

Chapter 4

Thermal Acclimation Studies

4.1 Introduction

Temperature has a profound effect on physiological rates and processes. The effect of long term temperature changes can be minimised by acclimation. Alternatively, an animal may modify or move to a more suitable microclimate to maintain a constant temperature range, or endure the temperature changes without altering its physiology or behaviour. The lack of a strong seasonal effect on the metabolic rate of *Urodacus manicatus* suggests that metabolic compensation does not occur in this species (Chapter 3). Seasonal changes in temperature selection, however, suggest that *U. manicatus* may behaviourally adjust to changes in microhabitat temperatures (Chapter 3). Permeability was different between the Black Mountain and Dubbo populations during the warmer months (Chapter 3). The similarity of the burrow temperature profiles, however, suggested that temperature was not the reason for the differences in permeability.

The aims of this chapter are to determine the effects of high and low acclimation temperatures that approximate summer and winter burrow temperatures on:

1. metabolic rate.
2. temperature selection and activity.
3. permeability of whole scorpions to evaporative water loss.

4.2 Materials and Methods

Adult female scorpions were collected from the Black Mountain site and were maintained at 20°C in the laboratory for two weeks until the following experiments were conducted.

The scorpions were divided into two groups to separately examine the effect of acclimation to two temperatures on VO_2 (Section 2.3) and temperature selection (Section 2.4) so that each could be measured at the same time. After 12 days, food was withheld for 48 hours and VO_2 at 30°C and temperature selection were measured. Each group was then randomly split into two groups which were maintained at either 10 or 30°C. Animals were watered but not fed during acclimation. Measurements were repeated after 7 and 14 days. In addition to 30°C, VO_2 was also measured at 20 and 40°C. Measurements of animals from each group were staggered over days as not all animals could be processed at the same time.

The effect of acclimation temperature on water loss at 30°C (Section 2.2) was investigated. After two weeks at 20°C, the animals were divided into three groups and their daily water loss was measured over 2 days. The animals were then watered at the new temperatures of 10, 20 and 30°C over 7 days after which water loss was measured over a further 2 days.

The ability of mass to adjust rate of water loss was first tested in covariate analysis. A 3 x 2 between-subjects ANCOVA was performed on second day rate of water loss ($\text{mg H}_2\text{O h}^{-1}$) with mass at the end of the second day as the covariate (g). The independent variables were acclimation temperature group (10, 20 and 30°C) and effect of acclimation (water loss after acclimation to 20°C, then 7 days after further acclimation). Mass failed to adjust water loss, $F(1, 41) = 0.00, P > 0.05$. Analysis was, therefore, performed on the second day permeability ($\text{mg H}_2\text{O cm}^{-1} \text{h}^{-1}$). A 3 x 2 between-subjects MANOVA was performed on the two dependent variables with the independent variables being acclimation temperature group and effect of acclimation.

Prior to analysis on the effect of thermal acclimation on RMR, the adjustment made by mass as a covariate on the log transformed oxygen consumption rates was first tested. A 2 x 2 between-subjects MANCOVA was performed on the three dependent variables associated with the

temperature at which oxygen consumption was measured (20, 30, and 40°C) with log mass as the covariate. The independent variables were acclimation group (10 and 30°C) and days acclimated (7 and 14). There were no univariate or multivariate within-cell outliers at $\alpha = 0.001$. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory. However, Wilk's criterion indicated that mass did not provide adequate adjustment of the combined dependent variables, $F(3, 23) = 2.34$. The size range of the adult females was judged to be too narrow for covariate analysis which was therefore abandoned in favour of analysis of variance of mass-specific rates. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory for the non-transformed data, thus no transformations were conducted for analyses.

Comparisons with pre-acclimation measurements were made with a 2 x 3 between-subjects ANOVA performed on the dependent variable associated with oxygen consumption measured at 30°C. The independent variables were acclimation group (10 and 30°C) and days acclimated (7 and 14).

The effect of thermal acclimation on temperature selection was analysed using three profile analyses performed on five repeated measure dependent variables associated with the time periods: 15-18:00 h, 18-21:00 h, 21-24:00 h, 24-03:00 h and 03-06:00 h. The grouping variables were acclimation group (20, 10 and 30°C) and days at the acclimation temperatures (0, 7 and 14 days). The three analyses separately examined average, maximum and minimum selected temperature. For each analysis, order of entry of the grouping variables was group, then day. There were no univariate or multivariate within cell outliers at $\alpha = 0.001$. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory for the non transformed data.

The effect of thermal acclimation on activity was examined with profile analysis performed on five repeated measure dependent variables associated with the time periods: 15-18:00 h, 18-21:00 h, 21-24:00 h, 24-03:00 h and 03-06:00 h. The grouping variables were acclimation group (20, 10 and 30°C) and days at the acclimation temperatures (0, 7 and 14 days). The order of entry of the grouping variables was group, then day. There were no univariate or multivariate

within cell outliers at $\alpha = 0.001$. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory for the non transformed data. *Post hoc* tests examined the cause of deviation of the activity profiles from flatness using simple effects analyses which examined the mean differences between adjacent periods in a series of one-way within-subjects ANOVAs. Activity was calculated for each group after 14 days of acclimation (Figure 4.7), as the proportion of animals that had turned in the gradient every 15 minutes.

4.3 Results

4.3.1 Evaporative Water Loss

Acclimation temperature did not affect permeability as measured at 30°C, 0% RH, but acclimation time (seven days) resulted in smaller permeabilities for each acclimation group (Figure 4.1). The permeability of the 10°C-acclimated group decreased from 0.083 (± 0.012) to 0.071 (± 0.006) mg H₂O cm⁻² h⁻¹ and the 30°C-acclimated group decreased from 0.086 (± 0.014) to 0.074 (± 0.001) mg H₂O cm⁻² h⁻¹ after seven days of acclimation. The decrease in permeability with time was not significant, $F(2, 41) = 1.11$, $P > 0.05$ and was the same for each acclimation group, $F(2, 41) = 0.02$, $P > 0.05$.

