Chapter 3

Microhabitat and Seasonality Studies

3.1 Introduction

Habitat selection influences water and temperature relations and regulatory behaviours of animals (Ahearn and Hadley 1969, Anderson et al. 1979). However, studies should ensure that the habitat in question is the microhabitat immediate to the animals being studied (Cloudsley-Thompson 1962b). *Urodacus manicatus* has a distribution that extends from cool-temperate ranges to more xeric plains. This does not automatically imply that different populations of *U. manicatus* experience different seasonal changes in their microclimates. Before comparing the physiological and behavioural responses of *U. manicatus* to changing seasons, the microclimatic conditions of the populations in question need to be examined.

The aims of this chapter are:

1. to examine the microclimates of two populations of *Urodacus manicatus* that inhabit different parts of the species' distribution.

2. to examine the effect of season on metabolic rate, temperature selection and activity for *U. manicatus* from both populations.

3. to examine the effect of population and season on the permeability of whole scorpions to evaporative water loss, their body water content and haemolymph osmolality.

4. to measure the supercooling point in *U. manicatus* from the more xeric population.

3.2 Materials and Methods

The two populations of *Urodacus manicatus* chosen for this study were from the rural property "Glücksberg" near Black Mountain (Lat. 30°19', Long. 151°39') (Plate 3.1a) and Goonoo State Forest near Dubbo (Lat. 31°57', Long. 148°53') (Plate 3.1b) both in New South Wales, Australia (Figure 1.1b). Monthly meteorologic il data was obtained from weather stations located at Black Mountain and Dubbo (Australia 1988). Monthly average daily temperature ranges (Figure 3.1), rainfall (Figure 3.2a) and rainday data (Figure 3.1b) indicated that the sites sufficiently differed in broad climatic conditions to warrant their selection for this comparative study. Oxygen consumption, temperature selection and activity were determined for adults of both sexes collected from both populations during each season.

Microhabitat temperatures were determined monthly at Black Mountain and seasonally at Dubbo. This was achieved by placing minimu n-maximum thermometers beneath basalt rocks at Black Mountain (Plate 3.2a) and fallen timber (Plate 3.2b) at Dubbo at the same depths as the living area of scorpion burrows. The rocks selected at Black Mountain were of similar size (each approximately 300 x 200 x 200 mm). Two of these were established home sites of two adult females with one located at the base of a mature *Eucalyptus* sp. in perpetual shade and the other located between tree canopies of the open woodland thus receiving both sun and shade. A third rock was a non home site rock lying completely exposed in an area of cleared vegetation approximately 10 m from the other two rocks. Shaded air temperature was measured by placing thermometers 2 m high on the trunks of trees.

When scorpions were collected, microhabitat temperatures were recorded electronically during summer and winter every 15 minutes over a few days by use of an eight channel temperature data logger powered by a 6 Volt car battery. The data logger was sealed within a rectangular plastic food storage container and the thermocouples connected via small sealed holes in the side of the container. A larger container was placed over both the battery and logger to protect the equipment from rain or interference by animals. The same parts of the microhabitat were measured in summer and winter and locations with similar aspect and surrounding shade were selected at each site. The microhabitat temperatures at Black Mountain were recorded at

the burrow and runway entrance of an established home site rock measuring approximately 280 x 180 x 190 mm, and also in the soil at a depth of 20 mm positioned 40 mm from the burrow entrance. Adjacent to the home site was a smaller diameter rock measuring approximately 130 x 110 x 190 mm under which a thermocouple was centrally placed. Similarly, temperatures at Dubbo were recorded in the living area and runway entrance of an established home site that was piece of fallen bark measuring approximately 2100 x 400 x 40 mm, as well as the soil at a depth of 20 mm positioned 40 mm from the burrow entrance. A thermocouple was also placed beneath an adjacent unoccupied smaller piece of bark measuring approximately 1300 x 130 x 40 mm.

The population density of *U. municatus* for all instars throughout the year was much greater at Black Mountain than at Dubbo. Thus many more animals were collected from the former site. Once the animals were collected, they were transported to the laboratory as soon as was possible. They were kept in the collecting jars overnight at the nightly temperatures experienced in the field and then the adults were placed into the temperature gradient (Section 2.4). The following morning the animals were removed and weighed and their oxygen consumption was measured at 20, 25, 30, 35 and 40°C (Section 2.3) The masses of all adults collected including those not used in the various measurements were recorded to obtain a seasonal profile of mass for each population. In most cases, animals were killed in boiling water and then dried at 80°C in an oven until their mass no longer decreased.

Temperature selection was not measured for winter males from Black Mountain, thus winter was excluded from an initial test on the effect of sex on temperature selection. A profile analysis was performed on six 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, 03-06:00 h and 06-09:00 h. The grouping variables were population (Black Mountain and Dubbo), season (summer, autumn and spring) and sex. Only average selected temperature was analysed. Order of entry of grouping variables was population, then season and sex. 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.

For the levels test, no significant difference was found between the sexes when temperature was averaged over all the periods, $F(\ , 65) = 1.15$, $P > 0.05$ nor did the profiles of the periods

between the sexes differ significantly from parallelism, $F_{(5, 61)} = 0.69$, $P > 0.05$. Female and male data were, therefore, combined to examine the effects of population and season (including winter) on temperature selection over the six time periods.

Three profile analyses were performed on six 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, 03-06:00 h and 06-09:00 h. The grouping variables were population (Black Mountain and Dubbo) and season (summer, autumn, winter and spring). The three analyses separately examined average, maximum and minimum selected temperature. For each analysis, order of entry of the grouping variables was population, then season 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.

Activity was not measured for winter males from Black Mountain, thus winter was excluded from an initial test on the effect of sex on temperature selection. A profile analysis was performed on six 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, 03-06:00 h and 06-09:00 h. The grouping variables were population (Black Mountain and Dubbo), season (summer, autumn and spring) and sex.

No significant difference was found between the sexes when temperature was averaged over all the periods, $F_{(1, 65)} = 0.86$, $P > 0.05$, nor did the profiles of the periods between the sexes differ significantly from parallelism, $F_{(5, 61)} = 0.72$, $P > 0.05$. Female and male data were therefore combined to examine the effects of population and season (including winter) on temperature selection over the six time periods. *Post hoc* tests were conducted to evaluate flatness and deviation from parallelism of the profiles with regard to season 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 population and season in Figures 3.14 to 3.17 as the proportion of animals that had turned in the gradient every 15 minutes.

The effect of population on season changes in RMR was initially examined utilising the allometric relationship between metabolic rate and body mass (Brody 1945). When metabolic rate is measured as oxygen consumption, the relationship is presented as:

$$VO_2 = a\,m^b$$

where VO_2 is the rate of oxygen consumption ($\mu l\ O_2\ h^{-1}$) and m is mass (g) with coefficient a and exponent b. The logarithmic form of this model is linear ($\log VO_2 = \log a + b \log m$), thus enabling the comparison of means of VO_2 after adjustment for mass by the use of ANCOVA where mass is the covariate. Analyses based on this form must have an adequate range of body size to obtain meaningful regressions As adults were used to gain more accurate measurements, the range could only be extended if there was no significant difference between male and female mass-specific VO_2 and males could therefore be treated as smaller females. To check for this, one way MANOVAs were conducted for each combination of population and season on the five dependent variables associated with temperature at which VO_2 was measured. Males collected in summer from Dubbo had a significantly higher VO_2 than females for the combined repeated measures, univariate $F(1, 12) = 5.26$, $P < 0.05$. Dubbo winter males at 40°C had a smaller VO_2 than females, univariate $F(1, 14) = 9.34$, $P < 0.01$. Also confounding was that, owing to time constraints, males in some cases were not measured at all temperatures and no adult males were found at Black Mountain during winter. All further analyses were, therefore, based on mass-specific VO_2, treating females and males separately.

For females, a 2 x 4 between-subjects MANOVA was performed on five dependent variables associated with the temperature at which VO_2 was measured: 20, 25, 30, 35 and 40°C. Independent variables were population (Black Mountain and Dubbo) and season (summer, autumn, winter and spring). Two analyses were conducted for males. Firstly, a 2 x 3 between-subjects MANOVA was performed on dependent variables associated with the temperature at which VO_2 was measured: 20, 30 and 40°C. Independent variables were population (Black Mountain and Dubbo) and season (summer, autumn and spring). Secondly, to include winter males from Dubbo, a single factor between-subjects univariate analysis of variance was performed on VO_2 measured at 40°C. The independent variable was season (summer, autumn, winter and

spring). Order of entry of independer t variables was population, then season. There were no univariate or multivariate within cell cutliers at $\alpha = 0.001$. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory in each analysis.

Values of temperature coefficient (Q_{10}) for each 5°C rise in temperature were calculated for each female. A 2 x 4 between-subjects MANOVA was performed on four dependent variables associated with each 5°C temperature difference at which oxygen consumption was measured: 20-25, 25-30, 30-35 and 35-40°C. Independent variables were population (Black Mountain and Dubbo) and season (summer, autumn winter and spring). To compare the Q_{10} values of females with males, Q_{10} was calculated for each 10°C rise in temperature. A 2 x 3 between-subjects MANOVA was performed on two dependent variables associated with each 10°C temperature difference at which VO_2 was measured: 20-30 and 30-40°C. Independent variables were sex and season (summer, autumn and spring).

The effects of population and season on the masses of adult *U. manicatus* used in the measurements of RMR and temperature selection were examined. Adult males were always smaller than females and were, therefore, treated separately. Female mass was examined with a 2 x 4 between-subjects ANOVA performed on mass as the dependent variable. Independent variables were population (Black Mountain and Dubbo) and season (summer, autumn, winter and spring). A similar 2 x 3 analysis was performed on male data that excluded winter. Winter data was included by performing a single factor analysis of variance on males collected only from Dubbo with season (summer, autumn. winter and spring) as the independent variable. The masses of adult females (of which VO_2 and temperature selection were not measured) were included with the above and the results were expressed in histograms to illustrate the change in distribution with season for each population. The cuticles of the summer males from both populations were prepared as described in Section 2.2 to examine whether the differences in body mass between the populations was due to current nutritional status or size. Seasons were combined and the means of each population were compared with Student's *t*-test.

The proportion of water mass of the total wet body mass was measured to ascertain whether water was responsible for the observed differences in mass. The effect of sex was

examined first and found to be non significant, $F(1, 63) = 0.61$, $P > 0.05$. Water contents of females and males were combined and, owing to limitations in the data, the following two analyses were conducted. A 2 x 3 analysis of variance was performed with water content as a proportion of wet body mass as the dependent variable excluding winter. Independent variables were population (Black Mountain and Dubbo) and season (summer, autumn and spring). A single factor analysis of variance was performed on Black Mountain data only with season as the independent variable (summer, autumn, winter and spring).

