

Chapter Three: Thermoregulation and Metabolic Rate Reduction During Hibernation and Daily Torpor

3.1 Introduction

By the means of torpor of various patterns, mammalian heterotherms are able to substantially reduce their MR and T_b in response to cold and/or food shortage (Lyman et al. 1982; Wang 1987, 1989). Although low MRs and T_b s have been described in many species, the physiological mechanisms causing the reduction of MR and the interrelations between the drop in MR and T_b during torpor remain controversial (Malan 1986, 1993; Geiser 1988a, 1993b; Heldmaier and Ruf 1992; Heldmaier et al. 1993).

Because the Q_{10} of MR reduction in many species is between 2 and 3, which is typical of the temperature dependence of many biological reactions, it was claimed that MR is reduced by the lowered T_b (Tucker 1965; Hammel et al. 1968; Snapp and Heller 1981). This widely held interpretation is presented in most animal physiology text books. However, MRs that are below that explained by temperature effects, and consequently Q_{10} values well above 2 - 3, have been observed in small hibernators, as well as during entry into torpor (Kayser 1964; Morrison and Ryser 1962; Henshaw 1968; Geiser 1988a). It has been concluded that metabolic inhibition, in addition to temperature effects, may cause the low MR during torpor (Malan 1986, 1988; Geiser 1988a; Storey and Storey 1990).

Recently, this view has been challenged by two new hypotheses. The first of these claims that MR during hibernation and daily torpor is actively downregulated, and T_b may have no influence at all in reducing MR during torpor. According to this hypothesis, MR during both normothermia and torpor is proportional to the

differential (ΔT) between T_b and T_a (Heldmaier and Ruf 1992, Heldmaier et al. 1993). The second hypothesis claims that MR is reduced as a function of a low C (Snyder and Nestler 1990). In these cases T_b would be only a consequence of the low MR (Snyder and Nestler 1990; Heldmaier and Ruf 1992; Heldmaier et al. 1993).

Since reduction of MR is much more pronounced in hibernation than in daily torpor (Geiser and Ruf 1995), it is likely that physiological processes determining MR during torpor differ between hibernators and daily heterotherms. To test these hypotheses in the two torpor categories, detailed measurements on hibernators and daily heterotherms that provide conclusive results on the thermal relations of MR reduction are required. In the past, such investigations were often difficult to interpret because most of the experiments were conducted on sciurid rodents which enter torpor only at low T_a s (Lyman et al. 1982), making a detailed analysis on the effect of T_a on steady-state T_b and MR and the interrelations between these variables difficult.

Previous studies on torpor of *S. macroura* and *C. nanus* showed that both of them display torpor over a relatively wide range of T_a (Geiser and Baudinette 1985; Geiser 1993a). Above approximately T_c 2°C in *C. nanus* and T_a 15°C in *S. macroura*, no apparent defence of T_b was observed during torpor. Below these critical T_a s, the torpid animals show proportional thermoregulation (Geiser and Baudinette 1985; Geiser 1993a).

In the present study, MR, T_b and T_a of hibernating *C. nanus* and during daily torpor of *S. macroura* were measured both below and above the T_{set} for T_b during torpor. The data were used to determine interrelations between variables in these two temperature ranges, and for comparison with values from normothermic individuals.

3.2 Materials and Methods

3.2.1 Measurements on *S. macroura*

Seven adult male *S. macroura* were used in the experiment. The mean BM determined at the time of MR measurements was 23.6 ± 2.6 g. The animals were kept at a T_a of $20 \pm 2^\circ\text{C}$ when not being measured. Food and water were not available during measurements.

For determination of TMR and RMR, animals were measured using system A of the respirometry equipment (chapter 2.2.1) with a flowrate of about 200 mL min^{-1} . Animals were also measured in system B (chapter 2.2.1) on several occasions to ensure the same values were obtained. Each channel was read for 3 min, thus \dot{V}_{O_2} and corresponding T_b and T_a for each individual were recorded simultaneously at 12-min intervals. Measurements were conducted at a constant T_a ranging from 7 to 30°C for about one day. Measurements of BMR were carried out separately (chapter 2.4.2) using the same respirometry equipment.

3.2.2 Measurements on *C. nanus*

Eight adult *C. nanus* (4 females, 4 males) were used in the experiment. The mean BM determined at the time of MR measurement was 36.2 ± 5.8 g. The animals were kept at a T_a of $20 \pm 2^\circ\text{C}$ for most of the time when not being measured. For the measurements at T_a s below 15°C they were acclimated at T_a 10°C for at least 6 weeks before measurements. Food and water were not available during measurement.

Measurements were conducted with the system B of the respirometry equipment because of its greater sensitivity. \dot{V}_{O_2} and corresponding T_b and T_a were read in 3-min intervals. TMR was measured at a constant T_a set between 5 and 30°C . Each measurement lasted over a complete torpor bout (1 - 20 days depending on the T_a). In addition, cooling experiments were conducted during the light phase, to determine the T_{set} for T_b . When the TMR had stabilised at a T_a of 6°C , the T_a was reduced in

1°C steps, until an increase in TMR and no further decline of T_b were observed. This T_a was then maintained until the steady-state TMR and T_b had been measured.

RMR was measured at constant T_a s ranging from 1 to 30°C. The TNZ and BMR were determined in separate measurements (chapter 2.4.2).

3.2.3 Statistical Methods

Since ΔT appeared to be constant at low T_a s above the T_{tc} , but was temperature-dependent at high T_a s, the correlations between T_a and ΔT during torpor above the T_{tc} were analysed by fitting the whole data set with two regressions starting at 30°C. Data were separated from the T_a at which the linear regression for the lower ΔT values became insignificant. $\dot{V}O_2$ values as function of T_b were also graphed as Arrhenius plot. These values were fitted with a single regression line and two regression lines. The smallest sum of the residual sum of squares of the two regression lines of TMR vs T_b was considered to be the best fit. Significance for the regressions was derived from considerations of probability less than 5%. Coefficient of determination (r^2) and sample size are also provided.

3.3 Results for *Sminthopsis macroura*

3.3.1 General Torpor Pattern

The MR and T_b of *S. macroura* showed pronounced daily fluctuations (Fig. 3.1). During the day, the animals were usually resting. After lights off, animals became active and both T_b and MR were generally elevated. The animals entered torpor between 2200h and 0500h. When entering torpor both MR and T_b were reduced and steady-state levels were reached after about 3 h. Torpor was terminated by spontaneous arousal, characterised by a steep increase of MR followed by a rise of T_b (Fig. 3.1).

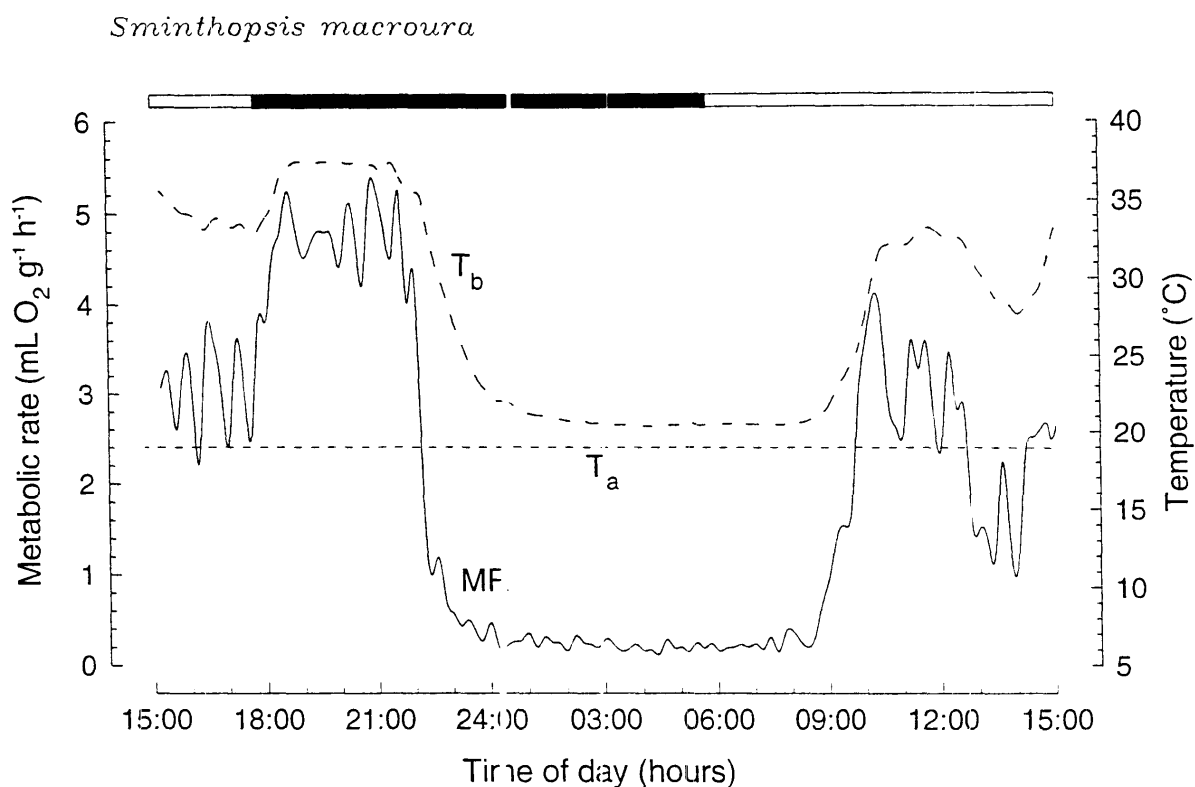


Fig. 3.1. Fluctuations of oxygen consumption ($\dot{V}O_2$) and body temperature (T_b) of a *S. macroura* at an air temperature (T_a) of 19°C. Food and water were not available. The animal entered torpor just before midnight and aroused at about 0900 in the morning. The dark bar indicates the dark phase.

3.3.2 Normothermia

S. macroura increased their RMR linearly with decreasing T_a ($r^2 = 0.92$, $p < 0.01$) when exposed to T_a s below the T_{lc} of $31.3 \pm 1.0^\circ\text{C}$ ($N = 7$, $n = 119$; Fig. 3.2a). The TNZ ranged from T_a 31.3 to 34.0°C , and BMR was $0.89 \pm 0.08 \text{ mL g}^{-1} \text{ h}^{-1}$ ($\text{BM} = 24.8 \pm 2.3 \text{ g}$, $N = 7$, $n = 19$; Fig. 3.2a).

At the T_{lc} the T_b of resting individuals was $34.3 \pm 0.6^\circ\text{C}$ ($N = 7$, $n = 18$). Below the T_{lc} , T_b was inversely related with T_a ($r^2 = 0.37$, $p < 0.01$). Above the T_{lc} , T_b increased with T_a ($r^2 = 0.65$, $p < 0.01$; Fig. 3.2b).

ΔT ($T_b - T_a$) increased linearly below the T_{lc} ($r^2 = 0.99$, $p < 0.01$). Above the T_{lc} , ΔT was also negatively correlated with T_a ($r^2 = 0.75$, $p < 0.01$), but the slope was less steep (Fig. 3.2c).

The C during normothermia was $0.20 \pm 0.04 \text{ mL g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ ($N = 7$, $n = 76$) below the T_{lc} . Above the T_{lc} , C increased markedly with T_a ($r^2 = 0.67$, $p < 0.01$; Fig. 3.2d).

3.3.3 Torpor

The T_{tc} of *S. macroura* occurred at T_a 14.3°C . From 30°C to the T_{tc} , torpid individuals showed no evidence of thermoregulation. Both steady-state TMR and T_b in this T_a range decreased with T_a ($r^2 = 0.64$, $p < 0.01$; $r^2 = 0.93$, $p < 0.01$, respectively; Fig. 3.2a, b), but ΔT remained constant at $2.1 \pm 0.9^\circ\text{C}$ ($N = 7$, $n = 31$; Fig. 3.2c). Therefore, the C decreased with T_a ($r^2 = 0.50$, $p < 0.01$) to the minimum value of $0.09 \pm 0.05 \text{ mL g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ ($N = 7$, $n = 13$) at T_a s of $14 - 19^\circ\text{C}$, which was significantly lower than the C of normothermic individuals in the same T_a range ($p < 0.01$; Fig. 3.2d).

Below the T_{tc} , the response of TMR to T_a was reversed and showed an increase with a drop of T_a ($r^2 = 0.58$, $p < 0.01$; Fig. 3.2a). The T_b in this T_a range did not fall with T_a , but was regulated above 16°C (Fig. 3.2b), which resulted in an increase of ΔT

($r^2 = 0.27$, $p < 0.05$; Fig. 3.2c). With the onset of thermoregulation, C gradually increased with the decreasing T_a ($r^2 = 0.43$, $p < 0.01$) to levels observed during normothermia (Fig. 3.2d).

In the T_a range in which T_b of torpid *S. macroura* was not metabolically defended, TMR was positively related to T_b (Fig. 3.3). Both linear ($r^2 = 0.79$) and exponential ($r^2 = 0.74$) fits appeared to be appropriate. However, the exponential fit provided the better model, because the r^2 for the regression of the predicted y-value for the exponential model versus the measured y-value ($r^2 = 0.83$) was larger than that for the linear model (Fig. 3.3). The Q_{10} for TMRs between T_b 16.0°C at a T_a of about 14°C and T_b 32.0°C at a T_a of about 30°C was 2.56, and the Q_{10} for MRs between the BMR at T_b 34.3°C and TMR at T_b 16.0°C at a T_a of about 14°C was 2.79. In contrast to T_b , ΔT was not related to the TMR in the T_a range in which T_b was not regulated ($p = 0.35$; Fig. 3.4).

In the T_a range below the T_{tc} where T_b was regulated, T_b was also correlated with TMR ($r^2 = 0.21$, $p = 0.04$; Fig. 3.5). In contrast to the situation at high T_a s, in this T_a range ΔT increased with decreasing T_a and TMR showed a significant relationship with ΔT (Fig. 3.6). Both the slope and the intercept for the regression of TMR versus ΔT were not significantly different from those for the regression of RMR versus ΔT ($p = 0.50$, $p = 0.31$, respectively; Fig. 3.6). The Q_{10} for MRs between RMR at T_b 34.9°C and TMR at T_b 22.7°C at a T_a of 10°C was 2.38.

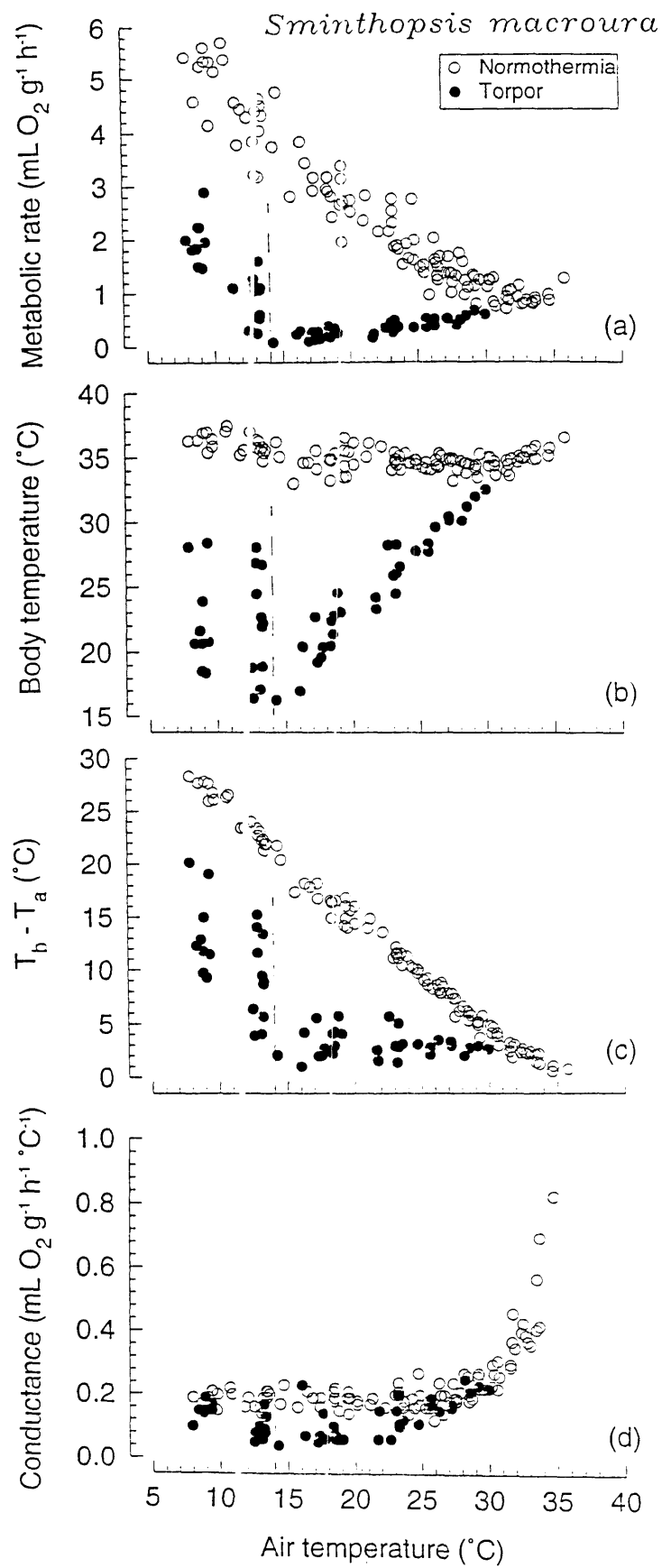


Fig. 3.2.

Fig. 3.2. (previous page) The effect of air temperature (T_a) on (a) metabolic rate (MR) measured as rate of oxygen consumption, (b) body temperature (T_b), (c) the temperature differential (ΔT) between T_b and T_a , and (d) apparent conductance (C) during daily torpor (filled circles) and normothermia (open circles) in *S macroura* ($N = 7$). Mean BM was 23.6 ± 2.6 g. Regression equations for physiological variables during normothermia below the T_{lc} were:

$$\text{RMR} = 6.68 - 0.19 \times T_a, r^2 = 0.92, p < 0.01.$$

$$T_b = 36.8 - 0.08 \times T_a, r^2 = 0.37, p < 0.01.$$

$$\Delta T = 36.7 - 1.08 \times T_a, r^2 = 0.99, p < 0.01.$$

$$C = 0.16 + 0.02 \times T_a, r^2 = 0.11, p > 0.05.$$

Regression equations for physiological variables during normothermia above the T_{lc} were:

$$\text{RMR} = 0.64 + 0.01 \times T_a, r^2 = 0.06, p > 0.05.$$

$$T_b = 20.5 + 0.44 \times T_a, r^2 = 0.65, p < 0.01.$$

$$\Delta T = 19.4 - 0.53 \times T_a, r^2 = 0.75, p < 0.01.$$

$$C = -3.25 + 0.11 \times T_a, r^2 = 0.67, p < 0.01.$$

Regression equations for physiological variables during torpor above 14°C were:

$$\log \text{TMR} = -1.41 + 0.04 \times T_a, r^2 = 0.64, p < 0.01.$$

$$T_b = 3.69 + 0.97 \times T_a, r^2 = 0.93, p < 0.01.$$

$$\Delta T = 3.69 - 0.03 \times T_a, r^2 = 0.01, p = 0.59.$$

$$C = -0.09 + 0.01 \times T_a, r^2 = 0.50, p < 0.01.$$

Regression equations for physiological variables during torpor below 14°C were:

$$\text{TMR} = 4.24 - 0.26 \times T_a, r^2 = 0.58, p < 0.01.$$

$$T_b = 22.8 - 0.04 \times T_a, r^2 = 0.001, p = 0.90.$$

$$\Delta T = 22.8 - 1.04 \times T_a, r^2 = 0.27, p < 0.05.$$

$$C = 0.26 - 0.01 \times T_a, r^2 = 0.43, p < 0.01.$$

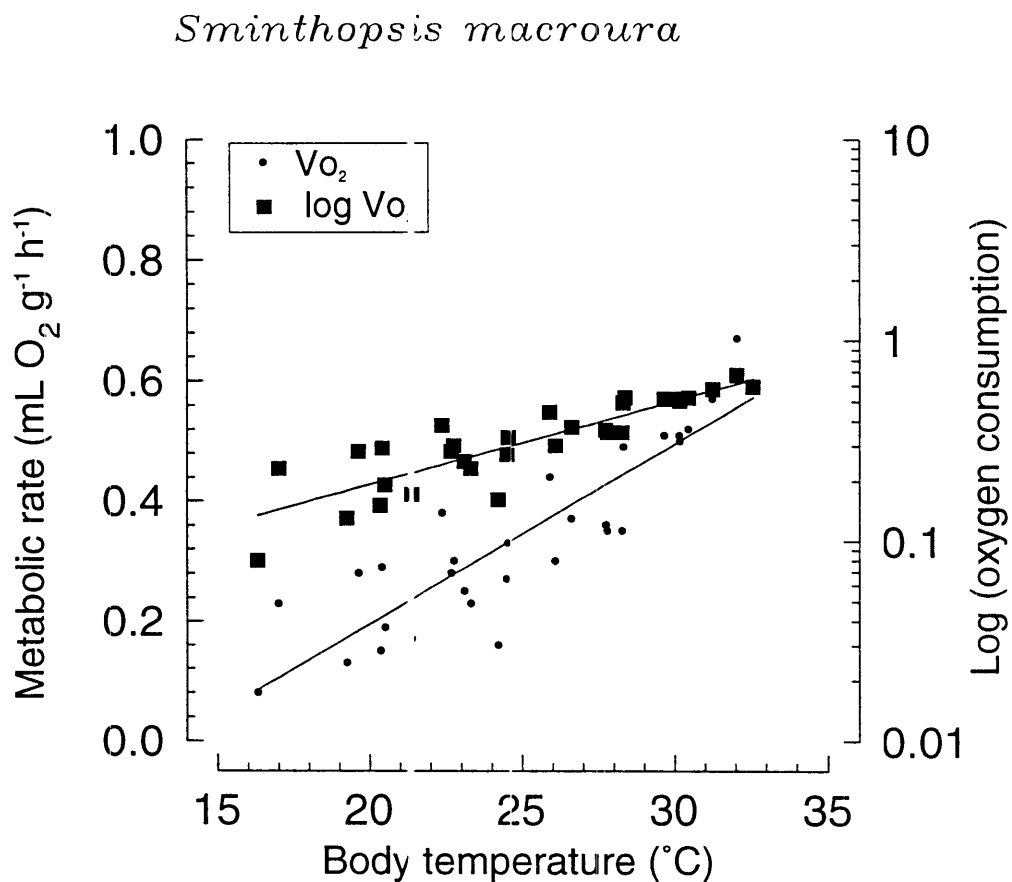


Fig. 3.3. The metabolic rate during torpor (TMR) as a function of body temperature (T_b) above the critical air temperature during torpor (T_{tc}) in *S. macroura*. Both linear fit (circles; $TMR = -0.41 + 0.03 \times T_b$, $r^2 = 0.79$, $p < 0.01$) and exponential fit (squares; $\log TMR = -1.56 + 0.04 \times T_b$, $r^2 = 0.74$, $p < 0.01$) were appropriate. However, the exponential fit provided the better model, because the r^2 for the regression of the predicted y-value for the exponential model versus the measured y-value ($r^2 = 0.83$) was larger than that for the linear model ($r^2 = 0.79$).