4.3.2 Oxygen Consumption

The VO_2 's measured at 30°C after 7 and 14 days acclimation to 10°C and 30°C were greater than when the VO_2 's were measured after pre-acclimation to 20°C (Figure 4.2). When measured at 30°C, the VO_2 's of the 10 and 30°C-acclimated scorpions were the same after 14 days, but when VO_2 was measured at 20 and 40°C, scorpions acclimated to 10°C had a greater VO_2 than that of 30°C-acclimated animals (Figure 4.3). The respective pre-acclimation and 14 days post-acclimation VO_2 's (as measured at 30°C) were 57.43 (± 10.80) and 78.73 (± 8.13) $\mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$ for

the 10°C group and $54.99 (\pm 7.15)$ and $71.92 (\pm 8.21)$ $\mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$ for the 30°C group. The effect of a change in acclimation temperature on VO_2 measured at 30°C was not significant, $F(2, 40) = 1.20, P > 0.05$. Pairwise comparisons with Bonferroni adjustment indicated that the increase in VO_2 after seven days acclimation to 10°C was not significant, $P > 0.05$. The VO_2 's measured at both 20 and 40°C were not significantly different between the 10 and 30°C acclimation groups after 7 or 14 days acclimation, $F(1, 28) = 0.22, P > 0.05$.

Acclimation temperature did not affect Q_{10} . The Q_{10} 's between 20 and 30°C for the 10°C and 30°C-acclimated groups respectively were $2.24 (\pm 0.19)$ and $3.18 (\pm 0.63)$ and between 30 and 40°C were $1.70 (\pm 0.11)$ and $1.63 (\pm 0.09)$. There was a significant difference in Q_{10} between the upper and lower temperature ranges, $F(1, 13) = 10.56, P < 0.01$, but not between the groups, $F(1, 13) = 2.51, P > 0.05$.

4.3.3 Temperature Selection

The average temperatures selected by *Urodacus manicatus* were dependent upon the time of day (Figure 4.4). The three hourly profiles of temperature averaged over all animals deviated significantly from flatness, $F(4, 59) = 26.97, P < 0.001, \eta^2 = 0.65$. All temperatures selected after 7 days of acclimation to 10°C were less than when pre-acclimated to 20°C and did not decrease any further after 14 days (Figure 4.4b). Selected temperature in the first 3 hours of the scotophase decreased in 7 days from $32.2^\circ\text{C} (\pm 1.3)$ to $22.4^\circ\text{C} (\pm 1.4)$ and that of the photophase decreased from $26.3^\circ\text{C} (\pm 1.8)$ to $21.7^\circ\text{C} (\pm 1.8)$. The control group, which were continuously held at 20°C, did not change in selected temperature after 14 days (Figure 4.4a). Selected temperature in the first 3 hours of the scotophase was initially $30.1^\circ\text{C} (\pm 1.3)$ and then $30.2^\circ\text{C} (\pm 1.3)$ after 7 days. Photophase temperatures after acclimation to 30°C were the same as when pre-acclimated to 20°C. However, temperatures in the first 6 hours of the scotophase had increased after 7 days with no further increase after 14 days (Figure 4.4c). Selected temperature in the first 3 hours of the scotophase increased in 7 days from $31.4^\circ\text{C} (\pm 1.3)$ to $34.4^\circ\text{C} (\pm 1.3)$ whereas equivalent photophase temperatures were $24.4^\circ\text{C} (\pm 1.2)$ and $24.1^\circ\text{C} (\pm 2.1)$. The different effects of different acclimation temperatures on average selected temperature were

significant, $F(4, 62) = 3.74, P < 0.01$. The temperature profiles over time of day for each acclimation group deviated significantly from parallelism, $F(8, 118) = 3.23, P < 0.01$, but not between days of acclimation, $F(8, 118) = 1.33, P > 0.05$.

Maximum temperatures recorded within the gradient were affected by acclimation temperature in a similar way to that of average selected temperature (Figure 4.5). The three hourly profiles of maximum temperature averaged over all animals deviated significantly from flatness, $F(4, 59) = 44.93, P < 0.001, \eta^2 = 0.75$. Scotophase maximum temperatures after 7 days of acclimation to 10°C were less than when pre-acclimated to 20°C and their profile over time of day became flatter after 14 days (Figure 4.5b). The maximum temperature of the photophase period was the same after 7 days acclimation to 10°C but decreased after 14 days. Maximum temperature in the first 3 hours of the scotophase decreased in 7 days from 36.6°C (± 1.3) to 30.9°C (± 1.4) and further decreased to 28.3°C (± 1.4) after 14 days. That of the photophase decreased from 31.8°C (± 1.8) to 26.0°C (± 1.7) at day 14. The control group, which were continuously held at 20°C, did not change in maximum temperature after 7 days but decreased slightly after 14 days (Figure 4.5a). Selected temperature in the first 3 hours of the scotophase was 35.9°C (± 1.3) after 7 days and 31.5°C (± 1.4) after 14 days. Photophase maximum temperatures after acclimation to 30°C were the same as when pre-acclimated to 20°C, but temperatures in the first 6 hours of the scotophase had increased after 7 days with no further increase after 14 days (Figure 4.5c). Maximum temperature in the first 3 hours of the scotophase increased in 7 days from 35.9°C (± 1.3) to 39.4°C (± 1.3). The change in maximum temperature with acclimation was significantly different between acclimation groups, $F(4, 62) = 3.74, P < 0.01$. The maximum temperature profiles over time of day for each acclimation group were not significantly different, $F(8, 118) = 1.92, P > 0.05$, although the profiles did change with day, $F(8, 118) = 2.15, P < 0.05, \eta^2 = 0.24$.