Evaporative water loss was measured at 30°C, 0% RH (Section 2.2). This temperature was selected because it was closest to the average temperatures normally selected by scorpions in the gradient. No food or water was provided between collection and placement in the desiccator. To account for differences in size of the animals collected from each population and season, second day water loss rates were expressed as permeability, that is, as per surface area (mg H_2O cm^{-2} h^{-1}). The values were normalised by log transformation. A single factor MANOVA with sex as the independent variable was performed on the dependent variable of permeability. Univariate analysis showed no significant effect of sex on permeability, $F(1, 69) = 0.16$, $P > 0.05$. Females and males were, therefore, combined to study the effect of population and season in a 2 x 3 between-subjects MANOVA performed on the dependent variable of permeability. Independent variables were population (Black Mountain and Dubbo) and season (summer, autumn and spring). A separate ANOVA was performed on Dubbo measurements only to include winter data from that population. Thus season became the only independent variable. Order of entry of independent variables was population, then season. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory.

Haemolymph osmolality was examined in adult animals collected during winter. Eight individuals of each sex were collected from Black Mountain and ten of each sex from Dubbo. The small size of *U. manicatus* and the need to keep other specimens vital in Chapter 5 made it necessary to devise a means of sampling haemolymph with the minimum of damage. The scorpions were restrained by placing a piece of adhesive putty over the metasoma as well as the cephalothorax and pedipalps and securing to a base. The scorpions did not struggle once their eyes were covered. The dorsal surface of the mesosoma was washed thoroughly with distilled

water and a cotton bud and then allowed to dry. The sixth dorsal sclerite of the mesosoma was punctured to one side of the heart with an insect minutin pin and a small droplet of haemolymph formed on the surface of the cuticle. This was immediately collected in a capillary tube by placing the end of the tube into the haemolymph and drawing it up by capillary action. Clear haemolymph was always obtained owing to the open circulatory system (Kimura et al. 1988). The osmolality of the sample was immediately measured using a Wescor 5100 B vapour pressure osmometer. Only one sample was collected from each specimen to reduce the impact on haemolymph volume. The scorpions did not appear to be detrimentally affected by this process and they rapidly regained weight when allowed to drink over night before the treatments started. Scorpions are capable of rapidly producing haemolymph (haemopoiesis) (Vachon 1953).

The effects of population and sex on the dependent variables of haemolymph osmolality and mass were examined with a 2 x 2 between-subjects MANOVA. The order of entry of the independent variables was population (Black Mountain and Dubbo), then sex. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory.

Supercooling points were measured for 11 females (0.234 ± 0.054 g) collected during winter from Dubbo. An aluminium, six-chambered specimen holder was mounted onto the surface of a cold stage, each chamber measuring 30 mm x 8 mm. Specimens were constrained within a suitably sized piece of PVC surgical tubing with cotton wool placed in the ends. A thermocouple was inserted into each tube and placed against the surface of the animal, the inner wall of the tube keeping it in place. The stage was cooled using a Peltier cell and the heat was removed by flowing water. The specimen holder was covered with a clear perspex lid and the whole cooling block insulated with a polystyrene-lined bakelite container. An automatic temperature controller provided a standard cooling rate of $1°C \, min^{-1}$ (Salt 1966). The thermocouple amplifier unit was connected to a Maclab/8. Maclab software plotted the progressive temperature drop against time with an accuracy of 0.1°C. The previous temperature recorded before a rise in temperature (assumed to be due to the release of the latent heat of crystallisation) was noted as the supercooling point.

3.3 Results

3.3.1 Microhabitat

Seasonal changes in the temperature ranges of typical home sites at Black Mountain and Dubbo (Figure 3.3b) generally reflected the pattern air temperature at each site (Figure 3.3a). The seasonal ranges in air temperature at Dubbo extended beyond the equivalent seasonal ranges at Black Mountain (Figure 3.3a). The highest air temperatures recorded at Black Mountain and Dubbo were 33.5 and 40.5°C and the lowest were -2.0 and -3.0°C, respectively. The maximum home site temperature for February a: Black Mountain was greater than for Dubbo but dropped by 12°C within a month. By April the minimum home site temperature at Black Mountain was 9°C lower than the same at Dubbo. From May to August, the magnitude of the ranges in home site temperature at Black Mountain remained the same, but dropped in overall temperature. The magnitude of the seasonal home site ranges of February to April were greater at Black Mountain, encompassing the Dubbo seasonal range for the same period. The magnitudes of the temperature ranges of the other three seasons were the same between the sites, with maximum and minimum temperatures at Dubbo generally 3 to 5°C higher than at Black Mountain and never falling below 0°C.

Potential rock home sites at Black Mountain that differed in the amount of shade they received, exhibited different seasonal patterns of temperature ranges (Figure 3.4). The continuously shaded rock experienced much smaller ranges contained within the ranges of the partially shaded and exposed rock during the warmest and coldest months of the year. Generally, the temperature below the tree-shaded rock remained above 3°C. The drop below 0°C recorded in September for all three of the rocks was due to a large and unseasonable snowfall that remained on the ground for two days. The ranges of the partially shaded and exposed rocks were similar as temperatures decreased in autumn. During winter, the minimum temperatures remained below 0°C but the maximum temperatures of the exposed rock were above 20°C and that of the partially shaded rock no higher than 15°C. As the months got warmer the monthly maximum temperatures of the exposed rock increased before hose of the partially shaded rock and also remained higher except for the maxima recorded for February.

Different parts of the microhabitat exhibited different daily temperature profiles at Black Mountain (Figure 3.5a) and Dubbo (Figure 3.5b) during mid summer and mid winter. The days selected for the microhabitat temperature measurements experienced similar amounts of sunshine and near average daily air temperatures for each site (the air maxima and minima recorded over the 48 hours are presented in Table 3 1). The burrow temperatures during the day at Black Mountain exhibited unusual high peaks that reached 24.5°C that were not present for the entrance temperature profile (Figure 3.5a). For the duration of the temperature recordings, the scorpions were not removed from their home si es. Although an effort was made to record only burrow temperature, it was possible that the l irge female under the home site rock at Black Mountain was active during the day within the burrow and caused the thermocouple junction to rest occasionally against the underside of the rock. Th is, the peaks in the profiles may represent rock temperature and not burrow temperature. The bases of these peaks followed the profile of the entrance temperatures and therefore represented the actual burrow temperatures.

The burrow temperature beneath the rock home site rose more sharply than that below the bark approximately one hour after sur rise in both seasons. There was little fluctuation in daily temperature in the bark burrow in winter (temperature range 11.0 to 13.3°C). This contrasts with the rock burrow (temperature range -1.8 to 15°C). The summer ranges in burrow temperature were similar for both populations: Black Mountain, 17.0 to 27.8°C and Dubbo, 19.0 to 29.0°C.

The soil below small but potent al home sites adjacent to larger occupied home sites exhibited diurnal fluctuations in their temperature profiles at Black Mountain (Figure 3.6a) and Dubbo (Figure 3.6b). The smaller rock at Black Mountain heated the soil below it to the same degree as the larger rock in both summer and winter, but its minimum temperatures at night in winter remained 3 to 4°C higher than the larger rock and didn't fall below 0°C. The temperature profile of the smaller home site at Dubbo followed the soil and entrance profiles in Figure 3.6a. As such it would not have afforded protection from high temperatures during summer.

3.3.2 Body Mass

Adult female scorpions from both populations and adult males from Black Mountain exhibited seasonal changes in body mass with the smallest masses occurring during winter, whereas the masses of adult males from Dubbo were independent of season (Figure 3.7). The masses of females from Black Mountain and Dubbo respectively, were 1.21 ($\pm$ 0.05) g and 1.14 ($\pm$ 0.04) g during winter and 1.56 ($\pm$ 0.06) g and 1.66 ($\pm$ 0.11) g during summer. The mass of spring-collected females from Black Mounta n was 1.52 ($\pm$ 0.12) g compared to 1.25 ($\pm$ 0.07) g for females from Dubbo. There was a significant effect of season, $F_{(3, 54)}$ = 8.83, $P < 0.001$, but not of population, $F_{(3, 54)}$ = 1.11, $P > 0.05$, nor their interaction, $F_{(3, 54)}$ = 1.46, $P > 0.05$.

The masses of males from Black Mountain and Dubbo respectively were 0.57 ($\pm$ 0.02) g and 0.79 ($\pm$ 0.02) g during winter and 0.72 ($\pm$ 0.03) g and 0.80 ($\pm$ 0.02) g during summer. The effect of population on male mass was significant, $F_{(1, 24)}$ = 34.27, $P < 0.001$, but neither season, $F_{(2, 24)}$ = 1.81, $P > 0.05$ nor the interaction, $F_{(2, 24)}$ = 2.27, $P > 0.05$ were significant. The inclusion of male winter masses for Dubbo seasonal effects did not affect the result of non-significance, $F_{(3, 18)}$ = 0.55, $P > 0.05$. The distributions of mass for all adult females changed with season with Black Mountain (Figure 3.8a) having a greater proportion of heavy females during spring than Dubbo (Figure 3.8b).

The cuticle mass of Dubbo males was 0.071 ($\pm$ 0.004) g which was significantly greater than 0.054 ($\pm$ 0.002) g for males from Black Mountain, $t(10)$ = 3.30, $P < 0.01$, indicating that Dubbo males grew to a greater size than Black Mountain males.

Water content, as a proportion of wet body mass, was independent of season for combined females and males (Table 3.2). Water content did not significantly differ between populations, $F_{(1, 53)}$ = 0.32, $P > 0.05$, nor seasons, $F_{(2, 53)}$ = 1.15, $P > 0.05$, and their interaction was also non significant, $F_{(3, 54)}$ = 1.46, $P > 0.05$. The inclusion of winter for Black Mountain also produced a non significant result for seasons, $F_{(3, 32)}$ = 0.94, $P > 0.05$.

3.3.3 Evaporative Water Loss

Scorpion permeability to evaporative water loss was affected by the population from which the scorpions were collected, but not by the season of collection (Figure 3.9). The permeability of scorpions from Black Mountain during the warmer seasons was greater than that of Dubbo, $F(1, 53) = 5.58$, $P < 0.05$. Mean permeabilities for Black Mountain scorpions collected in summer and spring were 0.045 ($\pm$ 0.003) and 0.050 ($\pm$ 0.006) mg H_2O cm^{-2} h^{-1} respectively, compared to Dubbo values of 0.030 ($\pm$ 0.004) and 0.040 ($\pm$ 0.002) mg H_2O cm^{-2} h^{-1}. With the inclusion of winter, there was no difference between the seasons in Dubbo scorpion permeability, $F(3, 36) = 2.08$, $P > 0.05$.

3.3.4 Haemolymph Osmolality and Supercooling Point

The haemolymph osmolalities of adult scorpions from Black Mountain were greater than those of scorpions from Dubbo and were independent of sex (Table 3.3). Males from Dubbo had greater masses than males from Black Mountain. Univariate analysis indicated that population did not significantly affect osmolality, $F(1, 32) = 2.87$, $P > 0.05$, but did affect mass, $F(1, 32) = 11.06$, $P < 0.01$. The sexes were significantly different with regard to mass, $F(1, 32) = 131.94$, $P < 0.001$, but not to osmolality, $F(1. 32) = 0.09$, $P > 0.05$. Sexual differences were not significantly affected by population for osmolality, $F(1, 32) = 0.08$, $P > 0.05$, but were affected for mass, $F(1, 32) = 7.83$, $P < 0.01$.

