpor (D_t) was determined as the period from $\dot{V}O_2$ falling below 75% of RMR during torpor entry to the start of arousal that was shown by a continuous increase of $\dot{V}O_2$ (illustrated in Fig. 5.1 for *S. macroura* and in Fig. 5.10 for *C. nanus*).

Duration of arousal (D_a) was determined as the period from the start of the continuous increase of $\dot{V}O_2$ to the time when $\dot{V}O_2$ reached RMR after a MR overshoot (illustrated in Fig. 5.1 for *S. macroura* and in Fig. 5.10 for *C. nanus*). In *C. nanus*, arousal occasionally was completed during the dark phase, and thus the MR overshoot during arousal could not be clearly separated from the following high activity values. In these cases, D_a was considered to be terminated when MR reached the lowest value after MR overshoot, while T_b had reached 33°C.

The total and the time-specific energy expenditure during these torpor phases were calculated by summing or integrating the MR over these periods (Fig. 5.1; Fig. 5.12). Thus both the total costs and the average MRs during torpor and arousal were obtained. The relative energy savings during a torpor bout, including torpor and arousal, were determined as the ratio of the average MR during the entire torpor bout (ATBDMR) to RMR (i.e. ATBDMR / RMR).

The torpor duration threshold (D_t') for energy saving, defined by the average MR during the entire torpor bout being equal to RMR, was calculated as follows:

$$AD_tMR \times D_t' + AD_aMR \times D_a = RMR \times (D_t' + D_a).$$

Thus: $D_t' = D_a \times (AD_aMR - RMR) / (RMR - AD_tMR)$.

(where D_t' is torpor duration threshold, D_a is arousal duration, AD_tMR is average MR during torpor, and AD_aMR is average MR during arousal.).

Data collected for thermoregulating and non-thermoregulating animals below and above the T_{tc} were presented separately to investigate possible differences in the two T_a ranges.

5.3 Results for *Sminthopsis macroura*

5.3.1 Energetics of Daily Torpor as a Function of T_a

The energy consumption of torpid *S. macroura* was substantially lower than the RMR (Fig. 5.1). The average MR during torpor (i.e. during D_t) of non-thermoregulating

animals above the T_{tc} increased with T_a and was best described by an exponential fit ($r^2 = 0.16$, $p < 0.05$, $n = 22$; Fig. 5.2). Below the T_{tc} , the average MR during torpor of thermoregulating animals increased linearly with decreasing T_a ($r^2 = 0.76$, $p < 0.001$, $n = 11$; Fig. 5.2).

Sminthopsis macroura

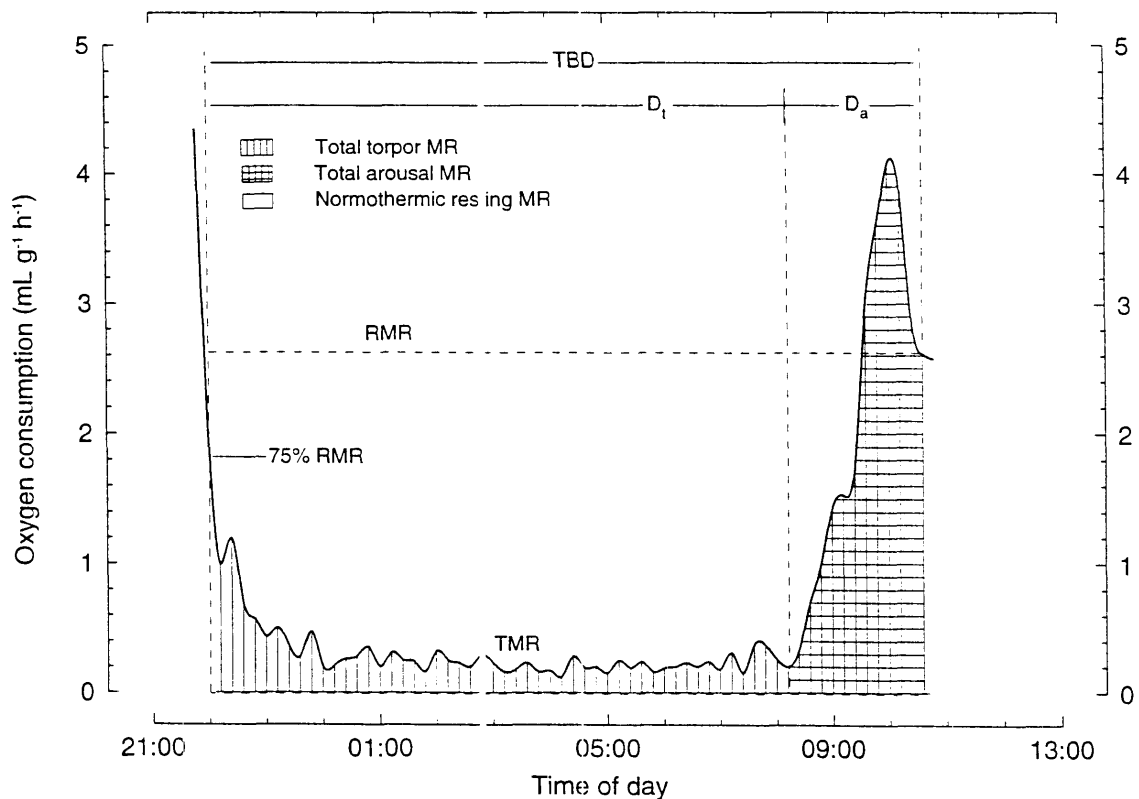


Fig. 5.1. An example for a bout of daily torpor of *Sminthopsis macroura* to illustrate how the calculations of torpor duration (D_t), arousal duration (D_a), total torpor MR, total arousal MR, and the equivalent cost during the same period of resting normothermia were performed.

During arousal, oxygen consumption increased rapidly and a MR overshoot above RMR was observed at all T_a s measured (Fig. 5.1). The average MR during arousal (i.e. during D_a) at T_a s above the T_{tc} was inversely correlated with T_a (Fig. 5.3). This

relationship was better described by an exponential ($r^2 = 0.33$, $p < 0.01$, $n = 22$), than a linear regression ($r^2 = 0.30$, $p < 0.01$, $n = 22$). Below the T_{tc} , the average MR during arousal was higher than above the T_{tc} , but the change of this variable with T_a was not significant ($r^2 = 0.08$, $p > 0.05$, $n = 11$; Fig. 5.3). Nevertheless, the changes of arousal cost in the two T_a range were consistent, thus the whole data set could be fitted with a single exponential equation ($r^2 = 0.69$, $p < 0.001$, $n = 33$; Fig. 5.3).

The average energy expenditure during the entire torpor bout (i.e. during TBD) decreased with increasing T_a (Fig. 5.4). Above the T_{tc} , the change of this cost with T_a ($r^2 = 0.27$, $p < 0.01$, $n = 22$; Fig. 5.4) was less pronounced than that below the T_{tc} ($r^2 = 0.68$, $p < 0.001$, $n = 11$; Fig. 5.4). This is apparently because above the T_{tc} , the decreased arousal costs with increasing T_a obliterated the temperature-induced increase of TMR, and therefore the average MR during the entire torpor bout decreased slightly. In contrast, below the T_{tc} , an increase of temperature decreased energy requirements for both torpor and arousal, thus the average MR during the entire torpor bout decreased substantially.

Although during a bout of daily torpor at T_a s above the T_{tc} , the average energy expenditure was lower at high than at low T_a s, this picture was reversed when related to RMR (Fig. 5.5). The ratio of average MR during the entire torpor bout to RMR was the smallest of about 30% at T_a s that was slightly above the T_{tc} . This ratio increased exponentially with T_a ($r^2 = 0.65$, $p < 0.001$, $n = 22$), showing that relative energy savings declining with the increase of T_a . At T_a s close to the T_{tc} , the energy savings during daily torpor were as little as about 15% of RMR. In contrast, below the T_{tc} , the ratio of the average torpor bout MR to RMR was inversely related to T_a ($r^2 = 0.29$, $p < 0.05$; Fig. 5.5), presumably because with decreasing T_a , torpor costs increased more than normothermic resting costs. Nevertheless, the average MR during the entire torpor bout was always smaller than RMR at every T_a , demonstrating that steady-state daily torpor plays an important role in energy conservation (Fig. 5.5).

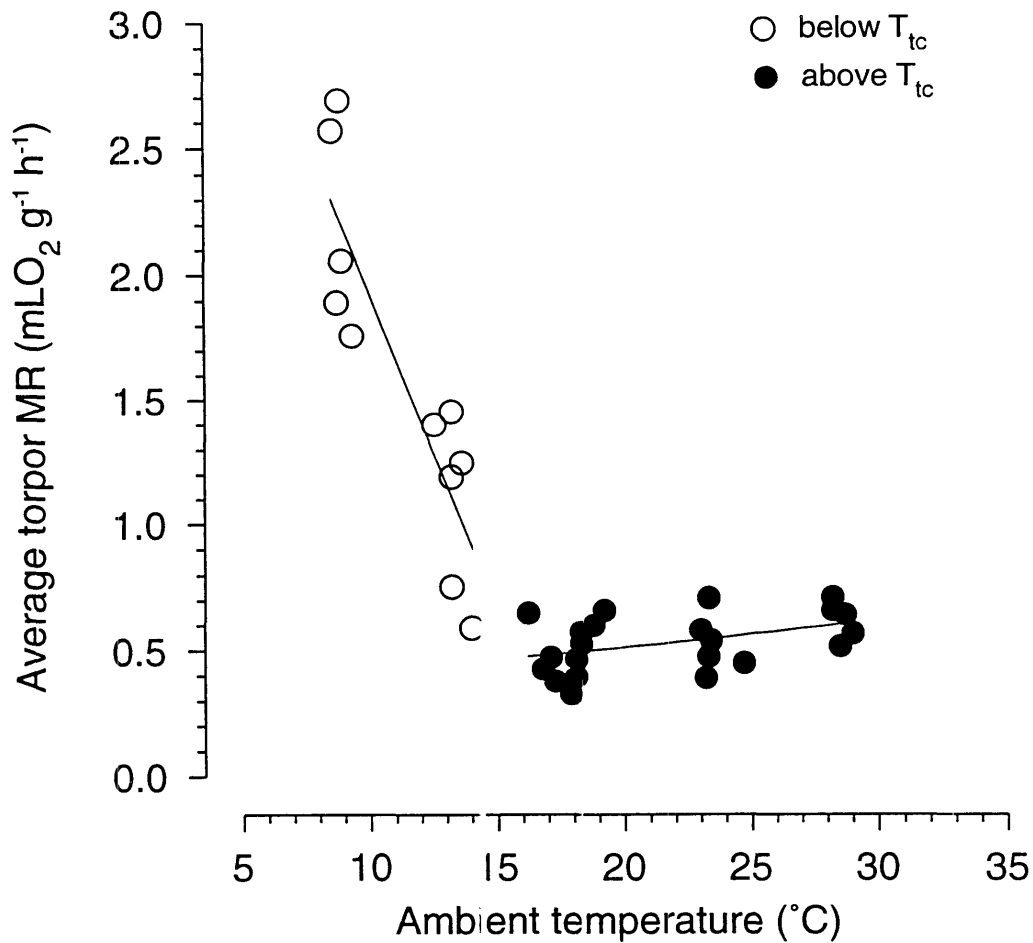
Sminthopsis macroura

Fig. 5.2. The average torpor MR (AD_tMR) of *Sminthopsis macroura* as a function of ambient temperature (T_a). At T_as above the T_{tc}, the AD_tMR was positively related to T_a (filled circles, N = 7, n = 22):
 $AD_tMR = 0.306 + 0.0103 \times T_a$ ($r^2 = 0.15$, $p < 0.05$) for linear fit.
 $AD_tMR = 10^{-0.477 + 0.009 \times T_a}$ ($r^2 = 0.16$, $p < 0.05$, solid line) for exponential fit.

The exponential fit was provided because it was slightly better than the linear fit, and the relationships between TMR and T_a was better described by an exponential fit (see chapter 3).