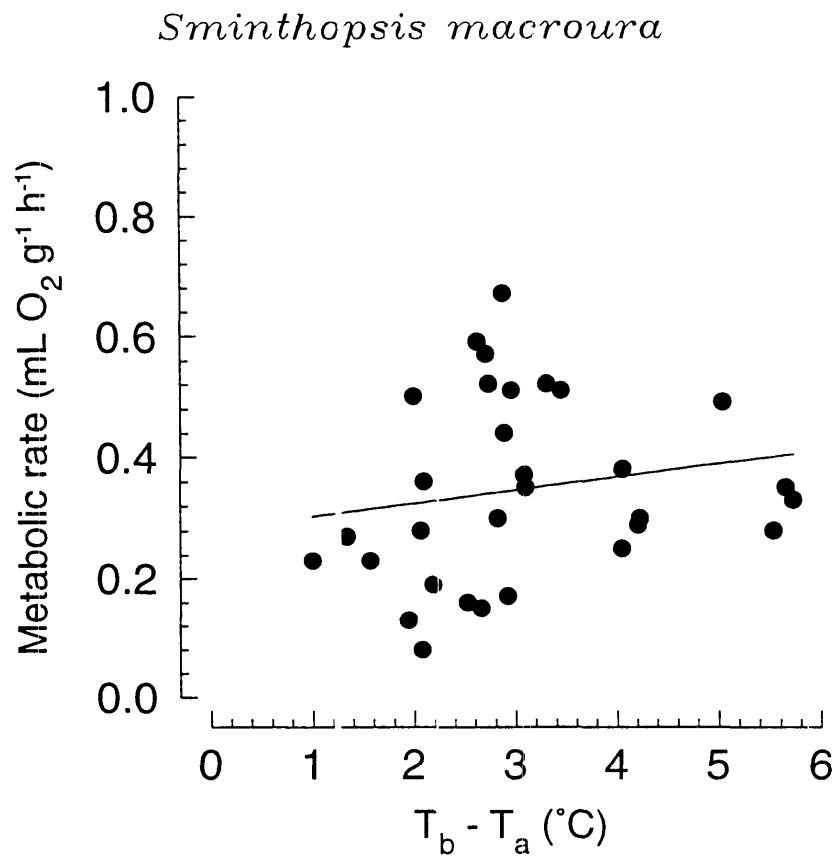


Fig. 3.4. The metabolic rate during torpor (TMR) as a function of the temperature differential (ΔT between body temperature (T_b) and air temperature (T_a) above the critical air temperature during torpor (T_{tc}) in *S. macroura*. The equation for the regression was: $TMR = 0.28 + 0.02 \times \Delta T$, $r^2 = 0.03$, $p = 0.35$.

Sminthopsis macroura

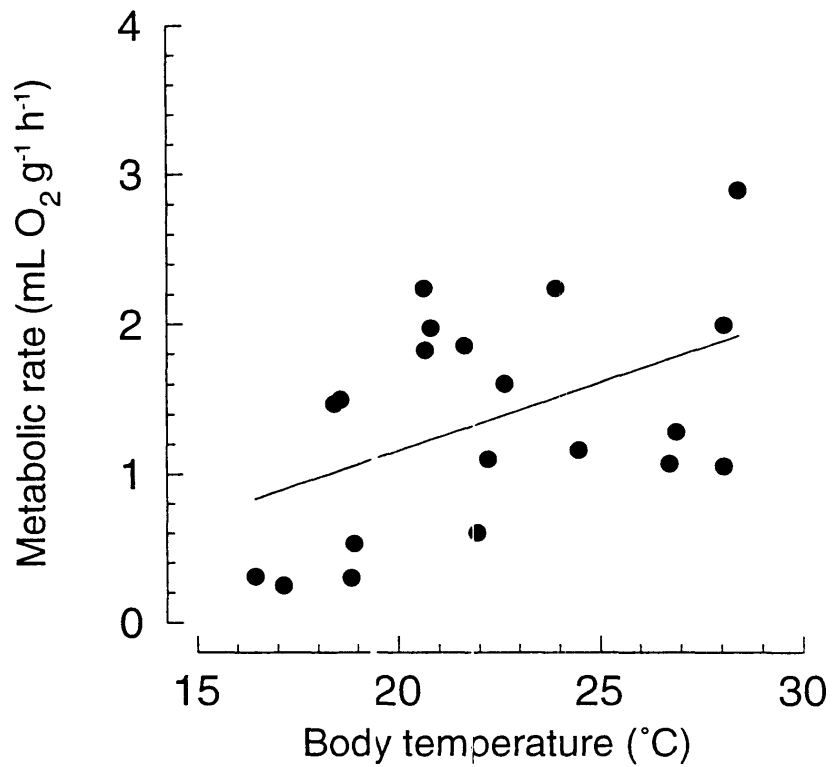


Fig. 3.5. The metabolic rate during torpor (TMR) as a function of body temperature (T_b) below the critical air temperature during torpor (T_{tc}) in *S. macroura*. The equation for the regression was:

$$\text{TMR} = -0.66 + 0.09 \times T_b, r^2 = 0.21, p = 0.04.$$

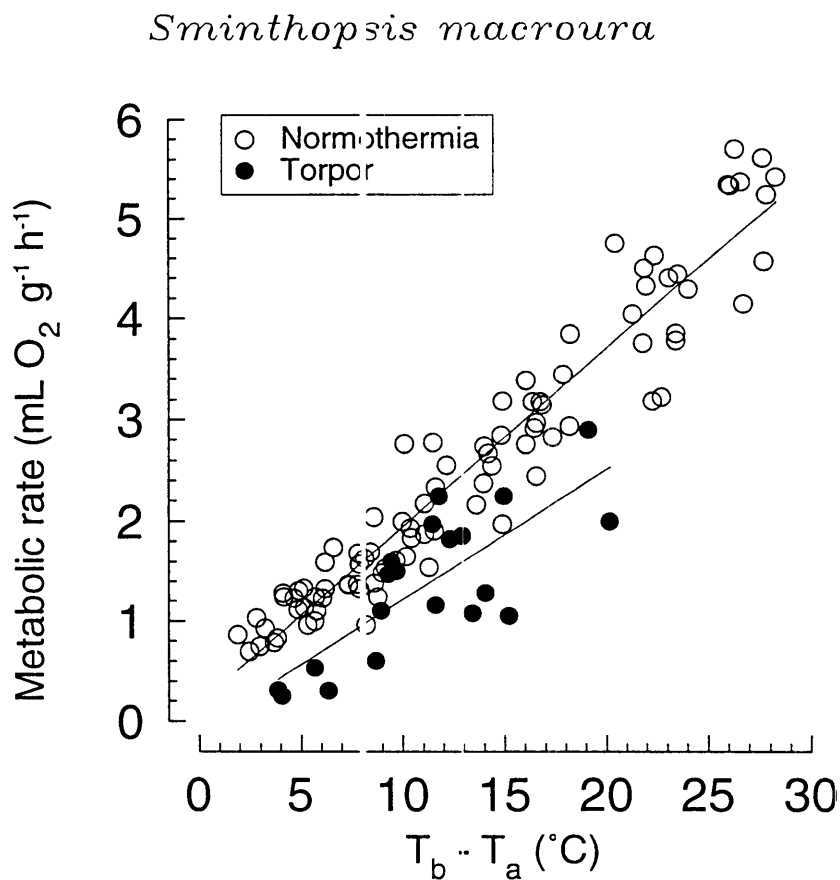


Fig. 3.6. The metabolic rate during torpor (TMR; filled circles) below the critical air temperature (T_c) and the resting metabolic rate of normothermic individuals (RMR; open circles) below the lower critical temperature (T_{lc}) as function of the temperature differential (ΔT) between body temperature (T_b) and air temperature (T_a). The equations for the regressions were $TMR = -0.08 + 0.13 \times \Delta T$, $r^2 = 0.61$, $p < 0.01$; $RMR = 0.17 + 0.18 \times \Delta T$, $r^2 = 0.92$, $p < 0.01$. Both the slope and the intercept of the two regressions were not significantly different ($p = 0.50$, $p = 0.31$, respectively).

3.4 Results for *Cercartetus nanus*

3.4.1 General Torpor Pattern

The MR and T_b of *C. nanus* showed pronounced fluctuations between high values during normothermia and low values during torpor (Fig. 3.7). Torpor usually started in the dark phase, after animals had been in the respirometry chambers for several hours. Entrance into torpor was initiated by a rapid decrease of MR, followed by a gradual decline in T_b . The steady-state TMR was usually reached after 3 - 5 h (Fig. 3.7). Torpor bout duration varied with T_a . Below T_a 25°C, torpor bouts usually lasted for several days (Fig. 3.7a). Above T_a 25°C, torpor bouts were usually shorter than one day, and T_b and TMR during steady-state torpor were more variable than at low T_a s (Fig. 3.7b). Torpor was periodically interrupted by spontaneous arousal, which often occurred in the afternoon, several hours before lights off. Arousal was characterised by a MR overshoot and a rise of T_b , followed by post-arousal with RMR and normothermic T_b of about 35°C usually lasting for only a few hours (Fig. 3.7).

3.4.2 Normothermia

The TNZ of normothermic *C. nanus* ranged from T_a $28.7 \pm 0.9^\circ\text{C}$ to $32.9 \pm 0.7^\circ\text{C}$, and the BMR was $0.66 \pm 0.17 \text{ mL g}^{-1} \text{ h}^{-1}$ ($\text{BM} = 36.0 \pm 7.5 \text{ g}$, $N = 7$, $n = 29$, Fig. 3.8a). The RMR increased linearly with a decreasing T_a ($r^2 = 0.86$, $p < 0.001$) between the T_{lc} and T_a 5°C (Fig. 3.8a). Below T_a 5°C, MR was more variable and increased at a higher rate because of the intensive shivering required for heat production (Fig. 3.8a). These values and corresponding T_b values were excluded from regression analyses.

T_b of normothermic individuals at rest both below and above the T_{lc} was relatively stable and independent of T_a (Fig. 3.8b). Below the T_{lc} , mean T_b was 33.9

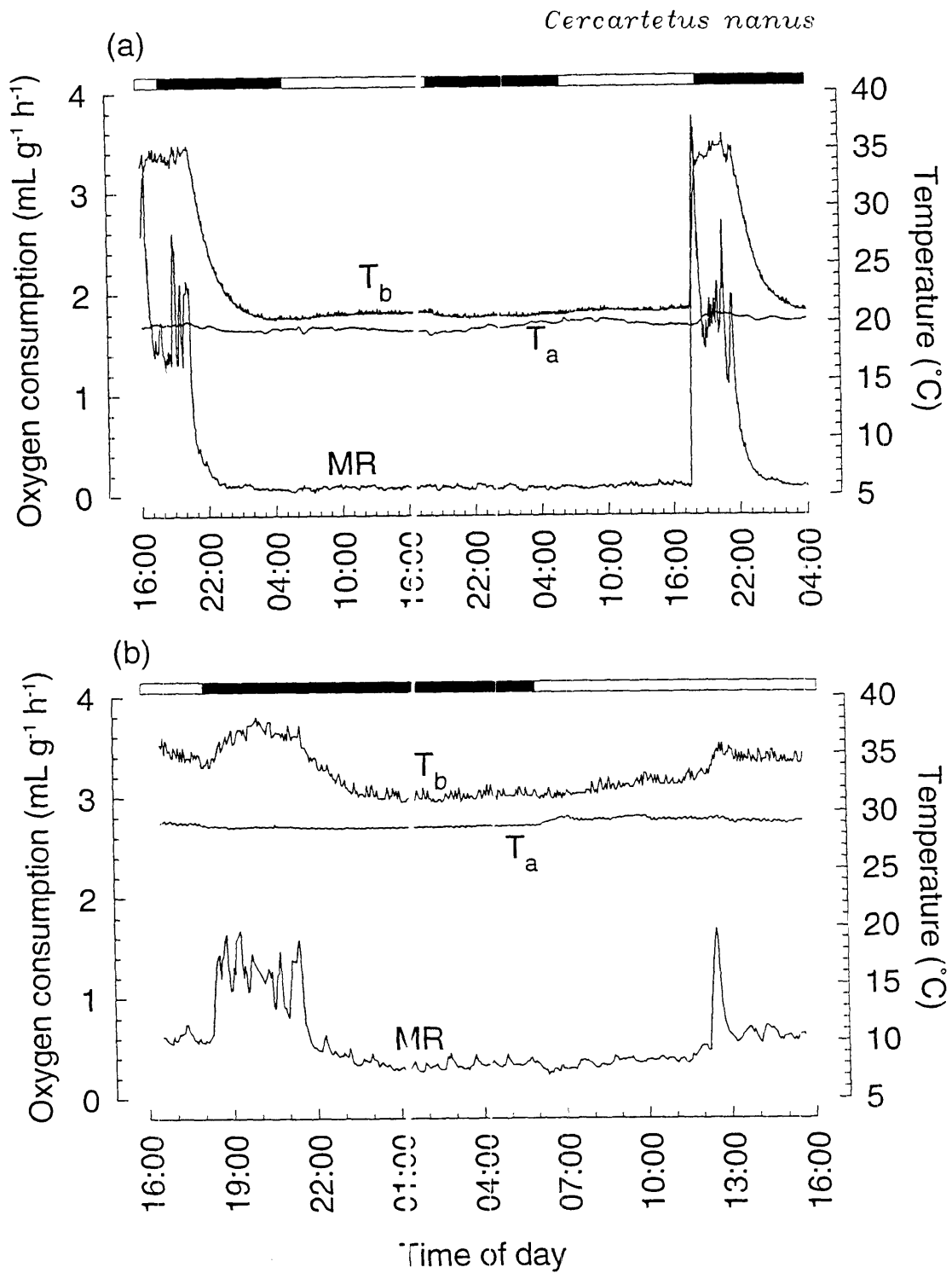


Fig. 3.7. Fluctuations of metabolic rate (MR) and body temperature (T_b) of a *C. nanus* at an air temperature (T_a) of (a) 20°C , and (b) 29°C . The dark bars indicate the dark phases.

$\pm 0.6^{\circ}\text{C}$ ($N = 8$, $n = 80$), and within the TNZ mean T_b was $34.3 \pm 0.4^{\circ}\text{C}$ ($N = 7$, $n = 29$; Fig. 3.8b).

ΔT ($T_b - T_a$) was negatively correlated with T_a both below the T_{lc} ($r^2 = 0.99$, $p < 0.001$) and within the TNZ ($r^2 = 0.80$, $p < 0.001$; Fig. 3.8c).

Above the T_{lc} , the C increased significantly with rising T_a ($r^2 = 0.75$, $p < 0.001$; Fig. 3.8d). Below the T_{lc} , C also showed a positive relationship with T_a , but the slope of the regression was much shallower ($r^2 = 0.16$, $p < 0.001$; Fig. 3.8d). The C at $T_a 5.8 \pm 0.5^{\circ}\text{C}$ was $0.110 \pm 0.015 \text{ mL g}^{-1} \text{ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$ ($N = 7$, $n = 14$). At low T_a s, C fluctuated markedly, consistent with variations of RMR (Fig. 3.8a, d).

3.4.3 Torpor

C. nanus displayed torpor during measurements at T_a s ranging from 5 to 30°C . When T_a was reduced below 5°C , animals remained torpid with a gradually decreasing T_b and MR. At the T_{tc} , MR increased to defend T_b . During steady-state torpor two different physiological responses to a change of T_a were observed. The animals showed proportional thermoregulation only below the T_{tc} of $4.8 \pm 0.7^{\circ}\text{C}$ ($N = 7$, $n = 87$; Fig. 3.8). The minimum T_b (T_{bmin}) at the T_{tc} was $5.9 \pm 0.7^{\circ}\text{C}$ and the corresponding minimum TMR (TMR_{min}) was $0.019 \pm 0.003 \text{ mL g}^{-1} \text{ h}^{-1}$ ($N = 7$, $n = 7$), which was 0.6% of the RMR at the same T_a .

Over the T_a s ranging from the T_{tc} to 30°C , TMR was positively related to T_a (Fig. 3.8a) and was better described by an exponential regression ($r^2 = 0.90$) than a linear fit ($r^2 = 0.74$). In this T_a range T_b linearly correlated with T_a ($r^2 = 0.99$, $p < 0.001$; Fig. 3.8b). The animals that entered torpor within the TNZ (above $T_a 28.7^{\circ}\text{C}$) had a TMR of $0.341 \pm 0.085 \text{ mL g}^{-1} \text{ h}^{-1}$ ($N = 6$, $n = 7$), and a T_b of $31.4 \pm 0.5^{\circ}\text{C}$ ($N = 6$, $n = 7$).

The ΔT also decreased with T_a above the T_{tc} ($r^2 = 0.26$, $p < 0.001$; Fig. 3.8c). However, this was mainly due to a significant relationship between ΔT and T_a at high

T_{as} from 23 to 30°C ($r^2 = 0.14$, $p < 0.05$). Below T_a 20°C to the T_{tc} , ΔT was not correlated with T_a ($r^2 = 0.0003$, $p > 0.05$, mean $\Delta T = 1.9 \pm 0.9^\circ\text{C}$), although in this T_a range TMR showed a significant decline ($r^2 = 0.58$, $p < 0.001$, $N = 8$, $n = 48$).

Above the T_{tc} , the C of torpid animals decreased with T_a (Fig. 3.8d). At the T_{tc} , the minimum C was $0.023 \pm 0.012 \text{ mL g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ ($N = 7$, $n = 7$), which was significantly lower than the minimum C of normothermic individuals in the same range of T_a ($p < 0.01$ t-test; Fig. 3.8d).

At T_{as} below the T_{tc} , TMR increased with decreasing T_a ($r^2 = 0.61$, $p < 0.01$; Fig. 3.8a). T_b was maintained relatively stable at $6.1 \pm 1.0^\circ\text{C}$ ($N = 7$, $n = 11$), which was 27.4°C lower than the normothermic value, and T_b was not correlated with T_a ($r^2 = 0.0004$, $p = 0.74$; Fig. 3.8b). ΔT increased with a decreasing T_a ($r^2 = 0.66$, $p < 0.01$; Fig. 3.8c). The C at T_a $1.3 \pm 0.6^\circ\text{C}$ (the lowest T_a measured) was $0.099 \pm 0.013 \text{ mL g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ ($N = 5$, $n = 5$). The regression coefficient for C vs T_a below the T_{tc} was not significant ($r^2 = 0.08$, $p > 0.1$; Fig. 3.8d). Moreover, the C at 1.3°C was similar to the mean C of resting individuals below the T_{tc} , although the TMR was only about 10% of RMR at the same T_a (Fig. 3.8a, d).

Above the T_{tc} at which animals in steady-state torpor showed no proportional thermoregulation, TMR was better explained by an exponential function of T_b ($r^2 = 0.92$, $p < 0.001$; Fig. 3.9a) than by a linear fit. Although the linear fit was also significant ($r^2 = 0.77$, $p < 0.001$; Fig. 3.9a). The Q_{10} for the reduction of steady-state MR over the T_{as} from the TNZ to the T_{tc} was 3.3. However, Q_{10} s varied at different T_{as} (Table 3.1). When TMR as function of T_b was presented and analysed in an Arrhenius plot, two linear regressions with a transition at T_b 20.2°C provided the best fit (Fig. 3.9b). Below T_b 20.2°C , Q_{10} was 1.9, above T_b 20.2°C , Q_{10} was 3.7.

The relationships between TMR and ΔT and between TMR and C measured above the T_{tc} differed at lower (from T_{tc} to 20°C) and higher (from 23 to 30°C) T_{as} (Fig. 3.10). In the lower T_a range, the TMR was not correlated with ΔT ($r^2 = 0.03$, $p >$

Table 3.1 Q_{10} s for MR between different T_a s in *Cercartetus nanus*

State	1 normothermic in the TNZ	2 torpid in the TNZ	3 torpid	4 torpid at the T_{tc}
T_a (°C)	30.5	29.0	19.8	5.0
T_b (°C)	34.3	31.4	21.0	6.1
MR (mL g ⁻¹ h ⁻¹)	0.655	0.341	0.106	0.022
	$Q_{10} (1 - 2) = 9.5$	$C_{10} (2 - 3) = 3.1$	$Q_{10} (3 - 4) = 2.8$	
	$Q_{10} (1 - 3) = 3.9$	$C_{10} (2 - 4) = 3.0$		
	$Q_{10} (1 - 4) = 3.3$			

0.1; Fig. 3.10a). The lack of the relationship between TMR and ΔT below T_a 20°C, where TMR declined with T_a (Fig. 3.8a), was most likely explained by the significant relationship between C and TMR ($r^2 = 0.43$, $p < 0.001$; Fig. 3.10b). In the higher T_a range, TMR was significantly related to ΔT ($r^2 = 0.34$, $p < 0.001$; Fig. 3.10a), most likely this was explained by the fact that C was not correlated with TMR ($r^2 = 0.01$, $p > 0.1$; Fig. 3.10b).

In the T_a range below the T_{tc} where T_b was regulated and relatively stable, TMR of hibernating individuals was not correlated with T_b ($r^2 = 0.07$, $p > 0.1$; Fig. 3.11a), as during normothermia ($r^2 = 0.03$, $p > 0.05$; Fig. 3.11a). The Q_{10} for MR between normothermic and hibernating thermoregulating animals during hibernation and during normothermia at a T_a of 1.5°C was 2.2 for values derived from regressions, and 2.7 for measured values (Fig. 3.12). Below the T_{tc} , TMR was a linear function of ΔT ($r^2 = 0.91$, $p < 0.001$; Fig. 3.11b). Both the slope and the intercept for the regression of MR vs ΔT in hibernating and normothermic animals were indistinguishable ($p > 0.05$, t-test; Fig. 3.11b). Below the T_{tc} , TMR was also positively related to C ($r^2 = 0.59$, $p < 0.01$; Fig. 3.11c), in contrast to the situation during normothermia where RMR showed no relationship with C ($r^2 = 0.04$, $p = 0.05$; Fig. 3.11c).

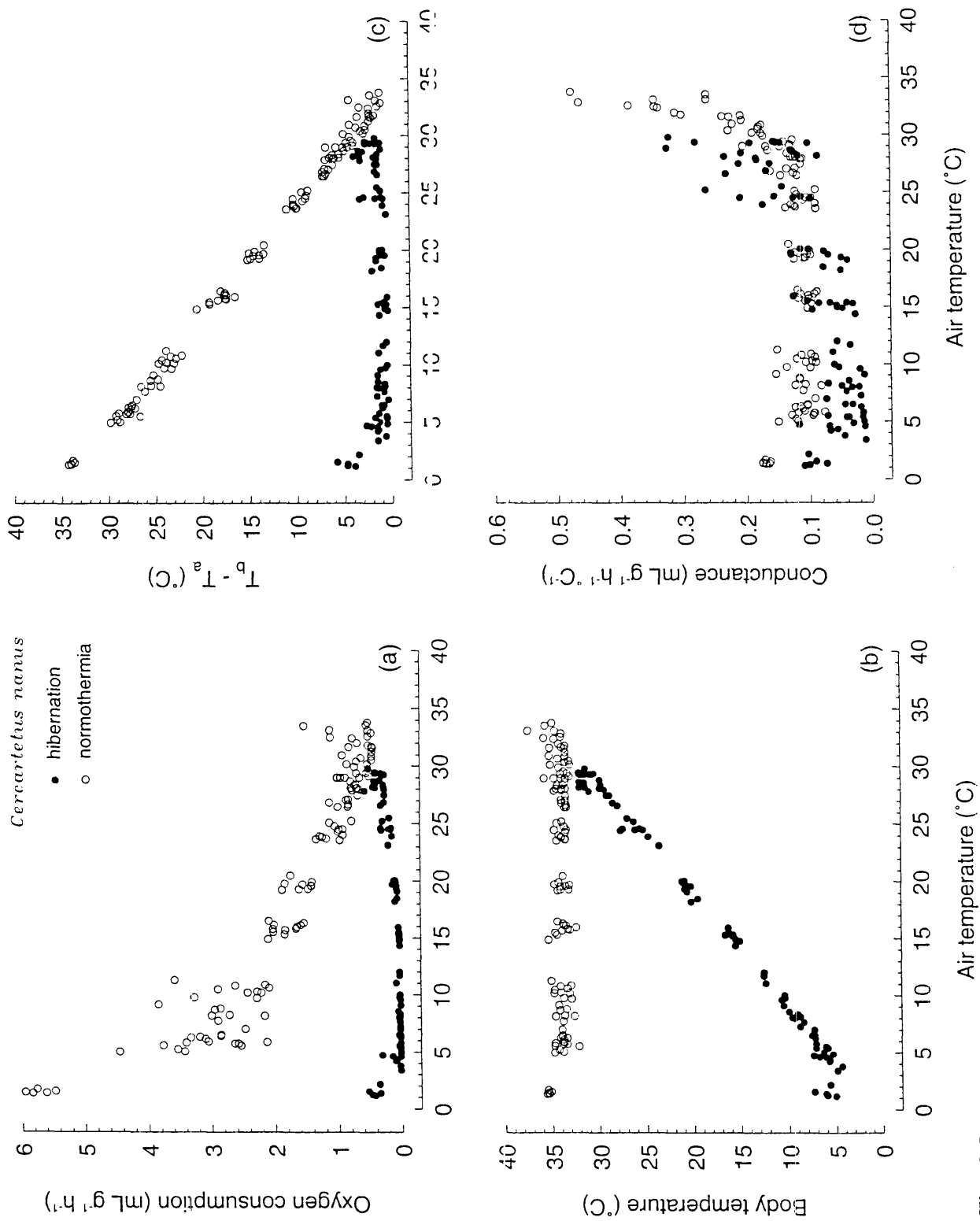


Fig. 3.8.