The mean ($\pm$ SE) supercooling point for winter-collected females from Dubbo was -5.4°C ($\pm$ 0.5). Supercooling points were not significantly affected by mass, $F(1, 9) = 0.01$.

3.3.5 Oxygen Consumption

Female (Figure 3.10a) and male (Figure 3.10b) mass-specific VO_2 increased with increasing temperature for every season. Although the mean values suggested that VO_2 increased exponentially with temperature, single degree of freedom polynomial contrasts indicated that the increase in VO_2 with temperature for all females was best described as being linear, $F(1, 51) = 548.21$, $P < 0.001$. Mean winter VO_2 s for females between 20 and 30°C were greater than summer values. However, at 40°C, summer females had the highest VO_2 of 152.11 ($\pm$ 11.47) $\mu l\ O_2\ g^{-1}\ h^{-1}$ and spring VO_2 was the lowest at 116.77 ($\pm$ 10.23) $\mu l\ O_2\ g^{-1}\ h^{-1}$. There was a significant effect of season on VO_2 averaged over all temperatures, $F(15, 130) = 2.79$, $P < 0.001$, but the populations did not differ, $F(5, 47) = 1.19$, $P > 0.05$, nor did the pattern of seasonal change between the populations, $F(15, 130) = 0.66$, $P > 0.05$. The effect of season on each of the dependent variables was investigated by univariate analysis and there were no significant differences at any of the temperatures. This was supported by the univariate between subjects result, $F(1, 51) = 0.30$, $P > 0.05$. The effect of temperature on VO_2 was significantly different between seasons, univariate within subjects $F(12, 204) = 3.83$, $P < 0.001$.

Males collected during spring had the highest VO_2 at 20°C of 43.19 ($\pm$ 2.05) $\mu l\ O_2\ g^{-1}\ h^{-1}$ compared to summer (34.42 $\pm$ 3.80 μ $O_2\ g^{-1}\ h^{-1}$) and autumn (33.49 $\pm$ 4.71 $\mu l\ O_2\ g^{-1}\ h^{-1}$). At 40°C, spring VO_2 (127.81 $\pm$ 6.67 $\mu l\ O_2\ g^{-1}\ h^{-1}$) was the lowest of these three seasons. Summer and autumn VO_2 were 151.22 ($\pm$ 8.56) and 158.32 ($\pm$ 6.92) $\mu l\ O_2\ g^{-1}\ h^{-1}$ respectively. Winter VO_2 was the lowest of all seasons at 40°C (85.28 $\pm$ 7.33 $\mu l\ O_2\ g^{-1}\ h^{-1}$). Season had a significant effect on VO_2, $F(6, 38) = 4.78$, $P < 0.001$, but population did not, $F(3, 19) = 1.27$, $P > 0.05$. The seasonal changes were not affected by population, $F(6, 38) = 1.25$, $P > 0.05$. The effect of season on each of the dependent variables was investigated by univariate analysis and there were significant differences at 20°C, $F(2, 21) = 3.73$, $P < 0.05$ and 40°C, $F(2, 21) = 5.53$, $P < 0.05$. The effect of temperature on male VC_2 was significantly different between seasons, univariate within subjects $F(4, 42) = 9.38$, $P < 0.001$ and the increase in VO_2 with temperature was best described as being linear, $F(1, 21) = 738.49$, $P < 0.001$. At 40°C, pairwise comparison with Bonferroni adjustment showed that Dubbo winter VO_2 was significantly lower than summer, $P < 0.001$, autumn, $P < 0.001$ and spring, $P < 0.01$.

The Q_{10}'s of females from each season were greatest at low temperatures although the Q_{10} of summer-collected females between 35 and 40°C was also high (Table 3.4). The Q_{10} of summer females increased from 1.83 (± 0.09))etweer 30 and 35°C to 3.36 (± 0.31) between 35 and 40°C. Autumn Q_{10} decreased from 2 58 (± 0.25) to 1.73 (± 0.08) for the same temperature ranges. Neither population, $F(4, 48) = 0.26$, $P > 0.05$ nor its interaction with season, $F(12, 127) = 0.98$, $P > 0.05$ had significant effects on the combined Q_{10}'s of the temperature ranges, but seasonal differences were significant, $F(12, 127) = 3.28$, $P < 0.001$. The interaction between temperature range and season was also significant, $F(9, 119) = 3.52$, $P < 0.001$. Univariate analyses revealed that the Q_{10} for 35 to 40°C made the largest contribution, $F(3, 51) = 6.71$, $P < 0.001$, to the combined result. The only other significant univariate result was for the range 30 to 35°C, $F(3, 51) = 3.47$, $P < 0.05$

Between 20 and 30°C, Q_{10} was indep ndent of season for females and males together but became dependent between 30 and 40°C (Table 3.5). The calculation of Q_{10} over 10°C reduced large changes in Q_{10} that were observed ov r 5°C increments in temperature. However, summer Q_{10}'s in the upper temperature range remaii ed elevated above those of autumn and spring. The Q_{10} of spring scorpions decreased from 2.35 (± 0.19) in the lower temperature range to 1.72 (± 0.14) in the upper temperature range. Decreas es in Q_{10} for summer and autumn were smaller. There was no significant effect of sex on the corr bined Q_{10}'s of both temperature ranges, $F(2, 65) = 1.75$, $P > 0.05$, but there was a significant effect of season, $F(4, 130) = 4.51$, $P < 0.01$. Univariate analyses showed that the combined si nificant result was entirely due to the temperature range of 30 to 40°C, $F(2, 66) = 6.99$, $P < 0.01$

3.3.6 Temperature Selection

The average temperatures selected by *Urodacus manicatus* were dependent upon the time of day (Figure 3.11). The three hourly profiles of temperature averaged over all animals deviated significantly from flatness, $F(5, 87) = 18.89$, $P < 0.001$, $\eta^2 = 0.52$. Seasonal variation in average selected temperature between 15:00 and 09:00 h was greater at Black Mountain (Figure 3.11a)

than at Dubbo (Figure 3.11b). Active temperatures in the first three hours of the scotophase at Black Mountain were the lowest durii g winter and the highest during spring. Spring average temperature in the first three hours of the scotophase was 32.7°C (± 1.2) and for winter it was 23.6°C (± 1.6). For Dubbo animals, active temperatures for summer, autumn, winter and spring respectively were 29.6°C (± 1.1), 28.5°C (± 1.3), 28.9°C (± 1.2) and 28.6°C (± 1.3). The populations did not differ in temperature, $F_{(1, 91)} = 1.91$, $P > 0.05$ but were significantly different in their seasonal changes of temperature, $F_{(3, 91)} = 3.01$, $P < 0.05$.

The time of day profile for Blac< Mountain spring was the flattest of the seasons over all periods. Black Mountain winter temperatures changed the least during the scotophase whereas that of Dubbo consistently decreased. The winter pre-dusk temperatures of both populations were the highest of the seasons with 32.6°C (± 2.2) for Black Mountain and 32.5°C (± 1.6) for Dubbo. The summer and autumn profiles of both populations across all periods were similar with decreasing temperature during the scotophase although the profile of autumn Dubbo animals was initially flat. Pre-dusk photophase temperatures for summer and autumn respectively were 28.2°C (± 1.6) and 27.5°C (± 1.8) compared o post-dawn temperatures of 21.3°C (± 1.7) and 18.1°C (± 1.3). Temperature selection was significantly dependent on season, $F_{(3, 91)} = 3.80$, $P < 0.05$ and the seasonal profiles deviated significantly from parallelism, $F_{(15, 240)} = 3.16$, $P < 0.001$, $\eta^2 = 0.39$. *Post hoc* testing revealed hat differences in the seasonal profiles were mostly attributable to the differing responses to the onset of the scotophase and also changes in temperature between the first and second periods of the scotophase (Table 3.6).

The results of maximum temperatures recorded in the gradient were the same as average selected temperature (Figure 3.12). T1e high avoidance temperature varied with time of day and deviated significantly from flatness, $F_{(5, 87)} = 31.55$, $P < 0.001$, $\eta^2 = 0.64$. Seasonal variation in maximum temperature between 15:0C and 09:00 h were greater at Black Mountain (Figure 3.12a) than at Dubbo (Figure 3.12b). Active high avoidance temperatures in the first three hours of the scotophase at Black Mountain were th e lowest during winter and the highest during spring. Spring temperature in the first three hours of the scotophase was 35.9°C (± 1.0) and for winter it was 30.6°C (± 1.3). For Dubbo scorpions, active temperatures in the first three hours were highest in winter and lowest in spring Scorpions during winter and spring encountered temperatures of 34.1°C (± 1.0) and 3 .0°C (± 1.1) respectively. The populations did not differ in

temperature, $F_{(1, 91)} = 0.49$, $P > 0.05$ and (unlike for average selected temperature) the effect of population on seasonal changes were not significant, $F_{(3, 91)} = 2.48$, $P > 0.05$.

The time of day profile for Black Mountain spring was the flattest of the seasons over all periods. For Black Mountain and Dubbo respectively, the pre-dusk high avoidance temperatures were 33.3°C (± 1.5) and 28.1°C (± 1.7) and the temperatures post-dawn were 30.5°C (± 2.2) and 28.2°C (± 2.2). The scotophase winter temperature profile was not flat like that of average selected temperature. Temperatures decreased during the scotophase in a similar pattern to that of Black Mountain winter-collected scorpions and the combined populations of summer and autumn-collected scorpions. The winter pre-dusk temperature of Black Mountain was the highest recorded temperature for all animals and was 36.0°C (± 2.0). Temperature selection was significantly dependent on season, $F_{(5, 91)} = 4.04$, $P < 0.01$ and the seasonal profiles deviated significantly from parallelism, $F_{(15, 240)} = 3.16$, $P < 0.001$, $\eta^2 = 0.39$. *Post hoc* testing revealed that differences in the seasonal profiles were attributable to both the responses to the onset of the scotophase and also the changes in high avoidance temperature between the first and second periods of the scotophase (Table 3.6).