The patterns in the minimum temperatures recorded were different to those of average and maximum temperatures (Figure 4.6). All minimum temperatures selected after 7 days of acclimation to 10°C were less than when pre-acclimated to 20°C and did not decrease any further after 14 days (Figure 4.6b). The minimum temperature in the first 3 hours of the scotophase decreased in 7 days from 23.9°C (± 1.6) to 16.5°C (± 1.7) and that of the photophase decreased from 24.4°C (± 1.3) to 18.9°C (± 2.0). The control group, which were continuously held at

20°C, did not change in selected temperature after 14 days (Figure 4.6a). Selected temperature in the first 3 hours of the scotophase was initially 22.5°C (± 1.9) and then 22.1°C (± 2.0) after 7 days. Temperatures in the first 6 hours of the scotophase had increased after 7 days acclimation to 30°C followed by a decrease in the first 3 hours of the scotophase after 14 days (Figure 4.6c). The profiles after acclimation to 30°C were the least flat of the acclimation groups with a rise in minimum temperature associated with the onset of the scotophase. Selected temperature in the first 3 hours of the scotophase increased in 7 days from 24.0°C (± 1.2) to 27.8°C (± 2.1). The different effects of different acclimation temperatures on minimum selected temperature were significant, $F(2, 62) = 3.36$, $P < 0.05$, although the effect of day alone was not significant, $F(2, 62) = 2.71$, $P > 0.05$. The temperature profiles over time of day for each acclimation group deviated significantly from parallelism, $F(8, 118) = 2.19$, $P < 0.05$, but not between days of acclimation, $F(16, 180) = 0.89$, $P > 0.05$.

4.3.4 Activity

The activity profiles after 14 days acclimation to each temperature were typical of *Urodacus manicatus* with activity increasing on dusk and then decreasing throughout the scotophase (Figure 4.7). Activity in the first 6 hours of the scotophase after acclimation to 10°C (Figure 4.7a) and 30°C (Figure 4.7c) was greater than in animals for which acclimation temperature had not changed from 20°C (Figure 4.7b). Activity between 21:00 and 24:00 h was 3.00 (± 0.72) turns for 20°C-acclimated animals compared to 4.25 (± 0.67) and 3.75 (± 0.67) for scorpions acclimated to 10°C and 30°C respectively. However, there were no significant differences in activity between the acclimation groups, $F(2, 62) = 2.26$, $P > 0.05$, or the days, $F(2, 62) = 1.41$, $P > 0.05$. The dependence of the amount activity on the time of day was significant, $F(4, 59) = 43.81$, $P < 0.001$, $\eta^2 = 0.75$. *Post hoc* tests indicated that activity was greatest in the first 3 hours of the scotophase, having increased with the onset of the scotophase, $F(9, 62) = 21.39$, $P < 0.001$, and decreased after 21:00 h, $F(9, 62) = 8.67$, $P < 0.001$. The profiles were not significantly different between the groups, $F(8, 113) = 1.72$, $P > 0.05$, and were not affected by day, $F(8, 118) = 1.96$, $P > 0.05$. Activity was, therefore, independent of thermal acclimation.

4.4 Discussion

This study clearly demonstrates a profound effect of thermal acclimation on the thermoregulatory behaviour of *Urodacus manicatus*. The temperature to which a scorpion was acclimated was positively reflected in the active temperatures selected in the gradient as well as the high and low avoidance temperatures. That acclimation is important to scorpions was supported by indirect evidence from other studies. Abushama (1964) found that the upper temperature at which *Leiurus quinquestriatus* reversed its direction within a gradient increased from 39 to 44°C with high temperature acclimation (> 39°C). In two different studies on temperature selection in *L. quinquestriatus*, Abushama (1964) measured a selected temperature of 20°C with a range of 15 to 39°C whereas Warburg and Ben-Horin (1931) found that field-collected *L. quinquestriatus* selected temperatures in the range 25-27°C. Although, differences in technique for measuring temperature selection may partially account for the different results of these studies, the lower temperatures selected in the study of Abushama (1964) were most likely due to acclimation in the laboratory whereby the animals were maintained at room temperatures lower than the field temperatures experienced by this desert grassland species.

Other studies on invertebrates have demonstrated a positive correlation between acclimation temperature and selected temperature. The crayfish, *Astacus astacus*, selected greater temperatures and had higher cold and warm avoidance temperatures when acclimated to 20°C than when acclimated to 5°C (Kivivuori 1994). Locusts and cockroaches also exhibited an increase in selected temperature following an increase in acclimation temperature (Chapman 1955, Coenen-Staß 1976).

Temperature selection in fish reflected their upper tolerance temperature (Tsuchida 1995). It was probable, therefore, that the effect of thermal acclimation on temperature selection in scorpions also affected their upper tolerance temperatures. This was further supported by the corresponding change in the maximum temperatures at which *Urodacus manicatus* reversed their direction within the gradient (upper avoidance temperatures). Other studies have found that pre-conditioning to higher temperatures than normal enhanced high temperature resistance in scorpions (Cloudsley-Thompson 1962a, Hadley 1990).

The active temperatures selected by the 10°C-acclimated *Urodacus manicatus* were the same as winter-collected scorpions from Black Mountain (Chapter 3) suggesting that during the colder months, the thermal strategy of *U. manicatus* changed in response to its cooler thermal environment. The decrease in selected temperature in response to a cooler thermal environment may depend on the threshold temperature at which the animals became active (Chapter 3). *U. manicatus* from Dubbo did not decrease their active selected temperatures in the gradient at a time when winter burrow temperatures were 12 to 15°C. Scorpions from Black Mountain, however, did decrease their active selected temperatures at a time when burrow temperatures were 2 to 6°C. Also, *U. manicatus* fed at 15°C but not at 10°C in captivity (pers. obs.). These observations suggest that 10 to 12°C may be the threshold temperatures at which *U. manicatus* becomes active in the field. By selecting lower temperatures in the gradient, *U. manicatus* reduced their metabolic expenditure and thus conserved their metabolic reserves during winter when feeding rates were low. The physiological change in *U. manicatus* with low temperatures was further supported by the movement of its range in temperatures to the cooler end of the gradient.