At T_as below the T_{tc}, the AD_tMR was inversely related to T_a (open circles, N = 6, n = 11):

$$AD_tMR = 4.46 - 0.254 \times T_a \quad (r^2 = 0.76, p < 0.001, \text{solid line}).$$

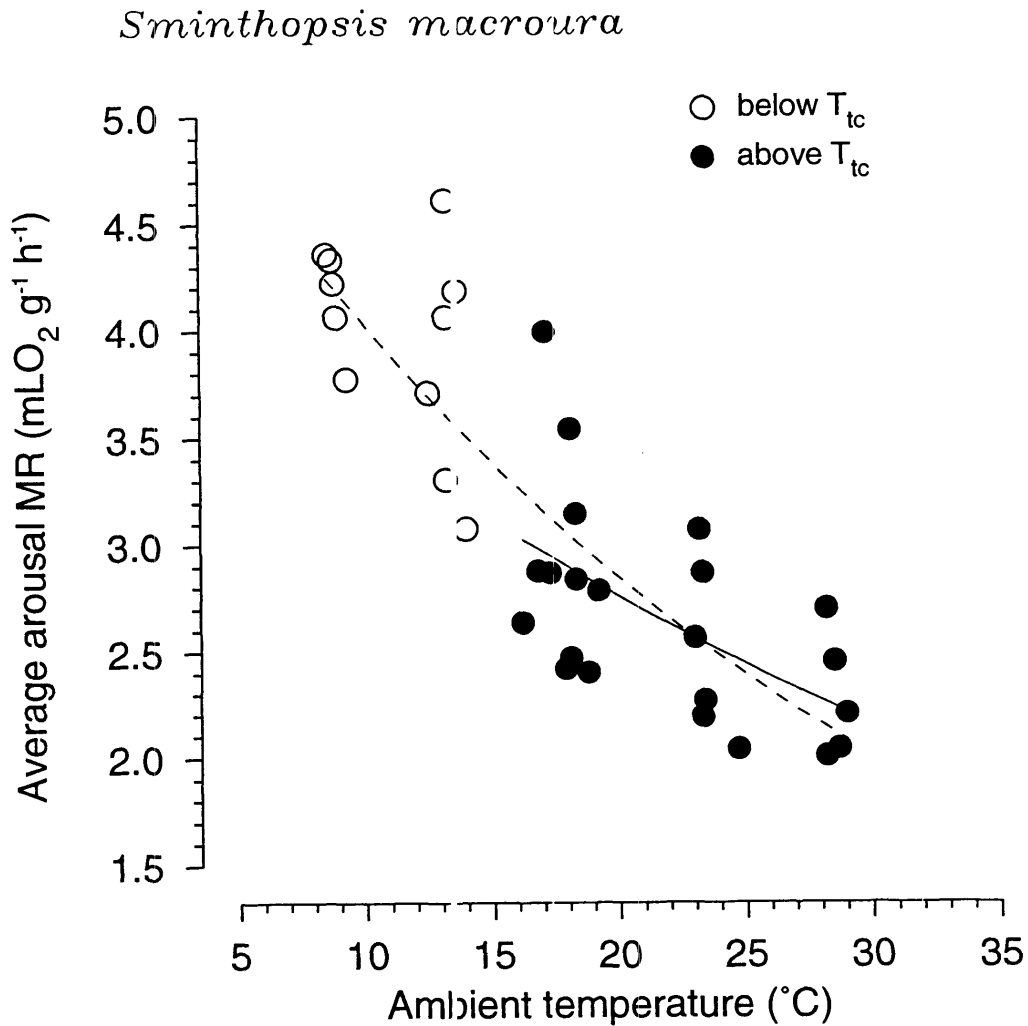


Fig. 5.3. The average arousal MR (AD_aMR) of *Sminthopsis macroura* as a function of ambient temperature (T_a). At T_a s above the T_{tc} , the AD_aMR was inversely related to T_a (filled circles, $N = 7$, $n = 22$):

$$AD_aMR = 4.03 - 0.063 \times T_a \quad (r^2 = 0.30, p < 0.01) \text{ for linear fit.}$$

$$AD_aMR = 10 (0.641 - 0.0103 \times T_a) \quad (r^2 = 0.33, p < 0.01, \text{ solid line}) \text{ for exponential fit.}$$

At T_a s below the T_{tc} , the AD_aMR was not correlated with T_a ($r^2 = 0.08$, $p > 0.05$, open circles, $N = 6$, $n = 11$).

The exponential equation for AD_aMR vs T_a for all animals both above and below the T_{tc} was

$$AD_aMR = 10 (0.742 - 0.0145 \times T_a) \quad (r^2 = 0.69, p < 0.001, \text{ dashed line}).$$

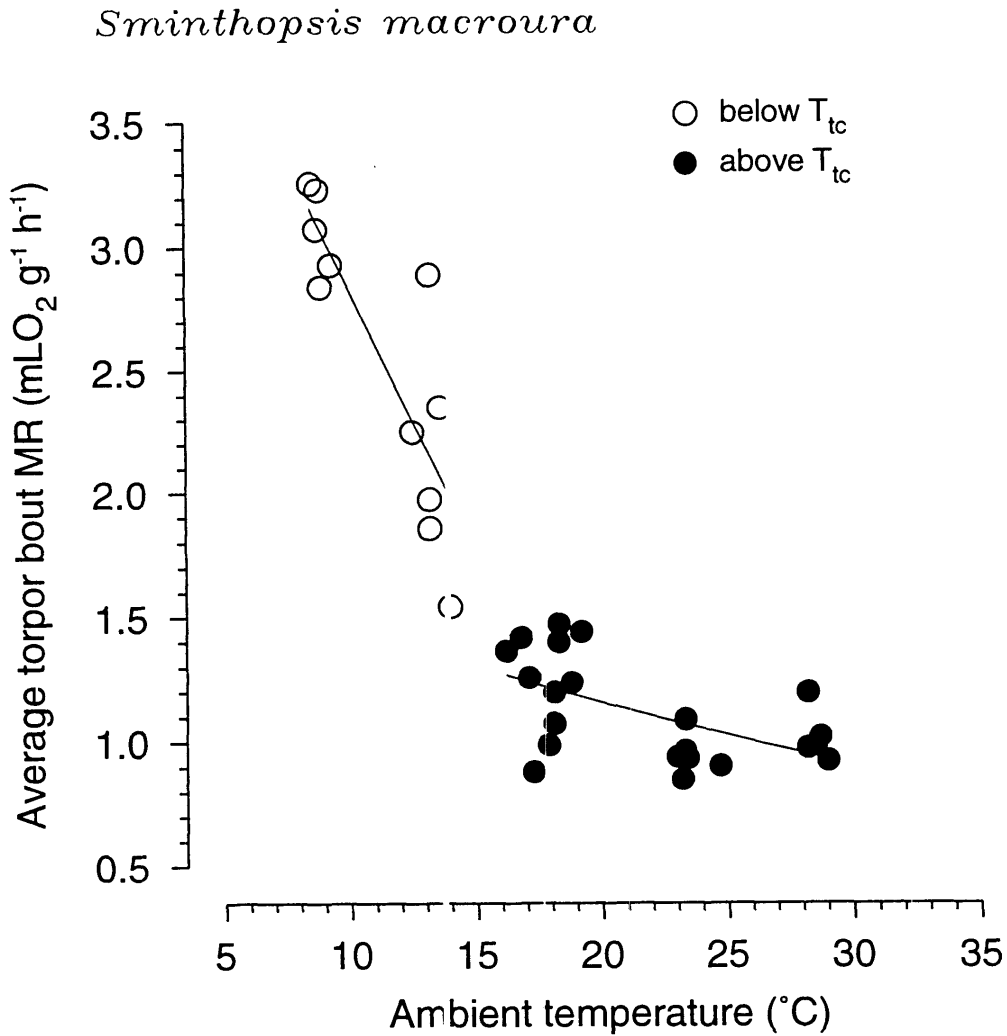


Fig. 5.4. The average torpor bout MR (ATBDMR) of *Sminthopsis macroura* as a function of ambient temperature (T_a). Above the T_{tc} , the ATBDMR was inversely related to T_a (filled circles, $N = 7$, $n = 22$):

$$\text{ATBDMR} = 1.66 - 0.025 \times T_a \quad (r^2 = 0.27, p < 0.01) \text{ for linear fit.}$$

$$\text{ATBDMR} = 10 (0.243 - 0.009 T_a) \times T_a \quad (r^2 = 0.26, p < 0.01, \text{ solid line}) \text{ for exponential fit.}$$

Below the T_{tc} , the ATBDMR was also inversely correlated with T_a (open circles, $N = 6$, $n = 11$):

$$\text{ATBDMR} = 4.96 - 0.213 \times T_a \quad (r^2 = 0.69, p < 0.001).$$

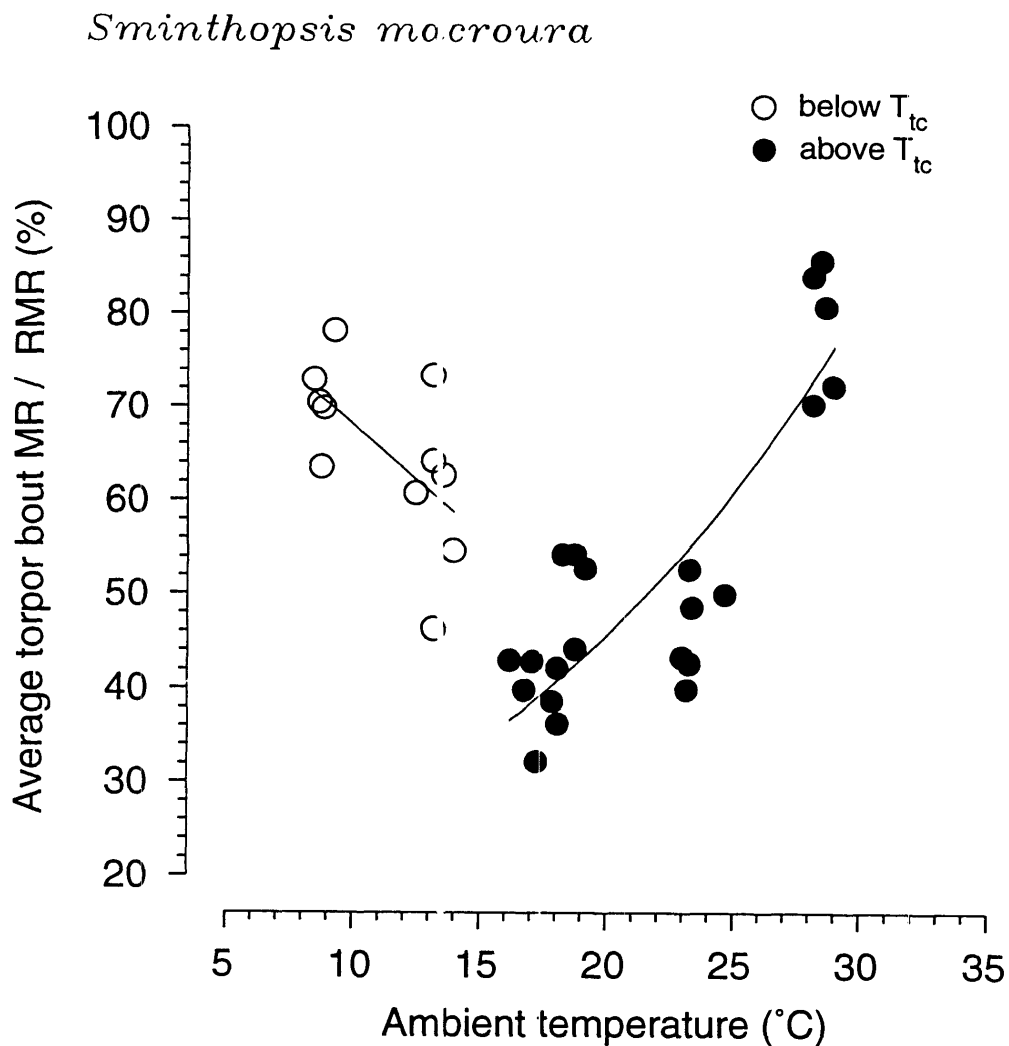


Fig. 5.5. Ratio of the average torpor bout MR (ATBDMR) to the resting metabolic rate (RMR) of *Sminthopsis macroura* as a function of ambient temperature (T_a). Above the T_{tc} the ATBDMR / RMR increased exponentially with T_a (filled circles, $N = 7$, $n = 22$):