The patterns in the minimum temperatures recorded were different from those of the average and maximum temperatures (Figure 3.13). The scotophase profiles of Black Mountain (Figure 3.13a) and Dubbo (Figure 3.13b) were flatter and animals travelled further into the cool end of the gradient with the onset of the scotophase during summer, autumn and winter. *Post hoc* tests revealed that the higher pre-dusk values accounted for the significant deviation of all profiles from flatness (Table 3.6). Spring minimum temperatures from both sites changed least over time. Pre-dusk spring temperatures of Black Mountain and Dubbo respectively were 27.8°C (± 1.9) and 23.1°C (± 2.1) compared to post-dawn values of 25.6°C (± 2.2) and 21.2°C (± 1.8). The highest active minimum temperature recorded in the first three hours of the scotophase was for Black Mountain spring (27.8°C ± 1.6). The populations, $F_{(1, 91)} = 5.73$, $P < 0.05$ and the seasons, $F_{(3, 91)} = 4.40$, $P < 0.01$ were significantly different in minimum temperature. The seasonal changes in temperature were significantly different between the populations, $F_{(1, 91)} = 3.06$, $P < 0.05$

3.3.7 Activity

The activity profiles showed an increase in activity with the onset of the scotophase and then decreased towards the end of the night and into the next photophase (Figures 3.14 to 3.17). The increase in activity at the time of dusk was lowest in spring animals from both Black Mountain (Figure 3.17a) and Dubbo (Figure 3.17b) with a combined increase of only 0.87 to 2.20 turns compared to summer (increase of 3.35 to 5.25 turns, Figure 3.14), autumn (increase of 3.62 to 5.54 turns, Figure 3.15) and winter (increase of 3.78 to 5.07 turns, Figure 3.16). Activity in all seasons except spring (which remained low at 1.70 turns) decreased after 24:00 h. Summer decreased from 2.30 to 2.07 turns, autumn by 1.5 to 2.75 turns and winter by 0.96 to 3.43 turns. *Post hoc* testing revealed that the activity profiles over time deviated from flatness mostly due to the difference between the first two periods during which the scotophase commenced, $F_{(8, 91)} = 23.24$, $P < 0.001$ with another large difference in activity occurring before and after 24:00 h (Table 3.7). Season had an effect on the overall amount of activity due to the low activity levels of spring animals, $F_{(3, 91)} = 11.13$, $P < 0.001$ and on the profiles, $F_{(15, 240)} = 3.81$, $P < 0.001$, $\eta^2 = 0.44$. The populations did not differ in the amount of activity, $F_{(1, 91)} = 1.48$, $P > 0.05$ or the seasonality of activity, $F_{(3, 91)} = 0.06$, $P < 0.05$.

Scorpions react to the onset of the scotophase by becoming more active and selecting higher temperatures. Generally, this increased activity is maintained for 3 to 6 hours after which the animals become less active and gradually retreat into the cooler parts of the gradient during the rest of the night. Since the animals do not usually react to the start of the photophase in any way, the second period of light (d2) was not included in the temperature selection studies of the remaining chapters to increase the degrees of freedom in the various subsequent analyses.

3.4 Discussion

Burrow structure and microclimates at Black Mountain and Dubbo

The microclimate study clearly shows that populations of the scorpion *Urodacus manicatus* encounter different microclimatic conditions on seasonal and diurnal scales. This depends on their location within the distribution range for the species and the means by which the scorpions from each population modify the microhabitat.

Initial comparisons of the home site burrows of *Urodacus manicatus* suggested that Black Mountain and Dubbo populations differed in their modification of the microhabitat. Some Black Mountain scorpions were found with an inclined burrow extending from the living area into the soil which was not present at any of the Dubbo home sites. There is evidence to suggest, however, that this extended part of the burrow was not constructed nor utilised by *U. manicatus*. The presence and size of the burrows at Black Mountain were not related to the size of the scorpion and on no occasion were scorpions found within the extended part of the burrow when initially disturbed, although occasionally they would attempt to retreat into the burrow to avoid capture. The small incidence of extended burrows at Black Mountain contrasts with the finding of (Smith 1966) who calculated that for *U. manicatus* near Canberra, Australian Capital Territory, 80% of the population had an extended burrow described as being "short, horizontal or somewhat inclined". The lengths of some extended burrows exceeded 20 cm which I did not regard as being "short". The home sites with inclined burrows occurred in areas where large subsurface rocks were present in the soil below the home site rock (i.e. on rocky outcrops) and the burrows tended to follow gaps some distance between the rocks. In doing so the burrows did not have the even walled form typical of the deep burrows of soil inhabiting species such as *U. yaschenkoi* and *U. hoplurus* (Koch 1978). Also, the burrows were usually much larger than the inhabitant suggesting that they were dug by other larger animals such as centipedes and small skinks that regularly occupied similar burrows beneath non-scorpion home sites. On the property "Palmerston" 5 km east of Armidale, New South Wales and approximately 33 km south of Black Mountain, Willmer (1967) recorded similar observations for *U. manicatus*. The extent of the burrows of *U. manicatus* populations in the Mount Lofty Ranges of South Australia could not be

determined from the brief description of Southcott (1954) who described them as "shallow tunnels under fairly large stones".

As with the Black Mountain population of *Urodacus manicatus*, *Diplocentrus peloncillensis* had burrows beneath rocks but never utilised them, not even for protection from sub zero temperatures (Crawford and Riddle 1974). Regardless of the form of the burrow, most species of scorpions construct their burrows in one day (Polis 1990). Thus, if the normal form of the burrow of *U. manicatus* included an extended burrow then most home sites would be of this form (c.f. Smith 1966). In conclusion, all *U. manicatus* in this study, regardless of population, were considered not to have utilised the extended burrow and that most activity occurred between the living area and the runway entrance. For the rest of the thesis, the combination of the "runway" and the "living area" shall simply be referred to as the "burrow".

The maximum-minimum thermometers placed beneath the home site shelters did not accurately reflect actual burrow temperatures. Excavations larger than a scorpion's burrow were necessary to accommodate the thermometers beneath the home sites. Undoubtedly, the disturbance of the soil influenced the temperatures recorded. Thus, the temperatures obtained from these thermometers must be treated with caution but do provide a useful comparison between different home sites and air temperature. A great deal of variation exists in the microclimate profiles of home sites within a population depending on the dimensions of the shelter, the extent of embedment of the shelter, the shade received and the aspect of the home site with respect to topography. Although microhabitat temperature generally reflected seasonal changes in air temperature, *Urodacus manicatus* selected and modified parts of the microhabitat such that microclimatic conditions were more inducible to survival. Heating of the timber shelter at Dubbo during winter prevented the minimum home site temperature from approaching that of air temperature which sometimes went below 0°C. During summer, the maximum timber home site temperature extended above the air temperature and approached the upper lethal temperatures of scorpions (45 to 47°C) (Cloudsley-Thompson 1962a and 1962c, Hadley 1970a and 1990). However, these temperatures were most likely aberrations resulting from the method by which home site temperatures were recorded. Actual burrow temperatures beneath the timber home sites were well below lethal temperatures (see below). The largest difference between rock home site temperature and air temperature at Black Mountain occurred during summer when the

home site maxima increased above the maxima of the air but remained well below the lethal temperatures of scorpions.

Willmer (1967) stated that *U. n anicatus* avoided areas lacking vegetation cover and instead selected home site rocks that differed only because they received shade from the surrounding trees. The same phenomenon was found at both sites in this study. At Black Mountain, rocks exposed to the sun were heated during the day such that soil temperatures below were greater than that of rocks receiving less direc solar radiation because of tree cover. Trees not only reduced the amount of radiation reach ing the ground but also transmitted the radiation lost from the ground ineffectively and thus served as an insulation layer (Shanks 1956). Home sites that were continuously shaded therefore had higher minima during winter but lower maxima during the warmer months of the year than home sites that were heated by the sun (Figure 3.4). *U. manicatus* tended to select rock shelters that were partially shaded in open woodland such that the burrow temperature was not excessively high during summer. During winter at Black Mountain, enough solar radiation was received by the rock during the day to raise temperatures to 15°C but nightly temperatures were below 0°C. Dubbo scorpions had supercooling points of -5°C even though they did not encounter sub zero temperatures. This suggests that the supercooling point is a reflection of the osmolality of the body fluids and is not an adaptive trait of *U. manicatus*. Assuming that the Black Mountain scorpions possessed the same supercooling point, then the temperature at which their body water froze was also less than the coldest microhabitat temperature at Black Mountain of -2°C. Thus, it is likely *U. manicatus* were able to survive the cold winter nights at Black Mountain without freezing. Similarly, the scorpion *Centruroides vittatus* had a supercool ng point that was lower than the minimum field temperatures it experienced in winter (Whitmore et al. 1985). The supercooling point of scorpions does not appear to change with season (Crawford and Riddle 1975, Whitmore et al. 1985).

The different thermal properties of the rock and timber shelters influenced the daily temperature profiles of the burrows beneath them (Figure 3.5). The thermal conductivity of rock is more than an order of magnitude greater than that of timber (Weast 1968). The rock shelters therefore absorbed solar radiation and conducted the heat to the burrow beneath it at a greater rate than the timber shelters. Diurnal summer burrow temperature below the rock at Black

Mountain peaked at 34°C, whereas the surrounding soil at the same depth peaked at 24°C. Similar results were recorded for the scorpion *Diplocentrus peloncillensis* by (Crawford and Riddle 1974). The temperatures below their mountain home site rocks were greater than that of the surrounding soil surface temperatures during autumn. Insulation is the reciprocal of thermal conductivity and the greater insulation of the timber shelter at Dubbo maintained living area temperatures below 28°C as the surrounding soil peaked at about 47°C during summer.

During the day time in winter, the high thermal conductance of the rock heated the burrow beneath it to 15°C, whereas the temperature of the surrounding soil temperature was 8°C. However, as the solar radiation diminished later in the day, the rock rapidly lost heat to the cooler air and the temperature of the burrow decreased to -2°C. During winter at Dubbo, the timber shelter did not conduct heat to its lower surface due to its high insulation and the burrow temperatures were similar to that of the surrounding soil.

During summer, the range of temperatures along the lengths of the burrows was also dependent upon the different thermal properties of the shelters. The entrance temperature of the timber home site at Dubbo was influenced by the surrounding soil, thus the insulation afforded by the timber shelter was lowest at its edge and greatest at its centre. Cooling and heating of the living area below the timber lagged behind that of the entrance and surrounding soil thus creating a gradient of temperatures along the length of the burrow. The entrance temperature of the burrow below the rock at Black Mountain was mostly influenced by the heat conducted by the rock and less on the surrounding soil temperature resulting in a smaller gradient of temperature along the length of the burrow at night.

Considering the dimensions of the shelter, the thermal conductivities of the rock and timber home sites were largely independent of their size, but the rate at which they exchanged heat with their surroundings was size-dependent. The smaller the timber shelter, the more important the edge effect of less insulation becomes in determining temperatures below the shelter. During summer, the soil temperature at the centre of the small shelter had a temperature profile similar to the entrance temperature profile of the larger shelter (Figure 3.6b). Some insulation was still apparent at the centre of the small shelter because peak day time temperatures were less than that of the entrance temperature of the larger shelter. During winter, the size of the timber shelter did not affect soil temperatures at its centre. The small rock at Black Mountain conducted heat to the

soil beneath it in less time than the large home site rock. As a result, during summer, the soil

temperature beneath the small rock increased before that of the burrow of the large rock at

sunrise and its peak day time temperature was 1 to 3°C higher (Figure 3.6a). The winter day time

temperature profile of the small rock was similar to that of the large rock. However, night

temperatures beneath the small rock were more dependent on the surrounding soil temperature

than the heat lost from the rock because of its small contact surface area with the soil. As a

result, the night time winter temperatures below the small rock remained above 0°C.