Fig. 3.8. (previous page) The effect of air temperature (T_a) on (a) metabolic rate (MR) measured as rate of oxygen consumption, (b) body temperature (T_b), (c) temperature differential (ΔT) between T_b and T_a , and (d) apparent thermal conductance (C) during hibernation (filled circle) and normothermia (open circle) in *C. nanus*. Mean body mass was 36.2 ± 5.8 g. Regression equations for physiological variables during normothermia between T_a 5°C and the lower critical temperature during normothermia (T_{lc}) ($N = 8$, $n = 80$) were:

$$\text{RMR} = 3.69 - 0.106 \times T_a, r^2 = 0.86, p < 0.001.$$

$$T_b = 33.9 - 0.005 \times T_a, r^2 = 0.004, p = 0.56.$$

$$\Delta T = 33.9 - 1.00 \times T_a, r^2 = 0.99, p < 0.001.$$

$$C = 0.076 + 0.002 \times T_a, r^2 = 0.16, p < 0.001.$$

Regression equations for physiological variables during normothermia in the thermoneutral zone (TNZ) ($N = 7$, $n = 29$) were:

$$\text{RMR} = 1.92 - 0.041 \times T_a, r^2 = 0.14, p < 0.05.$$

$$T_b = 30.2 + 0.129 \times T_a, r^2 = 0.05, p = 0.13.$$

$$\Delta T = 30.2 - 0.872 \times T_a, r^2 = 0.80, p < 0.001.$$

$$C = -1.00 + 0.041 \times T_a, r^2 = 0.75, p < 0.001.$$

Regression equations for physiological variables above the critical temperature during torpor (T_{tc}) ($N = 8$, $n = 76$) were:

$$\log \text{TMR} = -1.99 + 0.052 \times T_a, r^2 = 0.90, p < 0.001.$$

$$T_b = 0.527 + 1.05 \times T_a, r^2 = 0.99, p < 0.001.$$

$$\Delta T = 0.529 + 0.046 \times T_a, r^2 = 0.26, p < 0.001.$$

$$C = -0.029 + 0.008 \times T_a, r^2 = 0.17, p < 0.001.$$

Regression equations for physiological variables during torpor below the T_{tc} ($N = 7$, $n = 11$) were:

$$\text{TMR} = 0.557 - 0.094 \times T_a, r^2 = 0.61, p < 0.01.$$

$$T_b = 5.81 + 0.074 \times T_a, r^2 = 0.0004, p = 0.74.$$

$$\Delta T = 5.81 - 0.926 \times T_a, r^2 = 0.66, p < 0.01.$$

$$C = 0.103 - 0.007 \times T_a, r^2 = 0.08, p = 0.22.$$

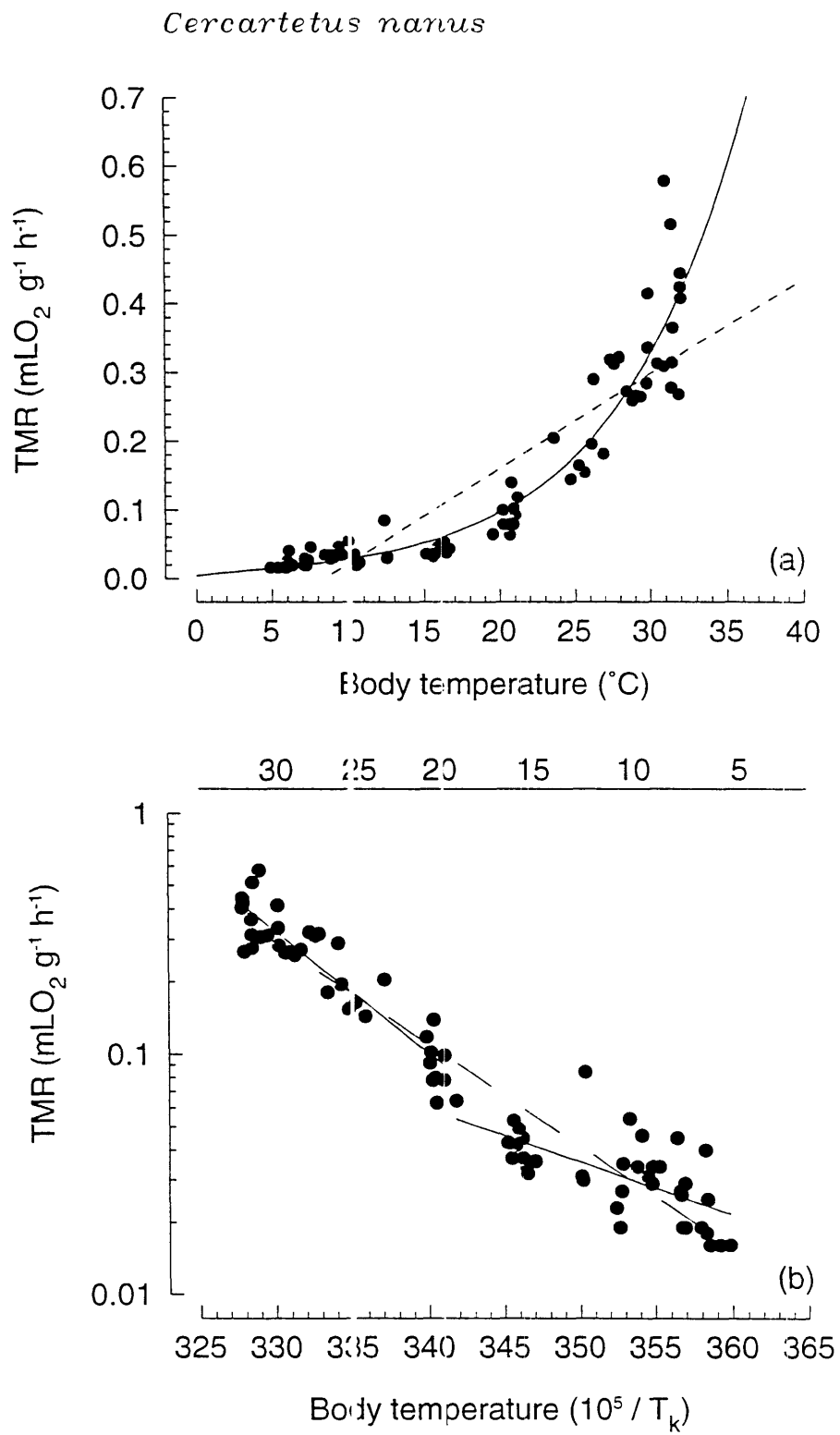


Fig. 3.9.

Fig. 3.9. (previous page) The metabolic rate during torpor (TMR) as a function of body temperature (T_b) above the critical air temperature during torpor (T_{tc}) in hibernating *C. nanus*.

(a) Both linear regression (dashed line: $TMR = -0.116 + 0.014 \times T_b$, $r^2 = 0.77$, $p < 0.001$) and exponential fit (solid line; $\log TMR = -2.02 + 0.05 \times T_b$, $r^2 = 0.92$, $p < 0.001$) were significant. However, the exponential fit provided the appropriate model, because the r^2 for the regression of the predicted TMR for the exponential model versus the measured TMR ($r^2 = 0.89$) was larger than that for the linear model ($r^2 = 0.77$).

(b) The slope of the regressor of TMR vs T_b in an Arrhenius plot (solid lines) was steeper at high T_b ($\log TMR = 16.2 - 0.0506 \times 10^5 / T_k$, $r^2 = 0.85$, $p < 0.001$, $N = 8$, $n = 39$) than at low T_b ($\log TMR = 6.22 - 0.0219 \times 10^5 / T_k$, $r^2 = 0.41$, $p < 0.001$, $N = 8$, $n = 37$). The dashed line shows the regression when the values were fitted with one regression ($\log TMR = 13.4 - 0.0422 \times 10^5 / T_k$, $r^2 = 0.91$, $p < 0.001$, $N = 8$, $n = 76$). The two line fit was better than the single line fit since the former had the smallest sum of the residual sum of squares.

Cercartetus nanus

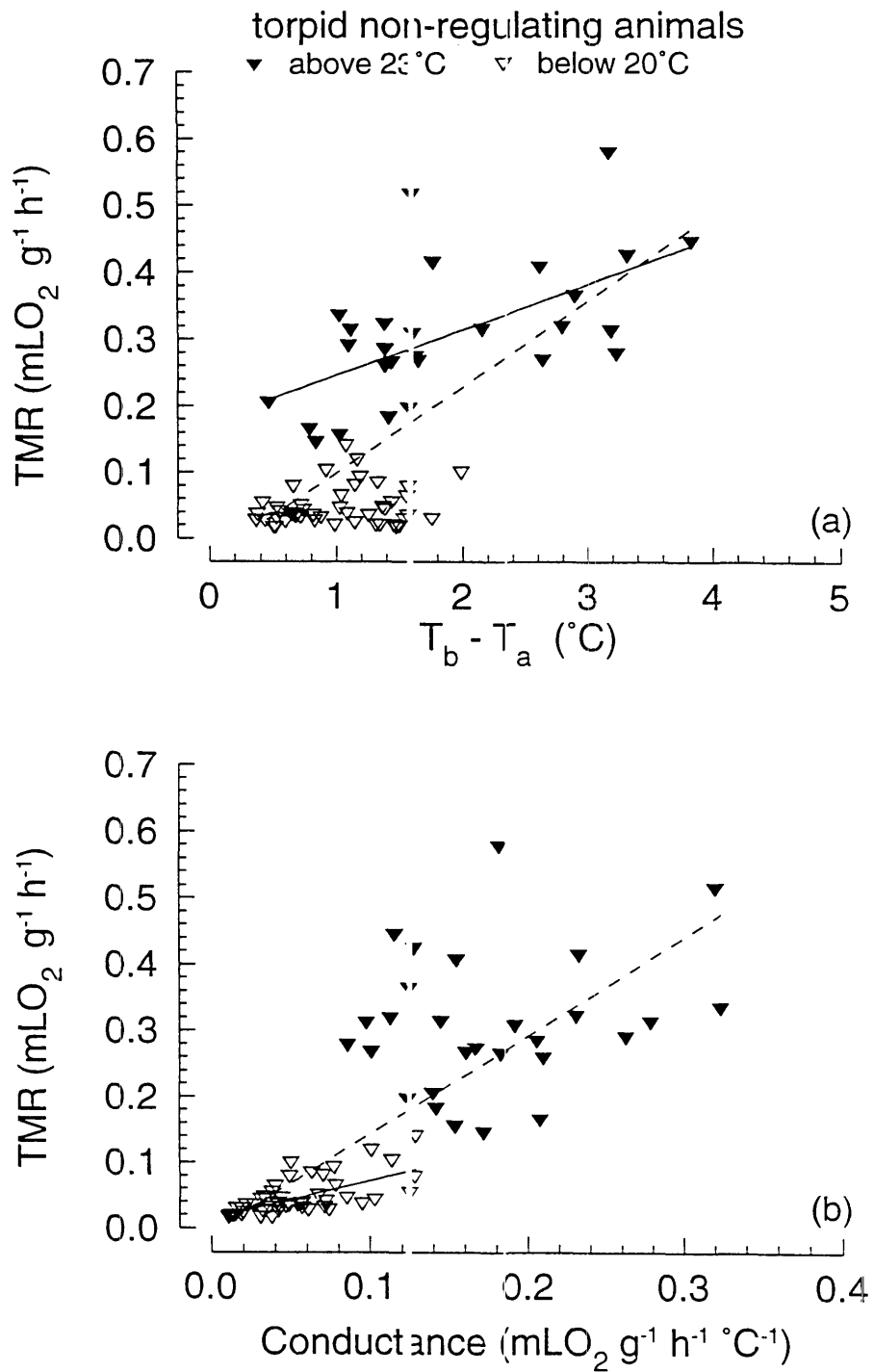


Fig. 3.10.

Fig. 3.10. (previous page) The metabolic rate during torpor (TMR) of *C. nanus* as a function of (a) temperature difference (ΔT) between body temperature (T_b) and air temperature (T_a), and (b) apparent thermal conductance (C) at T_a s above the critical temperature during torpor (T_{tc} ; both symbols, dashed line).

The regression equations were: $TMR = -0.03 + 0.129 \times \Delta T$ ($r^2 = 0.48$, $p < 0.001$, $N = 8$, $n = 76$). $TMR = 0.104 + 0.326 \times C$ ($r^2 = 0.14$, $p < 0.001$, $N = 8$, $n = 76$).

In the low T_a range (5 - 20°C open triangles) TMR was not related to ΔT : $TMR = 0.031 + 0.015 \times \Delta T$ ($r^2 = 0.03$, $p = 0.14$, $N = 8$, $n = 48$), but was significantly correlated with C : $TMR = 0.014 + 0.59 \times C$ ($r^2 = 0.43$, $p < 0.001$, $N = 8$, $n = 48$). In the high T_a range (23 - 30°C, filled triangle), TMR was significantly correlated with ΔT : $TMR = 0.177 + 0.068 \times \Delta T$ ($r^2 = 0.34$, $p < 0.001$, $N = 8$, $n = 28$), but was not related to C : $TMR = 0.319 - 0.057 \times C$ ($r^2 = 0.01$, $p = 0.49$, $N = 8$, $n = 28$).

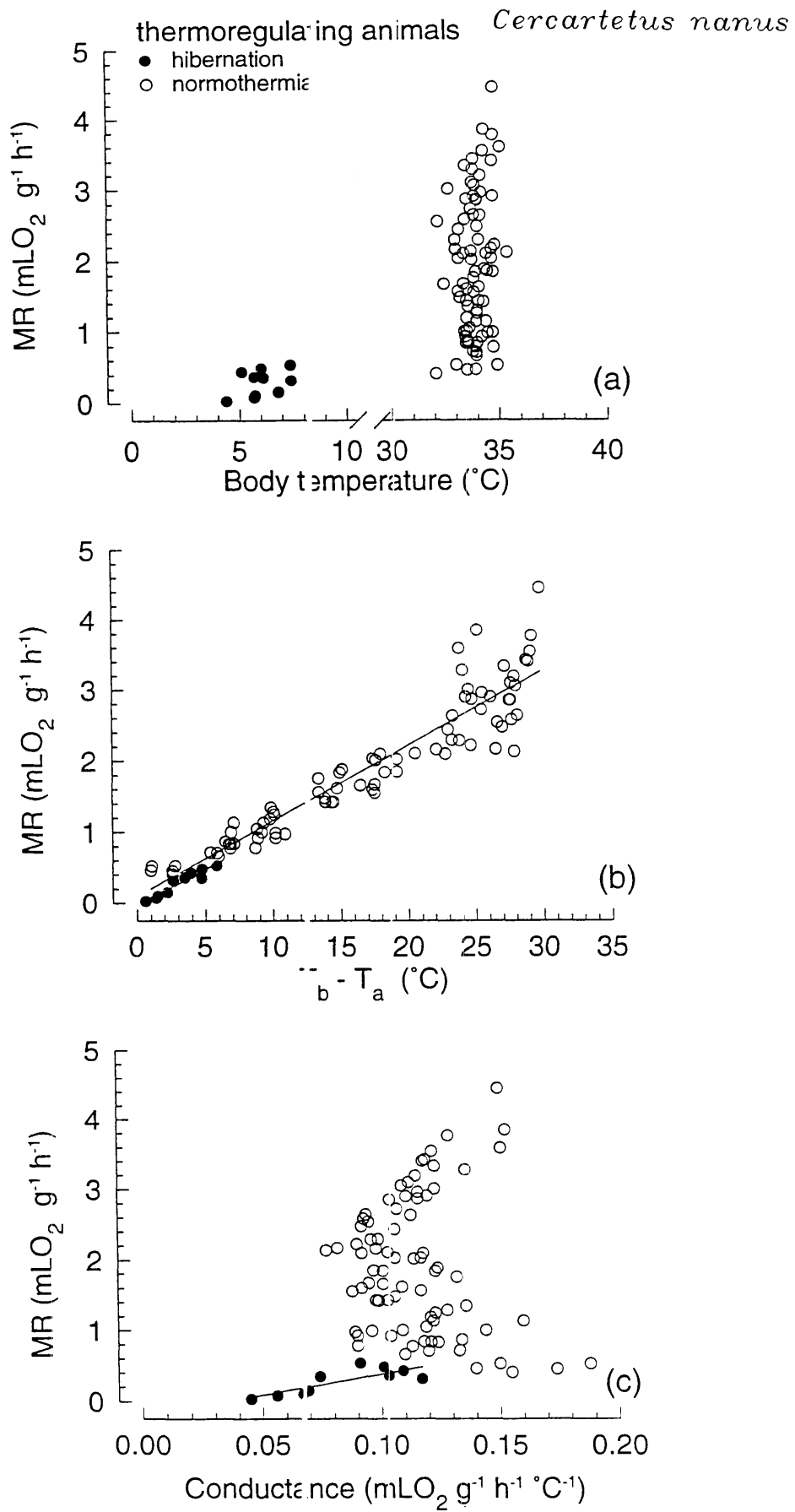


Fig. 3.11.

Fig. 3.11. (previous page) The metabolic rate during torpor (TMR) (filled circles, $N = 8$, $n = 11$) below the critical air temperature (T_{1c}), and the resting metabolic rate (RMR) of normothermic *C. nanus* (open circles, $N = 8$, $n = 80$) below the lower critical temperature (T_{lc}) as a function of (a) body temperature (T_b), (b) temperature differential (ΔT) between T_b and air temperature (T_a), and (c) apparent thermal conductance (C).

Neither TMR nor RMR of thermoregulating *C. nanus* was related to T_b :

$$\text{TMR} = -0.191 + 0.079 \times T_b \quad (r^2 = 0.07, p = 0.23).$$

$$\text{RMR} = -8.67 + 0.313 \times T_b \quad (r^2 = 0.03, p = 0.07).$$

Both TMR and RMR were significantly related to ΔT :

$$\text{TMR} = -0.032 + 0.101 \times \Delta T \quad (r^2 = 0.91, p < 0.001).$$

$$\text{RMR} = 0.096 + 0.106 \times \Delta T \quad (r^2 = 0.88, p < 0.001).$$

TMR was also positively related to C :

$$\text{TMR} = -0.208 + 5.89 \times C \quad (r^2 = 0.59, p < 0.01).$$

whereas RMR was not related to C :

$$\text{RMR} = 2.41 - 3.89 \times C \quad (r^2 = 0.04, p = 0.05).$$

3.5 Discussion

3.5.1 Two Responses of TMR to T_a

Both torpid *C. nanus* and *S. macroura* showed two clearly different responses of TMR to a change of T_a . Above the T_{tc} , when T_b of torpid animals was above the set-point for T_b during torpor, T_b was not defended and both TMR and T_b fell with T_a . Below the T_{tc} , when T_b of torpid animals was metabolically defended, TMR was inversely related with T_a . Similar observations have been reported for many mammalian hibernators (Hock 1951; Davis and Reite 1967; Heller and Colliver 1974; Geiser and Kenagy 1988; Song and Zeng 1991) and for daily torpor in mammals and birds (Hainsworth and Wolf 1970; Nagel 1985; Geiser and Baudinette 1987). This suggests that the TMRs of heterothermic endotherms in the two T_a ranges are due to different physiological responses. The differences between these two responses must be kept in mind when interrelations between physiological variables measured during torpor are to be investigated.

3.5.2 Thermoregulation during Torpor

Below the T_{tc} of about 6°C, torpid *C. nanus* showed the capacity for T_b regulation. Similarly, the core T_b of torpid *S. macroura* was also regulated below the T_{tc} , although at a much higher value of about 14°C. The metabolic heat production in this T_a range was proportional to ΔT in both species, demonstrating that torpid animals compensated for the increased heat loss at low T_a s by proportional thermoregulation.

Metabolic defence of T_b during torpor has also been demonstrated for other heterothermic endotherms (Hainsworth and Wolf 1970; Wolf and Hainsworth 1972; Heller and Hammel 1972; Florant and Heller 1977; Heldmaier and Ruf 1992). As in *C. nanus* and *S. macroura*, the onset of thermoregulation of torpid mammals is stimulated when the set-point for T_b is approached (Heller and Colliver 1974; Florant et al. 1978). This capacity for proportional thermoregulation during torpor is one of

the principal differences between torpor in endotherms and that in ectotherms (Lyman et al. 1982).

Since in each species used for this study, both the slope and the intercept of TMR as function of ΔT in torpid and normothermic animals were similar, it appears that the physiological processes underlying thermoregulation at low T_{as} during torpor are the same as those during normothermia. However, thermoregulation during torpor may differ in some aspects from that during normothermia. For example, although T_b of both normothermic and torpid thermoregulating *S. macroura* was independent of T_a , it elevated slightly at very low T_{as} . Heller and Colliver (1974) have reported a similar phenomenon in hibernating ground squirrels, *Spermophilus lateralis*. The T_b of torpid thermoregulating hummingbirds is also slightly higher at low T_{as} (Hainsworth and Wolf 1970). Thermoregulation during torpor seems less precise than during normothermia, since T_b during torpor was maintained over a relatively wide range. This response is especially obvious during daily torpor of *S. macroura* with a T_b regulated between 16 and 28°C. These observations suggest a relatively variable set-point for T_b during torpor, as has been reported for other species (Heller and Colliver 1974).

Another characteristic of torpid individuals is that the contribution of C to thermoregulation during torpor differs to its contribution during normothermia. Below the T_{lc} , C of normothermic resting individuals was low and decreased slightly with T_a . This ensures a minimum heat loss to keep a constant T_b in the cold. In contrast, in torpid thermoregulating individuals, C increased substantially, together with increasing thermogenesis. Since the curled-up posture of the animals at low T_{as} indicated that they were trying to minimise heat loss, it is most likely that shivering, and the increased respiratory and circulatory activities, which were concomitant with the increasing MR for thermoregulation, resulted in an unavoidable increase in C . This explanation also applies to normothermic *C. nanus* at T_{as} below 5°C, in which C

increased significantly with the onset of shivering, suggesting that shivering thermogenesis may be very important in marsupials, as they appear to possess no brown adipose tissue (Hayward and Lisson 1992). However, changes of C with thermoregulation in torpor could also involve changes in peripheral circulation, caused by elevation in blood pressure, and therefore increased blood distribution into the periphery during thermoregulation at low T_{as} .

Although for both species the C of torpid thermoregulating animals at low T_{as} was similar to that during normothermia, the TMR was only 7 - 12 % of RMR in *C. nanus* and 25 - 40 % of RMR in *S. macroura*. This demonstrates that a C below that found in normothermic individuals is not a prerequisite for a low TMR, or for thermoregulation during either hibernation or daily torpor. Nevertheless, C of non-thermoregulating torpid animals at low T_{as} is low, which prevents the onset of thermoregulation, and thus high TMRs of thermoregulation during torpor.

3.5.3 Reduction of MR during Daily Torpor

In the T_a range in which T_b was not metabolically defended, TMR of *S. macroura* showed an exponential relationship with T_b . Since the Q_{10} s between BMR and TMR over this T_a range was between 2 and 3, it is likely that the reduction of TMR below the BMR was mainly caused by a temperature effect (Fig. 3.12). Furthermore, the drop of MR from normothermia to torpor in the thermoregulating animals seems also to be explained by a T_b effect (Fig. 3.12). Therefore, the results of this study support the claim of Malan (1993) that the principle of thermodynamics should act as a general law that governs the biochemical processes of animals, since energetic processes of a living organism rely on enzyme catalysed reactions.