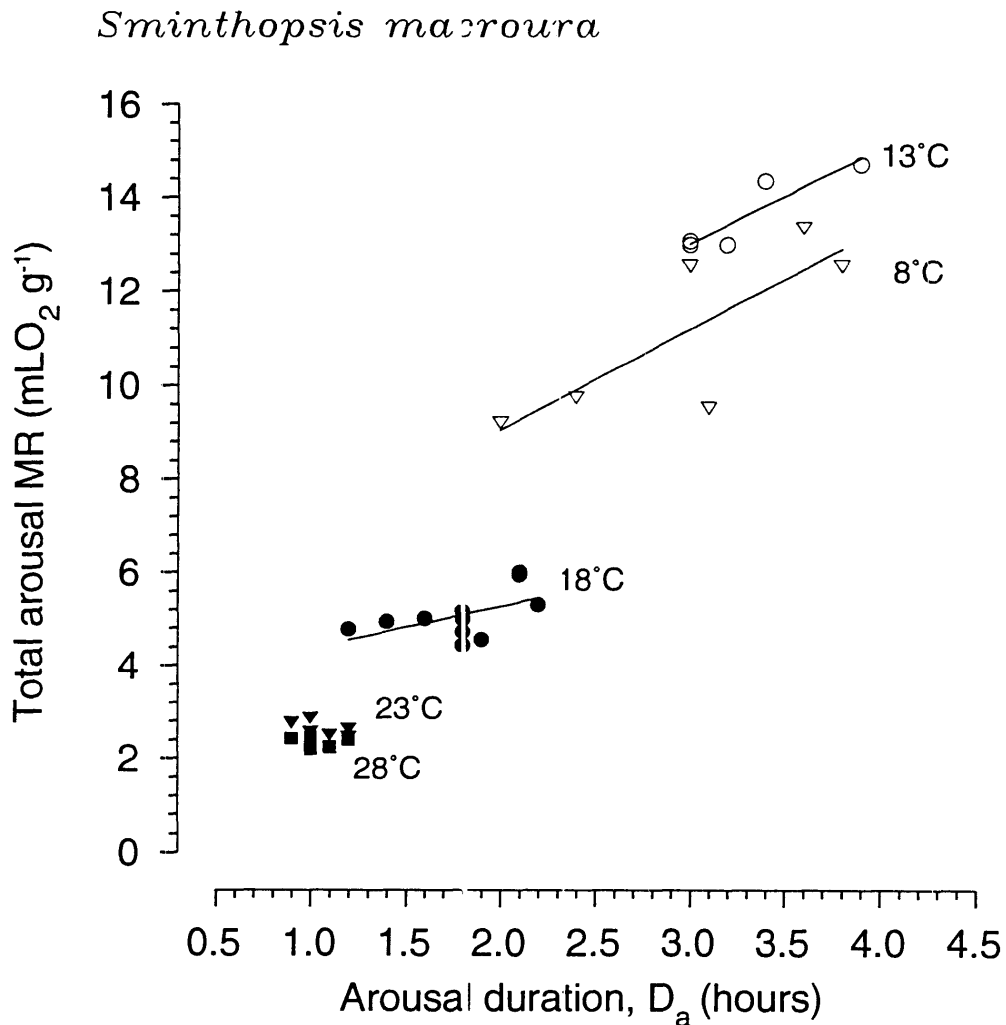
$$\text{ATBDMR} / \text{RMR} = 10 (-0.778 + 0.0219 \times T_a) \quad (r^2 = 0.65, p < 0.001).$$

Below the T_{tc} , the ATBDMR / RMR was inversely related to T_a (open circles, $N = 6$, $n = 11$):

$$\text{ATBDMR} / \text{RMR} = 0.916 - 0.0235 \times T_a \quad (r^2 = 0.29, p < 0.05).$$

5.3.2 Energetics in Relation to Duration of Arousal

Since both the average MR during arousal and arousal duration were affected by T_a (chapter 5.3.1, chapter 4), the relationship between energy consumption during arousal and the arousal duration (chapter 4) was analysed for each T_a (Fig. 5.6). The total



arousal duration (D_a) of *Sminthopsis macroura* at different ambient temperatures. The regression equations were:

$$D_a\text{MR} = -2.15 + 4.41 \times D_a \quad (r^2 = 0.91, p < 0.001, n = 33), \text{ for all data.}$$

$$D_a\text{MR} = 6.89 + 2.04 \times D_a \quad (r^2 = 0.78, p < 0.05, n = 5), \text{ at } 8^\circ\text{C}.$$

$$D_a\text{MR} = 4.80 + 2.13 \times D_a \quad (r^2 = 0.63, p < 0.05, n = 6), \text{ at } 13^\circ\text{C}.$$

$$D_a\text{MR} = 3.47 + 0.91 \times D_a \quad (r^2 = 0.29, p < 0.05, n = 11), \text{ at } 18^\circ\text{C}.$$

arousal MR increased with D_a (8°C: $r^2 = 0.78$, $p < 0.05$, $n = 5$; 13°C: $r^2 = 0.63$, $p < 0.05$, $n = 6$; 18°C: $r^2 = 0.29$, $p < 0.05$, $n = 11$; Fig. 5.6) except for T_{as} above 20°C. This suggests that at low T_{as} , shorter arousals were energetically favourable, although a higher rate of heat production had to be applied as illustrated by the inverse relationship between the time specific energy consumption and arousal duration (8°C: $r^2 = 0.77$, $p < 0.05$, $n = 5$; 18°C: $r^2 = 0.66$, $p < 0.01$, $n = 11$; 23°C: $r^2 = 0.88$, $p < 0.01$, $n = 6$; 28°C: $r^2 = 0.76$, $p < 0.05$, $n = 5$; not shown).

5.3.3 Energetics in Relation to Duration of Torpor

S. macroura showed remarkable variation in torpor duration (D_t), especially at low T_{as} (Fig. 5.7). Therefore D_t was not significantly related to T_a at T_{as} either below or above the T_{tc} ($r^2 = 0.09$, $p > 0.05$; Fig. 5.7), although torpor bouts longer than 8 h were only observed at T_{as} slightly above the T_{tc} . If only 2 longest torpor bouts observed at each T_a were considered, torpor durations decreased with increasing T_a above the T_{tc} ($p < 0.05$), but were shorter at T_{as} lower than the T_{tc} .

Although in non-thermoregulating animals above the T_{tc} , the relationship between torpor duration and TMR was significant ($r^2 = 0.22$, $p < 0.05$, $n = 22$; not shown), the average MR during torpor was a much better predictor for torpor length than the TMR ($r^2 = 0.51$, $p < 0.001$, $n = 22$; Fig. 5.8). In thermoregulating animals below the T_{tc} , neither TMR nor the average torpor MR was correlated with D_t ($r^2 = 0.09$, $p > 0.1$, $r^2 = 0.000$, $p > 0.5$; Fig. 5.8).

The torpor duration threshold (D_t'), below which torpor would not result in a reduction of energy expenditure, was temperature dependent. At T_{as} around the T_{tc} , D_t' was as short as 0.5 h, but it increased at both lower and higher T_{as} (Fig. 5.9). The D_t' s at T_{as} close to the T_{tc} were always longer than 1 h, suggesting that at high T_{as} very short term torpor might not be effective for saving energy. However, the D_t' at

every T_a was always below the actual D_t for steady-state torpor in both thermoregulating and non-thermoregulating animals (Fig. 5.7; Fig. 5.9).

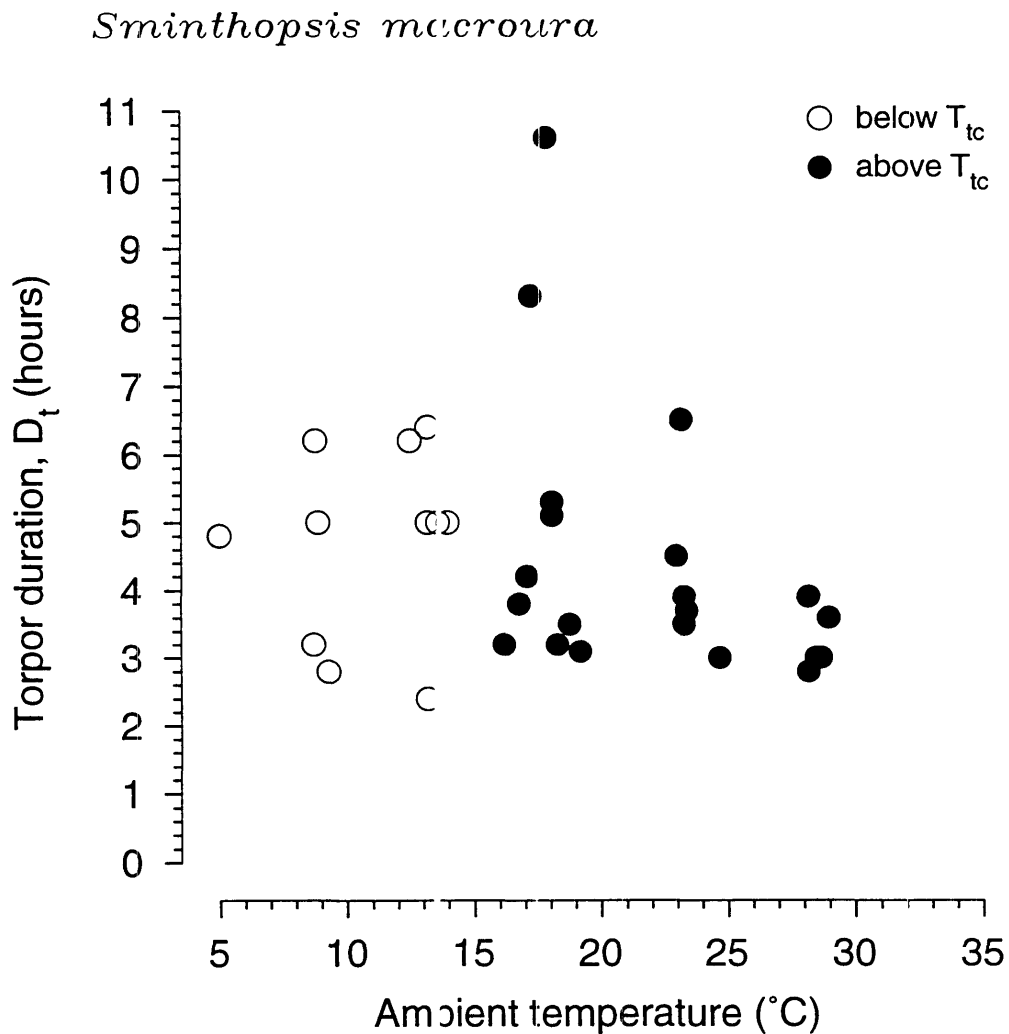


Fig. 5.7. Torpor duration (D_t) in *Sminthopsis macroura* as a function of ambient temperature (T_a). D_t was not correlated with T_a either above the T_{tc} (filled circles $r^2 = 0.09$ $p > 0.05$, $N = 7$, $n = 22$), or below the T_{tc} (open circles, $r^2 = 0.03$, $p > 0.5$, $N = 6$, $n = 11$).