Smith (1966) argued that the minimum size of a rock as a home site for *Urodacus*

manicatus was determined by the degree of insulation against extremes of temperature at the

centre of the rock as insulation decreased with size. However, this is only true for shelters of low

thermal conductivity (high insulation) and not those of high thermal conductivity (low insulation).

If rocks were used as shelters at Dubbo instead of timber, then the rocks, regardless of their size,

would not insulate the soil beneath them from the biologically inhibiting temperatures of the

surrounding soil. In a temperate climate though, the selection of larger rocks as shelters has the

advantage of further increasing the burrow temperature and, consequently, the body temperature

of the scorpion during the day which assists tissue maintenance and embryonic growth. However,

a large home site rock, unlike a small one does not protect the scorpion from sub zero

temperatures at night in the cooler months.

Potentially, an individual *Urodacus manicatus* could move between home sites that differed

in their seasonal and daily temperature profiles such that its thermal environment was optimised

throughout the year. Some scorpion species move up and down hill slopes or change their

burrowing behaviour depending on the season (Rosin and Shulov 1963, Zinner and Amitai 1969).

Smith (1966) claimed that only dispersing *U. manicatus* moved home sites and that adult females

were the most sedentary but his conclusions were based on the presence/absence of the extended

burrow as an indicator of time spent at a home site and not on actual observations of scorpion

movements. Willmer (1967) was not able to any draw conclusions about the movements of *U.*

manicatus in and out of sub populations from mark/recapture experiments. A more

comprehensive study is, therefore, required on the movements of *U. manicatus* and their selection

of home sites based on microclimate (Willmer 1967).

Body Mass

Urodacus manicatus from Black Mountain and Dubbo clearly differed in their seasonal profiles of mass for females and males (Figure 3.7). Relative mass can depend on the reproductive state in females and the nutritional state and body water content of both sexes. Females from both populations and males from Black Mountain increased in mass from winter through to summer whereas Dubbo males maintained a constant mass throughout the year. Smith (1966) stated that *U. manicatus* females from Canberra showed an increased mass during spring that was due to embryonic development and not due to increased feeding rates because the mass of the males did not increase at the same time. However, the hepatopancreas of scorpions is the major source of embryo nutrition (Subburam and Gopalakrishna Reddy 1978, Subburam and Gopalakrishna Reddy 1979, Subburam and Gopalakrishna Reddy 1980) and females must ensure that their reserves are large enough for the development of the young until parturition at the end of summer. Thus, the increased mass of females after winter was attributable to both an increase in feeding rate and embryonic development. Females from Black Mountain were larger during spring than females from Dubbo which may have been due to greater feeding rates or more rapid embryonic development or both at Black Mountain. Dubbo females, however, attained the same mass as Black Mountain females by midsummer suggesting a later increase in feeding rates and/or embryonic development. Oocyte growth (Warburg et al. 1995) and the length of gestation (Thornton 1956) have been shown to be temperature-dependent in scorpions so embryonic development is likely to be accelerated at higher temperatures. However, the microhabitat temperatures from spring to summer were similar between the two populations. Therefore, the differences in the rate of increasing mass after winter may be due to greater feeding rates and not faster embryonic development in Black Mountain females.

The seasonal constancy of body water content found in this study would dismiss an increase or decrease in water uptake as being responsible for the seasonal changes in mass. The overall mean ($\pm$ SE) of the percentage body water content for *Urodacus manicatus* was 67.93% ($\pm$ 1.26) which was consistent with the range of 60 to 70% determined by Warburg (1986) for *Scorpio maurus fuscus*, *Scorpio maurus palmatus*, *Leiurus quinquestriatus*, *Buthotus judaicus* and *Nebo heirochonticus*.

Water Balance

This study clearly demonstrates that t1e rate of evaporative water loss in *Urodacus manicatus*

was independent of season, but was d fferent between the Black Mountain and Dubbo

populations. During the warmer months, Black Mountain scorpions had higher rates of water

loss than those from Dubbo (Figure 3.9). These differences in permeability may have been due to

factors other than temperature becaus3 the burrow temperatures during summer were similar

between the populations (Figure 3.5). The cause of this difference in permeability may have been

phylogenetic or due to differences in water availability and/or the saturation deficit of the burrows'

air. Greater water availability and/or 1 smaller saturation deficit of the air within the burrows at

Black Mountain may have allowed this population to afford higher permeabilities than Dubbo

individuals. Two days after equivalert light rainfall during summer at both sites, the saturation

deficit of the air below the timber home sites at Dubbo was over five times that below the rocks at

Black Mountain. The high vapour de1sity of the burrows at Black Mountain resulted in the

condensation of water on the undersice of the rocks and occasionally on the dorsal surfaces of the

inhabiting scorpions (pers. obs.). Thus, during the warmer months at Black Mountain, the

scorpions there had a more mesic microclimate and a ready source of free water unlike the

scorpions from Dubbo.

The number of rain fall days at Black Mountain was greater and the rainfall was nearly

double that of Dubbo during the warmer months from October to March. Both sites however,

had similar rainfall statistics during th3 cooler months (Figure 3.2). Regions of higher rainfall

usually have a greater abundance of prey species for scorpions. Coupled with the greater amount

of ground vegetation and shelters, prey species were potentially more abundant at Black

Mountain than at Dubbo. High body water content, however, can be a problem if excessive water

is taken into the body. Scorpions exc·ete negligible amounts of water as part of their water-

conserving strategies (Horne 1969, Kanungo et al. 1962, Yokota 1984). Body water content

must be regulated largely by changes n their transcuticular evaporation. Alteration of

temperature and humidity selection to achieve greater rates of water loss can be assisted by

changing the permeability of the cuticle. Evidence for a mechanism that regulates body water

volume was suggested by the similarity of the haemolymph osmolality of female and male

Urodacus manicatus from the two populations during winter, a time when water availability was

lowest. (Kimura et al. 1988) also fou 1d no seasonal change in the haemolymph osmolarity of *Buthus martensi*. Since seasonal changes in cuticle permeability are achieved by changes in the lipids (Toolson and Hadley 1979), a difference in cuticle lipid composition may exist between the populations. Since different dietary lipids can affect the thermal tolerance of fly larvae (House et al. 1958) as well as the selected body temperatures of lizards (Geiser et al. 1992), they may alter cuticle lipid composition and therefore permeability of scorpions.

Activity

Urodacus manicatus is clearly a noct irnal species with increased activity triggered by the onset of the scotophase. Maximum movemen within the gradient was consistently within the first 3 h of darkness except for scorpions collected from Black Mountain during spring that maintained low levels of activity. The increase in act vity during the first hours of the scotophase within the gradient was supported by field observations made during summer at Black Mountain. The lifting of home site rocks revealed that all individuals were positioned in the living area two hours before dusk, whereas at dusk many had moved to the entrances of their burrows. Most nocturnal scorpions peak in activity during the first few hours after dusk (Abushama 1962, Benton 1992, Cloudsley-Thompson 1956, Cloudsley-Thompson 1967b, Cloudsley-Thompson and Constantinou 1983, Constantinou 1980, Crawford and Krehoff 1975, Dresco-Derouet 1960, Fet 1980, Fleissner 1977b, Hadley and Williams 1968, Locket 1986, Polis 1980b, Sasira Babu and Gopalakrishna Reddy 1988, Tourtlotte 1974). The seasonal occurrence of extra-burrow activity at night could not be examined directly with a UV lamp, a problem that Willmer (1967) also encountered using a torch. The failure to detect any animals throughout the year was due to concealment of the animals by undergrowth vegetation, leaf litter, rocks and general surface objects and was also due to the small percentage of time the scorpions spent away from the home site when they were either looking for a mate or a new home site.

U. manicatus is a "door-keeping" and "sit-and-wait" predator whose normal activity at night consists of moving to the burrow entrance and remaining in the alert immobility state (Tobler and Stalder 1988) ready to ambush passing prey. In the gradient, however, the animals became restless at dusk. How does this increase in activity relate to foraging behaviour? Scorpions in the alert immobility state are more sensitive to their surroundings than when they are

in the resting immobility state (Tobler and Stalder 1988). Thus the scorpions, when removed from their familiar burrows and placed within the gradient, entered the alert immobility state with the onset of the scotophase and became unsettled by the alien surroundings and perhaps by the attachment of a thermocouple to their cuticles. The degree of locomotion within the burrow would presumably have been far less than that exhibited within the gradient.

Activity periodically occurred during the photophase indicating that *Urodacus manicatus* did not continuously remain in the resting immobility state during the day. Some scorpion species are quite active during the day even though they do not venture out of the burrow (Alexander and Ewer 1958). Tilak (1970) found that disturbances near the mouth of the burrow during the day would quickly lure *Hormurus nigripes* to the entrance. On several occasions during this study animals have been found in an alert immobility state upon removal of the home site shelter with the characteristic raised pedipalps and lifted mesosoma (Tobler and Stalder 1988). These scorpions were quick to respond to disturbance. More often, however, *U. manicatus* appeared to be in the relaxed immobility state during the day, their arousal threshold being much higher. Scorpions in this state would quite often continue "sleeping" after being picked up by the metasoma and placed into a collecting jar.

Nocturnal scorpions are generally photonegative and have additional photoreceptors apart from the eyes (Bali and Pampathi Rao 1973, Torres and Heatwole 1967b). *Urodacus manicatus* is probably no exception. *Urodacus novaehollandiae* occurs in the same species group as *U. manicatus* (Koch 1977). The metasoma of this species is light sensitive (Zwicky 1968, Zwicky 1970b) and may play a role in cuing nocturnal activity (Zwicky 1970a). Like *U. manicatus*, most activity of *U. novaehollandiae* occurs in the first hours of the night although this rhythm is not endogenous as it disappears after three days of constant darkness. Zwicky (1970a) also found that blinded *U. novaehollandiae* maintained an activity rhythm. However, the burst of activity immediately following dusk disappeared.

Forest and woodland scorpions such as *Urodacus manicatus* tend to remain relatively inactive within their sheltered burrow and their movements are less rhythmic than that of desert species which are more strictly nocturnal and more active (Cloudsley-Thompson 1986). As to whether the rhythm is endogenous or exogenous depends on the life style of the species. Species that live in constant darkness due either to deep burrowing or dark rainforest inhabitation, tend to

have strong endogenous nocturnal rhythms (Cloudsley-Thompson 1967b, Constantinou 1980, Fleissner 1977b, Sasira Babu and Gopalakrishna Reddy 1988, Toye 1970). Entrainment of the rhythm in one such species, *Androcto ius australis*, is facilitated by the lateral eyes (Fleissner 1977a, Fleissner 1977b, Fleissner 1977c, Fleissner 1986, Fleissner and Fleissner 1986, Fleissner and Heinrichs 1982). Day time foragers or species that live under shelters such as *U. manicatus*, tend to have exogenous rhythms (Cloudsley-Thompson 1956, Crawford and Krehoff 1975, Toye 1970) and an increase in their activity is cued by the commencement of the scotophase (Cloudsley-Thompson and Constantinou 1983, Cloudsley-Thompson and Constantinou 1985, Constantinou 1980). The size of the shelter may influence the degree of foraging activity if the scorpions are receiving a stronger cue of light change (Crawford and Krehoff 1975). Since the commencement of the scotophase and the concomitant increase in activity in *U. manicatus* always occurred at 18:00 h in the gradient, the circadian rhythm of *U. manicatus* is most likely controlled exogenously. Examination of the persistence of the rhythm under continuous light or dark would confirm the nature of the circadian rhythm in *U. manicatus*.