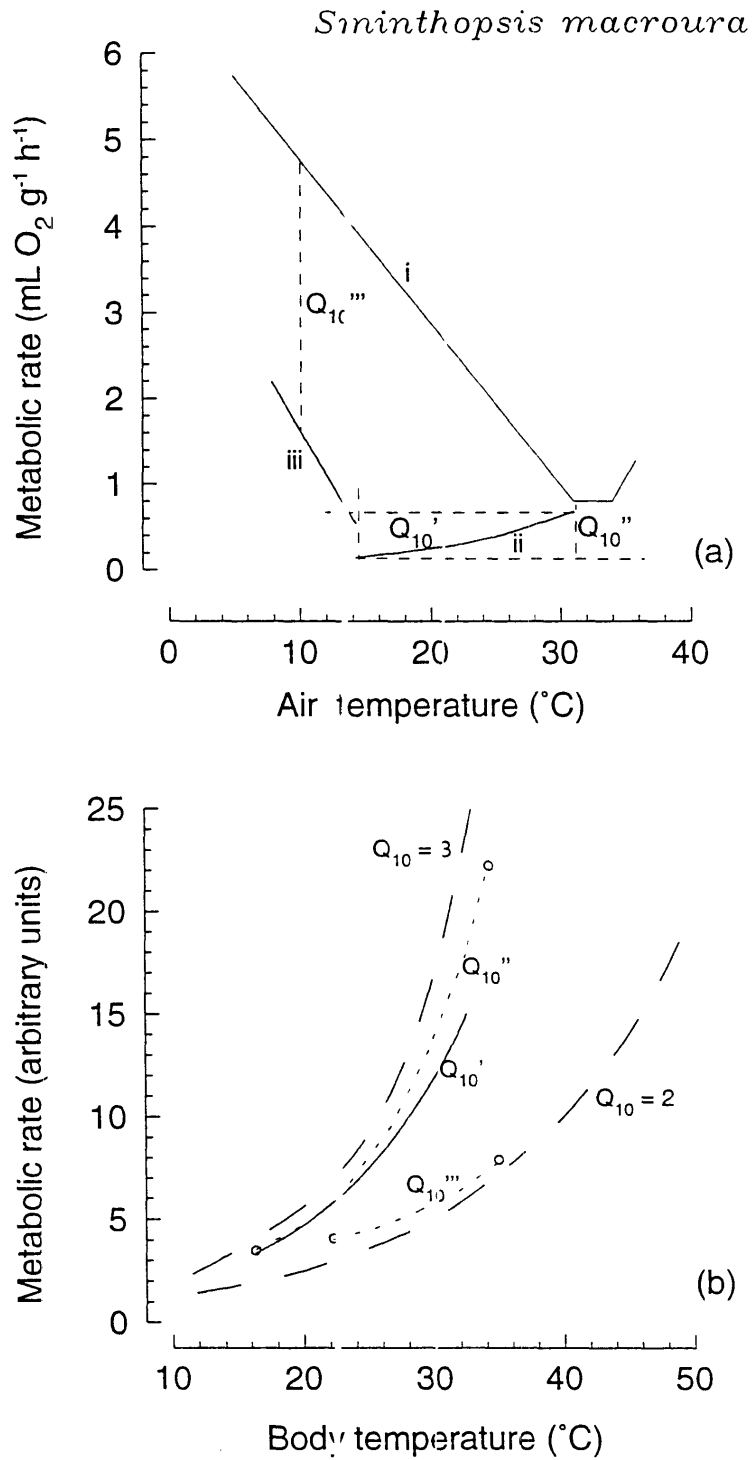


Fig. 3.12.

Fig. 3.12. (previous page)

(a) Regressions describing changes of metabolic rate (MR), measured as mass-specific oxygen consumption, as function of air temperature (T_a) of *S. macroura*. The metabolic rate of normothermic resting individuals (RMR) increased linearly with decreasing T_a : $RMR = 6.68 - 0.19 \times T_a$

(i). The metabolic rate of torpid individuals (TMR) above the critical T_a decreased exponentially with decreasing T_a : $TMR = 0.039 \times 10^{0.04 \times T_a}$

(ii). TMR of thermoregulating animals increased linearly with decreasing T_a : $TMR = 4.24 - 0.26 \times T_a$ (i i).

(b) The effect of temperature on MR increasing with a Q_{10} of 2 or 3 in comparison with the effect of T_b on MR of *S. macroura*. As illustrated in (a), Q_{10}' for TMR from body temperature (T_b) 16.0°C at a T_a of about 14°C to T_b 32.0°C at a T_a of about 30°C; Q_{10}'' for BMR between the basal metabolic rate (BMR) at T_b 34.3°C and TMR at T_b 16.0°C at a T_a of about 14°C; Q_{10}''' for MR between RMR at T_b 34.9°C and TMR at T_b 22.7°C at a T_a of 10°C. Open circles for BMR and RMR values; closed circles and solid line for Q_{10}' s between TMRs; dashed lines for Q_{10}'' s between RMR and TMR or between BMR and TMR.

The effect of temperature on enzyme and tissue metabolism is well documented for both ectothermic and endothermic organisms (Roberts and Smith 1967; Geiser and McMurchie 1984; Aloia and Raison 1989). In agreement with the values determined *in vitro*, the Q_{10} s of mammalian TMRs are generally within the range of 2 - 3 (Tucker 1965; Tähti 1978; Snapp and Heller 1981), as are the thermal responses of most ectotherms and that of a poikilothermic mammal (Buffenstein and Yahav 1991; Hislop and Buffenstein 1994). Nevertheless, Q_{10} s above this range have been observed for TMRs, and metabolic inhibition, in addition to temperature effects, has been proposed to explain the further reduction of TMR (Malan 1986, 1988; Geiser 1988a). However, as has been pointed out by Snapp and Heller (1981), some of the Q_{10} values above the "normal" range can also be due to experimental artifacts, such as "BMRs" measured below the TNZ or determination of TMR during apnea. Moreover, illogical or inconsistent calculations comparing thermoregulating and non-thermoregulating individuals for calculating Q_{10} s for TMRs as practised by Snyder and Nestler (1990) may lead to values that are not in the predicted range. The logic behind applying Q_{10} to endotherms is unsound only when basic rules for calculation of Q_{10} as an expression of the direct effect of temperature on rates (Schmidt-Nielsen 1990) are violated.

The ΔT at T_{as} above the T_{tc} of *S. macroura* was constant and was not related to TMR. These findings do not support the interpretations of Heldmaier and Ruf (1992) who suggested that TMR does not depend on T_b but is always a function of ΔT . However, in their study all values of TMR, most of which were determined below the set-point for T_b during torpor, were analysed as a single data set and, therefore, do not explain the thermal response of TMR of non-thermoregulating animals above the set-point.

The minimum C of both normothermic and torpid *S. macroura* were close to those calculated for similar sized rodents (Snyder and Nestler 1990). The C of torpid

animals was similar to that of normothermic inactive animals at high T_a s below the T_{lc} , but it decreased with T_a in non-thermoregulating torpid animals. As mentioned above, this lowered C may prevent T_b from reaching the set-point during torpor, which would induce an increase of TMR. Superficially, these findings contrast with those by Heldmaier and Ruf (1992), who reported similar conductance in torpid and normothermic hamsters, *Phodopus sungorus*. However, their measurements concentrated on T_a s below the set-point for T_b . At very low T_a s, C of torpid *S. macroura* was also similar to that of normothermic individuals as is the case of *P. sungorus* (Heldmaier and Ruf 1992).

3.5.4 Reduction of MR during Hibernation

The decrease of TMR and T_b with T_a in torpid *C. nanus* above T_{tc} clearly differed from that of thermoregulating individuals. In this T_a range, TMR was an exponential function of T_b , suggesting that the reduction of TMR is dependent of T_b . This is similar to the situation of non-thermoregulating *S. macroura* during daily torpor. Furthermore, a Q_{10} of 2.2 to 2.7 (Fig. 3.13) for MR decline from normothermic to torpid thermoregulating animals at the same T_a suggests that even this response to temperature is caused by the reduction of T_b , as found in other hibernators (Heller and Colliver 1974; Florant et al. 1978) and in daily torpor of *S. macroura*.

However, the reduction of MR during hibernation in *C. nanus* was more pronounced than in torpid *S. macroura*. Thus the overall reduction of MR from BMR to TMR_{min} of *C. nanus* revealed a Q_{10} (Table 3.1) that was above the range for typical biochemical reactions (i.e. Q_{10} above 3.0), suggesting that temperature effects alone are not sufficient to explain all of the reduction of TMR during hibernation. This is different from *S. macroura*, in which the overall MR reduction appears to be largely a function of the lowered T_b .

Q_{10} values above the range of 2 - 3 have also been reported in other hibernating species (Morrison 1962; Henshaw 1968; Geiser 1988a), and are evident for a synergistic effect of metabolic inhibition and temperature effects on TMR (Malan 1986; Geiser 1988a; Storey and Storey 1990). Since Q_{10} s greater than 2 - 3 were observed in *C. nanus* only at higher T_b s, especially in the TNZ (Table 3.1), it seems that Q_{10} s for MRs differ at different T_b s (Henshaw 1968; Geiser 1988a), and that the metabolic inhibition may be more pronounced during the transient state between normothermia and torpor when T_b is high (Geiser 1988a; Malan 1993).

The use of metabolic inhibition at low T_b s appears to be more pronounced in small than in large hibernators, because the extent of metabolic reduction is mass-dependent (Geiser 1988a). The apparent metabolic inhibition in hibernators could be caused by a number of mechanisms. The extensive respiratory acidosis and pH alterations have been proposed to be critical for the initiation of the metabolic inhibition during hibernation (Malan 1986; Milsom 1993). Alternatively, the reversible phosphorylation, and switching to different metabolic pathways also play important roles in reducing metabolism during hibernation (Storey and Storey 1990; Guppy et al. 1994). In addition, part of the substantial reduction of TMR during hibernation may also be explained by the higher activation energy, and thus Q_{10} , of some enzymes of hibernators than those of daily heterotherms (Raison and Lyons 1971; Geiser and McMurchie 1984). Differences in the response of cell membrane to cold between hibernation and daily heterotherms have been demonstrated and provide a possible explanation as to why hibernators allow their T_b drop to T_a while daily heterotherms only allow a much smaller drop of T_b (Geiser and McMurchie 1984).

Interestingly, *C. nanus* displayed torpor in the TNZ. The TMR of torpid individuals dropped by nearly 50% from BMR, while T_b dropped by only 3°C and therefore, a very large Q_{10} was observed (Table 3.1). This is clear evidence for largely temperature-independent metabolic inhibition in endotherms. This result also

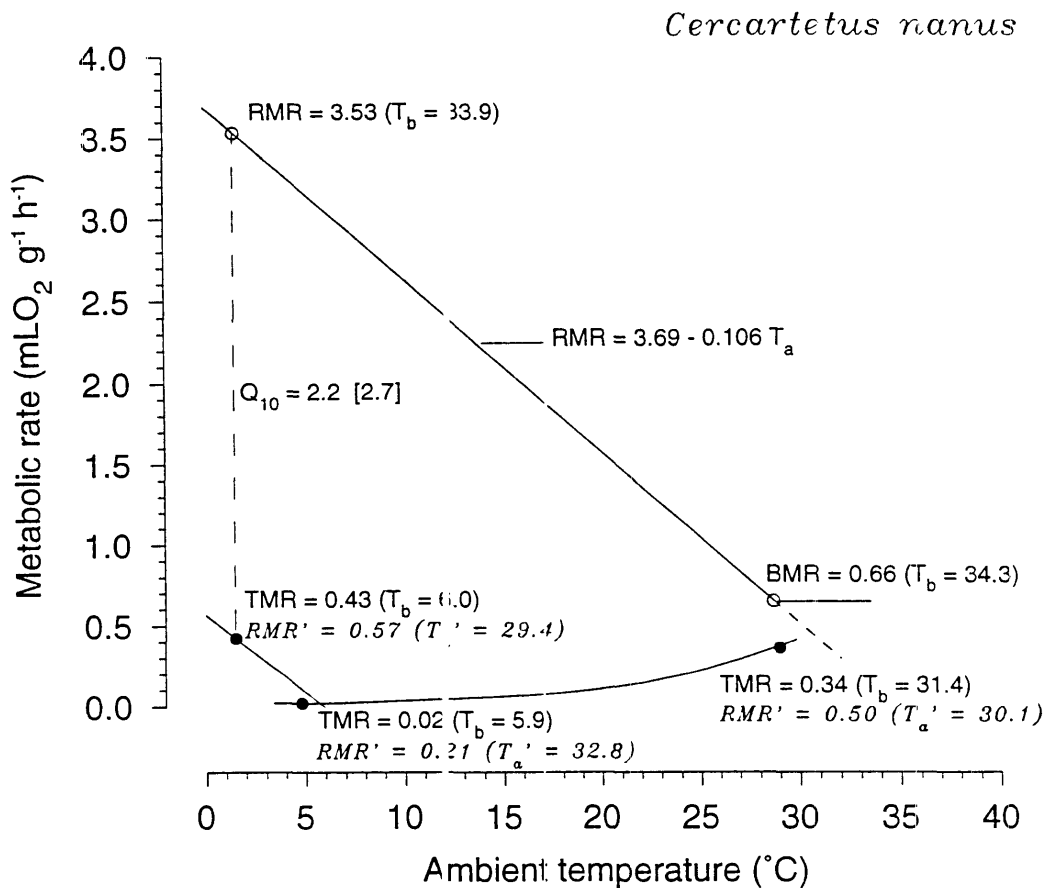


Fig. 3.13. Comparison between the metabolic rate during torpor (TMR). The predicted metabolic rate (RMR' , as shown in italics) for these TMR given the same differential between body temperature (T_b) and air temperature (T_a) of *C. nanus*. RMR' was calculated from the equation: $RMR' = 3.69 - 0.106 \times T_a'$. The T_a' was determined by using the formula: $T_a' = T_b' - (T_{b1} - T_{a1})$, where T_b' is the normothermic value, T_{b1} and T_{a1} the values during torpor, at which TMR was measured. Examples for the measurements below the set-point, at the critical air temperature, and in the thermoneutral zone showed that all derived RMR' were significantly larger than the TMR. The Q_{10} s for MR between thermoregulating animals during normothermia and during torpor were calculated for both measured data ($Q_{10} = 2.7$) and the data derived from regressions of MR vs T_a given in Fig. 3.8a ($Q_{10} = 2.2$).

shows that hibernation is not always initiated by cessation of heat production for normothermic thermoregulation and the following lowered T_b as commonly accepted (Bartholomew 1982; Withers 1992). In contrast, hibernation can also be initiated by the reduction of MR from BMR. It suggests that metabolic inhibition may play a very important role in the transition from normothermic thermoregulation to MR reduction during entry into hibernation.

The observation that ΔT of *C. nanus* was stable over a range of T_a in which TMR showed a significant decline clearly rejects the hypothesis that TMR is determined by ΔT (Heldmaier and Ruf 1992; Heldmaier et al. 1993). This hypothesis also fails to explain the temperature-dependence of TMR above the T_{tc} and the reduction of MR from RMR to TMR (Fig. 3.13). If the TMR was essentially a downregulated RMR with a low ΔT (Heldmaier 1992; Heldmaier et al. 1993), it should be a linear function of T_b , which it is clearly not. Moreover, a decrease of T_b should influence MR in the same manner as an increase in T_a by the same value (i.e. RMR and TMR with the same ΔT should be equal; Fig. 3.13). When values were derived using this assumption (Fig. 3.13) for TMR below the T_{tc} , the TMR_{min} , and the TMR within the TNZ, all were significantly higher (33%, 10-fold, 47%, respectively) than the measured values.

In the high T_a range (T_a 23 - 30°C), C of *C. nanus* was high and did not change with the fall of TMR (Fig. 3.8d; Fig. 3.10b). This implies that while thermogenesis was inhibited, heat loss was probably also facilitated via a high C at high T_b s. In the low T_a range, however, TMR was positively related to C (Fig. 3.10b). Nevertheless, it is most likely that C did not cause the TMR decline, but changed passively as a consequence of the changing TMR. The low TMR should result in a significantly decreased peripheral circulation and a decreased respiratory heat loss in association with the low T_b . This interpretation is supported by the finding that exposure to the highly conductive medium, He-O₂ (79% Helium and 21% Oxygen), of torpid *S. macroura* resulted in a fall of TMR but not a change of C . In this case a lowered

peripheral circulation appeared to compensate for the direct effect of the more conductive medium on C (Geiser et al. 1996). However, as during daily torpor in *S. macroura*, the lowered C at the low TMR in *C. nanus* may prevent T_b from reaching the set-point during torpor.

The increase of TMR with T_a during torpor of *C. nanus* above T_{tc} also differs from the thermoregulatory response of normothermic individuals above T_{lc} , although both show an increase of C . Above the T_{lc} , ΔT decreased while C showed a steep increase with T_a to avoid overheating and to maintain T_b at a normothermic level. In contrast, the increase of C during torpor above T_{tc} was accompanied by a rise in both T_b and TMR. A high C at high T_a s during torpor is not used for maintenance of a constant T_b but appears to reduce T_b , and thus TMR.

In summary, during torpor at T_a s below and above the T_{tc} , two different physiological processes are responsible for the responses of MR to T_a . Below the T_{tc} , torpid animals regulated their T_b with an increase of MR, in proportional to ΔT . This is similar to the thermoregulation during normothermia, although T_b during torpor was regulated at substantially low levels. Above the T_{tc} , the reduction of MR with T_a in *S. macroura* largely reflects the effect of T_b on biological reaction rates. In *C. nanus*, T_b and metabolic inhibition may cause the more pronounced metabolic rate reduction. The decrease of ΔT cannot satisfactorily explain the reduction of TMR. A decrease of thermal conductance does not appear to be a prerequisite for the reduction of MR or for thermoregulation during torpor. However, the low conductance with a lowered TMR may prevent T_b from reaching the T_{bmin} , thus prevent the increases of TMR for thermoregulation.

Chapter Four: Interrelations between Metabolic Rate and Body Temperature during Entry into and Arousal from Hibernation and Daily Torpor

4.1 Introduction

Heterothermic mammals are capable of withstanding a substantial reduction of T_b during a bout of torpor. Since major physiological adjustments associated with alterations of T_b occur in the relatively short phases of torpor entry and arousal, it is possible that these transient states involve more complex processes than steady-state torpor. For instance, it has been suggested that during torpor entry a temperature-independent metabolic inhibition may be involved in reducing MR (Malan 1986; Geiser 1988; Storey and Storey 1990), although in many species MR during steady-state torpor can be largely explained by the effect of the lowered T_b on biological reactions (Tucker 1965; Snapp and Heller 1981). Since both torpor entry and arousal are short-term events and their timing is difficult to predict, data for these processes are relatively rare, and detailed investigations on physiological variables during both torpor entry and arousal are thus needed. The present study focuses on the interrelations between MR and T_b during entry into and arousal from torpor in *C. nanus* and *S. macroura*. It addresses the following questions in regard to both hibernation and daily torpor:

1. What is the time course for the reduction of MR and T_b during entry into torpor?
2. How does the reduction of MR and T_b during torpor entry differ at T_{a_s} below and above the T_{tc} for T_b during torpor?

3. Does the reduction of MR during torpor entry involve an intensive metabolic inhibition in addition to T_b effects and how do the two torpor patterns differ in this aspect?
4. How is cooling rate during torpor entry influenced by MR?
5. What are the rewarming rates during arousal from torpor at different T_{as} and do the rewarming rates differ from other endotherms?
6. How are MR and rewarming rate interrelated?
7. Does the maximum MR during arousal from torpor reflect the capacity to generate maximum heat during normothermia and is the maximum MR influenced by T_b ?

4.2 Materials and Methods

Eight adult male *S. macroura* (25.2 ± 2.5 g) and seven adult *C. nanus* (4 males and 3 females, 36.4 ± 5.5 g) were used in this study. Measurements were conducted at T_{as} both above and below the T_{tc} s (14.3°C for *S. macroura* and 4.8°C for *C. nanus*, chapter 3). *S. macroura* were measured at the mean T_{as} of $10.3 \pm 1.0^\circ\text{C}$, $18.2 \pm 1.2^\circ\text{C}$, and $24.6 \pm 1.1^\circ\text{C}$ (referred as 10, 18 and 25°C in the following text). *C. nanus* were measured at the mean T_{as} of $5.1 \pm 0.8^\circ\text{C}$, $15.4 \pm 1.4^\circ\text{C}$, $25.0 \pm 0.5^\circ\text{C}$, and $29.5 \pm 0.6^\circ\text{C}$ (referred as 5, 15, 25, and 30°C in the following text). Both systems A and B of the respirometry equipment (chapter 2.2) were used for the measurements of *S. macroura*, while *C. nanus* were only measured in system B. The flowrates through the respirometry chamber were usually 200 mL min^{-1} . Occasionally during early arousal flowrates were 100 mL min^{-1} .

As pointed out in chapter 2.4.1, no steady-state MR values can be measured during torpor entry and arousal, due to the mixing of the gases in the respirometry chamber. Thus all \dot{V}_{O_2} readings were converted into instantaneous \dot{V}_{O_2} by using the correction factors for the washout (chapter 2.4.1, Table 2.1) before analysis.

To compare the maximum MR during arousal with that during activity, the maximum metabolic rate was determined during the arousal and activity peaks at different T_a s (see chapter 2.4.7 for details). For a comparison, the cold-induced thermogenic capacity ($\dot{V}O_{2\max}$) of normothermic individuals determined in He-O₂, at a T_a between 8 and 16°C for *S. macroura*, and between 2 and 10°C for *C. nanus*, were included (see chapter 2.4.7 for details). The cooling rates and the rates of rewarming were calculated as described in chapter 2.4.6. Determinations of other physiological variables are also given in chapter 2.4. Curves of cooling of living individuals were compared with those of freshly dead individuals and both were measured with temperature-sensitive transmitters. The timings of torpor entry and arousal were tested by Circular statistics (Raleigh-test).

4.3 Results for *Sminthopsis macroura*

4.3.1 Time Course of MR and T_b Reduction during Entry into Daily Torpor

Torpor in *S. macroura* was always initiated in the dark phase, between midnight and lights on in the morning (preferred time: $03:12 \pm 1:42$ h, Raleigh-test, $r^2 = 0.75$, $p < 0.001$, $n = 20$). The entrance into torpor was initiated by a drop of MR from active or resting levels, which was followed by a decrease of T_b .

Above the T_{tc}

At T_a s of 25 and 18°C, T_b and MR decreased concurrently during most of the entry phase (Fig. 4.1a, b). While torpor entry was occasionally interrupted by several sudden increases in MR, these increases were most regularly observed when MR was 0.86 ± 0.04 mL g⁻¹ h⁻¹ which approximates the BMR of 0.88 ± 0.09 mL g⁻¹ h⁻¹. MR decreased by over 86% from the active to the BMR level within 42 ± 15 min at T_a 25°C and 39 ± 13 min at T_a 18°C. During this initial drop of MR, T_b fell by only 1.5 ± 0.6 °C at T_a 25°C, and 1.9 ± 1.0 °C at T_a 18°C. However, the reduction of MR below the BMR level to the steady-state TMRs accounted for only a small fraction of the

overall MR reduction, although the T_b decreased greatly by a further $6.2 \pm 1.7^\circ\text{C}$ at T_a 25°C , and $11.5 \pm 2.3^\circ\text{C}$ at T_a 18°C (Fig. 4.1a, b).

When MR had reached TMR after about 3 hours, T_b was still $0.5 \pm 0.3^\circ\text{C}$ at T_a 25°C , and $0.9 \pm 0.7^\circ\text{C}$ at T_a 18°C higher than the lowest T_b (T_{b1}). The Q_{10} between BMR and this beginning of TMR was 3.7 ± 1.3 at T_a 25°C and 3.4 ± 1.7 at T_a 18°C (Fig 4.1a, b). Usually T_{b1} was reached within another hour (Fig. 4.1a, b). When both TMR and T_b had reached the steady-state lowest values, the Q_{10} between MR at the BMR and TMR, was 2.9 ± 1.1 at T_a 25°C , and 2.7 ± 0.9 at T_a 18°C . Thus the entire MR reduction can be explained by temperature effects upon MR (Fig. 4.2).