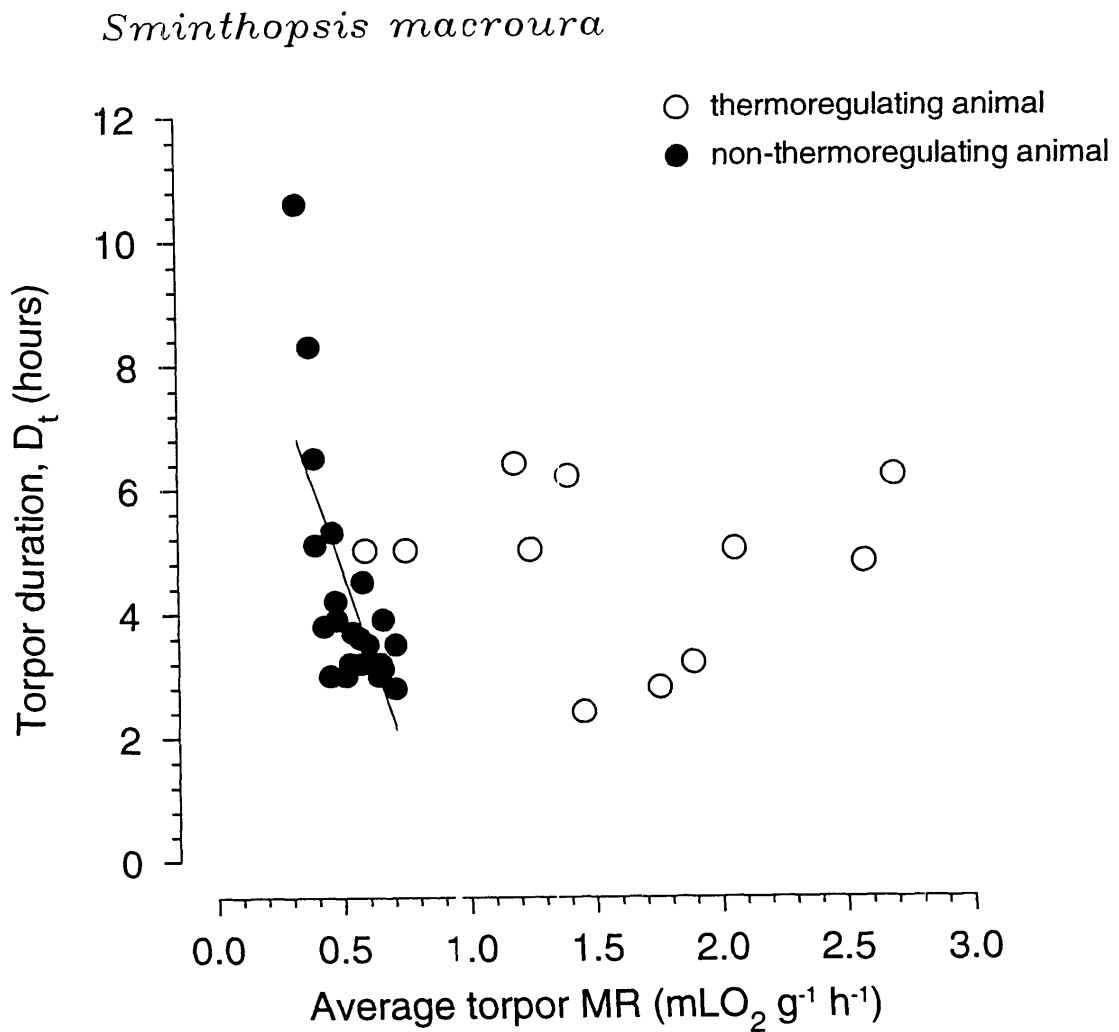


Fig. 5.8. Interrelations between torpor duration (D_t) and the average torpor MR (AD_tMR) of *Sminthopsis macroura*. Above the T_{tc} , D_t was inversely related to AD_tMR (filled circles, $N = 7$, $n = 22$):

$$D_t = 10.90 - 12.3 \times AD_tMR \quad (r^2 = 0.51, p < 0.001).$$

Below the T_{tc} , the D_t was not a function of the AD_tMR ($r^2 = 0.001$, $p > 0.5$, open circles, $N = 6$, $n = 11$).

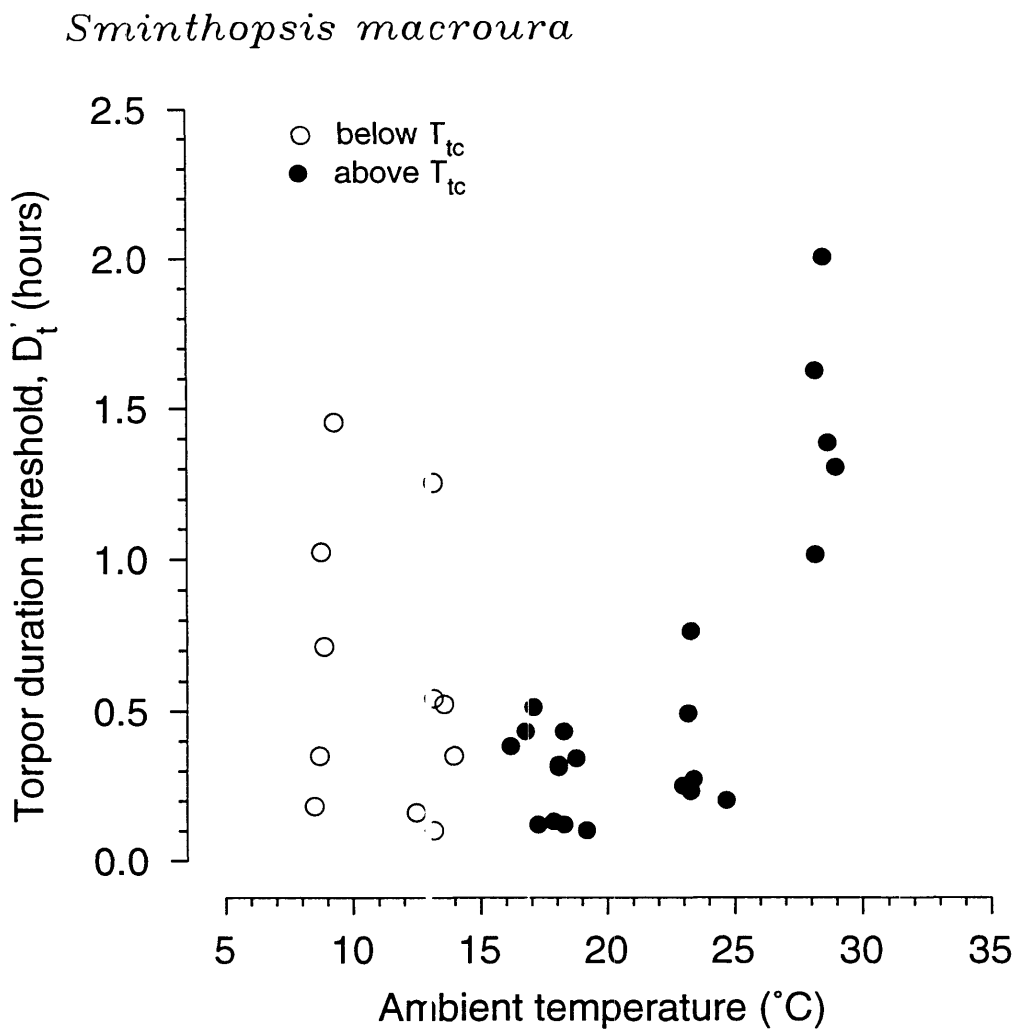


Fig. 5.9. The threshold of torpor duration (D_t') of *Sminthopsis macroura* as a function of ambient temperature for animals above the T_{tc} (filled circles) and below the T_{tc} (open circles). The D_t' was calculated with the assumption that the average MR during the entire torpor bout (ATBDMR) was equal to RMR, and the D_t' was calculated as:

$$D_t' = D_a \times (AD_a MR - RMR) / (RMR - AD_t MR)$$

(where D_a is arousal duration, $AD_t MR$ is the average MR during torpor, and $AD_a MR$ is the average MR during arousal). The D_t' increased at T_{as} both below and above the T_{tc} during torpor, but it was shorter than 2 h at all T_{as} .

5.4 Results for *Cercartetus nanus*

5.4.1 Energetics of Hibernation as a Function of T_a

During steady-state torpor which usually lasted from half a day to many days, the cost of hibernating *C. nanus* was always substantially lower than RMR (Fig. 5.10). Above

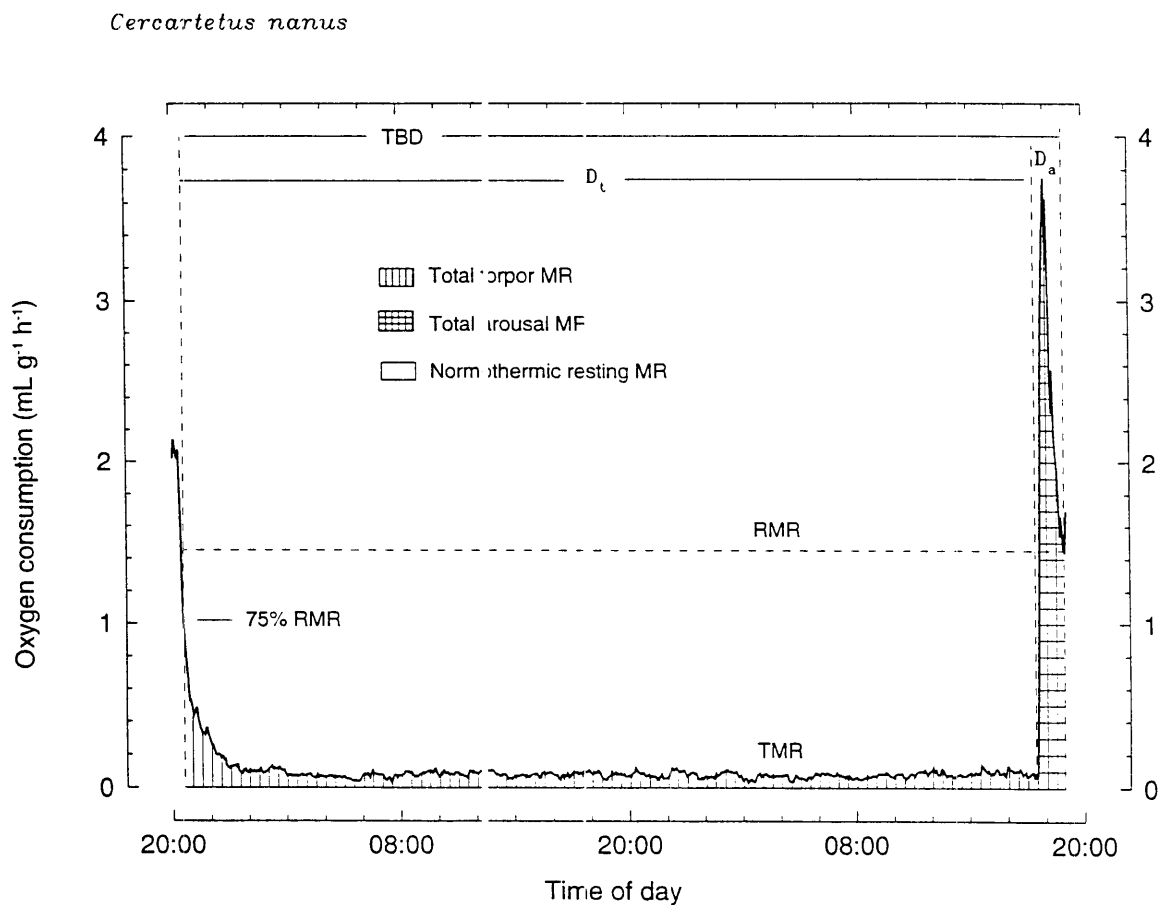


Fig. 5.10. An example for a bout of hibernation of *Cercartetus nanus* to illustrate how the calculations of torpor duration (D_t), arousal duration (D_a), total torpor MR, total arousal MR, and the equivalent cost during the same period of resting normothermia were performed.

the T_{tc} , the average torpor MR (determined over D_t) of non-thermoregulating animals increased exponentially with T_a ($r^2 = 0.82$, $p < 0.001$, $n = 34$; Fig. 5.11). Below the T_{tc} , when animals started to thermoregulate, the average MR during torpor was significantly higher, approaching values that were slightly lower than the maxima in non-thermoregulating individuals at high T_a s (Fig. 5.11).

During arousal MR increased and eventually exceeded RMR (MR overshoot) for a short period of time (Fig. 5.10). The average MR during arousal (determined over D_a) decreased significantly with an increasing T_a , and was similar for animals above and below the T_{tc} ($r^2 = 0.86$, $p < 0.001$, $n = 34$ for animals above the T_{tc} ; $r^2 = 0.86$, $p < 0.001$, $n = 37$ for all animals; Fig. 5.12).

Above the T_{tc} , average MR during the entire torpor bout (determined over TBD) increased exponentially with T_a ($r^2 = 0.64$, $p < 0.001$, $n = 34$; Fig. 5.13). This was because the TMR reduction with decreasing T_a during prolonged torpor had the strongest influence upon the average torpor bout MR, and the high arousal cost at low T_a s was overridden. In contrast, below the T_{tc} , the average energy expenditure during a torpor bout increased, because both the arousal MR and torpor MR increased with decreasing T_a (Fig. 5.13).

When related to RMR, the average torpor bout MR at any T_a was always below 70% RMR measured at T_a s close to the T_{tc} , and the relative energy savings increased exponentially with decreasing T_a at T_a s above the T_{tc} ($r^2 = 0.87$, $p < 0.001$, $n = 34$; Fig. 5.14). Energy savings were the highest from the T_{tc} to T_a 10°C with the average torpor bout MR being only 2% to 3% of RMR (Fig. 5.14).

Even in thermoregulating animals below the T_{tc} , the average MR during the entire torpor bout was only between 20% - 40% of RMR (Fig. 5.14), demonstrating that energy expenditure during torpor was always significantly lower than if the animal would have remained normothermic for the same period of time at the same T_a .