Female and male *Urodacus manicatus* were the least active during spring (Figure 3.17). The activity profiles of scorpions collected in summer (Figure 3.14), autumn (Figure 3.15) and winter (Figure 3.16) were similar to one another. Spring is the season during which courtship and mating take place (Smith 1966) and it is also marked by an increase in prey abundance and microhabitat temperature. Willmer (1967) observed that only male *U. manicatus* left their burrows to rove the surface at night in search of mates but there was no difference in the level of activity between the sexes during spring in this study. The observations of Willmer (1967) were also made later in spring than when the animals were collected for this study. It was possible that mating and courtship behaviour had not yet commenced in this study. The low levels of activity may have been associated with the nutritional state of *U. manicatus*. Temperature and the amount and incidence of rainfall usually increase during spring. Insect abundance can be correlated with these increases (Polis 1980b). Thus the feeding rates of *U. manicatus* increased after winter which was associated with a corresponding increase in mass (Figures 3.7 and 3.8). The surface foraging activity of the desert scorpion *Paruroctonus utahensis* decreased for up to 20 days following a meal (Bradley 1982), the scorpions presumably spending more time at night in the relaxed immobility state within their burrows. Thus, a recent feeding event prior to

collection would explain why the reaction to the commencement of the scotophase and the level of activity were reduced in spring-collected *U. manicatus*. The abundance of prey species decreases over summer (Polis 1980b) and temperatures during the cooler months inhibit feeding opportunities even further. The time since the last meal may therefore have been greater in *U. manicatus* collected during summer, autumn and winter. As a result, these animals alternated between the alert immobility state and activity within the gradient.

Thermal Relations

There was statistically no seasonality in the RMR's of *Urodacus manicatus* from Black Mountain and Dubbo over the temperature range normally encountered by the scorpions in the field (Figure 3.10). However, there was significant seasonality in Q_{10}. The Q_{10}'s between 20 and 25°C (Table 3.4) for females collected during all seasons were about 3. However, between 25°C and 35°C, the Q_{10}'s of summer, autumn and winter scorpions were lower at about 2 (Table 3.4). The Q_{10} of spring-collected females decreased to approximately 2 above 30°C. Values of Q_{10} equal to 2 or lower impart a degree of independence from changes in temperature (Dutton and Fitzpatrick 1975). When *U. manicatus* were most active, they selected temperatures in the range of 24 to 29°C (Figure 3.11) and travelled to the hot end of the gradient to temperatures of up to 31 to 34°C during summer, autumn and spring (Figure 3.12). Their low Q_{10} over these greater active temperatures enabled the scorpions to be little affected by temperature while higher body temperatures facilitate locomotion and prey capture (Hadley 1970a, May 1979, May 1985). As activity decreased during the night, the temperatures selected by *U. manicatus* also decreased, reaching their lowest values after dawn. The temperatures selected during the inactive periods (excluding the pre-scotophase period) were below 24°C for summer, autumn and winter scorpions and below 28°C for the spring scorpions. These temperatures corresponded to the temperatures at which Q_{10} increased. Therefore, when *U. manicatus* were inactive, a high Q_{10} plus a low body temperature resulted in an energy saving for the animals especially during the cooler months when feeding rates were low (King and Hadley 1979, Polis 1979) and metabolic reserves needed to be conserved.

During winter, males from Dubbo had VO_2's at 40°C that were much lower than all other *U. manicatus* measured in this study. Inverse metabolic compensation is an energy saving strategy

when feeding opportunities are low and temperatures are constantly moderate. These conditions are experienced by scorpions from Dubbo. It was not clear why Dubbo females did not inversely compensate metabolically. Perhaps adult males and females selected home sites that differed in their burrow temperature profiles such that the males experienced slightly higher temperatures than the adult females during winter. The increased ability of the males to conserve their metabolic stores may in part explain why males from Dubbo had a greater mass than those from Black Mountain.

Urodacus manicatus was opportunistic in the temperatures it selected during the cooler seasons. Within the gradient, available temperatures and those selected by the scorpions were greater than temperatures normally encountered in the field, especially at night when the scorpions were most active. For example, Black Mountain scorpions during winter selected an active temperature of 24°C in the gradient (Figure 3.11a) whereas the burrow entrance temperature was 2 to 6°C (Figure 3.5a). Similarly, Dubbo scorpions selected an active temperature of 29°C (Figure 3.11b) when the entrance to their burrow was 12 to 15°C (Figure 3.5b). This observation, however, doesn't negate the usefulness of temperature gradient experiments to predict physiological changes in scorpions from the field. The active temperature of scorpions collected from Black Mountain during winter was the lowest of all seasons and of the two populations and reflected the coldest temperatures recorded at their site. It is possible that the threshold temperature below which animals were not active represented the minimum temperature that did not induce a reduction in the temperatures selected by *U. manicatus*. For example, during winter at Dubbo, burrow temperature ranged from 12 to 15°C in the first hours after dusk which was greater than the activity threshold temperatures that have been recorded for surface foraging species (Polis 1980b, Tourtlotte 1974). *U. manicatus* collected from Dubbo during winter therefore selected the same active temperatures in the gradient as did summer, autumn and spring-collected animals. The burrow temperatures of Black Mountain winter scorpions were lower than the threshold temperatures which induced a lowering in their temperature selection in the gradient. Tourtlotte (1974) recorded a threshold temperature of 10°C for *Paruroctonus boreus* and Polis (1980b) observed no *Paruroctonus mesaensis* on the surface below 4°C although adults were absent below 20°C.

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The maxima and minima encountered within the gradient reflected changes in the average selected temperatures with season and the time of day. This shows that the animals did not simply move to the extremes of the gradient and then turned around to spend most of their time at a selected temperature. Instead, they actually experienced changes in their sensitivity to high and low temperatures. Thus, differences in the physiological constraints of *U. manicatus* were related to changes in the scorpions' biotic and abiotic environment. Evidence for a set point for temperature regulation in scorpions was provided by Cabanac and Le Guelte (1980) when a fever (selection of higher temperatures) was induced in the animals.

The high temperatures recorded before the onset of the scotophase for *Urodacus manicatus* collected in summer, autumn and winter were not representative of inactive temperatures. Although the animals were inactive during this period, the elevated temperatures were probably an aberration resulting from the animals' recent collection from field conditions. Animals that were maintained in captivity under constant conditions (see following chapters) usually had lower pre-scotophase temperatures that increased with the onset of the scotophase. All further discussion in this thesis regarding inactive temperatures shall refer to temperatures selected at the end of the scotophase.

The degree to which metabolic compensation is important to the survival of *Urodacus manicatus* was not established in this study. Metabolic rate and temperature selection may be dependent upon a number of factors such as previous thermal history, nutritional state, water balance and reproductive state (Kenagy and Stevenson 1982). Crawford and Riddle (1975) found that the metabolic rate of female *Diplocentrus peloncillensis*, a montane species, was relatively independent of season and reasoned that the constancy in metabolic rate was due to high prey abundance and regular feeding during the colder months as well as reduced energy expenditure during the warmer months. An increase in the metabolic rate of *D. peloncillensis* from mid to late winter was explained as being due to embryonic development (Crawford and Riddle 1975). Interestingly though, winter *D. peloncillensis* of both sexes metabolically compensated when acclimated to summer temperatures (Crawford and Riddle 1975). The metabolic rates of males of the desert scorpion *Paruroctonus utahensis* decreased in late summer (possibly due to decreasing prey abundance) but increased in early autumn at the time of surface roving activity (Riddle 1978). A number of studies on scorpions have reported thermal acclimation in captivity at both

whole animal and biochemical levels Kalarani et al. 1992, Kalarani et al. 1991a, Kalarani et al. 1991b, Punzo 1991, Riddle 1978, Riddle 1979). Other studies reported no long term change in the metabolic rate of scorpions when moved to different maintenance temperatures (Crawford and Riddle 1975, Dresco-Derouet 1967, Robertson et al. 1982). In this study, male *U. manicatus* collected during winter from Dubbo appeared to inversely compensate at high temperatures but unfortunately their VO_2 was not measured at lower temperatures. Moreover, summer scorpions from both populations exhibited elevated Q_{10}'s at temperatures above 35°C.

The range of temperatures selected by *Urodacus manicatus* in the gradient was larger than the ranges observed for other species of scorpion. A broad temperature selection range may result in *U. manicatus* having a greater potential to shift into neighbouring microclimates of different temperature thus enabling coexistence with species of more restricted ranges (Warburg and Ben-Horin 1981). Many other species, both invertebrate and vertebrate, utilised the rock and timber shelters at Black Mountain and Dubbo, with which *U. manicatus* must compete for suitable home sites. In this study, interspecific competition with the buthid *Lychas marmoreus* occurred at Dubbo but the temperature relations of this species were not examined.

Overall, *U. manicatus* traversed a range of 23 to 33°C in the gradient with a mean selected temperature of 29°C. This value was greater than the range of 25-27°C recorded for the desert grassland buthids *Leiurus quinquestriatus* and *Buthotus judaicus* (Warburg and Ben-Horin 1981). The temperature range of these two xeric species was considered to be adaptive compared to the lower range of 23-25°C recorded for the more mesic species *Nebo heirochonticus* and *Scorpio maurus fuscus* (Warburg and Ben-Horin 1981). The arid zone scorpion *Opisthophthalmus latimanus* selected a burrow temperature range of 32-38°C (Alexander and Ewer 1958). Temperatures selected by *U. manicatus* suggest that this species is more xeric than mesic adapted despite its mostly temperate distribution. A discussion regarding the evolutionary history of *U. manicatus* with respect to climate is presented in Chapter 8.

In conclusion, the distribution of *Urodacus manicatus* is partially determined by the avoidance of high temperatures, but apparently not by low temperatures. By selecting shelters of appropriate thermal conductivity, this shallow burrowing species can either increase burrow temperatures to

optimal activity temperature ranges ir a cool temperate climate or prevent burrow temperatures from reaching biologically inhibiting temperatures in more xeric climates. Assuming that the possession of a low supercooling poir t is a trait of the species, then the distribution of *U. manicatus* can extend into cool temperate regions where temperatures may become sub zero at night during winter.