Acclimation to 30°C occurred within seven days because there was no difference in temperature selection between 7th and 14th days of acclimation. By selecting higher temperatures, *Urodacus manicatus* maintained normal activity patterns induced by their acclimation to 30°C. Temperature selection during periods of inactivity was not affected by acclimation suggesting that the physiological effects of a higher acclimation temperature were associated with foraging and feeding behaviour. The 30°C-acclimated scorpions selected higher active temperatures than summer-collected animals, the temperatures of which were the same as when captive scorpions were acclimated to 20°C (Chapter 3). This suggests that 20°C more closely represents the average summer burrow temperatures of *U. manicatus*. Scorpions from both Black Mountain and Dubbo were never continuously exposed to 30°C in the field as they were when acclimated to that temperature in captivity. Burrow temperatures were greater than 30°C during summer, but for only a very short duration, no more than a few hours per day (Figure 3.5). Both summer and autumn-collected *U. manicatus* from Black Mountain selected higher active temperatures than winter animals (Chapter 3) suggesting that thermal acclimation to higher burrow temperatures did affect temperature selection in *U. manicatus*.

Thermal acclimation did not affect the levels of activity in *Urodacus manicatus*. The 10 and 30°C-acclimated scorpions had similar activity profiles to that of the summer, autumn and winter-collected scorpions from both Black Mountain and Dubbo (Chapter 3). This suggests that the low activity profiles of the spring-collected scorpions were not due to their thermal environment but to some other factor.

Metabolic compensation to changing temperature did not occur in captive *Urodacus manicatus*. The VO_2 's of the high and low temperature acclimated scorpions were within the range of the VO_2 's of the field-collected scorpions from all seasons (Figure 3.10). The elevated Q_{10} 's of the summer-collected scorpions (Tables 3.4 and 3.5) were not apparent in the 30°C-acclimated group and both the 30 and 10°C groups had the same Q_{10} 's after acclimation.

Metabolic compensation was also absent from the xeric species *Parabuthus villosus* when acclimated to 10 and 30°C (Robertson et al. 1982). The general lack of metabolic compensation in arachnids has been attributed to their low metabolic rates (Anderson 1970). Animals with low Q_{10} 's (less than 2) have metabolic rates that are insensitive to temperature change (Dutton and Fitzpatrick 1975). *Urodacus manicatus* have low Q_{10} 's over their optimal temperature range, suggesting that a change in acclimation temperature over the same range of temperature would not induce metabolic compensation. In this study, *U. manicatus* collected from the field selected active temperatures of about 30°C, a temperature at which this species exhibited low Q_{10} 's (Chapter 3). Thus, metabolic compensation by *U. manicatus* did not occur after acclimation to 30°C.

Urodacus manicatus also did not metabolically compensate after acclimation to 10°C. Instead, the animals selected lower active temperatures in the gradient and foraging activity decreased (see above). This alternative strategy to metabolic compensation suggests that *U. manicatus* reduced energy expenditure by selecting lower temperatures rather than elevate their metabolic rate such that normal activities could be continued. The desert grassland species *Paruroctonus utahensis* inversely compensated when acclimated to 14°C, the temperature at which this species did not forage (Riddle 1979). Inverse compensation resulted in a further saving of energy when foraging activity ceased for *P. utahensis* during the colder months (Riddle 1979). It was not known whether or not *P. utahensis* selected lower temperatures after cold acclimation.

Inverse metabolic compensation may be an alternative strategy to the selection of lower temperatures if cooler temperatures were not available in the field. A third metabolic strategy was exhibited by the scorpion *Heterometrus fulvipes* in which partial metabolic compensation occurred when the animals were acclimated to their average winter temperature of 18°C. This temperature may have been high enough to allow for foraging activity to continue through winter. As a result, the RMR of *H. fulvipes* were preserved, although at lower levels than during summer. It is not known what effect acclimation temperature has on the thermoregulatory behaviour of *Heterometrus fulvipes*.

Acclimation temperature did not affect the cuticle permeability of *Urodacus manicatus* and this result was in agreement with the finding of a lack of seasonal change in permeability (Chapter 3). The results of this study contrast with the findings on the xeric adapted *Centruroides exilicauda* which, when collected in winter, had a higher permeability than when collected in summer (Toolson and Hadley 1979). The lack of change in permeability in this study may have been due to the provision of water *ad libitum* and the high humidity (approximately 85% RH) of the scorpions' containers. This interpretation is supported by the finding that captive *U. manicatus* had permeabilities two to three times that of the field-collected animals.

This chapter supports the conclusion of Chapter 3 that *U. manicatus* utilises behavioural mechanisms to counter changes in their thermal environment. Temperature selection and activity are influenced by the threshold temperatures at which foraging activity ceases. Chapter 6 examines whether feeding above the threshold temperature influences the behaviour and physiology of *U. manicatus*. Foraging activity is also connected with water availability because scorpions obtain most of their water from their prey (Yokota 1984). The next chapter deals with the effects of water availability in the behaviour and physiology of *U. manicatus* and also examines the effects of water availability on cuticle permeability in lieu of a thermal effect.