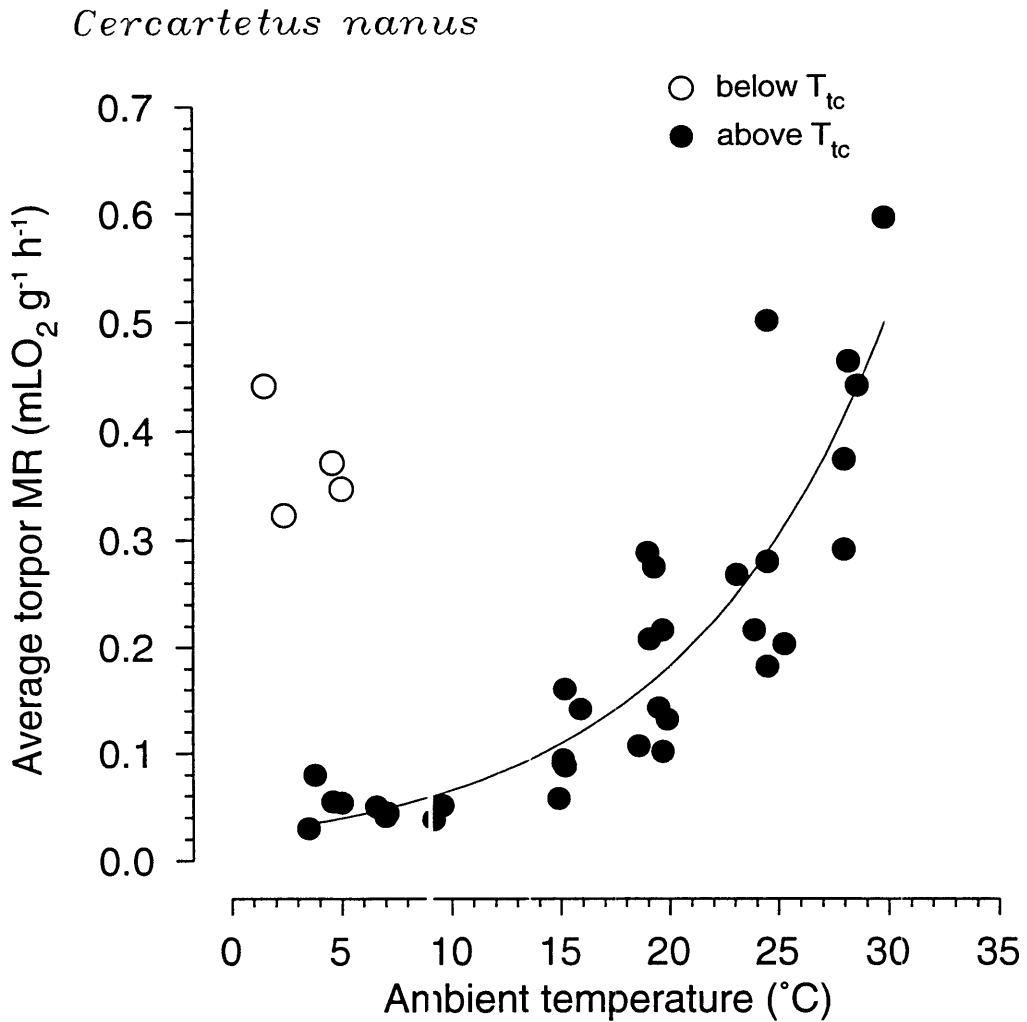


Fig. 5.11. The average torpor MR (AD_tMR) of *Cercartetus nanus* as a function of ambient temperature (T_a). Above the T_{tc} , the AD_tMR was positively related to T_a (filled circles. $N = 7$, $n = 34$):

$$\text{AD}_t\text{MR} = -0.078 + 0.015 \times T_{a1} \quad (r^2 = 0.67, p < 0.001) \text{ for linear fit.}$$

$$\text{AD}_t\text{MR} = 10 (-1.58 + 0.04 \times T_a) \quad (r^2 = 0.82, p < 0.001, \text{ solid line}) \text{ for exponential fit.}$$

Open circles show values below the T_{tc} .

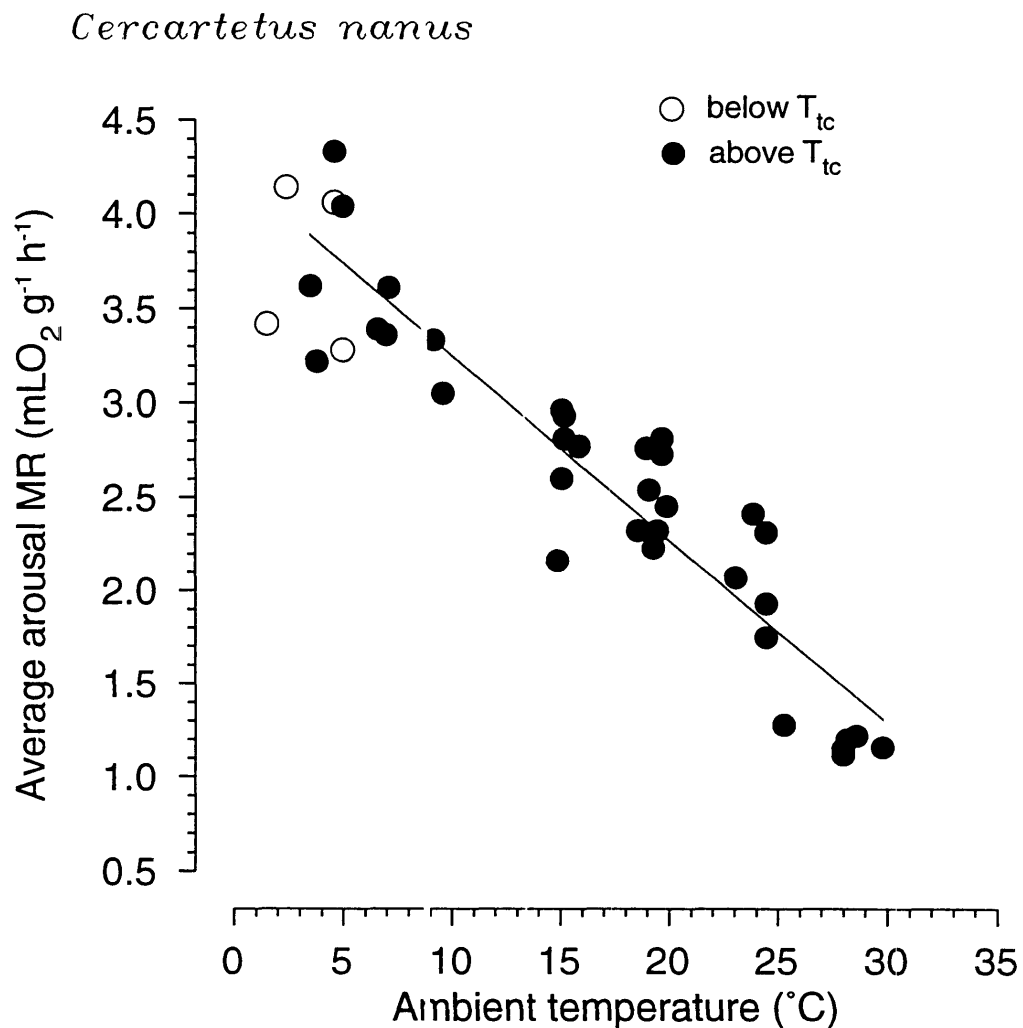


Fig. 5.12. The average arousal MR (AD_aMR) of *Cercartetus nanus* as a function of ambient temperature (T_a). Open circles show values below the T_{tc} .

Above the T_{tc} , the AD_aMR was inversely related to T_a (filled circles, $N = 7$, $n = 34$):

$$AD_aMR = 4.23 - 0.098 \times T_a \quad (r^2 = 0.86, p < 0.001, \text{ solid line}).$$

The regression equation for the animals both above and below the T_{tc} was:

$$AD_aMR = 4.16 - 0.095 \times T_a \quad (r^2 = 0.86, p < 0.001, n = 38).$$

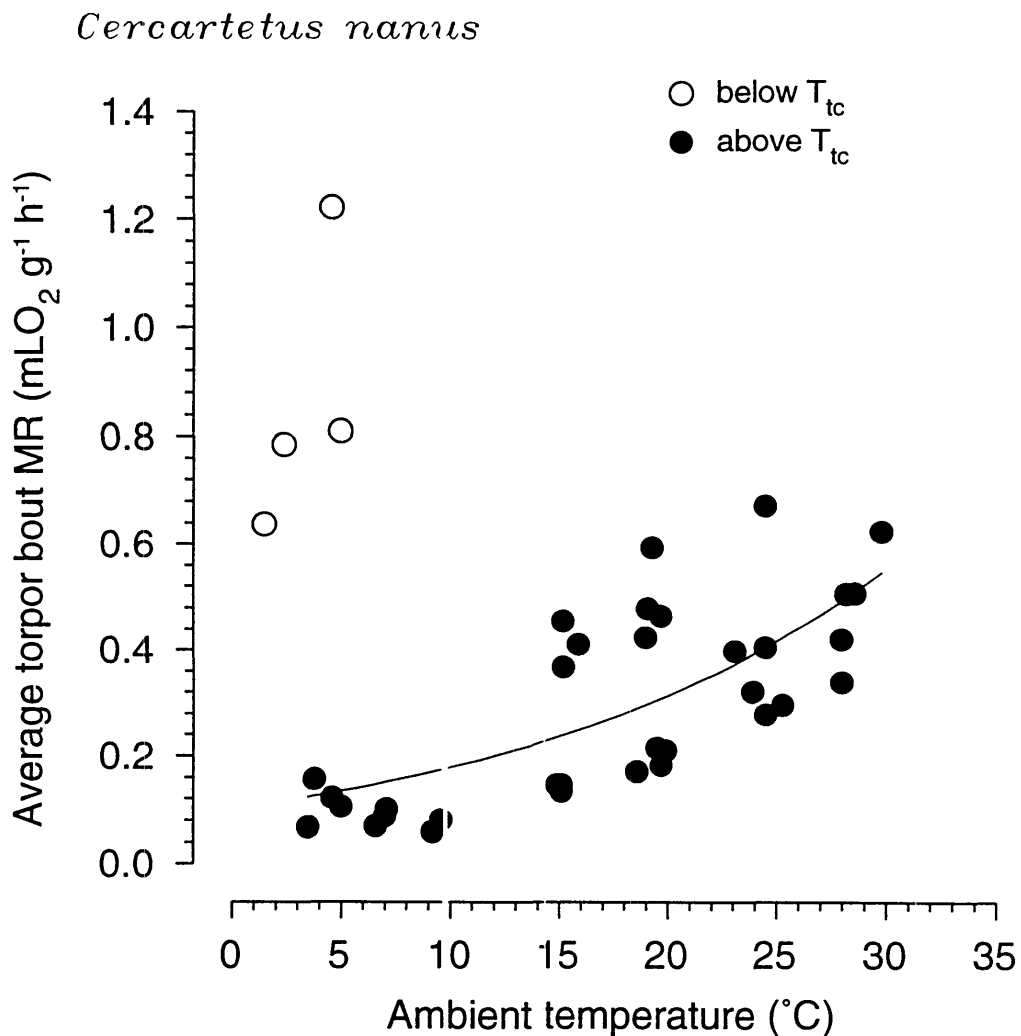


Fig. 5.13. The average torpor bout MR (ATBDMR) of *Cercartetus nanus* as a function of ambient temperature (T_a). The ATBDMR at T_a s above the T_{tc} was positively related to T_a (filled circles, $N = 7$, $n = 34$):

$$\text{ATBDMR} = 0.004 + 0.017 \times T_a \quad (r^2 = 0.54, p < 0.001) \text{ for linear fit.}$$

$$\text{ATBDMR} = 10^{-1.18 - 0.032 \times T_a} \quad (r^2 = 0.64, p < 0.001, \text{ solid line}) \text{ for exponential fit.}$$

Open circles show values below the T_{tc} .