Below the T_{tc}

At T_a 10°C , the time course of the reduction of MR and T_b during torpor entry followed different patterns (Fig. 4.1c). Within the initial 52 ± 17 min, MR declined to a value of $0.5 \pm 0.2 \text{ mL g}^{-1} \text{ h}^{-1}$, which was below BMR (i.e. a MR undershoot). The undershoot value was about 60% of the BMR and 50% of the steady-state TMR at the same T_a , which was reached after a gradual increase of MR, after about another 1.5 hours. Over the same time interval T_b usually showed a continuous decline. When MR dropped from active or resting MR to the BMR levels, T_b decreased by $3.1 \pm 1.8^\circ\text{C}$. The reduction of MR from EMR to the MR undershoot was accompanied by a $6.3 \pm 3.2^\circ\text{C}$ drop in T_b (Fig. 4.1c). The Q_{10} between MR at the BMR and the undershoot TMR at 10°C was 2.5 ± 0.9 , is consistent with the situation above the T_{tc} (Fig. 4.2).

4.3.2 Cooling Rates during Entry into Daily Torpor

As in all physical bodies, the cooling rate during entrance into daily torpor in *S. macroura* was proportional to ΔT . However, the log-transformed ΔT did not fit a

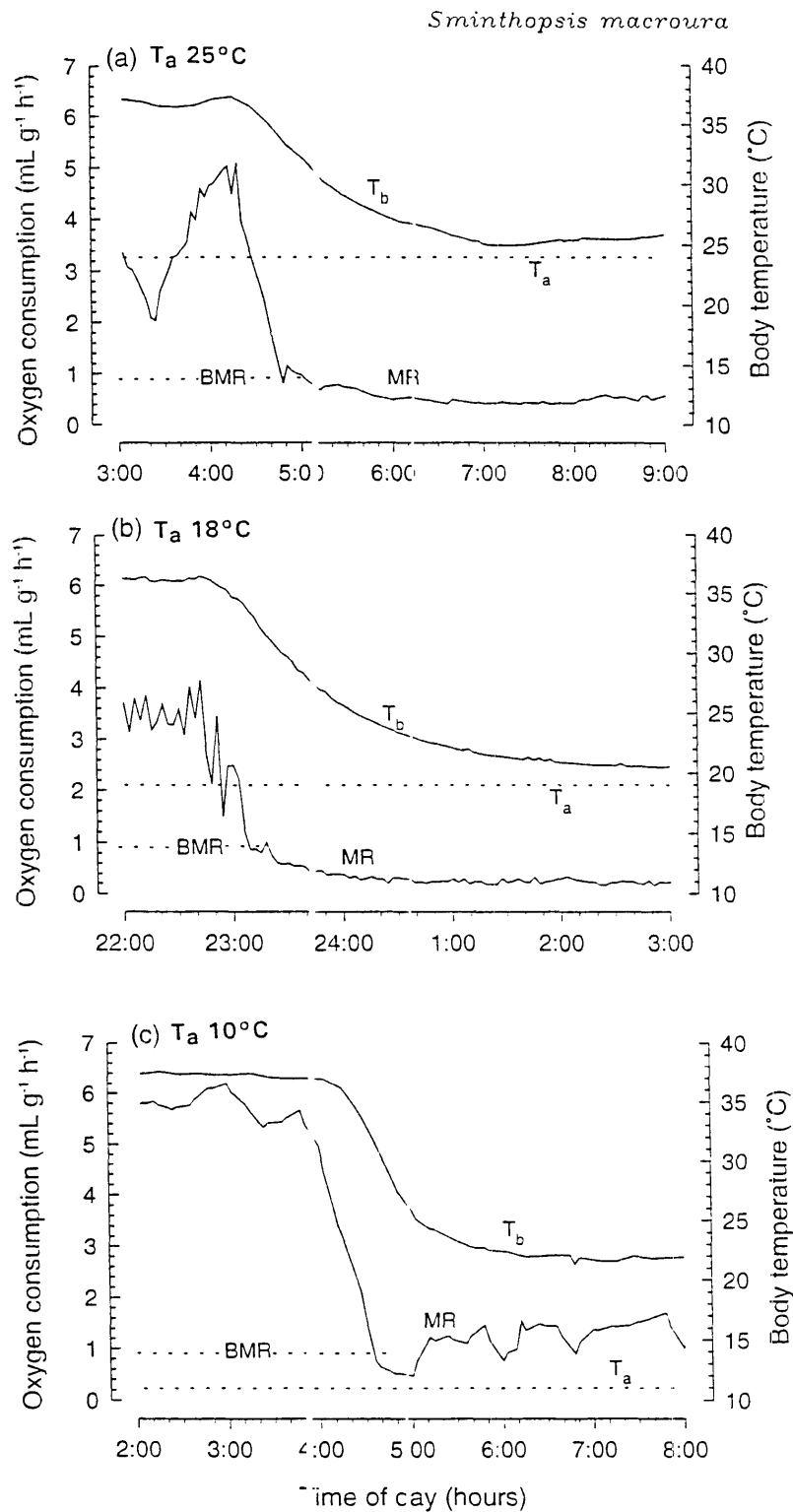


Fig. 4.1. Patterns of reduction of metabolic rate (MR) and body temperature (T_b) during entry into daily torpor of *Sminthopsis macroura* at air temperature; (T_a) of (a) 25°C, (b) 18°C, (c) 10°C (below the critical air temperature for thermoregulation during torpor).

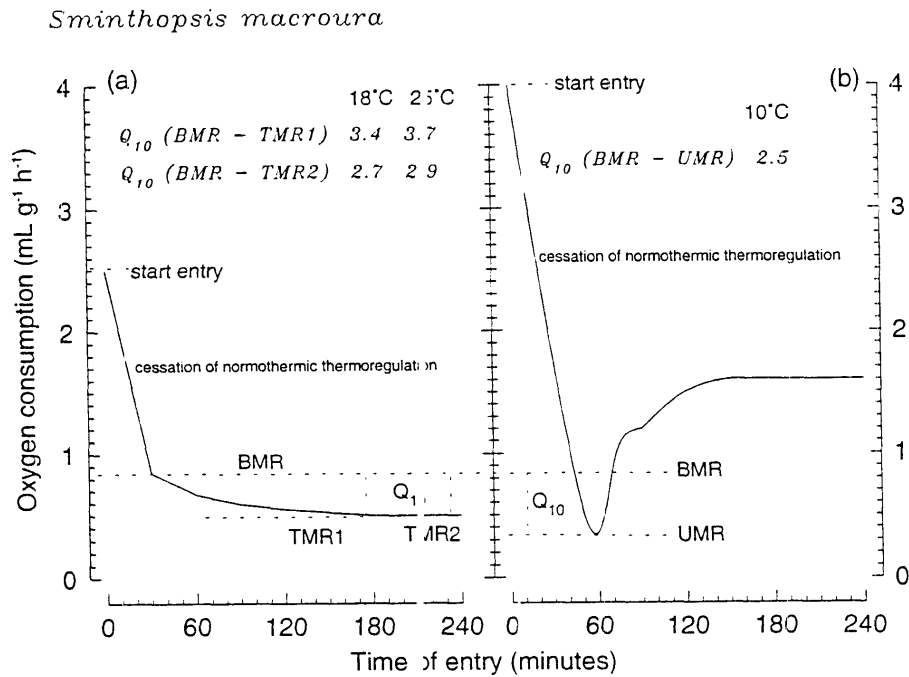


Fig. 4.2. Q_{10} s calculated for MRs during entry into daily torpor of *Sminthopsis macroura*. (a) above the critical temperature for thermoregulation during torpor and (b) below the critical temperature for thermoregulation during torpor. UMR represents the MR undershoot.

linear model. In contrast to the pure passive exponential cooling that was observed in a dead individual of the same BM as the living animals, the cooling curve during torpor entry showed three different phases (Fig. 4.3a, b). At all T_a s measured, during the initial entry phase when MR was still relatively high, the animal cooled slowly. In the second phase, rates of cooling accelerated as MR fell below about 60% and 70% of the pre-entry value at T_a 10°C and 18°C, respectively. During the third phase cooling rates were reduced. At T_a 10°C, the reduced cooling rate towards the end of torpor entry was largely caused by the thermoregulatory increase of TMR (Fig. 4.3a). However, at T_a s 18 and 25°C, cooling rates were also reduced, although MR continued to decline

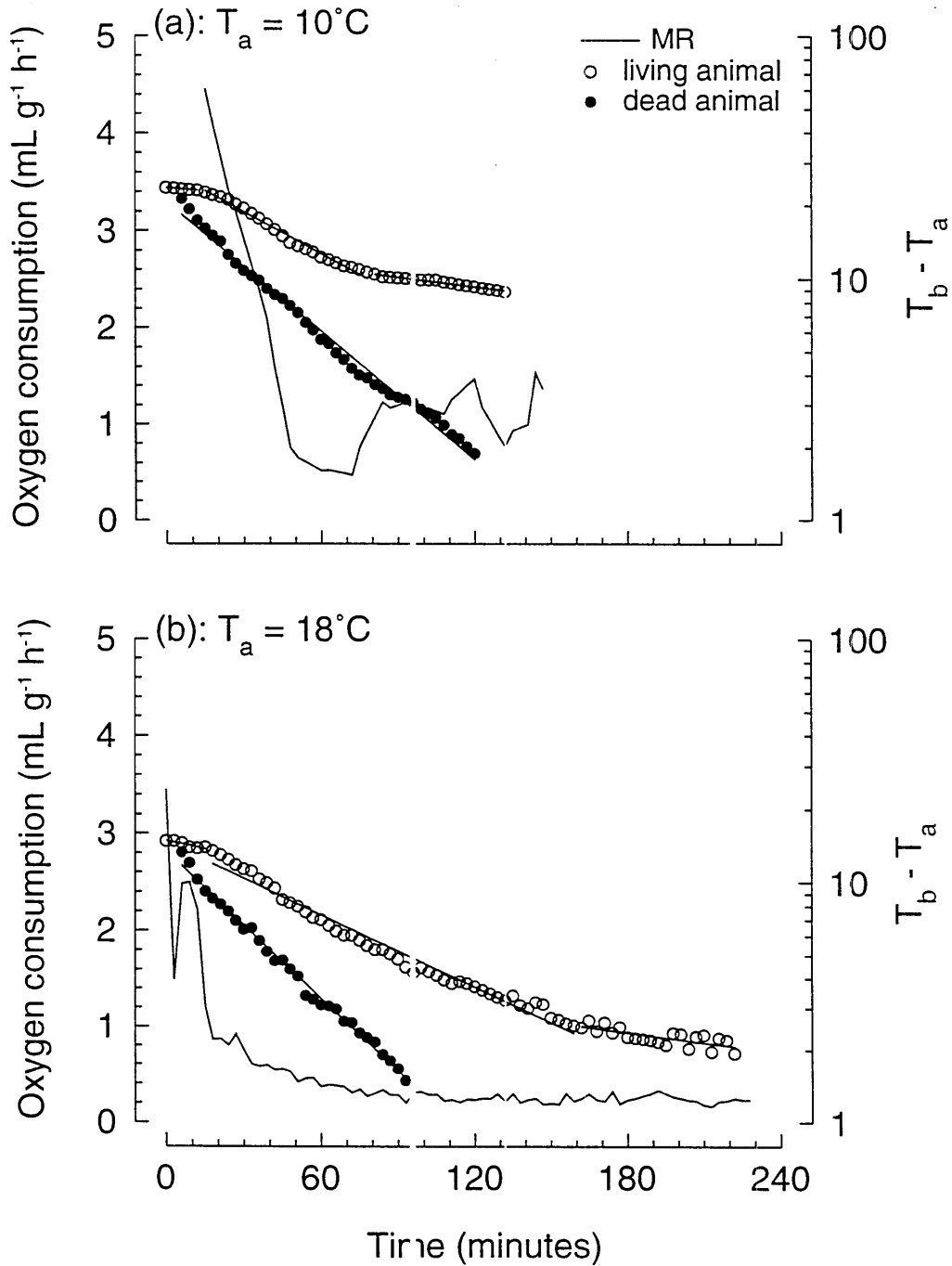


Fig. 4.3. Reductions of metabolic rate (MR) and the thermal differential (ΔT) between body temperature (T_b) and air temperature (T_a) during entry into daily torpor of *Sminthopsis macroura* at T_a s of (a) 18°C, (b) 10°C. The cooling rate of living animals (open circles) could be divided into three phases and was lower than that of a dead animal (closed circle) at any given T_a .

during this phase (Fig. 4.3b). It appeared that overall insulation had improved when MR approached TMR. Due to heat production and, most likely, also a smaller conductance, even the maximum cooling rates of living individuals were less than half of that of the dead animal (Fig. 4.3a, b).

4.3.3 Time Course of Arousal from Daily Torpor

Arousal from daily torpor in *S. macroura* was not random and was initiated preferentially in the morning ($08:07 \pm 1:09$ h, Raleigh-test, $r^2 = 0.88$, $p < 0.001$, $n = 20$). Arousal was always initiated by an increase of MR (Fig. 4.4). Under all T_a conditions, measurable changes in core T_b were usually not observed in the first 12 minutes of the rise in MR. This time-lag between MR and T_b was observed throughout the arousal phase even after arousal was well under way. During arousal MR increased continuously to a value that exceeded RMR (MR overshoot) and declined thereafter. With the initial increase of MR, T_b increased gradually. A rapid increase of T_b was observed when MR approached the overshoot value. Finally, rise of T_b slowed when it approached normothermic levels while the MR decreased, usually to RMR (Fig. 4.4).

4.3.4 Rewarming Rates during Arousal from Daily Torpor

S. macroura regained its normothermic T_b from the level of steady-state torpor within 192.5 ± 27.1 min at T_a 10°C, 91.7 ± 19.2 min at T_a 18°C, and 82.1 ± 17.3 min at T_a 25°C (Fig. 4.5). The influence of T_a on the rewarming time was significant ($p < 0.001$, ANOVA; Fig. 4.5). A longer rewarming process was observed at T_a 10°C, apparently due to the initial larger temperature differential between the animal and its environment, and a higher heat loss at low T_a s (Fig. 4.5).

During the process of rewarming, the T_b of *S. macroura* increased by an average of $0.09 \pm 0.02^\circ\text{C min}^{-1}$ at T_a 10°C, $0.13 \pm 0.03^\circ\text{C min}^{-1}$ at T_a 18°C, and $0.12 \pm$

$0.02^{\circ}\text{C min}^{-1}$ at T_a 25°C (Fig. 4.6). The maximum rewarming rates measured over 12 min were $0.21 \pm 0.04^{\circ}\text{C min}^{-1}$ at T_{i_c} 10°C , $0.29 \pm 0.06^{\circ}\text{C min}^{-1}$ at T_a 18°C , and $0.29 \pm 0.06^{\circ}\text{C min}^{-1}$ at T_a 25°C (Fig. 4.6), and these were observed shortly before T_b reached the normothermic level. Both the overall and the maximum rewarming rates differed among T_a s ($p < 0.05$, ANOVA for both overall rewarming rate and maximum rewarming rate, $n = 20$; Fig. 4.6). For both overall and maximum rewarming rates, a significantly lower rewarming rate was observed for animals at T_a 10°C , in comparison with that at T_a 18°C (Tukey's pairwise comparison; Fig. 4.6).

The rewarming rates of *S. macroura* were neither related to the initial T_b before arousal began, nor the T_b when maximum rewarming rates were obtained ($p > 0.05$; Fig. 4.7; Fig. 4.8).

4.3.5 Maximum MR during Arousal from Daily Torpor

The maximum MR during arousal was negatively related to the T_b at which arousal was initiated ($r^2 = 0.34$, $p < 0.01$, $n = 20$; Fig. 4.9), but it was not a function of the T_b at which the maximum MR was reached ($r^2 = 0.04$, $p > 0.1$; Fig. 4.9).

The maximum MR during arousal was $5.76 \pm 0.94 \text{ mL g}^{-1} \text{ h}^{-1}$ at T_a 10°C , $5.01 \pm 0.46 \text{ mL g}^{-1} \text{ h}^{-1}$ at T_a 18°C , and $4.20 \pm 0.61 \text{ mL g}^{-1} \text{ h}^{-1}$ at T_a 25°C . As the maximum MR measured during the activity peak ($r^2 = 0.88$, $p < 0.001$, $n = 20$; Fig. 4.10), the maximum MR during arousal peak increased linearly with decreasing T_a ($r^2 = 0.82$, $p < 0.001$, $n = 20$; Fig. 4.10). Despite the differences in T_b in these two physiological states, no significant difference was observed between the regressions of maximum MR vs T_a during arousal peak or activity peak, since both the slope and the intercept of the two regressions were similar ($p > 0.05$, t-test for both slope and elevation). In addition, the $\dot{V}_{O_{2\text{max}}}$ measured in He- O_2 was not significantly different from the maximum MR during the arousal peak at 10°C ($t = 0.76$, $p > 0.2$ paired t-test, $N = 6$),

suggesting that animals used their maximum capacity of heat production during arousal to increase T_b as quickly as possible.

In addition, the slopes of the regressions of maximum MR vs T_a during activity and arousal peaks were the same as that for RMR vs T_a ($p > 0.05$, t-tests; Fig. 4.10a).

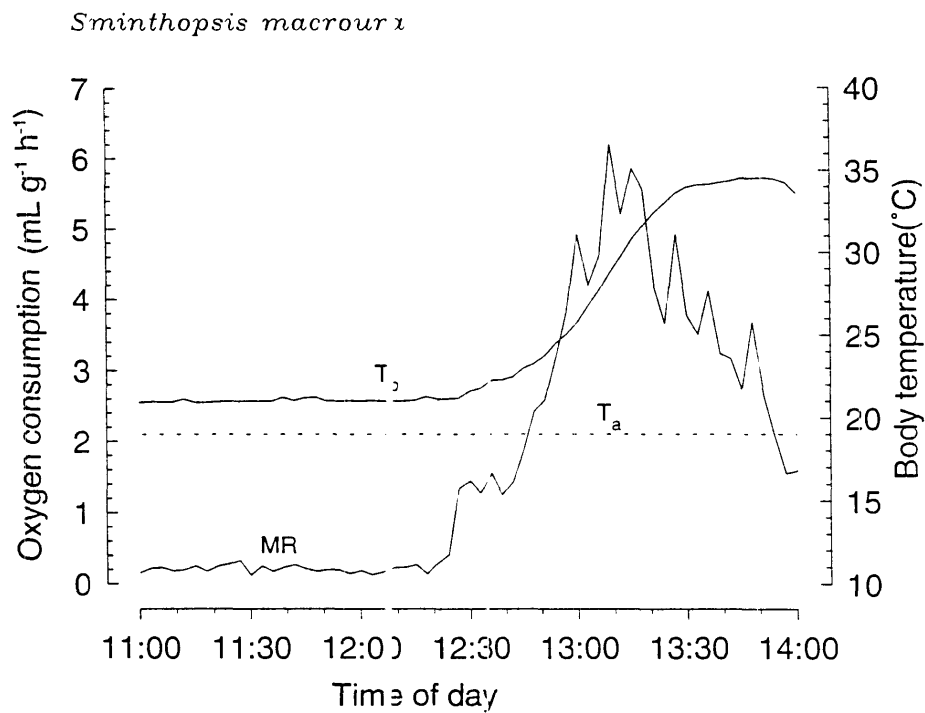


Fig. 4.4. Patterns of increase of metabolic rate (MR) and body temperature (T_b) of *Sminthopsis macroura* during arousal from daily torpor at an ambient temperature (T_a) of 20°C.

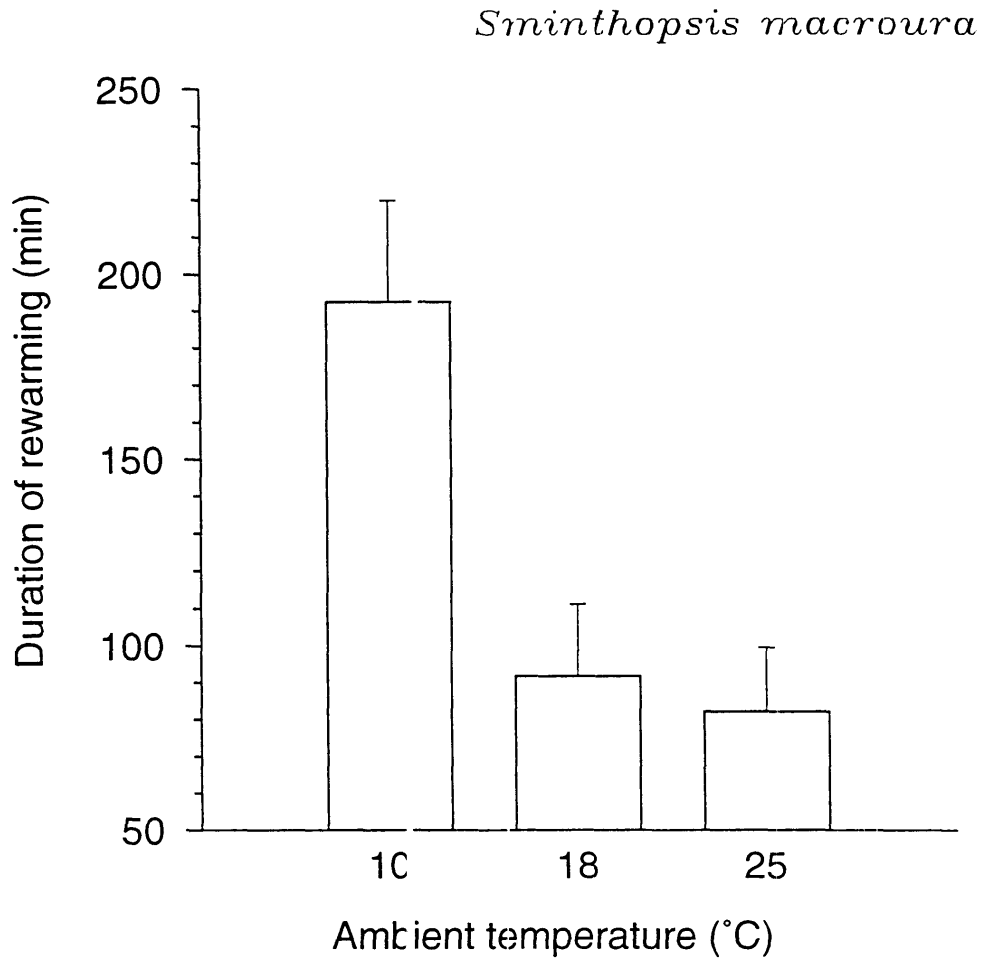


Fig. 4.5. Duration of rewarming from daily torpor of *Sminthopsis macroura* at different air temperatures (T_a). Error bars indicate the standard deviations for $N = 7$ at T_a 18 and 25°C, $N = 6$ at T_a 10°C. Rewarming time was significantly affected by T_a ($F = 52.16$, $p < 0.001$, ANOVA).