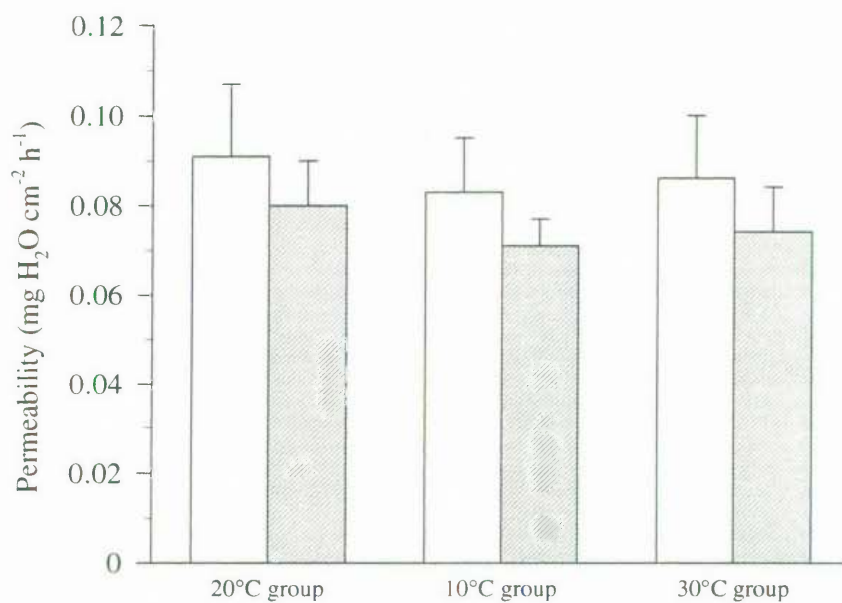


Figure 4.1. Effect of thermal acclimation on second day permeability (mg H₂O cm⁻² h⁻¹) measured at 30°C, 0% RH of adult females. Three groups of scorpions were pre-acclimated to 20°C (open bars) followed by acclimation to either 10, 20 and 30°C for 7 days (striped bars). For all cases $n = 8$.

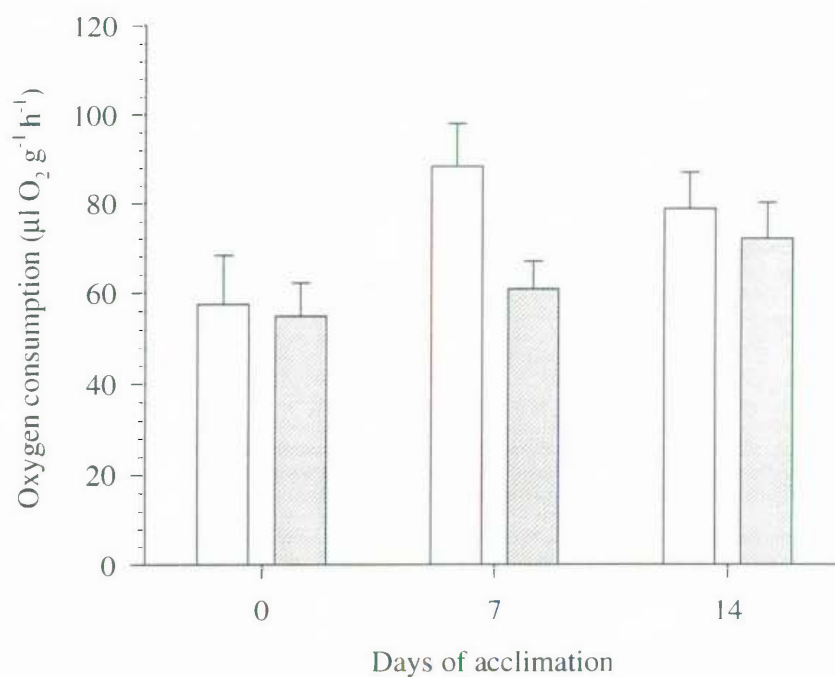


Figure 4.2. Effect of thermal acclimation on mass-specific oxygen consumption ($\mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$) measured at 30°C of adult females. Two groups of scorpions were pre-acclimated to 20°C (0 days) followed by acclimation to either 10°C (open bars; $n = 8$ for each day) or 30°C (striped bars; $n = 8$ for each day) for 7 and 14 days. Bars represent mean values \pm SE.

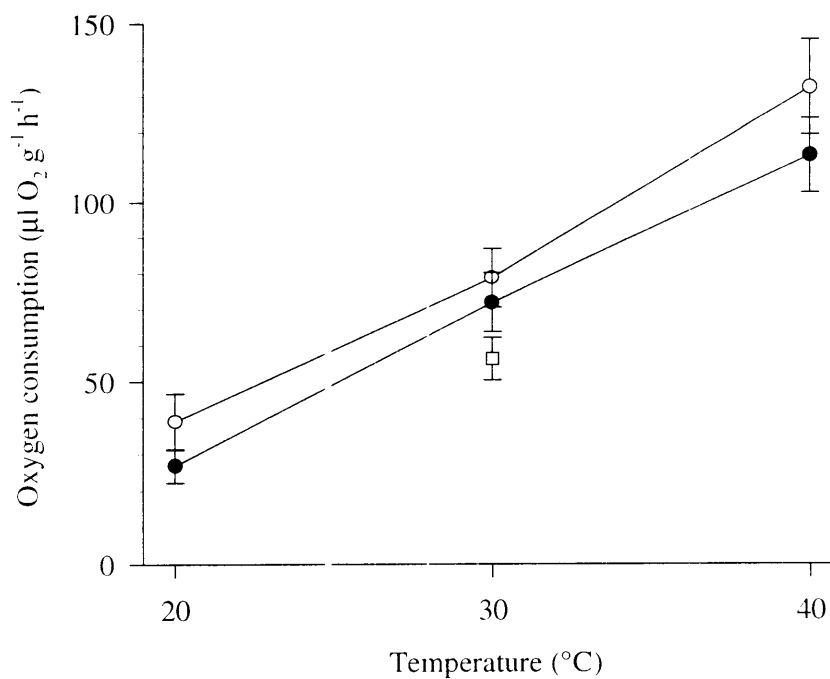


Figure 4.3. Effect of thermal acclimation on mass-specific oxygen consumption ($\mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$) of adult females. Two groups of scorpions were pre-acclimated to 20°C and their VO_2 measured at 30°C only (open square; $n = 16$) followed by acclimation to either 10°C (open circles; $n = 8$ for each day) or 30°C (closed circles; $n = 8$ for each day) for 14 days. Points represent mean values \pm SE.