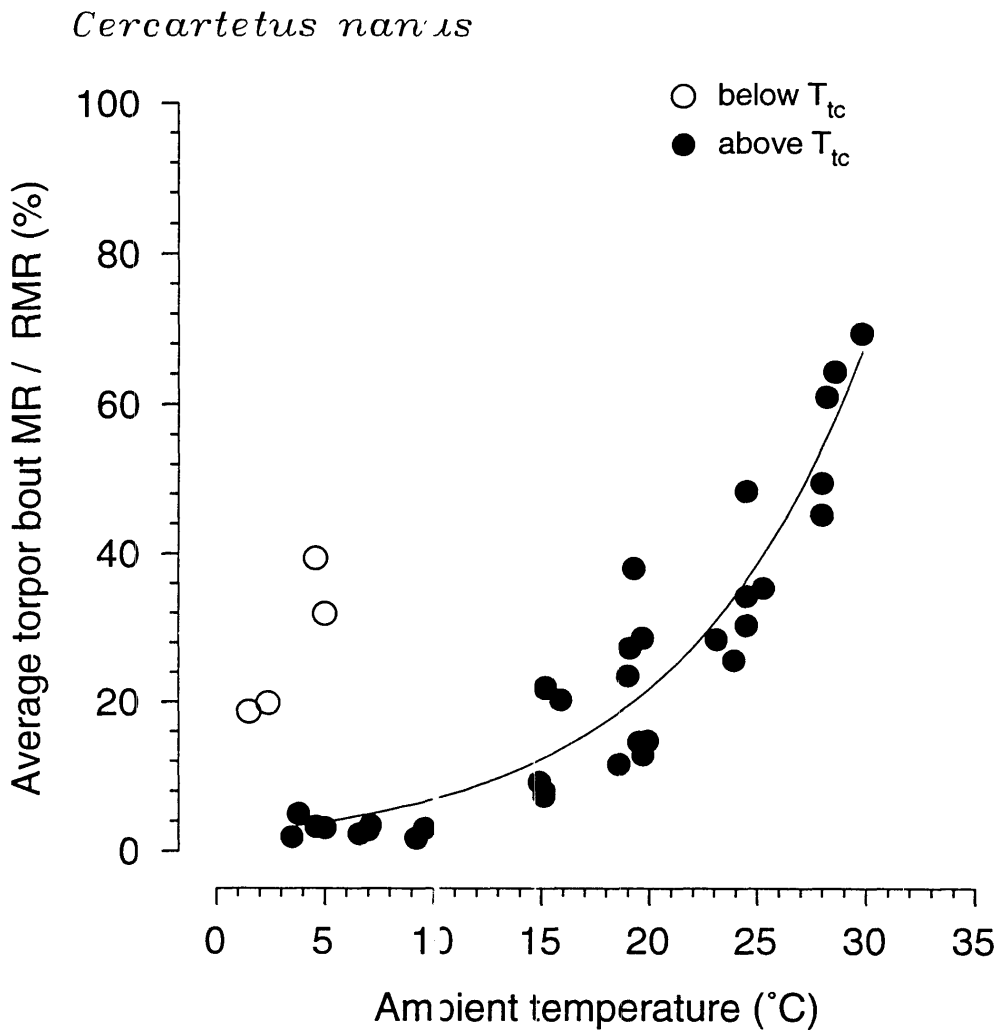


Fig. 5.14. Ratio of average torpor bout MR (ATBDMR) to resting metabolic rate (RMR) *Cercartetus nanus* as a function of ambient temperature (T_a). At T_a s above the T_{tc} ATBDMR / RMR increased exponentially with T_a (filled circles, $N = 7$, $n = 34$):

$$\text{ATBDMR} / \text{RMR} = 10^{-1.83 + 0.057 \times T_a} \quad (r^2 = 0.87, p < 0.001).$$

Open circles show values below the T_{tc} .

5.4.2 Energetics in Relation to Duration of Arousal

Since both arousal duration and the average MR during arousal of *C. nanus* were influenced by T_a (chapter 4; chapter 5.4.1), the relationship between energy expenditure during arousal and D_a for each T_a was examined separately (Fig. 5.15).

Cercartetus nanus

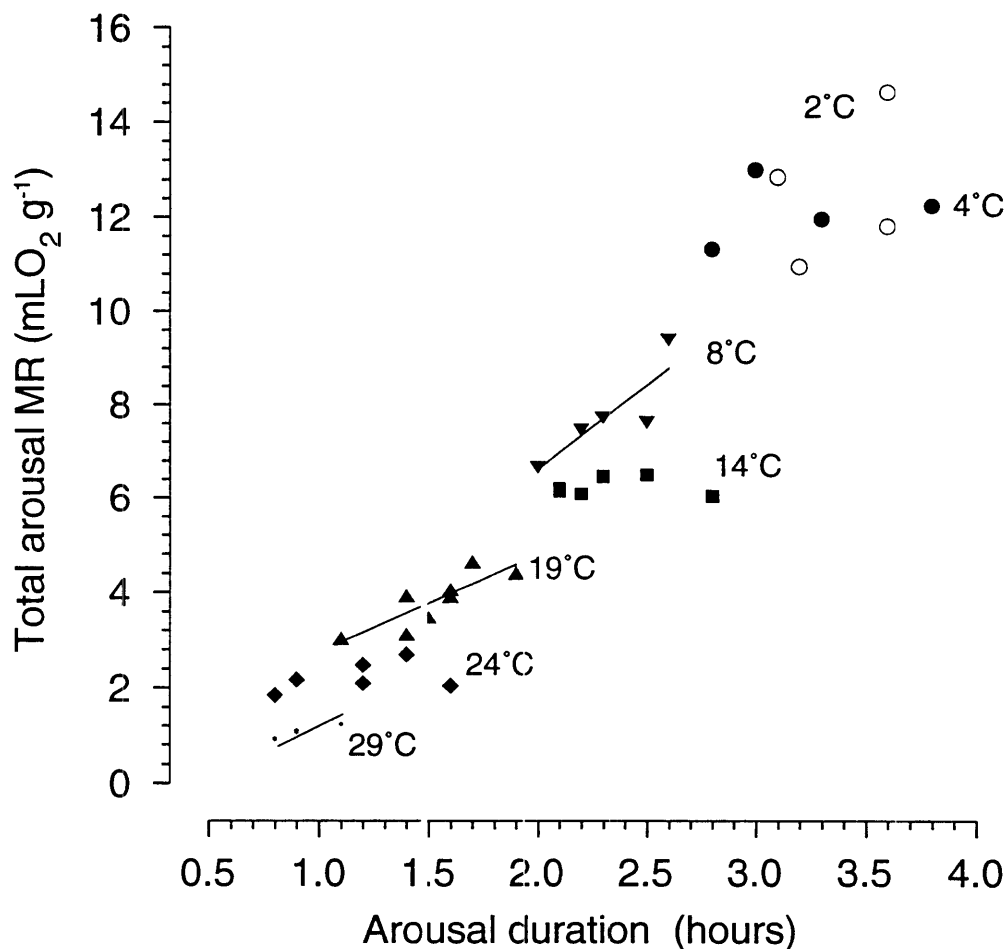


Fig. 5.15. Interrelations between total arousal MR (D_a MR) and arousal duration (D_a) of *Cercartetus nanus* at different ambient temperatures. The regression equations were:

$$D_a\text{MR} = -3.60 + 4.76 \times D_a \quad (r^2 = 0.91, p < 0.001, n = 38), \text{ for all data.}$$

$$D_a\text{MR} = -0.57 + 3.60 \times D_a \quad (r^2 = 0.74, p < 0.05, n = 5), \text{ at } 8^\circ\text{C.}$$

$$D_a\text{MR} = -0.72 + 2.04 \times D_a \quad (r^2 = 0.66, p < 0.01, n = 8), \text{ at } 19^\circ\text{C.}$$

$$D_a\text{MR} = 0.128 + 1.03 \times D_a \quad (r^2 = 0.91, p < 0.01, n = 5), \text{ at } 29^\circ\text{C.}$$

The total energy expenditure during arousal appeared to be positively correlated with D_a (Fig. 5.15), but due to the limited data set this relationship was significant only at 8, 19, and 29°C (8°C: $r^2 = 0.74$, $p < 0.05$, $n = 5$; 19°C: $r^2 = 0.66$, $p < 0.01$, $n = 8$; 29°C: $r^2 = 0.91$, $p < 0.01$, $n = 5$; Fig. 5.15). The relationship between the two variables shows that shorter arousals were energetically cheaper than longer arousals, although a higher rate of average heat production usually had to be applied in a short arousal.

5.4.3 Energetics in Relation to Duration of Torpor

At low $T_{a,s}$ above the T_{tc} , *C. nanus* showed long bouts of torpor. The D_t lasting from over 10 h at high $T_{a,s}$ above 23°C to about 390 h at a T_a of 7°C (Fig. 5.16a). Torpor bouts at low $T_{a,s}$ below the T_{tc} were always shorter than 50h (Fig. 5.16a).

Above the T_{tc} , D_t was inversely related to T_a . However, log-transformed data revealed that a single equation did not adequately describe the relationship of the two variables (linear fit: $r^2 = 0.65$, $p < 0.001$; Fig 5.16a; logarithmic fit: $r^2 = 0.74$, $p < 0.001$, $n = 34$; Fig. 5.16b). The D_t at high $T_{a,s}$ above 23°C remained constant ($p > 0.5$, $n = 11$; Fig. 5.16b). In contrast, below T_a 23°C, $\log D_t$ was closely related to T_a ($r^2 = 0.71$, $p < 0.001$, $n = 23$; Fig. 5.16b)

A correlation was found between D_t and the average torpor MR in non-thermoregulating *C. nanus* above the T_{tc} , when expressed as a double-logarithmic plot ($r^2 = 0.76$, $p < 0.001$, $n = 34$; Fig. 5.17a, b). Similarly, D_t was also correlated to TMR ($r^2 = 0.62$, $p < 0.001$, $n = 34$; not shown). Nevertheless, the better r^2 for the regression between D_t and the average torpor MR than between D_t and TMR shows that the average torpor MR is a better predictor for D_t than the TMR.

The average torpor MR was strongly correlated with D_t at values less than 0.27 mL g⁻¹ h⁻¹ ($r^2 = 0.79$, $p < 0.001$, $n = 24$; Fig. 5.17b). However, when the average torpor MR was above 0.27 mL g⁻¹ h⁻¹, which was observed at high $T_{a,s}$, D_t was not correlated with this average torpor cost ($r^2 = 0.04$, $p > 0.5$, $n = 10$; Fig. 5.17b). In a

similar way, the correlations between D_t and TMR ($r^2 = 0.68$, $p < 0.001$, $n = 26$; not shown) became insignificant above TMR of $0.14 \text{ mL g}^{-1} \text{ h}^{-1}$ ($p > 0.5$).

Below the T_{tc} , *C. nanus* always had an average torpor MR higher than $0.32 \text{ mL g}^{-1} \text{ h}^{-1}$, and the average torpor MR was not correlated with D_t in this T_a range (Fig. 5.17a, b).

The torpor duration threshold (D_t'), below which torpor would not result in a reduction of energy expenditure, was only 0.2 h at T_a s around the T_{tc} (Fig. 5.18). The D_t' increased with both the decrease and the increase of T_a below $T_a 20^\circ\text{C}$, but it became shorter again at T_a s close to the T_{lc} (Fig. 5.18). Nevertheless, even the maximum D_t' observed at $T_a 15^\circ\text{C}$ was only about 1.6 h, thus D_t' was always well below the actual D_t for both thermoregulating and non-thermoregulating animals. This shows that torpor in *C. nanus* always results in a net energy saving at any T_a .