U. manicatus exhibits strong temporal and spatial changes in its activity and thermoregulatory behaviour. Adaptive behavioural mechanisms are the principle means by which this species regulates its metabolic rate and evaporative water loss. Although this chapter principally dealt with changes in the thermal environment of *U. manicatus*, other factors may be responsible for the observed changes in physiology and behaviour. In the following chapters I have attempted to discern which factors, both abiotic (temperature and water) and biotic (nutritional state, reproductive state, sex and size) have an effect on *U. manicatus* and relate any effects to the ecology of this species.

Plate 3.1. General habitat of *Urodacus manicatus* at (a) "Glücksberg" near Black Mountain and (b) Goonoo State Forest near Dubbo, New South Wales.
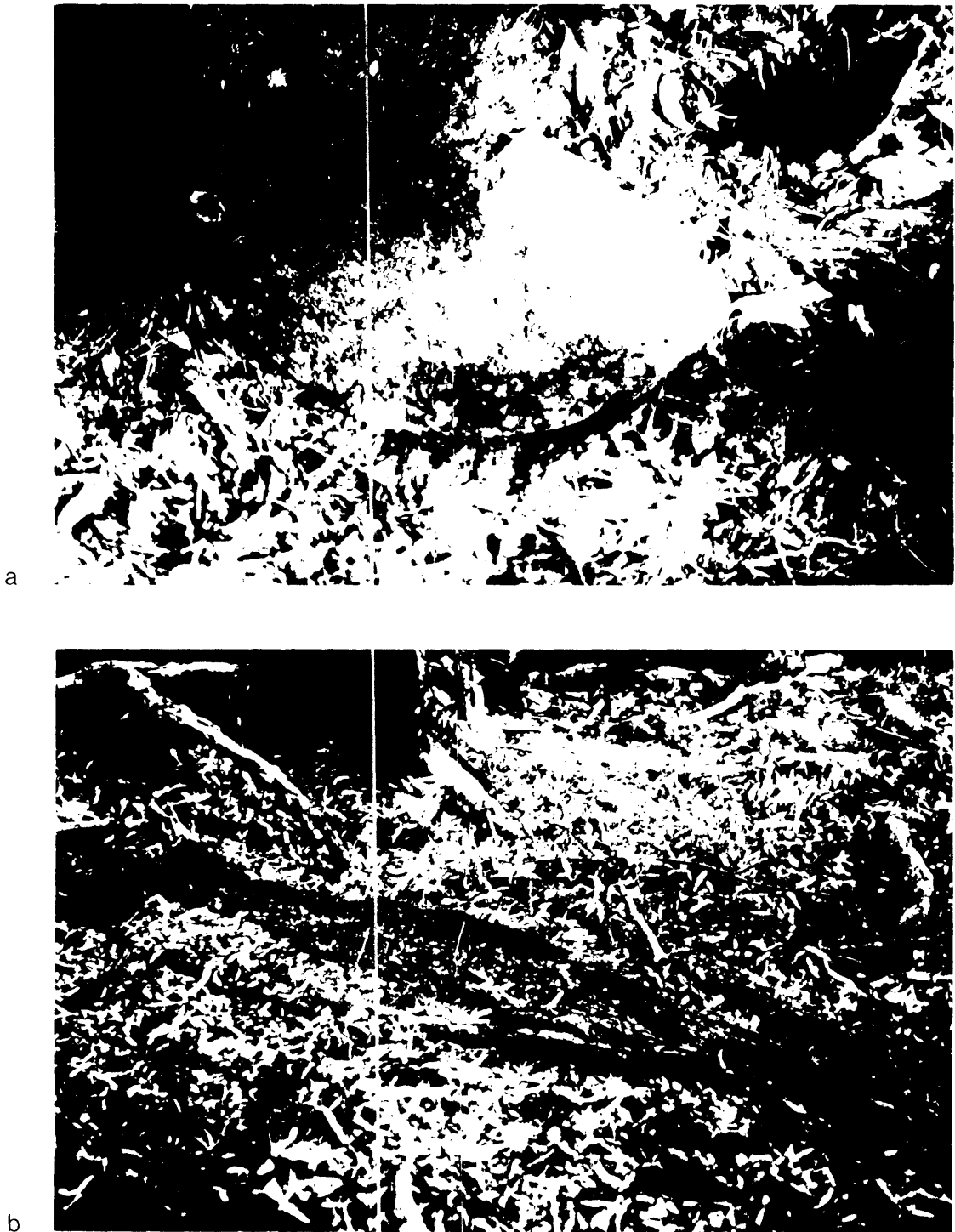
Plate 3.2. Typical homesites of *Urodacus manicatus* showing (a) basalt rock approximately measuring 320 x 200 x 170 mm near Black Mountain and (b) fallen sheets of bark approximately measuring 1900 x 460 x 40 mm near Dubbo.

Table 3.1. Maximum and minimum ai* temperatures recorded over 48 hours during the recording of microhabitat temperatures.

Site	Month	Max (°C)	Min (°C)
Black Mountain	January	24.0	11.3
	July	11.3	-1.8
Dubbo	January	33.0	12.3
	July	18.8	4.0

Table 3.2. Water content as the proportion of total wet body mass of combined adult females and males collected during different seasons.

Season	Body water content $(g \; H_2O \; g^{-1})$	n
Summer	0.693 ± 0.031	14
Autumn	0.650 ± 0.014	11
Winter	0.665 ± 0.013	7
Spring	0.698 ± 0.026	12

Values are mean $\pm$ SE, with the number of observations (n).

Table 3.3. Mass (g) and haemolymph osmolality (mOsmol kg^{-1}) of male and female scorpions collected during winter from Black Mountain and Dubbo. Osmolalities are not significantly different, but male masses are significantly different, P < 0.01.

Population	Sex	n	Mass	Osmolality
Black Mountain	Female	10	1.09 ± 0.04	489.40 ± 10.59
	Male	10	0.57 ± 0.02	481.30 ± 17.67
Dubbo	Female	8	1.11 ± 0.05	462.13 ± 9.80
	Male	8	0.79 ± 0.02	462.00 ± 13.42

Values are mean ± SE, with the number of observations (n).

Table 3.4. The effect of season on adult female temperature coefficients (Q_{10}) calculated for each increment in the temperature range fro n oxygen consumption rates.

Temperature range (°C)	Summer Q_{10}	Autumn Q_{10}	Winter Q_{10}	Spring Q_{10}	df	F
20-25	3.22 ± 0.38 (16)	2.76 ± 0.41 (14)	2.95 ± 0.48 (14)	3.27 ± 0.58 (16)	3, 51	0.24
25-30	2.14 ± 0.15 (16)	2.19 ± 0.18 (14)	1.85 ± 0.14 (15)	3.02 ± 0.81 (16)	3, 51	1.48
30-35	1.83 ± 0.09 (16)	2.58 ± 0.25 (14)	1.72 ± 0.15 (16)	1.83 ± 0.23 (16)	3, 51	3.47*
35-40	3.36 ± 0.31 (16)	1.73 ± 0.08 (13)	2.16 ± 0.21 (16)	2.03 ± 0.37 (16)	3, 51	6.71***

Values are mean ± SE, with the number of observations (n) and with univariate F values (*** $P < 0.001$, * $P < 0.05$).

Table 3.5. The effect of season on Q_{10} s calculated for each increment in the temperature range from oxygen consumption rates for combined females and males.

Temperature range (°C)	Summer Q_{10}	Autumn Q_{10}	Spring Q_{10}	df	F
20-30	2.46 ± 0.13 (26)	2.45 ± 0.14 (21)	2.35 ± 0.19 (26)	2, 66	0.53
30-40	2.32 ± 0.11 (28)	2.07 ± 0.07 (21)	1.72 ± 0.14 (26)	2, 66	6.99**

Values are mean $\pm$ SE, with the number of observations (n) and with univariate F values (** $P < 0.01$).

Table 3.6. Results of ANOVAs examining the mean differences in average, maximum and minimum temperatures between adjacent time periods. Only the significant results of flatness and parallelism for the three measures of temperature selection were tested.

Tests	Period contrasts	df	Univariate F		
			Average	Maximum	Minimum
Period	ll-dl	8, 91	4.25**ᵃ	3.20**	5.16***
(flatness)	dl-d2	8, 91	4.23**ᵃ	6.69***	0.59
	d2-d3	8, 91	2.55*	5.35***	0.44
	d3-d4	8, 91	3.29**	5.29***	0.86
	d4-l2	8, 91	4.54**ᵃ	3.25**	2.40*
Period by	ll-dl	3, 91	9.66**ᵃ	5.82***	3.85*
Season	dl-d2	3, 91	3.78*	6.49***	0.10
interaction	d2-d3	3, 91	0.11	0.37	0.90
(parallelism)	d3-d4	3, 91	0.73	1.30	0.59
	d4-l2	3, 91	1.58	0.92	1.96

*** P < 0.001, ** P < 0.01, * P < 0.05

Table 3.7. Results of ANOVAs exami*ing the mean differences in activity between adjacent time periods. Only the significant results of flatness and parallelism were tested.

Tests	Period contrasts	df	Univariate F
Period (flatness)	ll-dl	8, 91	23.24**⁵
	dl-d2	8, 91	2.11*
	d2-d3	8, 91	6.51**⁵
	d3-d4	8, 91	2.78**
	d4-l2	8, 91	0.38
Period by Season interaction (parallelism)	ll-dl	3, 91	9.13**⁵
	dl-d2	3, 91	0.77
	d2-d3	3, 91	4.44**
	d3-d4	3, 91	2.13
	d4-l2	3, 91	0.32

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$
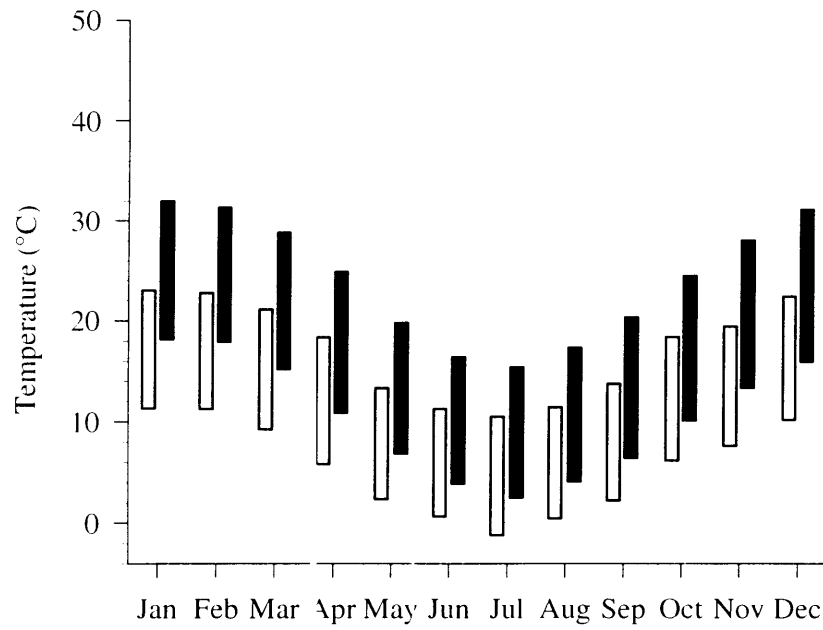
Figure 3.1. Average daily temperature ranges for each month obtained from local meteorological stations at Guyra (open bar) and Dubbo (closed bar).