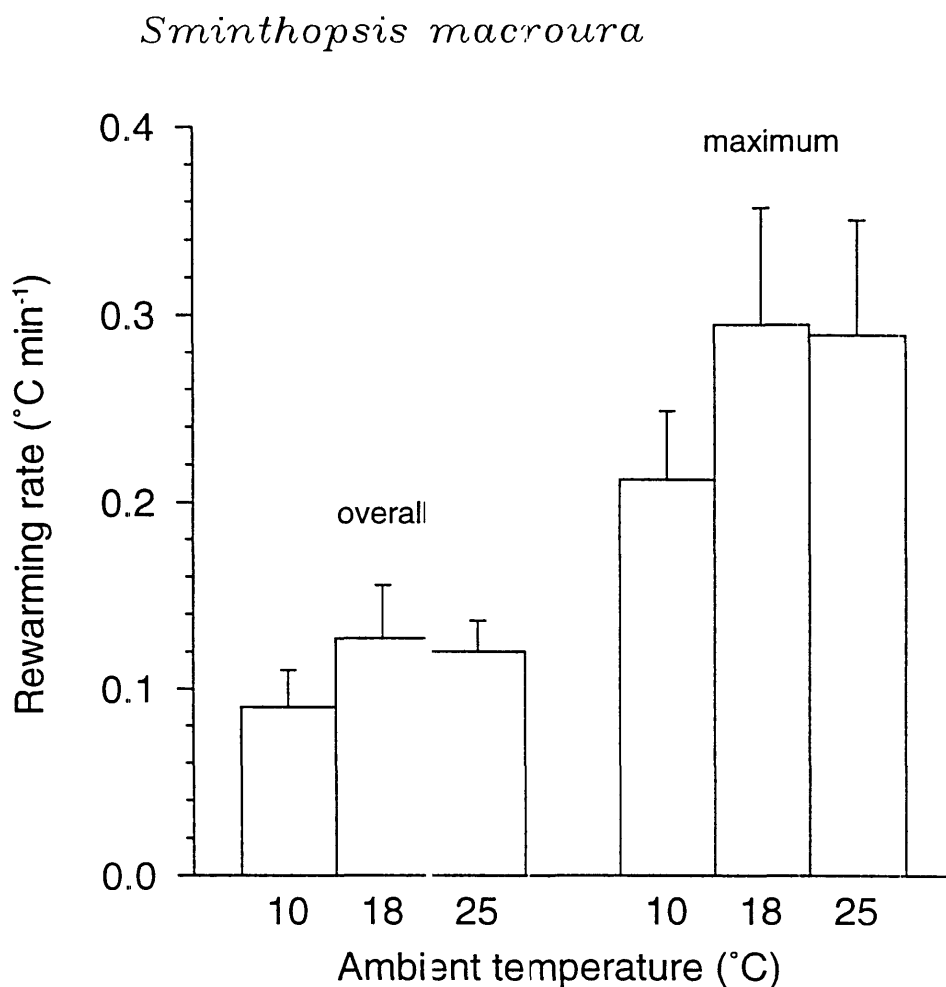


Fig. 4.6. The overall rewarming rates and the maximum rewarming rates of *Sminthopsis macroura* during arousal from daily torpor at different air temperatures (T_a). Error bars indicate the standard deviations for $N = 7$ at T_a 18 and 25°C, $N = 6$ at T_a 10°C. Both the overall and the maximum rewarming rates were affected by T_a ($F = 5.00$, $p < 0.05$, $n = 20$ for overall rewarming rate; $F = 4.39$, $p < 0.05$, $n = 20$ for maximum rewarming rate).

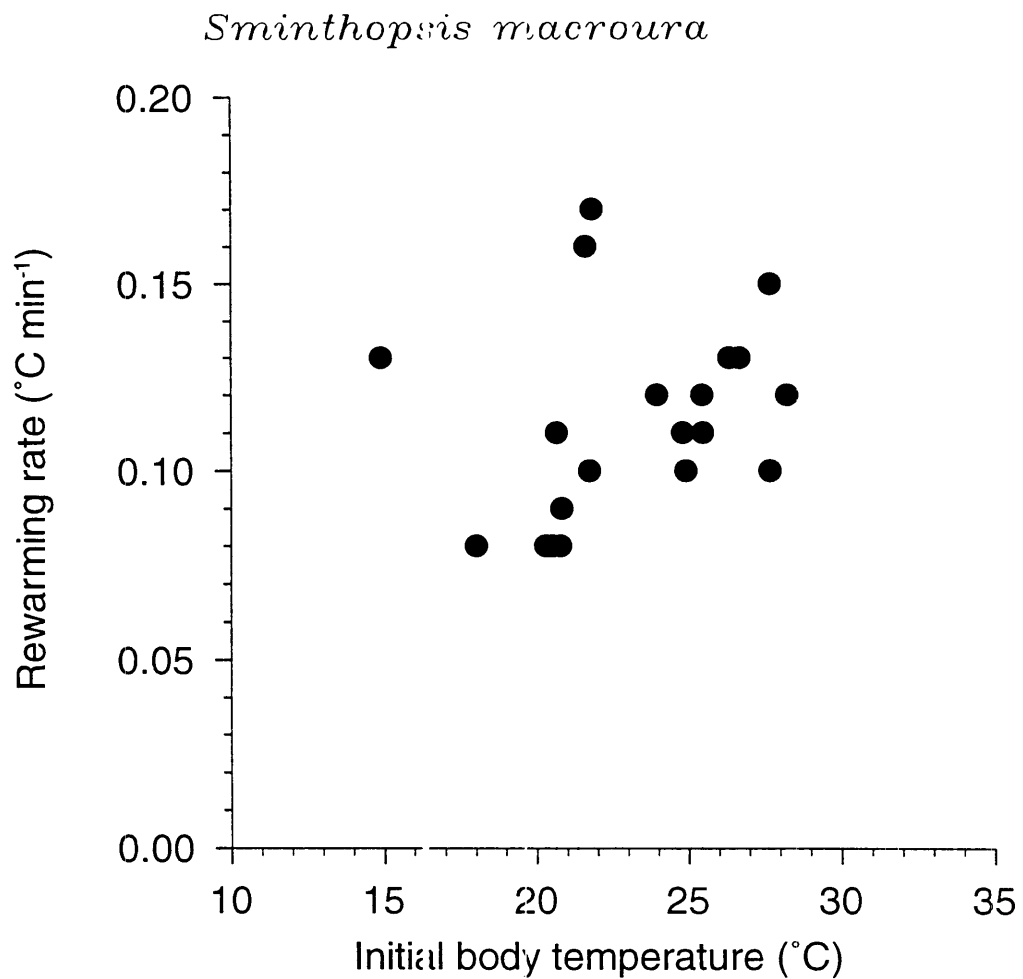


Fig. 4.7. The overall rewarming rate of *Sminthopsis macroura* during arousal from daily torpor as a function of the initial body temperature before arousal. The two variables were not correlated ($r^2 = 0.02$, $p > 0.2$, $n = 20$).

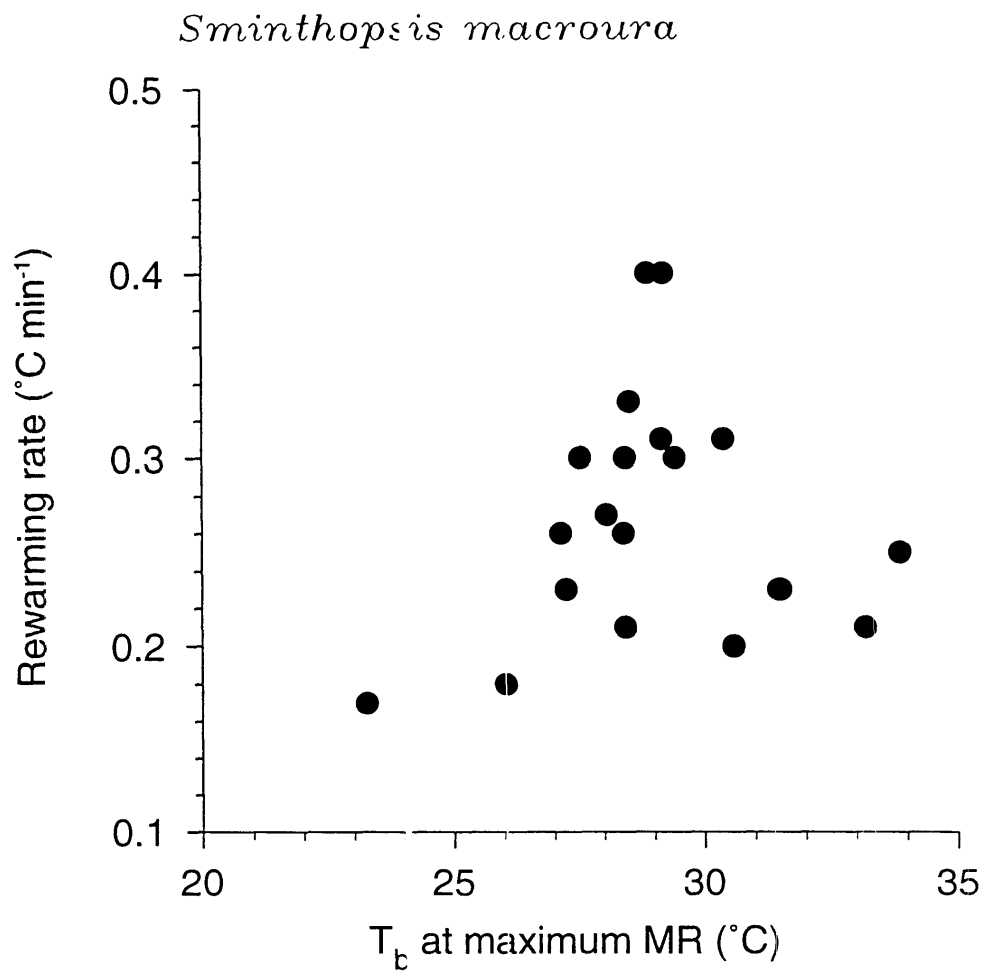


Fig. 4.8. The maximum rewarming rate of *Sminthopsis macroura* during arousal from daily torpor as a function of the T_b at which the maximum rewarming rate was obtained. The two variables were not correlated ($r^2 = 0.01$, $p > 0.6$, $n = 20$).

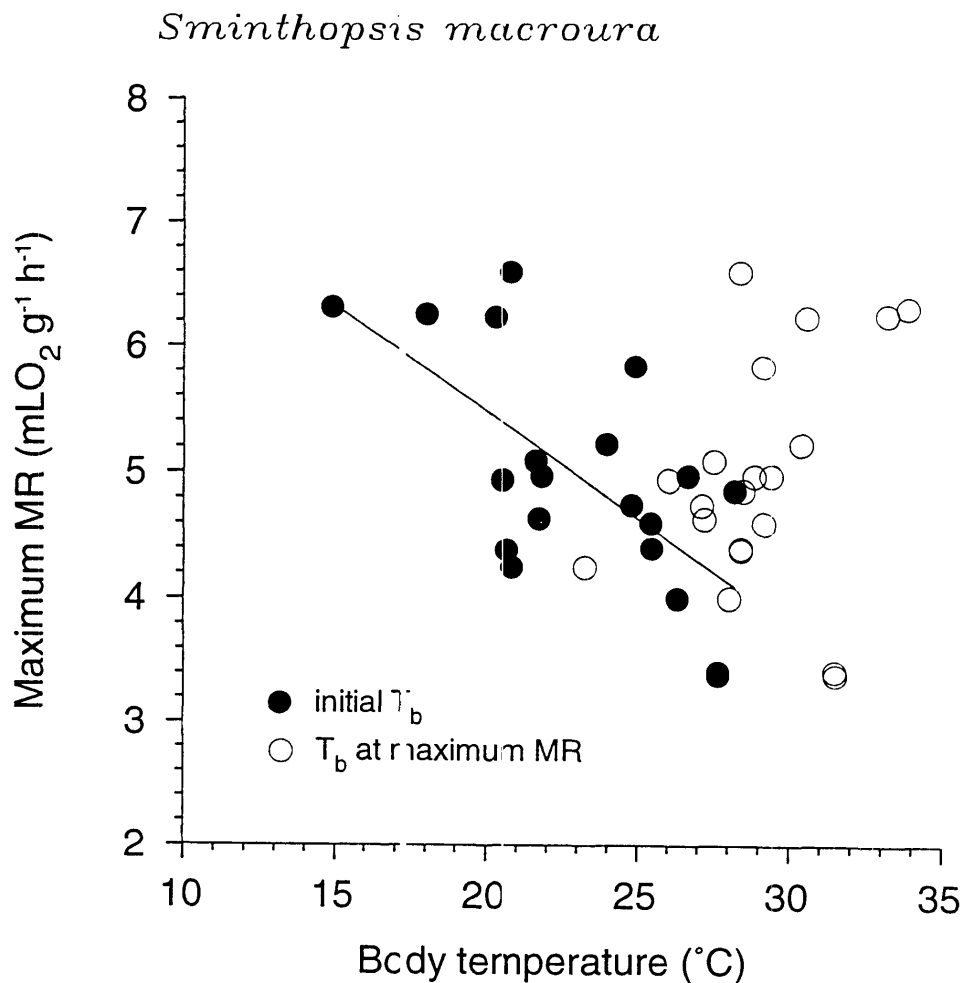


Fig. 4.9. Maximum metabolic rate of *Sminthopsis macroura* during arousal from daily torpor as a function of the body temperature (T_b) at which arousal was initiated (closed circles), and the T_b at which the maximum MR was reached (open symbols). The maximum MR was closely related to the T_b at which arousal was initiated:

$$\text{Maximum MR} = 8.57 - 0.157 \times T_b \quad (r^2 = 0.34, p < 0.005, n = 20).$$

However, the maximum MR was not a function of the T_b at which the maximum MR was reached ($r^2 = 0.04, p > 0.2, n = 20$).

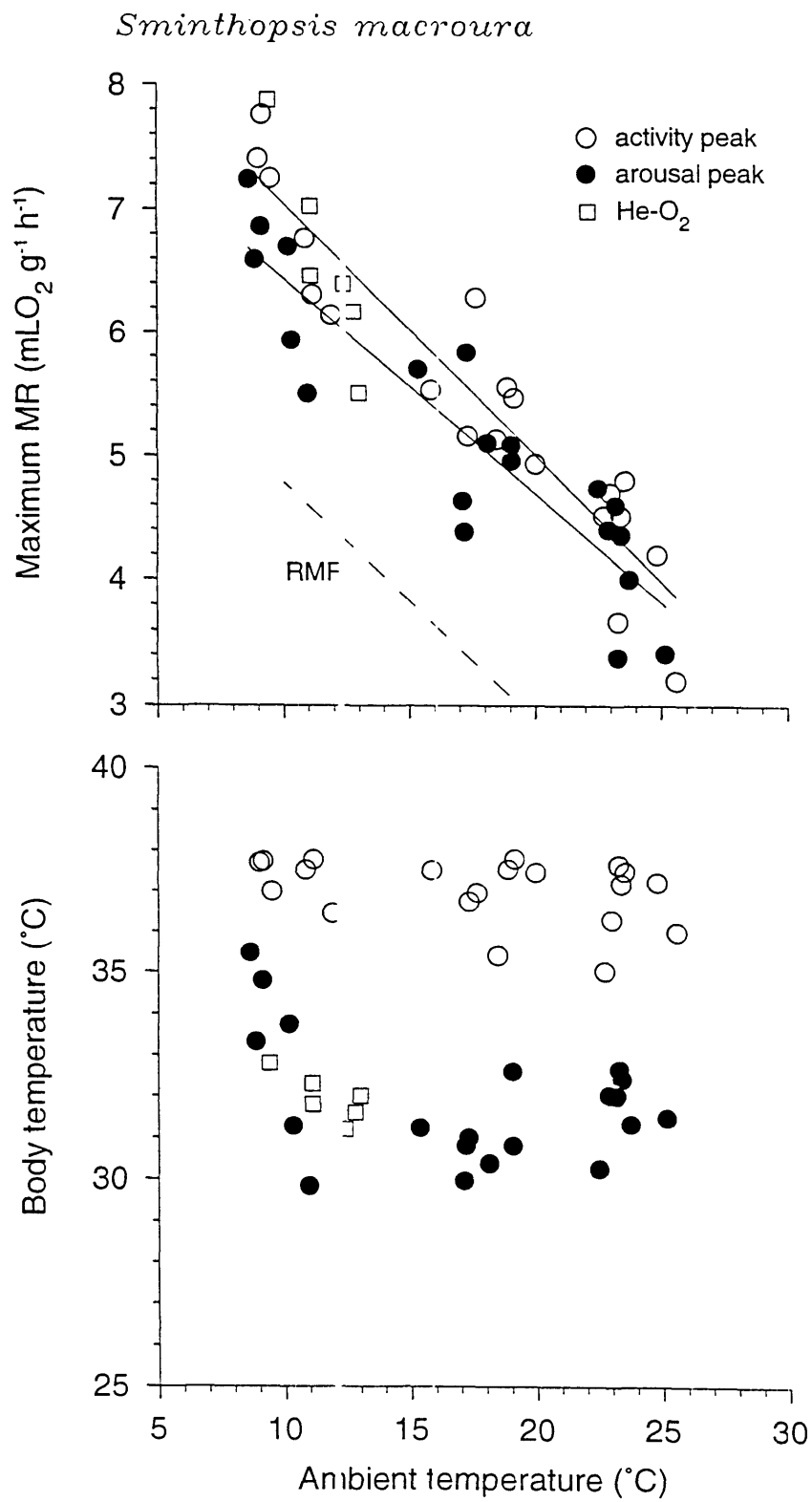


Fig. 4.10.

Fig. 4.10. (previous page) **(a)** Maximum metabolic rate of *Sminthopsis macroura* during arousal from daily torpor (closed circles) and during activity (open circles) as a function of air temperatures (T_a), and the cold-induced $\dot{V}O_{2max}$ measured in He-O₂ exposure (squares). The maximum MR during both arousal and activity peaks was closely related to T_a .

Arousal peak:

$$\text{Maximum MR} = 8.18 - 0.175 \times T_a \quad (r^2 = 0.82, p < 0.001, n = 20).$$

Activity peak:

$$\text{Maximum MR} = 9.10 - 0.205 \times T_a \quad (r^2 = 0.88, p < 0.001, n = 20).$$

The dashed line indicates the regression between RMR and T_a of the species.

(b) Body temperature (T_b) of *S. macroura* at which the maximum MRs and the $\dot{V}O_{2max}$ were obtained.

4.4 Results for *Cercartetus nanus*

4.4.1 Time Course of MR and T_b Reduction during Entry into Hibernation

The preferred time for the initiation of torpor entry in *C. nanus* was in the evening at $21:45 \pm 1:52$ h (Rayleigh-test, $r^2 = 0.62$, $p < 0.001$, $n = 25$). At each T_a , entrance into hibernation was always initiated by a dramatic drop of MR, followed by a gradual decline of T_b . This is shown by the examples of changes of MR and T_b during entry into hibernation at T_a s of 30°C (within the TNZ), 25 and 15°C (above the T_{tc}), and at T_a about 5°C (below the T_{tc} , Fig. 4.11a-d).

Above the T_{tc}

During early entry when T_b was still above 32°C , MR dropped rapidly to a value which was about 15% higher than the TMR (initial MR drop). After this initial drop MR always showed a sudden increase before declining again. Thus the MR value before this MR increase was taken as the endpoint of the initial MR drop. The reduction of MR to TMR after this sudden MR increase was much slower than the initial MR drop and often was accompanied by irregular MR fluctuations.

At T_a 30°C , the initial MR drop took 14.0 ± 2.5 min. During this time MR fell to the value of 0.446 ± 0.185 $\text{mL g}^{-1} \text{h}^{-1}$. At T_a 25°C , this process took 17.1 ± 7.3 min, and MR fell to the value of 0.516 ± 0.238 $\text{mL g}^{-1} \text{h}^{-1}$. At T_a 15°C , this process took 18.9 ± 7.4 min, and MR fell to the value of 0.403 ± 0.172 $\text{mL g}^{-1} \text{h}^{-1}$. At all the above T_a s, the initial MR drop resulted in MR values that were well below the BMR of 0.66 $\text{mL g}^{-1} \text{h}^{-1}$ (chapter 3.4), and there was no evidence for an increase of MR near the BMR (Fig. 4.11a-c).

The initial MR drop was accompanied by only a small decline of T_b of $0.5 \pm 0.2^\circ\text{C}$ at T_a 30°C , $0.6 \pm 0.4^\circ\text{C}$ at T_a 25°C , and $1.0 \pm 0.8^\circ\text{C}$ at T_a 15°C (Fig. 4.11a-c).

Because of the exponential relationship between MR and T_b , the further reduction of MR to the steady-state TMR, when both MR and T_b had reached the lowest values at that T_a , accounted for only a small proportion of the total MR reduction during

torpor entry (7% at 30°C, 15% at 25°C and 13% at 15°C). However, this small reduction of MR was accompanied by a drop of T_b of $3.5 \pm 0.8^\circ\text{C}$ at T_a 30°C, $8.8 \pm 1.4^\circ\text{C}$ at T_a 25°C, and $18.2 \pm 1.5^\circ\text{C}$ at T_a 15°C. Therefore, the Q_{10} for MR reduction between the endpoint of the initial MR drop and the steady-state TMR was 1.7 (T_a 30°C), 3.0 (T_a 25°C), and 3.5 (T_a 15°C). When the Q_{10} for MR reduction was calculated from BMR, the reduction of MR from BMR to the steady-state TMR resulted in higher Q_{10} values of 4.7 at T_a 30°C, 3.7 at T_a 25°C, and 4.5 at T_a 15°C.

The entire process of MR reduction to the steady-state TMR during torpor took an average of 315 ± 49 min at T_a 15°C, and 303 ± 48 min at T_a 25°C. In comparison, the decline of T_b at the above T_a s was completed later. After MR reached TMR, T_b continued to fall by $1.6 \pm 0.3^\circ\text{C}$ within another 72 ± 26 min at T_a 15°C, and $1.5 \pm 0.3^\circ\text{C}$ within another 66 ± 20 min at T_a 25°C, to reach the T_{bl} during the steady-state torpor. However, it is possible that this time difference was partially due to a better resolution of the equipment in determining T_b than MR.

At T_a 30°C, the steady-state TMR and T_{bl} were approached at almost the same time after 235 ± 116 min and 240 ± 114 min from the beginning of the MR reduction.

Below the T_{tc}

At T_a s below the T_{tc} , the time course of the reduction of MR and T_b differed from that above the T_{tc} . Within the initial 45 ± 7.8 min, MR dropped to an undershoot value of $0.154 \pm 0.112 \text{ mL g}^{-1} \text{ h}^{-1}$, which was 30% - 40% of the TMR. The further gradual increase of MR to steady-state TMR shows that metabolic thermogenesis was employed by the animal to maintain T_b . During the whole process of MR change, T_b decreased gradually and was finally maintained at about 6°C (Fig. 4.11d).

The Q_{10} for MR reduction between MR at the BMR and the MR undershoot was 4.4. This suggests that the undershoot during entry into hibernation can not be explained by temperature effects alone. This is consistent with the situations during entry into hibernation at T_a s above the T_{tc} .

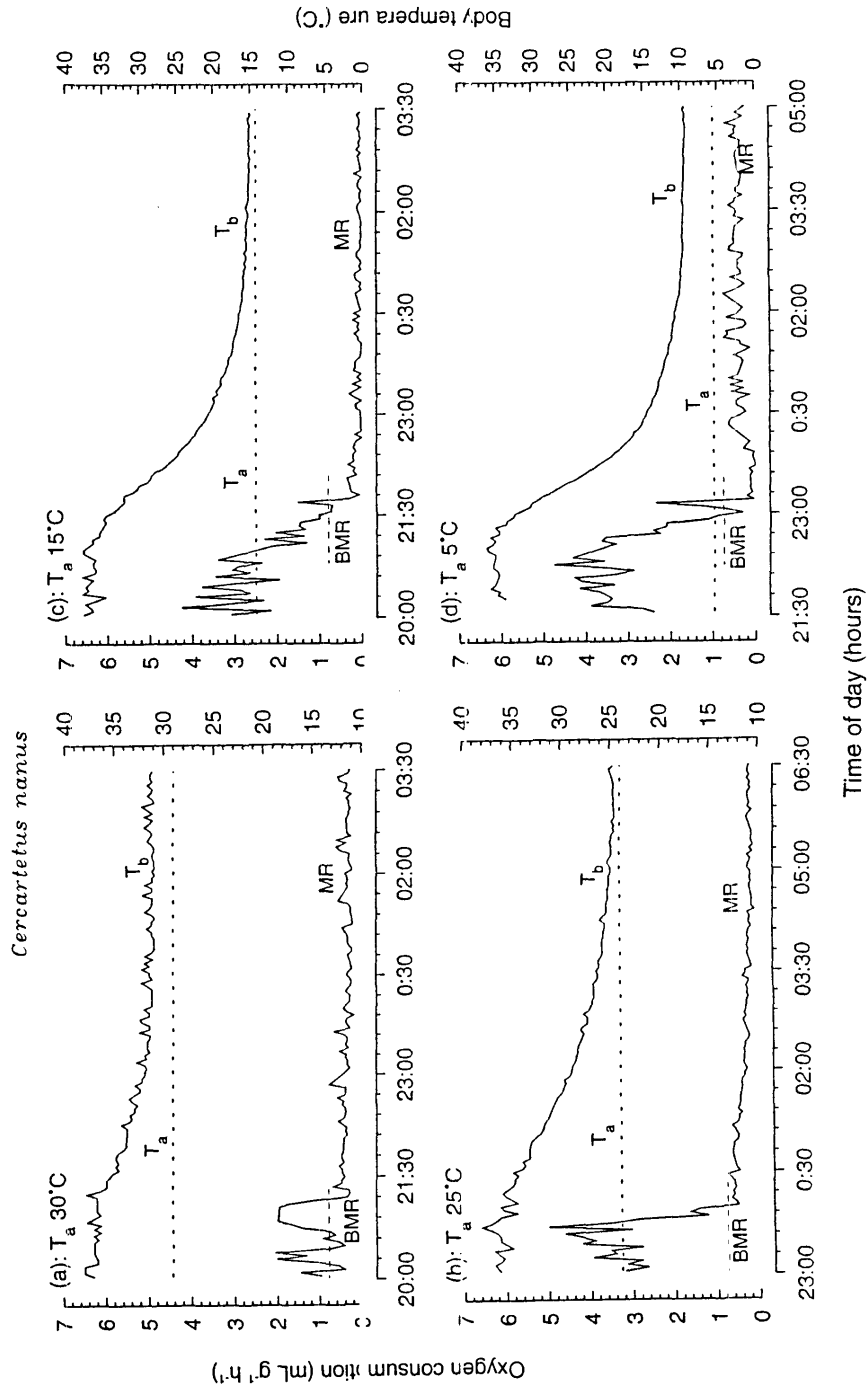


Fig. 4.11. Patterns of reduction of metabolic rate (MR) and body temperature (T_b) during entry into hibernation of *Cercartetus nanus* at air temperatures (T_a) of (a) 30°C (in the thermoneutral zone), (b) 25°C, (c) 15°C and (d) 5°C (below the critical air temperature for thermoregulation during torpor).