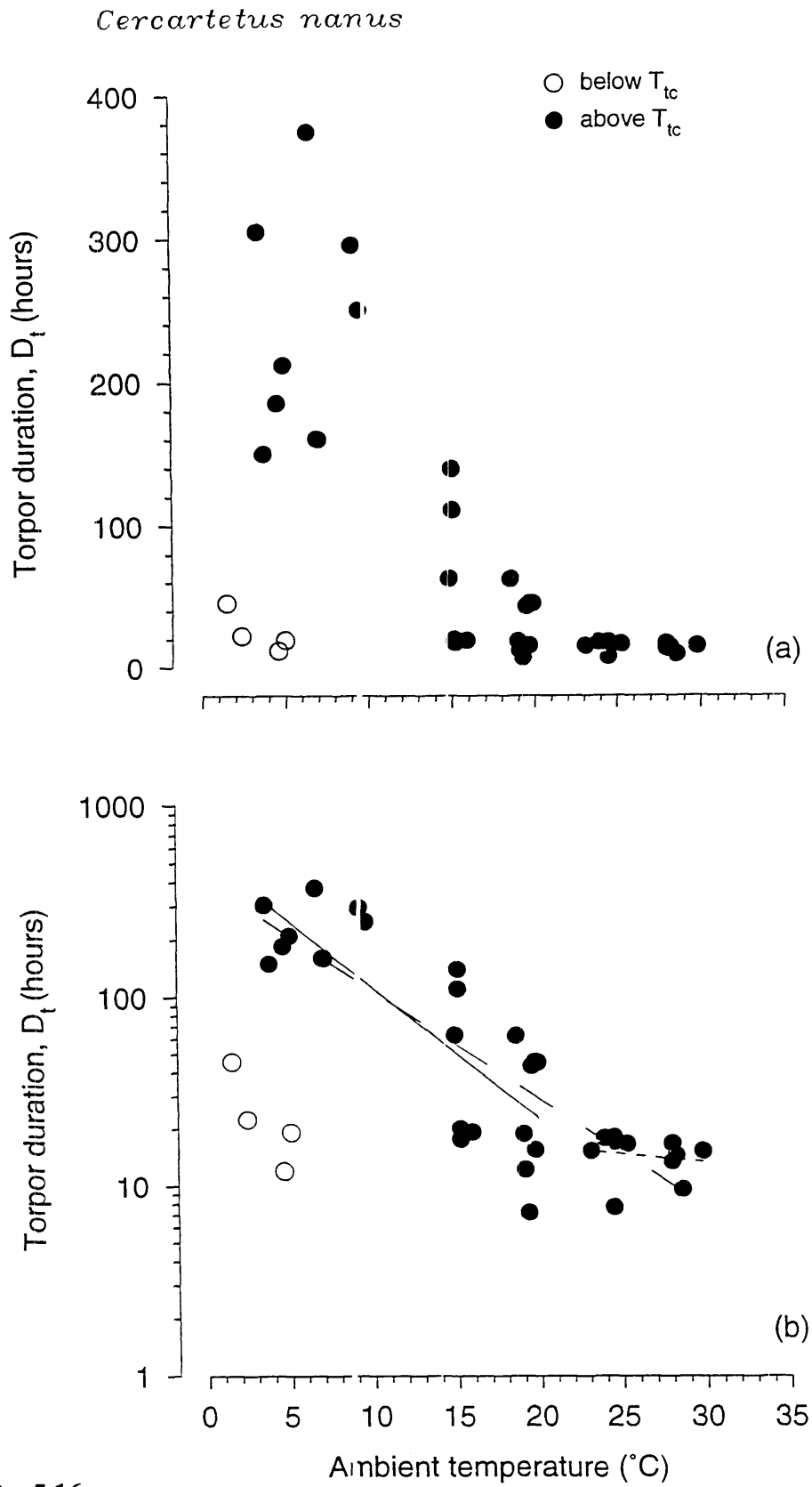


Fig. 5.16.

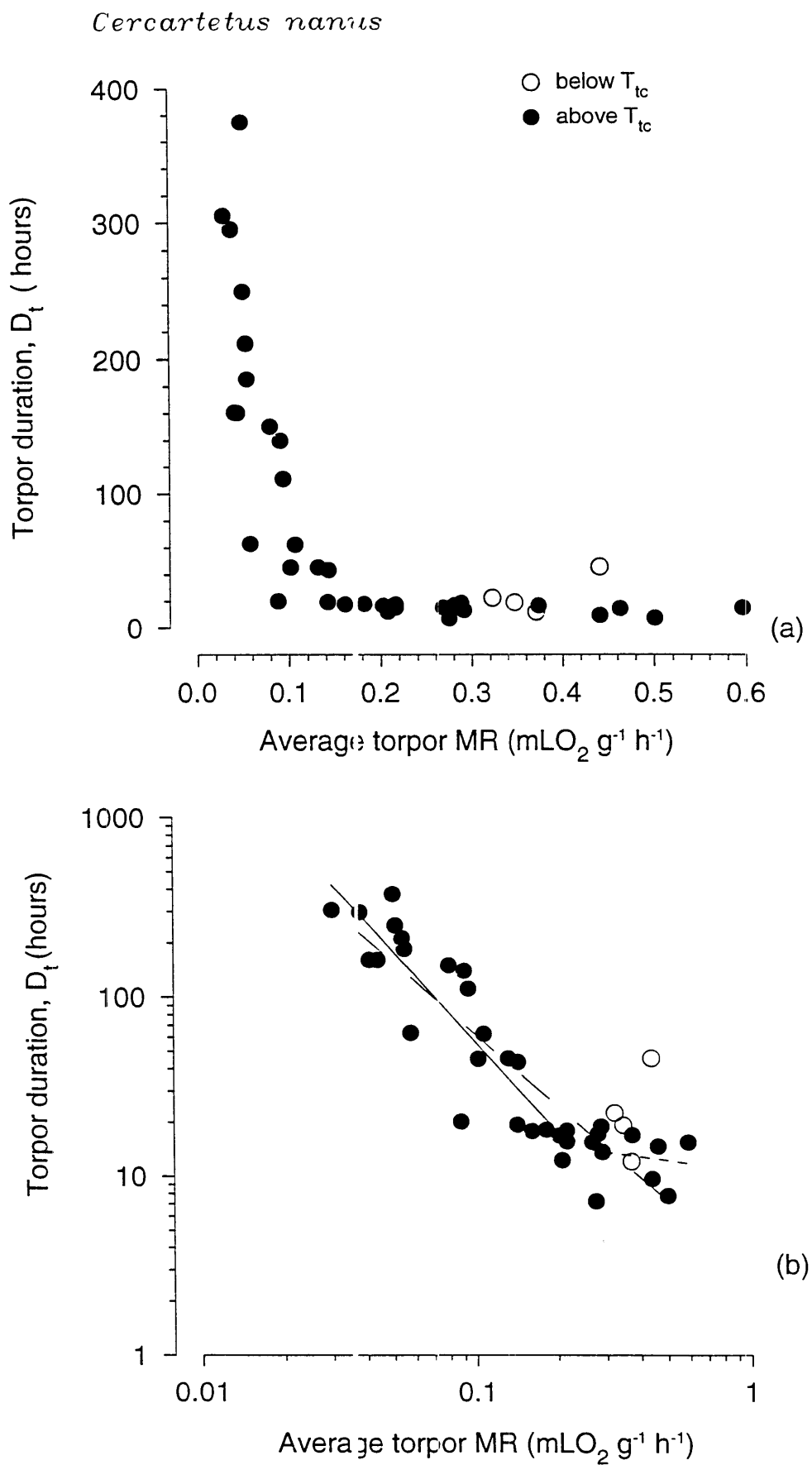


Fig. 5.17.

Fig. 5.16. (page 139)

(a) Torpor duration (D_t) of *Cercartetus nanus* as a function of ambient temperature (T_a). Above the T_{tc} , D_t was inversely correlated with T_a (filled circles, $N = 7$, $n = 34$):

$$D_t = 264 - 10.3 \times T_a \quad (r^2 = 0.65, p < 0.001).$$

(b) The log-transformed D_t was inversely correlated with T_a :

$\log(D_t) = 17.1 - 0.537 \times T_a$ ($r^2 = 0.74$, $p < 0.001$, $n = 34$, long-dashed line) for all data.

$\log(D_t) = 19.1 - 0.717 \times T_a$ ($r^2 = 0.74$, $p < 0.001$, $n = 23$, solid line) for data at T_a s below 23°C .

Above T_a 23°C , the two variables were not correlated ($r^2 = 0.04$, $p > 0.5$, $n = 11$, short-dashed line).

Open circles represent values below the T_{tc} .

Fig. 5.17. (previous page)

(a) Torpor duration (D_t) of *Cercartetus nanus* as a function of the average torpor MR (AD_tMR)

(b) Double logarithmic plot of D_t vs AD_tMR during torpor for animals above the T_{tc} (filled circles, $N = 7$, $n = 34$). The regression equations were:

$\log(D_t) = -2.73 - 12.1 \times \log(AD_tMR)$ ($r^2 = 0.76$, $p < 0.001$, $n = 34$, long-dashed line) for all data.

$\log(D_t) = -8.18 - 16.9 \times \log(AD_tMR)$ ($r^2 = 0.79$, $p < 0.001$, $n = 24$, solid line) for data of AD_tMR less than $0.27 \text{ mL g}^{-1} \text{ h}^{-1}$.

When AD_tMR was larger than $0.27 \text{ mL g}^{-1} \text{ h}^{-1}$, the two variables were not correlated ($r^2 = 0.04$, $p > 0.1$, $n = 10$, short-dashed line).

Open circles represent values below the T_{tc} .

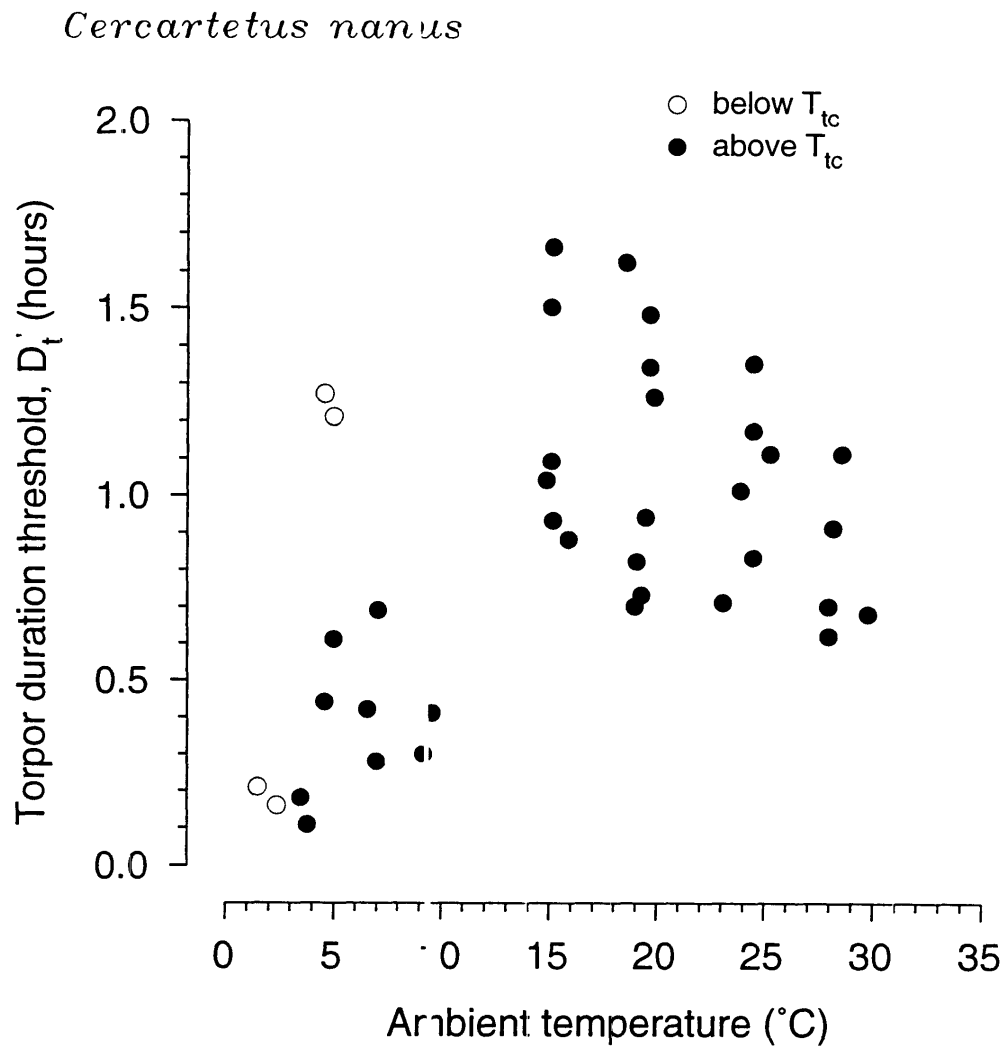


Fig. 5.18. Torpor duration threshold (D_t') of *Cercartetus nanus* as a function of ambient temperature at T_{as} above the T_{tc} (filled circles, $N = 7$, $n = 34$) and below the T_{tc} (open circles, $N = 4$, $n = 4$). The D_t' was calculated with the assumption that the average MR during the entire torpor bout (ATBDMI) was equal to RMR, and the D_t' was calculated as:

$$D_t' = D_a \times (AD_aMR - RMR) / (RMR - AD_tMR)$$

(where D_a is arousal duration, AD_tMR is the average MR during torpor, and AD_aMR is the average MR during arousal). The D_t' was shorter than 1.7 h at all T_{as} .