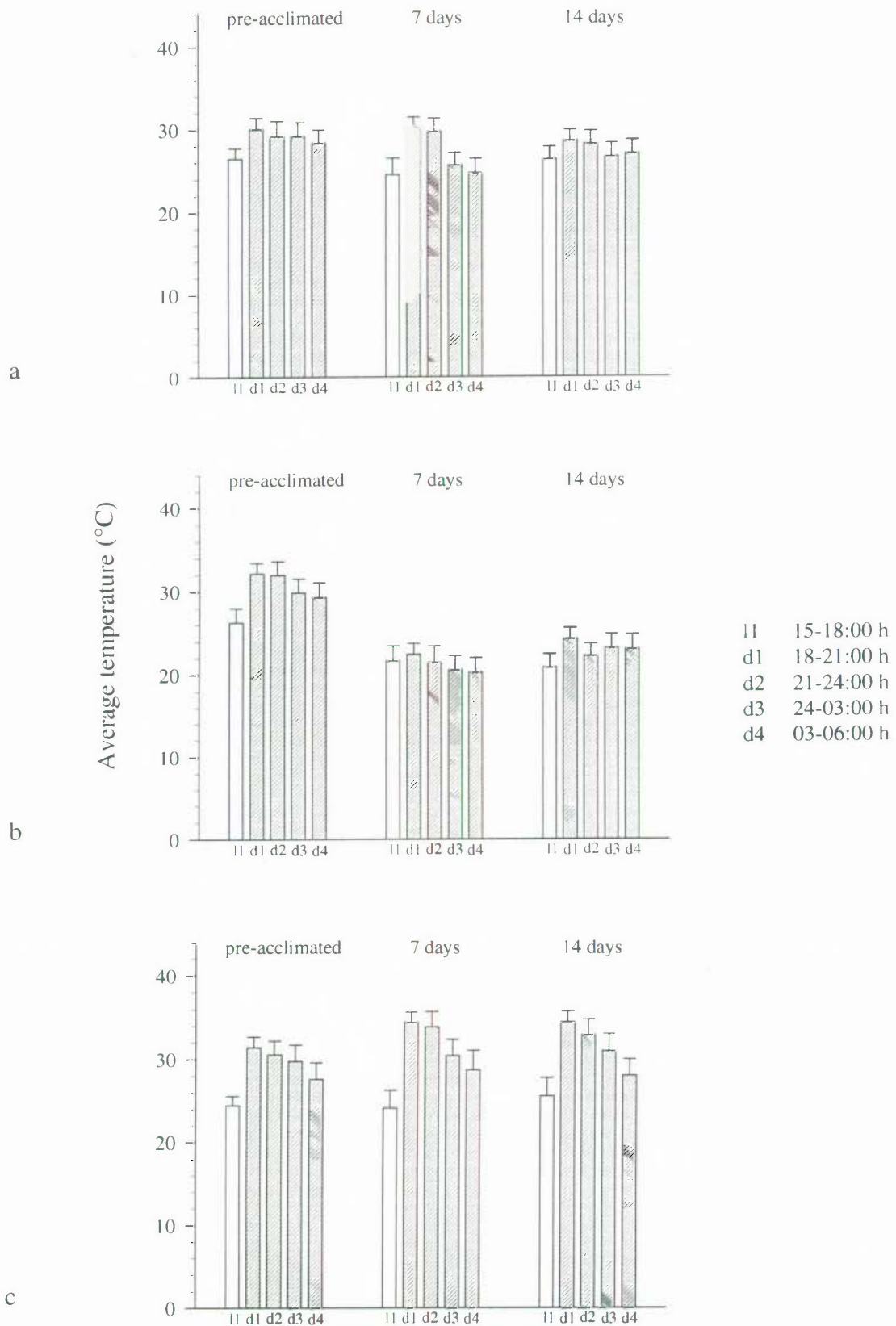


Figure 4.4. Effect of 7 and 14 days thermal acclimation on average selected temperature (°C) of adult females: (a) continuously acclimated to 20°C; (b) pre-acclimated to 20°C then 10°C; and (c) pre-acclimated to 20°C then 30°C. Averages are of 12 measurements per animal within the 3 hour periods of 15-18:00 h (l1), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3) and 03-06:00 h (d4). Bars represent mean values \pm SE (all $n = 8$). Shaded bars highlight the scotophase periods.