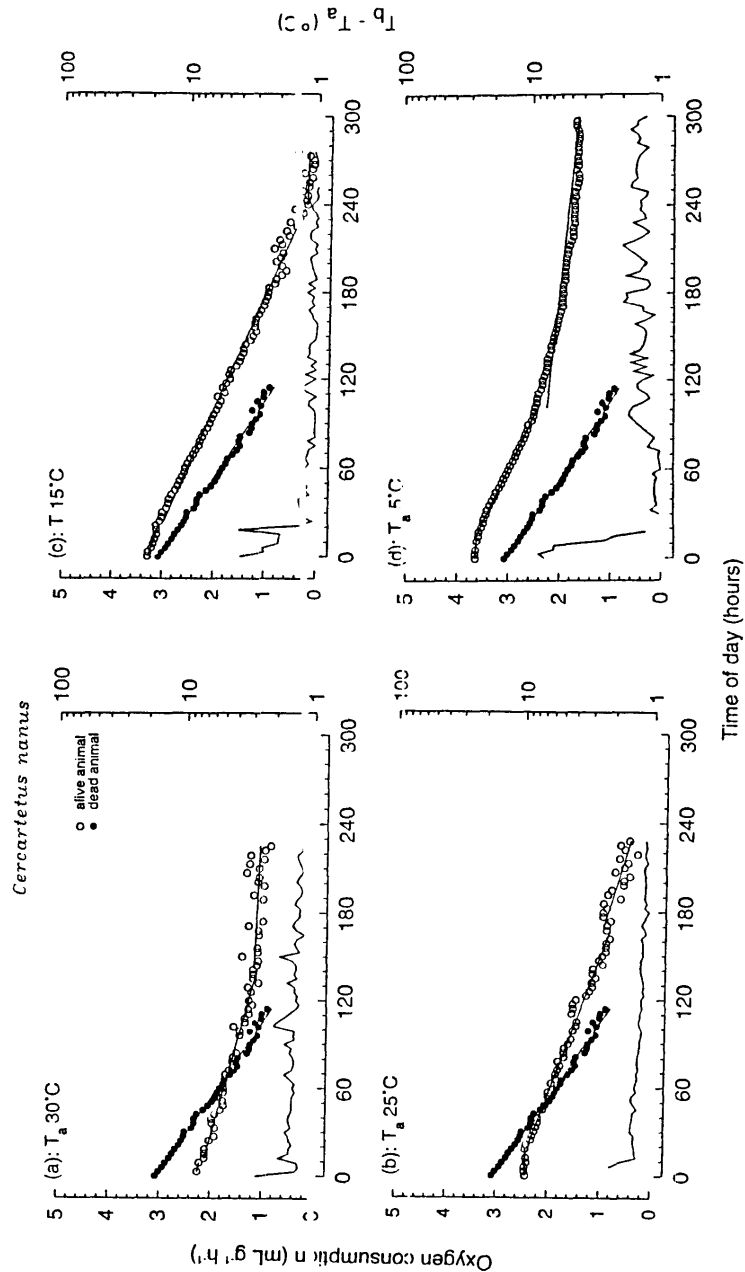


Fig. 4.12. Reductions of metabolic rate (MR) and the thermal differential (ΔT) between body temperature (T_b) and air temperature (T_a) during entry into hibernation of *Cercartetus nanus* at T_a s of (a) 30°C (in the thermoneutral zone), (b) 25°C, (c) 15°C and (d) 5°C (below the critical air temperature for thermoregulation during torpor). The cooling rate of living animals (open circles) could be divided into different phases, and was lower than that of a dead animal (closed circles) at any given T_a .

4.4.2 Cooling Rates during Entry into Hibernation

Cooling of *C. nanus* during entry into hibernation was proportional to ΔT (Fig. 4.12a-d). Nevertheless, the log-transformed data for the reduction of ΔT for the entire entry process did not fit a single linear model, unlike the pure passive exponential cooling measured for a dead individual of the same BM (Fig. 4.12). Below the TNZ, cooling rates were shallower at the beginning and the termination of entry than those during the main entry phase. In the TNZ, the cooling rate was relatively even. The cooling rate of living animals was always slower than that of a dead animal of similar BM, apparently due to heat production and a smaller C (Fig. 4.12a-d).

4.4.3 Time Course of Arousal from Hibernation

In *C. nanus*, the preferred time for spontaneous arousal was in the afternoon at 14:22 \pm 2:01 h (Raleigh-test, $r^2 = 0.56$, $p < 0.001$, $n = 25$). Arousal was initiated by an increase of MR, followed by a gradual increase of T_b . The rate of rewarming was not uniform throughout the arousal process. At the beginning of the arousal when T_b s were low, rewarming rates were slow. The increase of T_b accelerated when MR was over 50% of the maximum MR during arousal. The increase of T_b declined again when T_b approached normothermic values, and when the MR started to decrease (Fig. 4.13).

The time for rewarming from torpor in *C. nanus* was significantly affected by T_a ($p < 0.001$, ANOVA; Fig. 4.14). Rewarming required between 40.0 \pm 11.8 min at T_a 30°C to 169.8 \pm 61.2 at T_a 5°C. In addition, The time for rewarming process varied considerably among individuals, especially at low T_a s.

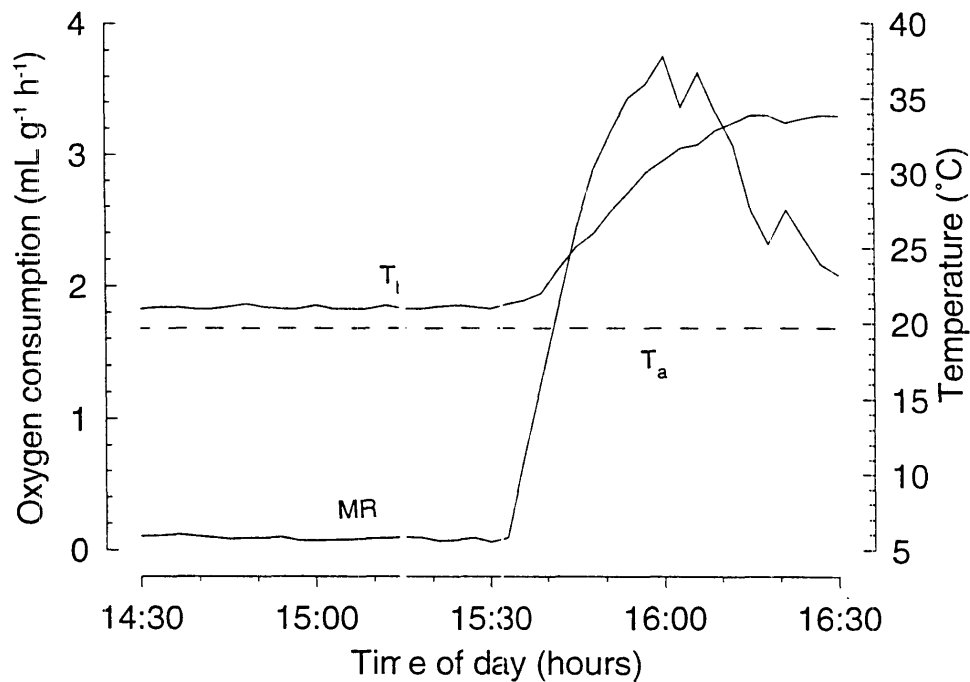
Cercartetus nanus

Fig. 4.13. Patterns of increase of metabolic rate (MR) and body temperature (T_b) of *Cercartetus nanus* during arousal from hibernation at an air temperature (T_a) of 20°C.

4.4.4 Rewarming Rates during Arousal from Hibernation

Both the overall and the maximum rewarming rates from torpor were influenced by T_a ($p < 0.001$, ANOVA for both overall and maximum rewarming rates; Fig. 4.15). Above the T_{tc} , the rates for both overall rewarming and maximum rewarming declined with increasing T_a . Below the T_{tc} , both the overall and the maximum rewarming rates were slower than those above the T_{tc} (Fig. 4.15). Rewarming in the TNZ was significantly slower than at any other T_a s (Tukey's pairwise comparison, Fig. 4.15).

The overall rewarming rate was negatively related to the initial T_b before arousal began ($r^2 = 0.33$, $p < 0.005$, $n = 25$; Fig. 4.16). The maximum rewarming rates measured over 12 min were negatively related to the T_b at which the maximum rewarming rate was observed ($r^2 = 0.20$, $p < 0.05$, $n = 25$; Fig. 4.17).

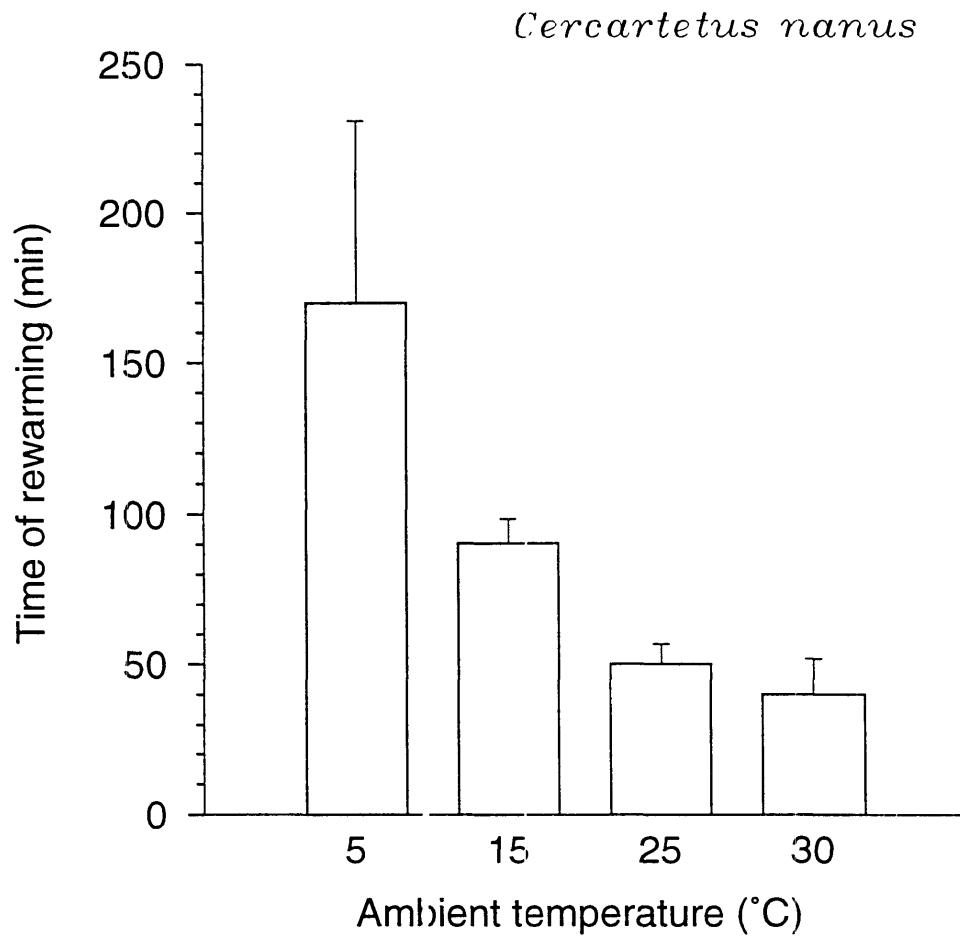


Fig. 4.14. Duration of rewarming from hibernation of *Cercartetus nanus* at different air temperatures (T_a). Error bars indicate the standard deviations for $N = 7$ at T_a 15 and 25°C, $N = 6$ at T_a 30°C, and $N = 5$ at T_a 5°C. The rewarming duration was effected by T_a ($F = 24.29$, $p < 0.001$, $n = 25$, ANOVA).

4.4.5 Maximum MR during Arousal from Hibernation

The initial T_b before arousal was initiated strongly affected maximum MR during arousal ($r^2 = 0.67$, $p < 0.001$, $n = 25$; Fig. 4.18). The maximum MR was also, though less significantly, related to the T_b at which the maximum rewarming rate was reached ($r^2 = 0.22$, $p < 0.05$, $n = 25$; Fig. 4.18), indicating that the maximum MR during arousal is probably not determined by T_b , but reflects how much T_b needs to be raised during rewarming.

The maximum MR during arousal was also effected by T_a ($p < 0.001$, ANOVA). In the TNZ, the maximum MR was significantly lower than at any other T_a (Tukey's pairwise comparison). As during the activity peak, the maximum MR during the arousal peak was linearly related to T_a (activity peak: $r^2 = 0.81$, $p < 0.001$, $n = 25$; arousal peak: $r^2 = 0.71$, $p < 0.001$, $n = 25$; Fig. 4.19a). When the maximum MRs during the two states were compared, no significant difference was observed in either the slope or the intercept of the regressions ($p > 0.05$ t-test for both slope and elevation), even though the T_b for the maximum MRs differed significantly (Fig. 4.19b). However, the cold-induced $\dot{V}O_{2max}$ measured in He-O₂ (6.710 ± 1.395 mL g⁻¹ h⁻¹) was significantly higher than those measured during the activity ($p < 0.05$ paired t-test, $N = 5$) and arousal peaks ($p < 0.01$ paired t-test, $N = 5$), despite the lower T_b for $\dot{V}O_{2max}$ in He-O₂. An extrapolation of the regressions between maximum MR during arousal and T_a , to the $\dot{V}O_{2max}$ in He-O₂ suggests that the cold-induced $\dot{V}O_{2max}$ would be reached at a T_a of about -10°C (Fig. 4.19a). It thus appears that the thermogenic capacity of *C. nanus* is higher than the rates they employed for rewarming during arousal.

The slopes for the regressions between the maximum MR and T_a during arousal and activity peaks were similar to that for RMR vs T_a ($p > 0.05$ for both, t-tests; Fig. 4.19a).

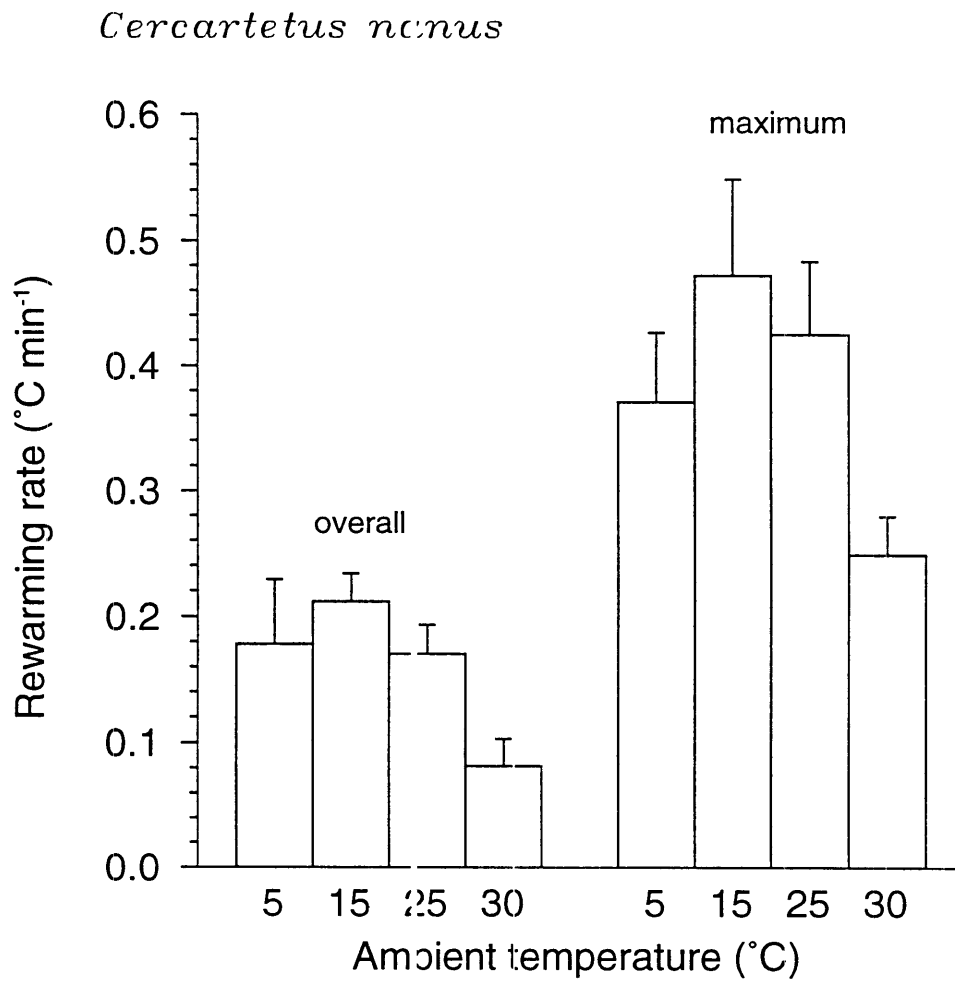


Fig. 4.15. The overall rearming rates and the maximum rearming rates of *Cercartetus nanus* during arousal from hibernation at different air temperatures (T_a). Error bars indicate the standard deviations for $N = 7$ at T_a 15 and 25°C, $N = 5$ at T_a 30°C, and $N = 5$ at T_a 5°C. Both the overall and the maximum rearming rates were affected by T_a ($F = 23.18$, $P < 0.001$, $n = 25$, ANOVA for overall rearming; $F = 19.87$, $p < 0.001$, $n = 25$, ANOVA for maximum rearming).

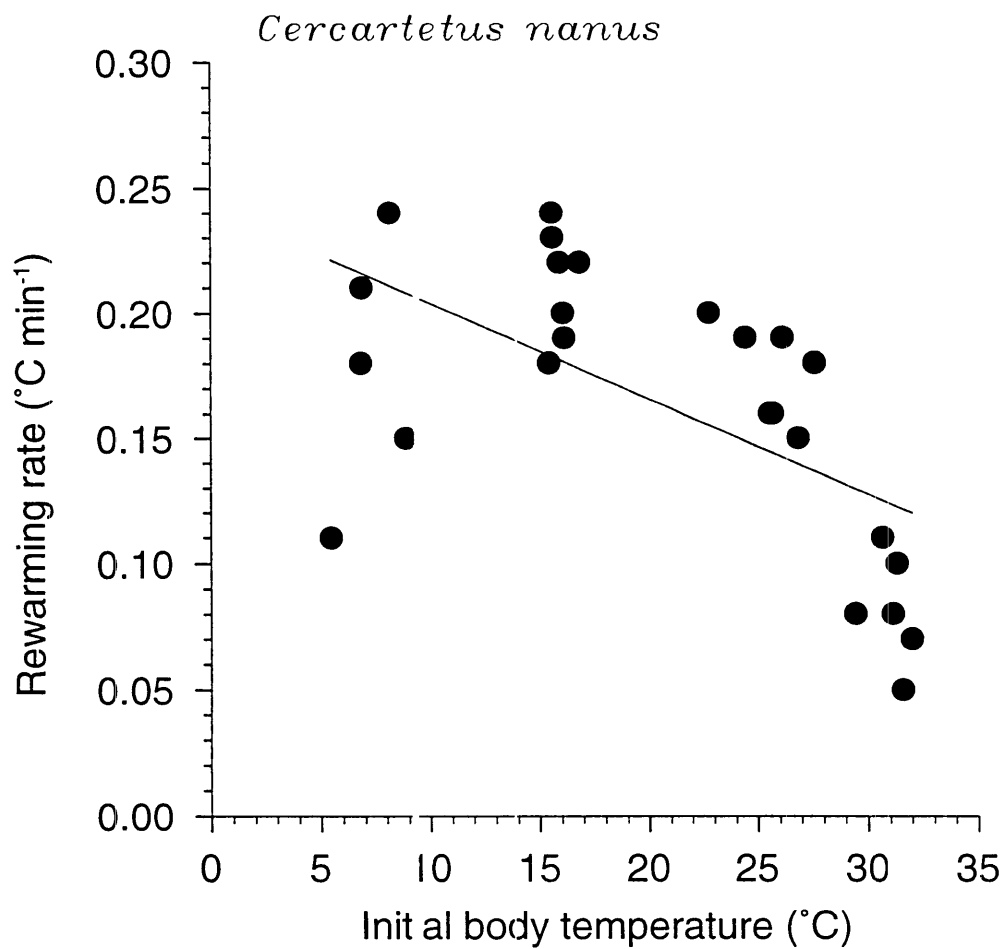


Fig. 4.16. The overall rewarming rates of *Cercartetus nanus* during arousal from hibernation as a function of the initial body temperature (T_b) when arousal began. The overall rewarming rate was inversely related to T_b :

$$\text{Rewarming rate} = 0.242 - 0.0038 \times T_b \quad (r^2 = 0.33, p < 0.005, n = 25).$$