5.5 Discussion

5.5.1 Arousal and Related Energetics

The high energetic cost of arousal has been reported for many hibernators. For the dormouse (*Glis glis*), the energy expenditure during a single arousal lasting for several hours was equivalent to that used during 10 days of hibernation (Kayser 1953). For the ground squirrel (*Spermophilus richardsonii*), over 71% of the energy used during hibernation season is consumed during periodic arousals and subsequent intervals of normothermia (Wang 1979). Similar to these species, in *C. nanus*, the average MR (i.e. MR per gram hour) during arousal accounted for up to 60-fold of that during the hibernating state. In *S. macroura*, the average arousal MR (MR per gram hour) was up to 6-fold of that during the torpid state. Since *S. macroura* stayed in torpor for only several hours while *C. nanus* remained in torpor for up to several hundred hours, the total cost for arousal is a larger proportion of the total energy expenditure during torpor in the daily heterotherm than in the hibernator.

The total arousal cost of both *S. macroura* and *C. nanus* was inversely related to T_a . This relationship is apparently due to the influences of T_a on both arousal duration (chapter 4) and the average energy consumption during arousal. This influence of T_a on arousal cost consistently held true at T_{as} both below and above the T_{tc} . In agreement with the observations made on *S. macroura* and *C. nanus*, an inverse relationship between the total arousal cost and T_a has also been reported for the hummingbird, *Selasphorus rufus* (Hiebert 1990). However, in the hummingbirds, the increase of total arousal cost below the T_{tc} seems to result only from an increasing average arousal MR, since arousal duration of the hummingbirds becomes shorter at low T_{as} (Hiebert 1990).

When the data of energy expenditure during arousal at the same T_a were analysed, it was found that the total energy consumption during arousal is a function of arousal time. In both *S. macroura* and *C. nanus*, a low total energy expenditure

during arousal was frequently associated with a short arousal, while long arousals were more costly. It thus appears that a fast arousal is energetically more favourable than a slow arousal. In addition, a fast arousal allows an animal to regain the normothermic T_b quickly. This ensures the animal to have more time to forage, feed, and fulfil other important requirements that are associated with the necessity of arousal. Therefore, a fast arousal is not only related to energy conservation, it also means time conservation.

5.5.2 Torpor Duration and Related Energetics

It is known that torpor bout duration varies among different hibernators and among individuals of the same species (Lyman et al. 1982). Nevertheless, an inverse relationship between torpor duration and T_a above the T_{tc} has been suggested for some hibernators (Twente and Twente 1965; Twente et al. 1977; French 1982a; Geiser and Kenagy 1988). A decrease of torpor duration below the T_{tc} in thermoregulating animals has also been proposed (Geiser and Kenagy 1988). These observations are in agreement with those made on *C. nanus* in this study. However, this relationship does not appear to exist in daily torpor of *S. macroura*, although the two longest torpor bouts at each T_{tl} were correlated to T_a .

The processes that determine torpor bout duration have been, and still are, subject to speculation. It seems torpor bout durations of hibernators are affected by many factors. Torpor bout length changes with the time of year. Longer bouts occur usually in mid-winter while short bouts occur at the beginning and end of the hibernating season (Twente and Twente 1967; French 1985; Barnes et al. 1993). Torpor bout duration seems also to be influenced by body size, by the method of energy storage as food or body fat, and even by the amount of food storage (Lyman et al. 1982; French 1986). Moreover, the concentration of essential polyunsaturated fatty acid appears to be critical in determining torpor bout duration (Geiser and Kenagy 1987; Florant et al.

1990; 1993). Several hypotheses have been proposed to explain how torpor duration is determined. These include accumulation of metabolic wastes and other metabolic imbalances (Twente and Twente 1967; Pengelley et al. 1971; Willis et al. 1971; Galster and Morrison 1975; French 1985; Geiser and Kenagy 1988), circadian and circannual rhythms (Strumwasser et al. 1967; Twente and Twente 1987; Zucker et al. 1993; Canguilhem et al. 1994), and sleep deprivation during torpor (Daan et al. 1991; Trachsel et al. 1991; Deboer and Tobler 1994).

In the past, a correlation between TMR and/or T_b and torpor bout duration for hibernators has been used to favour the hypothesis of metabolic determinations (Geiser and Kenagy 1988). Recent studies suggest that this hypothesis may be also valid for daily heterotherms, as has been shown by the results made on the rodent *Saccostomus campestris* (Ellison 1993, but see Ellison 1995). However, the correlations between torpor bout duration and TMR for both hibernators and daily heterotherms are often weak, as was the case for *C. nanus* and *S. macroura* in this study. Nevertheless, the result of this study shows the interrelations between torpor bouts and metabolic rate are remarkably improved if the average MR during torpor, from torpor entry to the beginning of arousal, rather than the minimum TMR, is taken into account. Both species investigated here showed strong correlations between energy expenditure and time in torpor, supporting the view that the two variables are somewhat linked (French 1985; Geiser and Kenagy 1988).

Although it is obvious that rate of metabolism during torpor is a relatively good predictor for torpor bout length, this is true only for *C. nanus* and *S. macroura* within certain temperature ranges. Above and below the range from the T_{tc} to 23°C in *C. nanus*, and below the T_{tc} in *S. macroura*, torpor duration was neither correlated with TMR, nor the average torpor MR. This suggests that factors other than metabolism must be involved in determining torpor bout length in both hibernation and daily torpor.

As is shown in chapter 4, both torpor entry and arousal of *C. nanus* occurred preferentially at a certain time of the day. The timing was especially precise at high T_{as} . Animals aroused with reasonable precision in the afternoon, and this ensured that they regained their normothermic T_b at the time around the lights-off, and to be ready to begin their phase of activity. Hence, the time of entry and arousal, and thus torpor duration, appear to be controlled by a circadian rhythm rather than the light-dark cycle as is also known for rodents and bats (Strumwasser 1967 et al.; Canguilhem et al. 1994). These studies support the view that an internal clock functions both during normothermia and hibernation, and that this biological clock is resynchronized by the photoperiod during arousal periods (Canguilhem et al. 1994). It is thus possible that the metabolic accumulation or imbalances during torpor sets the upper limit for torpor bout duration at low T_{bs} , but arousal may be triggered prematurely by the circadian rhythm, particularly at high T_{bs} .

The determination of torpor bout duration during daily torpor appears somehow different from that during hibernation. In *S. macroura*, all longer torpor bouts were observed at T_{as} at, or slightly above, the T_{tc} . However, the overall torpor duration varied markedly at different T_{as} . In addition, MR appeared to be a much weaker predictor for torpor bout duration during daily torpor in *S. macroura* than during hibernation in *C. nanus*. These all suggest a stronger effect of factors other than metabolism in determining torpor length in *S. macroura*. It has been proposed since daily heterotherms are not able to undergo prolonged torpor, their restricted daily arousals seem to be obligatory (Lyman 1982a, b). Thus torpor bout duration of daily heterotherms is most likely under the control of circadian rhythms. The preferred timing of torpor entry and arousal (chapter 4) and locomotor activity rhythms of *S. macroura* (O'Reilly et al. 1984; Francis and Coleman 1990) support this point of view. Similar observations have also been made on *Saccostomus campestris* (Haim et al. 1988) and on *Phodopus sungorus* (Ruf et al. 1989).

However, *S. macroura* occasionally terminated torpor earlier than the preferred time, demonstrating that torpor length of daily heterotherms is also sensitive to other factors. Animals that exhibit daily torpor usually do not retreat to well-insulated hibernacula as commonly associated with hibernation. Thus daily heterotherms have a reasonable chance to be exposed to external cues and various acute environmental variations. In addition, T_b during daily torpor does not usually fall to levels as low as that exhibited during hibernation. Thus daily heterotherms are able to sense and to respond to these external cues and interferences even during torpor. Therefore, the torpor length of daily heterotherms should be more sensitive to various interferences, and subject to a greater degree of fluctuations than that of hibernators. This view is supported by the fact that torpor bout duration of *S. macroura* changes at different food regimes, suggesting torpor length may be sensitive to body energy reserves (Song and Geiser 1997). Torpor bout length variations associated with varying energy reserves have also been reported for other heterothermic endotherms such as the wood mouse, *Apodemus sylvaticus* and hummingbirds (Walton and Andrews 1981; Hiebert 1990). Because of the high sensitivity to various influences, the chance for a torpor bout reaching the potential maximum as set by either metabolism or a biological clock should be smaller in daily heterotherms than in hibernators. This flexibility of torpor duration during daily torpor is important for daily heterotherms to adapt to their generally more changeable, thus less predictable environments, as compared to the more predictable seasonal changes for deep hibernation.

5.5.3 Energy Benefits by the Use of Torpor

This study shows that at any given T_a , the use of steady-state torpor always results in a reduction of energy expenditure from normothermic resting levels. This is true during both hibernation in *C. nanus* and daily torpor in *S. macroura* at all T_a s investigated. Since the duration of torpor for both species was always longer than the torpor

duration threshold for energy savings, use of torpor in these animals always results in a net decrease of energy expenditure, despite the high arousal costs that generally exceeds RMR. Obviously, compared with hibernation of *C. nanus*, energy savings by the use of daily torpor are significantly smaller in *S. macroura*.

The ratio of the total cost of torpor to RMR varied with T_a in both *S. macroura* and *C. nanus*. The lowest ratios were achieved at the T_{TC} , and smallest energy savings were observed at T_a s close to the T_{LC} . Previous studies on other species also suggest that energy saving by the use of torpor is higher at low, than at high T_a s (Tucker 1966; Vogt and Lynch 1982; Frey 1991; Holloway and Geiser 1995). This large potential reduction in energy expenditure may be the reason why many small mammals employ torpor more frequently when they are exposed to a low than to a high T_a (Brown and Bartholomew 1969; Webb and Skinner 1996). It has been reported that at a high T_a , torpor may even result in a net increase of energy expenditure in the daily heterotherm, *Graphiurus murinus*, since MR and T_b of some animals may be reduced only briefly (Webb and Skinner 1996). Nevertheless, there are other reports which show that only slight drops of T_b and MR substantially reduce energy expenditure in bats (Studier 1981).

At T_a s above the T_{TC} , the interrelations between the absolute energy expenditure during torpor and T_a differ between *S. macroura* and *C. nanus*. In *C. nanus*, although arousal costs increased with the lowering of T_a during torpor, the prolongation of torpor bout and the associated decrease of energy consumption are more pronounced. This resulted in a lower overall cost of torpor at low T_a s than at high T_a s. In contrast, the overall cost of torpor in non-thermoregulating *S. macroura* at a low T_a was not lower, but higher, than at a high T_a . Although energy consumption during torpor decreased at low T_a s, this reduction is apparently not sufficient to compensate for the increase of arousal costs although the torpor bouts were somewhat longer. Therefore, at T_a s that are only several degrees below the T_{TC} , the combined costs of torpor and arousal

during daily torpor may be higher than the BMR in *S. macroura*. In addition, energy expenditure of *S. macroura* is always higher at a low than at a high T_a , both during torpor and normothermia.

In the field, both *S. macroura* and *C. nanus* are, from time to time, exposed to a T_a that is well below the T_{tc} . It is clear from this study that torpor at T_a s below the T_{tc} , with the onset of thermoregulation is very costly for both *S. macroura* and *C. nanus*. Even at T_a s just below the T_{tc} , the average cost of torpor is already higher than the maximum values of non-thermoregulating animals. The average cost of arousal below the T_{tc} is also higher. This results in a very expensive overall energy expenditure in torpid thermoregulating animals even at T_a s just below the T_{tc} . This cost of thermoregulating animals increased drastically with a decreasing T_a . Therefore, in *S. macroura*, torpor at 10°C can be more expensive than remaining normothermic at T_a s above 18°C. In thermoregulating *C. nanus*, torpor is more expensive than remaining normothermic at T_a s close to or within the TNZ. This relationship between T_a and energy expenditure may provide an explanation as to why most heterothermic mammals have adjusted their T_{set} s during torpor above the lowest T_a that they often encounter in the field (Geiser et al. 1990). By avoiding torpidity by thermoregulating, the energy expenditure during torpor can be significantly reduced.

In summary, the function of torpor in reducing energy expenditure is more obvious at low T_a s, but above the T_{tc} . Because of the different proportional contributions of arousal costs between hibernation and daily torpor, the overall energy expenditure of daily torpor is low at high T_a s, while hibernation uses less energy at low T_a s. Torpor bout duration appears to be determined by many factors. Although metabolically related processes may set the maximum torpor length, biological rhythms and other influences may terminate torpor bouts prematurely, especially during daily torpor. Shorter rewarming processes are energetically more favourable.