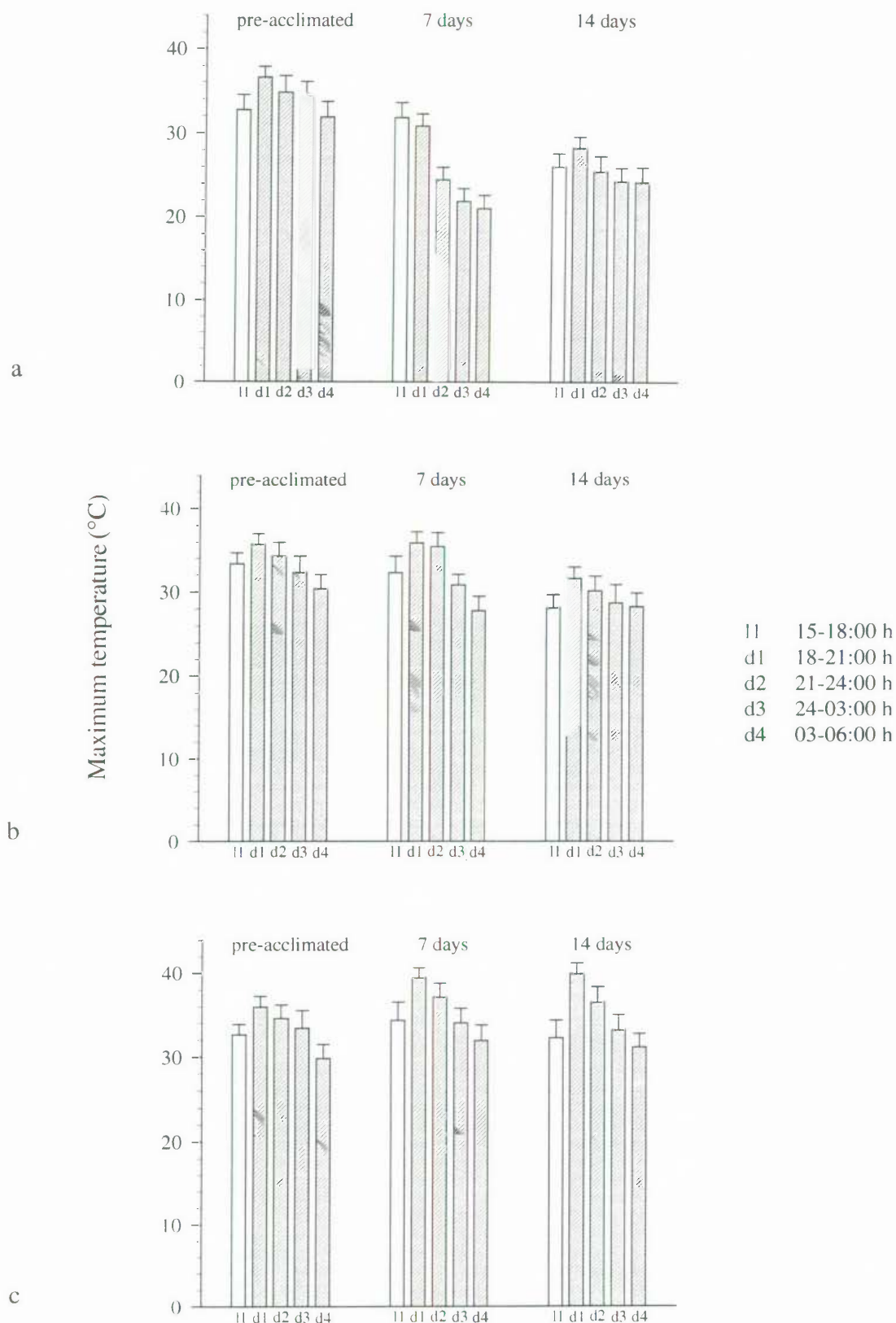


Figure 4.5. Effect of 7 and 14 days thermal acclimation on maximum temperature (°C) recorded in the gradient for adult females: (a) continuously acclimated to 20°C; (b) pre-acclimated to 20°C then 10°C; and (c) pre-acclimated to 20°C then 30°C. Maxima are from 12 measurements per animal within the 3 hour periods of 15-18:00 h (l1), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3) and 03-06:00 h (d4). Bars represent mean values \pm SE (all $n = 8$). Shaded bars highlight the scotophase periods.