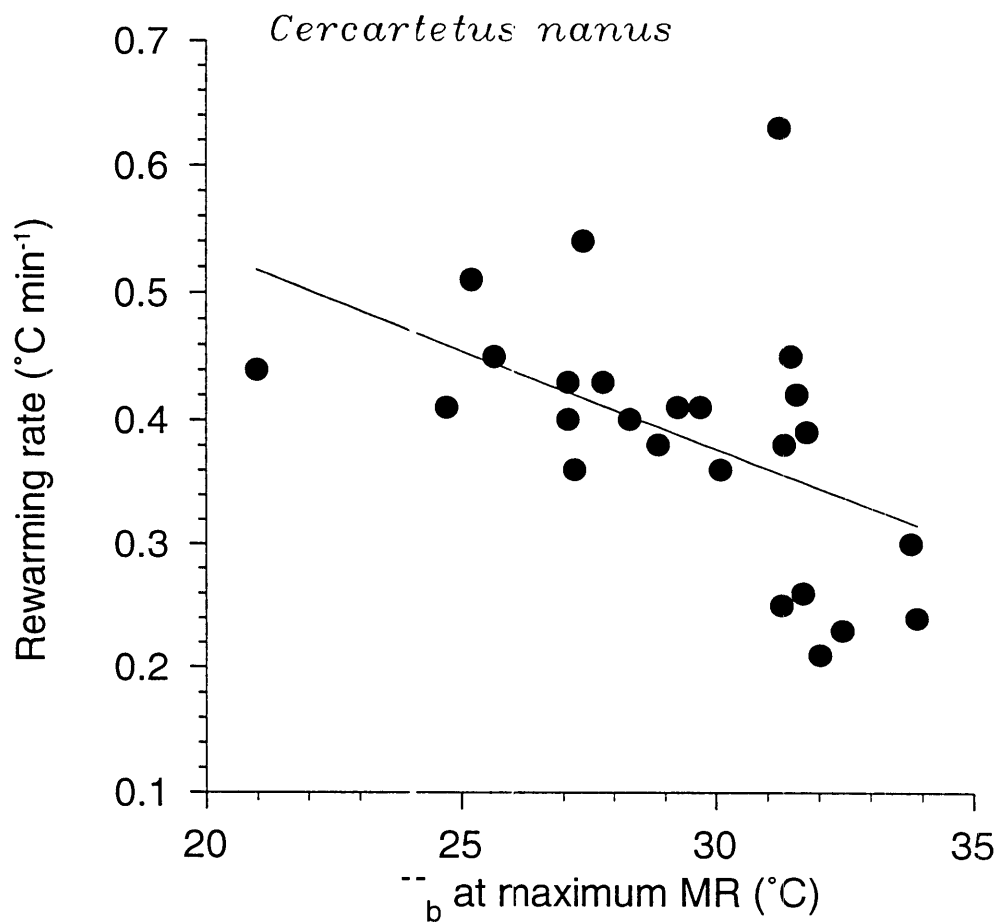


Fig. 4.17. The maximum rewarming rates of *Cercartetus nanus* during arousal from hibernation as a function of the T_b at which the maximum rewarming rate was reached. The maximum rewarming rate was inversely related to T_b :

$$\text{Rewarming rate} = 0.845 - 0.016 \times T_b \quad (r^2 = 0.20, p < 0.05, n = 25).$$

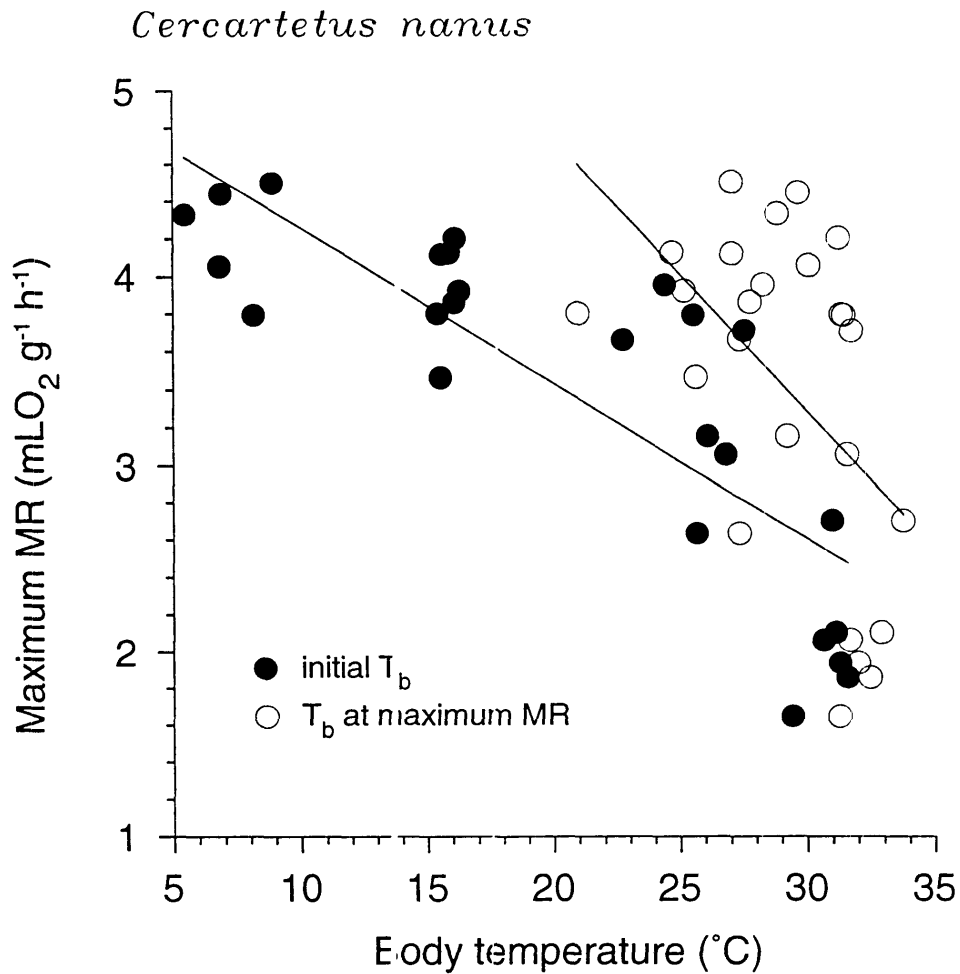


Fig. 4.18. Maximum heat production of *Cercartetus nanus* during arousal from hibernation as a function of the body temperature (T_b) at which arousal was initiated (closed circles), and the T_b at which the maximum MR was obtained (open circles). The maximum MR was inversely related to the T_b at which arousal was initiated:

$$\text{Maximum MR} = 5.09 - 0.0829 \times T_b \quad (r^2 = 0.67, p < 0.001, n = 25).$$

The $\dot{V}O_{2\max}$ was also inversely related to the T_b at which the maximum MR was obtained:

$$\text{Maximum MR} = 7.65 - 0.146 \times T_b \quad (r^2 = 0.22, p < 0.05, n = 25).$$

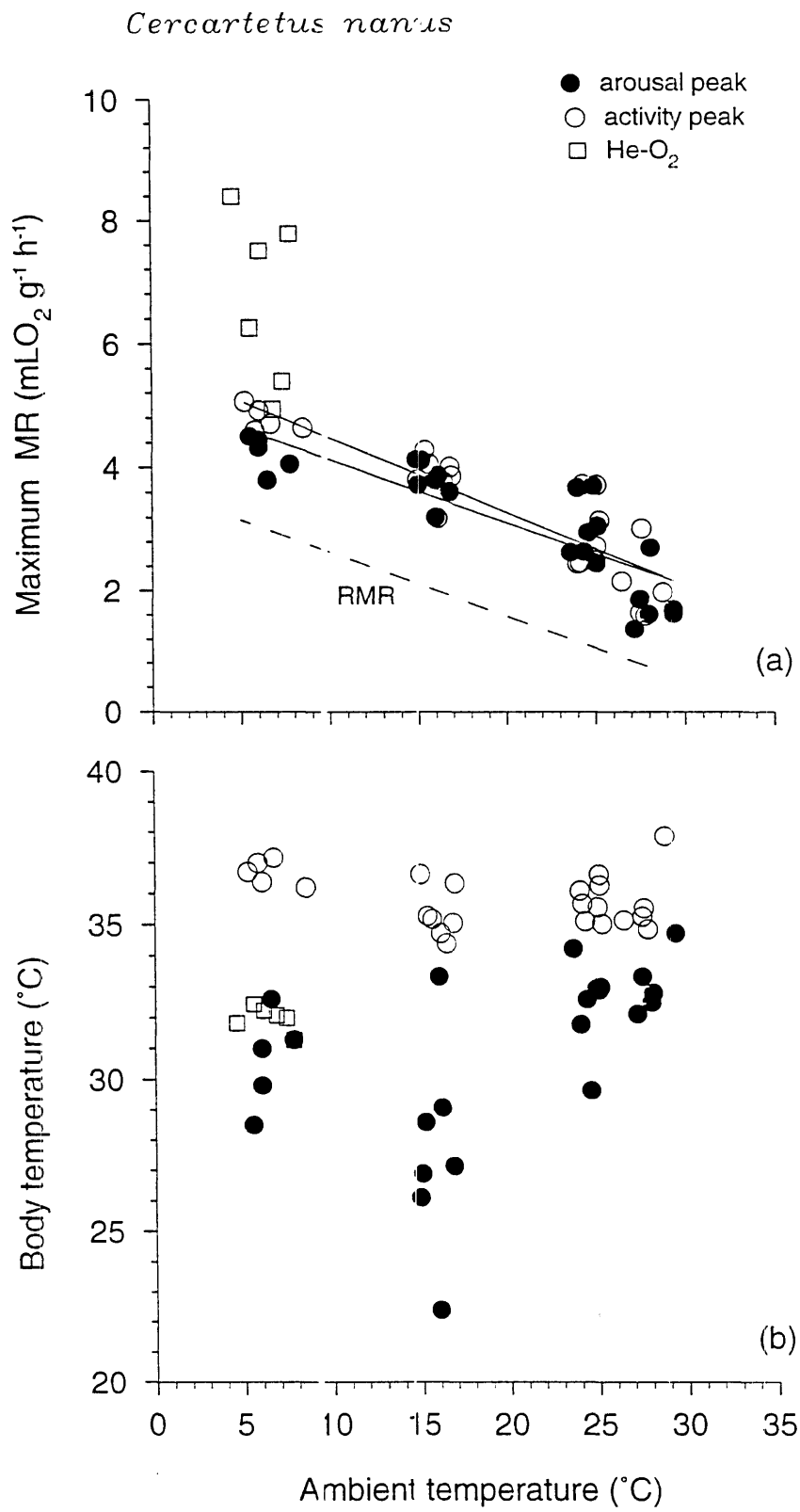


Fig. 4.19.

Fig. 4.19. (a) (previous page) Maximum metabolic rate of *Cercartetus nanus* during arousal from hibernation (closed circles), during activity (open circles) as a function of air temperatures (T_a), and the cold-induced $\dot{V}O_{2\max}$ measured in He-O₂ exposure (squares). The maximum MR during both arousal and activity peaks was closely related to T_a .

Arousal peak:

$$\text{Maximum MR} = 5.67 - 0.119 \times T_a \quad (r^2 = 0.80, p < 0.001, n = 25).$$

Activity peak:

$$\text{Maximum MR} = 5.14 - 0.102 \times T_a \quad (r^2 = 0.71, p < 0.001, n = 25).$$

The dashed line indicates the regression between RMR and T_a of the species.

(b) Body temperature (T_b) of *C. nanus* at which the maximum MRs were obtained.

4.5 Discussion

This study shows that both entry into and arousal from hibernation in *C. nanus* and daily torpor in *S. macroura* are complex phenomena. The relationships between MR and T_b during entry into torpor differ substantially from that during arousal from torpor. Although there are many similarities between hibernation and daily torpor during the entry or arousal phase, both torpor patterns have their idiosyncrasies.

4.5.1 Reduction of MR and T_b during Torpor Entry

It has been proposed that the reduction of MR during entrance into torpor of heterothermic endotherms consists of two separate processes. One is the drop of MR from RMR to BMR, caused mainly by the cessation of normothermic thermoregulation, which causes a decrease of T_b (Withers 1992). The other is a further drop of MR below BMR, due to the substantial decrease of T_b (Withers 1992), until a new equilibrium is reached (Bartholomew 1982). *S. macroura* always initiated torpor by a rapid reduction of MR that was followed by a fall of T_b supporting this interpretation. Of course, the two processes of MR reduction cannot be clearly separated, since MR is not reduced in a step-wise fashion from active or resting levels to BMR. In contrast, MR is affected by the fall of T_b from the beginning of entrance into torpor.

The Q_{10} for the reduction of MR below BMR during torpor entry in *S. macroura* after both TMR and T_b had reached steady-state was between 2 and 3. It is characteristic for temperature effects on rates of enzymatic reactions. This illustrates that the lowered T_b is an important factor in causing the reduction of MR during entry into daily torpor. This applies also to the MR undershoot at low T_{as} , suggesting a consistent influence of T_b on MR reduction at T_{as} both above and below the T_{tc} . While MR is affected by T_b , T_b itself can be precisely controlled during the whole entry phase (Heller et al. 1977; Wang 1989), as shown by the brief periodic increases

of MR which occurred throughout the entry phase. In addition, T_b regulation was also demonstrated by the fact that during torpor entry at T_{as} below the T_{tc} , TMR was approached at a stage where a ΔT was maintained with an increased MR. These observations are consistent with the theory of a sliding set-point for T_b during entry into hibernation (Heller & Colliver 1974; Florant and Heller 1977; Heller et al. 1977).

Although temperature effects upon MR can explain the steady-state TMR of *S. macroura*, TMR was reached when T_b was still in the process of decline. Hence the Q_{10} for the MR reduction between BMR and the start of TMR was slightly above the range for biological reactions. This indicates that superficially a combination of abandonment of regulatory thermogenesis and temperature effects on biochemical reactions is probably not sufficient to entirely explain the reduction of MR during early entrance into daily torpor in *S. macroura*. However, when TMR was reached, the core T_b of *S. macroura* was less than 1°C above the final T_b during entry, and thus most tissues had probably already reached the temperature minimum. Because of this temperature difference between the tissues, a measure of the core T_b might prevent a precise comparison of time course of the reduction of MR and T_b . Thus, if any, only a small fraction of MR reduction in *S. macroura* cannot be explained by temperature effects. Nevertheless, in other species, there appears to be some additional temperature-independent processes during entrance into torpor (Malan 1986, 1993; Geiser 1988a; Storey and Storey 1990; Milsom 1993; Guppy et al. 1994). This is further supported by recent interpretation *in vitro* measurements which show reductions of substrate binding of some glycolytic enzymes in the heart and skeletal muscle of deer mice during entry into daily torpor (Nestler et al. 1997).

In comparison to entry into daily torpor in *S. macroura* which may only involve a small proportion of metabolic inhibition, a large proportion of metabolic inhibition appears to be involved in the intense MR reduction during entry into hibernation in *C. nanus*. In contrast to the situation in *S. macroura*, in which the initial fast drop of MR

seems to terminate at a level only slightly below BMR, MR in *C. nanus* fell to 60% - 78% of the BMR during the initial MR drop. Consequently, the Q_{10} for the overall MR reduction from the BMR level during entry into hibernation was obviously well out of the range that is typical for the temperature effects on biochemical reaction rates. This suggests that the reduction of MR during torpor entry of *C. nanus* cannot be satisfactorily explained by a combination of cessation of heat production for normothermic thermoregulation and the effect of declining T_b . It is thus most likely that MR was substantially inhibited during entry into hibernation in *C. nanus*. This intense metabolic inhibition may explain the smaller TMR during hibernation than during daily torpor at the same T_b (Malan 1986; Geiser 1988).

From the extremely low MR value that was obtained shortly after torpor entry began, it seems that metabolic inhibition is more important in initiating torpor than during steady-state torpor. This initial metabolic inhibition is especially distinct at T_a s within the TNZ, since in this T_a range it is unlikely that MR can fall below BMR without any temperature-independent inhibition.

The significant metabolic inhibition during torpor entry may be to some extent related to a hypoventilation which leads to a retention of CO_2 and thus respiratory acidosis during the transient state from normothermia to torpor (Blickler 1984; Nestler 1990; Bharma and Milsom 1993). Respiratory acidosis occurs before changes of other physiological functions (Sjapp and Heller 1981; Malan 1986; 1988; Milsom 1993). Excess CO_2 may retard energy metabolism by affecting directly the neural control of thermoregulation such as facilitating the lowering of T_{set} , and thus cause T_b to decline progressively (Wünnenberg and Baltruschat 1982; Kuhnen et al. 1983; Heller 1988). Alternatively, functions of a number of enzymes that govern the rates of ATP synthesis and usage could be modified (Hand and Somero 1983; Hochachka and Guppy 1987).

4.5.2 Cooling Rate during Torpor Entry

In many physiological text books cooling during torpor entry in mammals and birds has been simplified and is described to be a pure Newtonian cooling curve (Lasiewski & Lasiewski 1967; Bartholomew 1982). However, animals differ considerably from a simple physical object. In contrast to a physical object without metabolic heat production, a mammal during entry into torpor shows a constantly changing thermogenesis. Cooling is slowed by internal heat production, and changes of MR affect cooling rates. Therefore, the log-transformed ΔT expressed as a function of time did not fit a single linear model in either *S. macroura* or *C. nanus*, but rather showed two to three phases that could be distinguished. The effect of MR on cooling rate was most significant at the beginning and mid entry phases, as well as when TMR was increased for thermoregulation at very low T_{as} . As expected, cooling in these instances was relatively slow since MR was high. Cooling accelerated when MR was decreasing after the initial MR drop during early entry into torpor.

Another important variable that affects rate of cooling is the thermal conductance. In contrast to an inanimate object which has a uniform C throughout the whole course of cooling, the C of an animal that is entering torpor can vary passively with MR apparently via changes of peripheral circulation and/or respiratory evaporation. The C may be also influenced actively by changes of posture, piloerection and circulation. The alteration of C is especially necessary at the end of the entry phase when MR approaches TMR. The C is high at high T_b s when MR is high which facilitates heat loss and a reduction of T_b , and it is low when the steady-state TMR is approached.

4.5.3 Rewarming Rates during Arousal from Torpor

The rewarming rate during arousal from torpor of heterothermic endotherms is mass-dependent, and general inverse allometric relationships between BM and rewarming rate have been established for different groups of mammals, including marsupials

(Heinrich and Bartholomew 1971; Heldmaier 1978; Geiser and Baudinette 1990). Much of the data for deduction of these allometric relationships were collected relying different experimental approaches and often within a relatively wide T_a range. Both hibernators and daily heterotherms were usually grouped together, and in some cases pre-flight warm-up of insects were also included (Heinrich and Bartholomew 1971). Nevertheless, the present study and the less than perfect fit of the allometric relationship ($r^2 = 0.55$ for 86 mammal species, Geiser and Baudinette 1990) show that body mass is only one of many factors that affect rewarming rates.

Firstly, the rewarming rate differs between spontaneous arousal and induced arousal. Thus, in this study, rewarming during spontaneous arousals of both *S. macroura* and *C. nanus* were slower than those of similar-sized animals during induced arousal (Geiser and Baudinette 1990). The differences in rewarming rates between induced and spontaneous arousals are apparently due to different rates of heat production that are applied during these arousals.

Secondly, rewarming rate changed significantly with T_a . For both *S. macroura* and *C. nanus*, rewarming rates at T_{a_s} below the T_{tc} were slow and do not fit an allometric equation. This is primarily because that in the cold heat loss of small animals increases more than in larger animals (Geiser and Baudinette 1990). Above the T_{tc} , rewarming rate of the two species decreased at high T_{a_s} . This change of rewarming rate with T_a was more pronounced in *C. nanus* than in *S. macroura*. *C. nanus* that were torpid within or close to the TNZ showed even slower rewarming rates than below the T_{tc} . The decrease of rewarming rates at high T_{a_s} was most likely due to a reduced thermogenic effort at the high T_{a_s} .

When compared at a similar T_a ($24.6 \pm 1^\circ\text{C}$ in *S. macroura* vs $25.0 \pm 0.5^\circ\text{C}$ in *C. nanus*), rewarming rate of the smaller species, *S. macroura*, was slower than that of the somewhat bigger species, *C. nanus*. This is in contrast to the prediction from the allometric relationship and, again, suggests the influence of factors other than BM on

rewarming rate. One consideration is that the differences in rewarming rate may be related to different torpor patterns of the two species, perhaps similar to the differences of TMR and T_b between hibernators and daily heterotherms. It has also been proposed that hibernators and daily heterotherms may be different during arousal in the aspect of vasomotor control of blood distribution (Wang and Hudson 1970, 1971). During rewarming, temperature differentia between anterior and posterior parts of daily heterotherms is not as obvious as that observed in hibernators (Wang and Hudson 1970). Nevertheless, the rewarming rates of 26 hibernators and 27 daily heterotherms appear similar (Geiser and Baudinet 1990). Therefore, despite the difference in TMR and the difference in differential rewarming pattern, rewarming rates do not differ between hibernators and daily heterotherms.

Alternatively, the different rewarming rates between *C. nanus* and *S. macroura* may reflect the marked differences of their conductance. Because *C. nanus* has abundant subcutaneous fat storage, and thus insulation, its heat loss is low. Therefore, a larger proportion of the heat produced during arousal is used for raising the T_b in *C. nanus* than in *S. macroura*. Presumably due to this greater effectiveness of the body heat storage during rewarming, *C. nanus* achieved the higher rewarming rate even at a lower rate of maximum MR compared with *S. macroura*.

4.5.4 Maximum Heat Production during Arousal

The maximum MR during arousal in both species was also T_a dependent, and the slopes of the increase of maximum MR with decreasing T_a were similar to those of RMR vs T_a . However, in *C. nanus*, even at the lowest T_a measured, the maximum MR during rewarming was still significantly lower than the $\dot{V}O_{2\max}$ measured in He-O₂, suggesting that the animal did not make a full use of their thermogenic capacity for arousal. Sub-maximal heat production during arousal has also been observed for other hibernators from cold environments, and energy conservation for other important

physiological tasks after arousal has been suggested (Wang and Abbotts 1981). Nevertheless, it is also possible that the $\dot{V}O_{2\max}$ in hibernators is mainly important to cope with colder environments during normothermia rather than for arousal.

In contrast to *C. nanus*, the maximum MR of *S. macroura* measured during arousal peaks at low T_{as} was not different from its cold-induced $\dot{V}O_{2\max}$ measured in He-O₂. This suggests that *S. macroura* may apply the highest rate of cold-induced heat production to ensure fast arousals.

In both species the maximum MR during arousal was inversely related to the T_b when arousal was initiated although rewarming rate did not seem to be a function of T_b , suggesting that the rate of heat production is a function of how much T_b needs to be raised from the torpid level to the normothermic level. This supports the view that the high rate of thermogenesis during arousal is activated by changes in the central regulation of T_b during the transition from torpor to arousal (Hammel 1986). In this process heat is produced proportional to ΔT via a negative feedback of thermoregulation, so that animals employ appropriate rates of heat production in proportion to the gradient between torpid and normothermic T_b s during the transient state from torpor to normothermia (Hammel 1986). Therefore, animals can accumulate the appropriate amount of heat during arousal to regain normothermia starting from different T_b s.

In consistency with the study on ground squirrels (Hammel et al., 1968), the rate of heat production during arousal continued to increase during rewarming. However, despite the increase of MR at high T_b s, the maximum MR of *S. macroura* was not related to the T_b at which the maximum MR was obtained, although the two variables were slightly correlated in *C. nanus*. Thus, the maximum MR that is achieved during arousal does not seem to be strongly affected by T_b . This interpretation is further supported by the fact that animals showed similar maximum MRs during arousal and activity, although their T_b s in the two physiological states were different.

The $\dot{V}O_{2\max}$ of *C. nanus* and *S. macroura* as well as that of other similar-sized dasyurids (Baudinette 1982) was 20% - 30% lower than that measured for rodents of similar size (Lechner 1978; Hinds and Rice-Warner 1992). However, the BMRs predicted from the equation derived for these rodents are much higher than the BMRs of the two marsupial species. Hence, the thermogenic scopes for both *C. nanus* and *S. macroura* were higher than that of these rodents. This supports the view that an animal that has a low BMR does not necessarily have a low thermogenic scope (Hinds and MacMillen 1984; Hinds et al. 1993). High thermogenic scopes together with low BMR have also been found for many other small marsupials and for heteromyid rodents (Dawson and Dawson 1982; Smith and Dawson 1985; Hinds and Rice-Warner 1992).

In summary, both changes of T_b during cooling into and rewarming from torpor are T_a dependent, apparently due to the effect of T_a on heat loss. However, changes of T_b are closely related with the change of MR during both entry and arousal. In both *S. macroura* and *C. nanus*, the initiation of torpor with a drop of MR before the decline of T_b demonstrates that cessation of normothermic thermoregulation is essential in the transient state from normothermia to torpor. The further reduction of MR from BMR to TMR during daily torpor entry of *S. macroura* can be largely explained by the progressively decreasing T_b . Nevertheless, metabolic inhibition appears to play an important role in reducing MR during hibernation in *C. nanus* in addition to T_b effects, especially during the initial entry phase. Both species possess a high thermogenic capacity. Nevertheless, *C. nanus* did not use its $\dot{V}O_{2\max}$ for spontaneous arousal, while *S. macroura* fully applied the $\dot{V}O_{2\max}$. However, despite the sub-maximal heat production, *C. nanus* achieved faster rewarming rates than *S. macroura*.