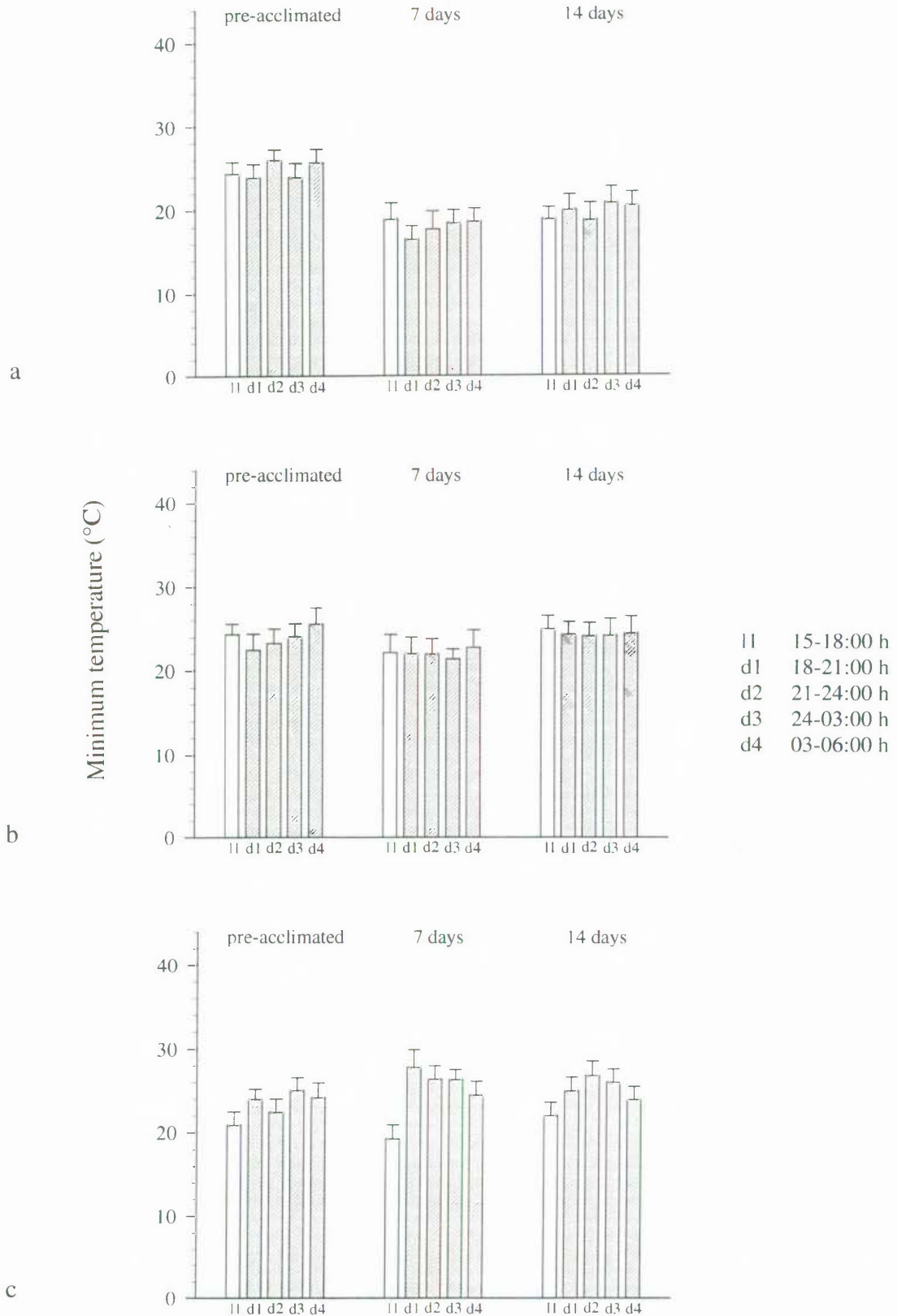


Figure 4.6. Effect of 7 and 14 days thermal acclimation on minimum temperature ($^{\circ}\text{C}$) recorded in the gradient for adult females: (a) continuously acclimated to 20°C ; (b) pre-acclimated to 20°C then 10°C ; and (c) pre-acclimated to 20°C then 30°C . Minima are from 12 measurements per animal within the 3 hour periods of 15-18:00 h (l1), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3) and 03-06:00 h (d4). Bars represent mean values \pm SE (all $n = 8$). Shaded bars highlight the scotophase periods.

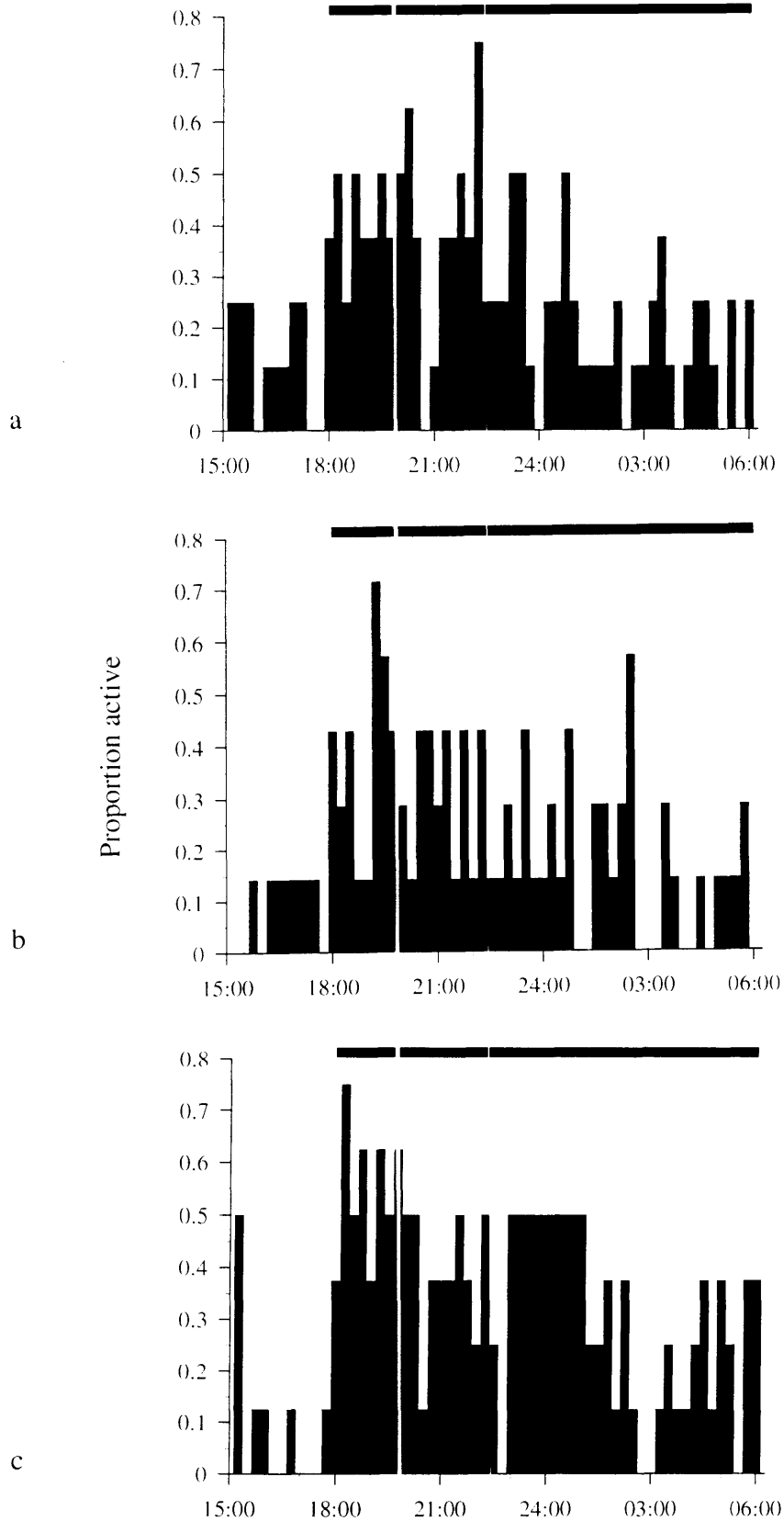


Figure 4.7. Activity profiles of adult females acclimated for 14 days to (a) 10°C, (b) 20°C, and (c) 30°C. Each bar represents the proportion of animals that had turned in the gradient resulting in a 5°C or greater change in selected temperature from the preceding temperature. Temperature was measured every 15 min. Horizontal bar represents scotophase from 18:00 to 06:00 hours. For all cases $n = 8$.