Figure 3.2. Rain statistics from local meteorological stations at Guyra (open bar) and Dubbo (closed bar): (a) average monthly rainfall, and (b) average monthly number of raindays.
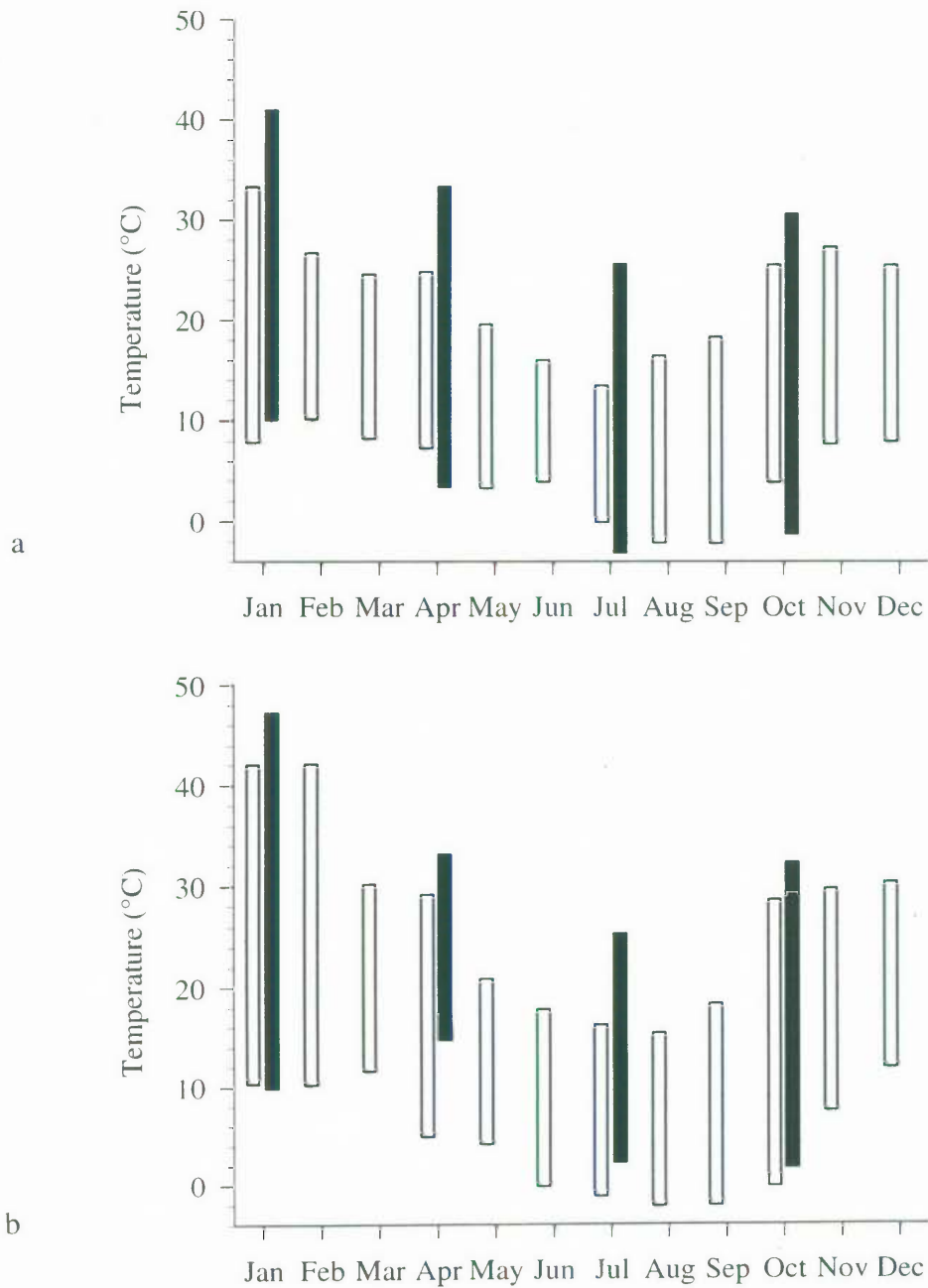
Figure 3.3. Monthly Black Mountain (open bar) and seasonal Dubbo (closed bar) habitat temperatures: (a) air temperature ranges measured 2.5 m above ground level, and (b) temperature ranges of typical *Urodacus manicatus* home sites.
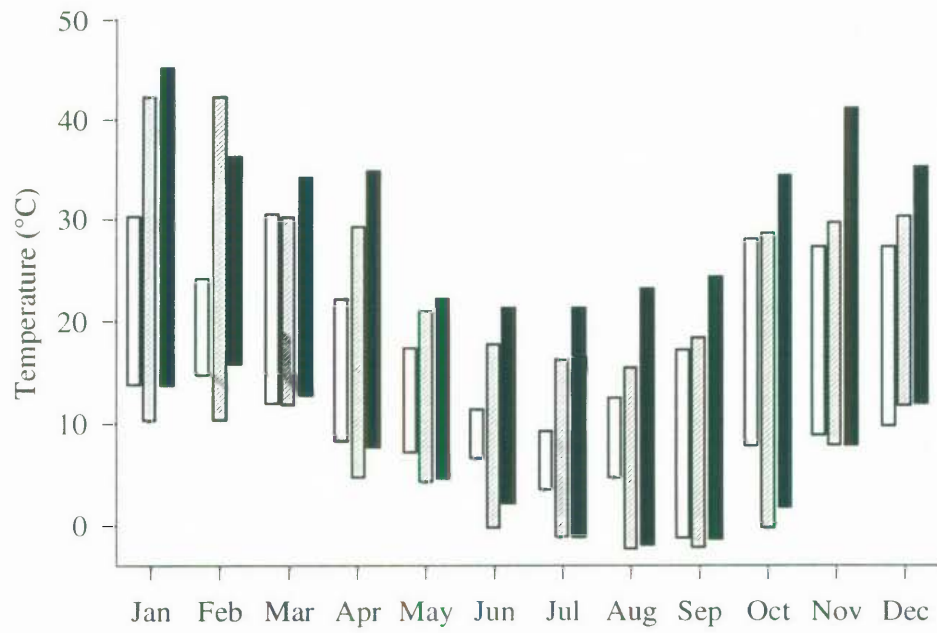
Figure 3.4. Monthly tree-shaded (open bar), typical partially shaded (striped bar) home site and exposed non home site (closed bar) temperature ranges at Black Mountain for similar sized rocks.
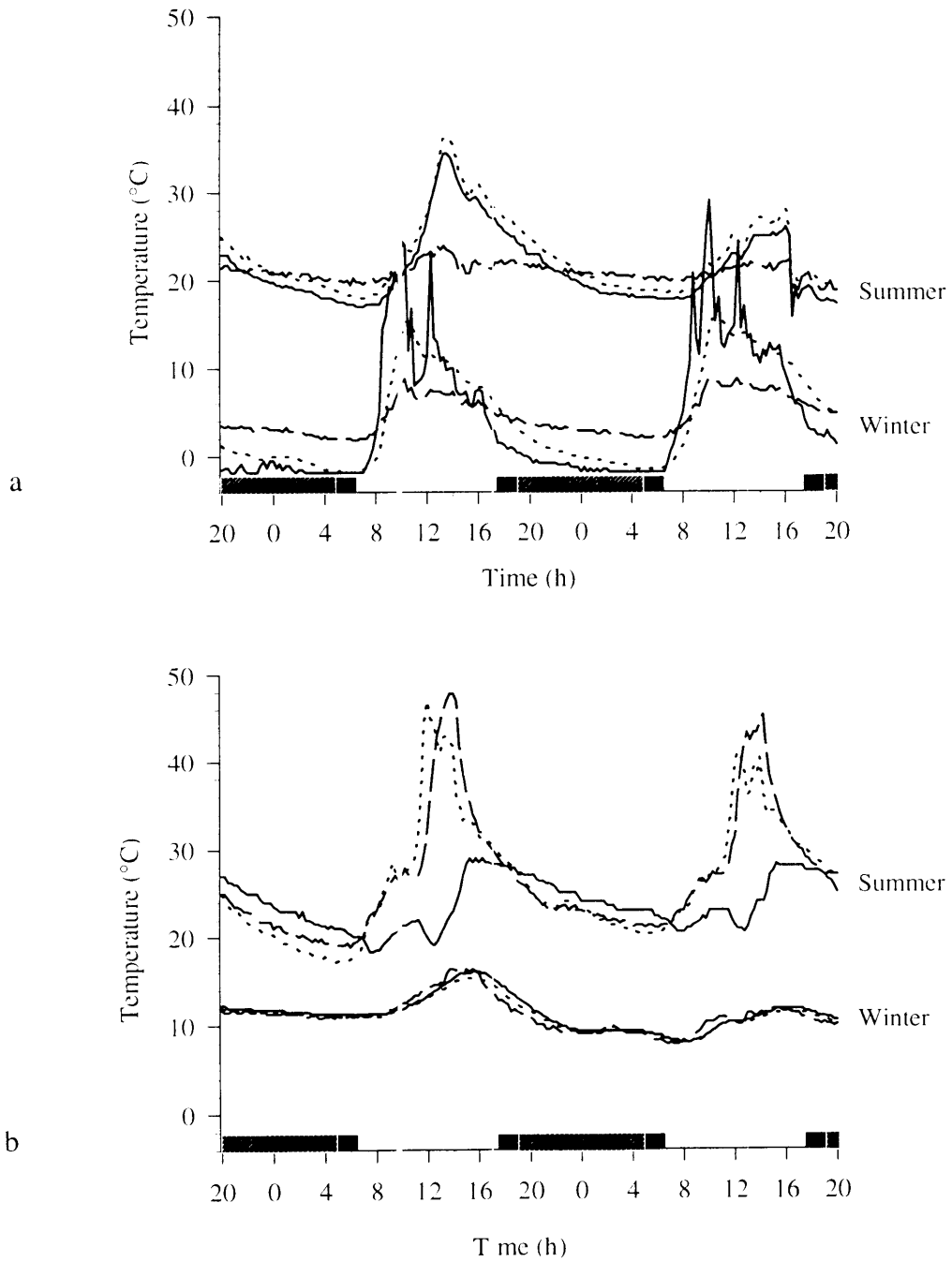
Figure 3.5. Daily temperature changes for typical scorpion microhabitats at (a) Black Mountain and (b) Dubbo. Lines indicate temperatures in living area (solid), within burrow entrance (dotted) and in soil at 2 cm depth near the homesite (dashed) over 24 hours during mid summer and mid winter. Horizontal bars indicate scotophases during mid summer (diagonal) and mid winter (solid) in Eastern Standard Time.
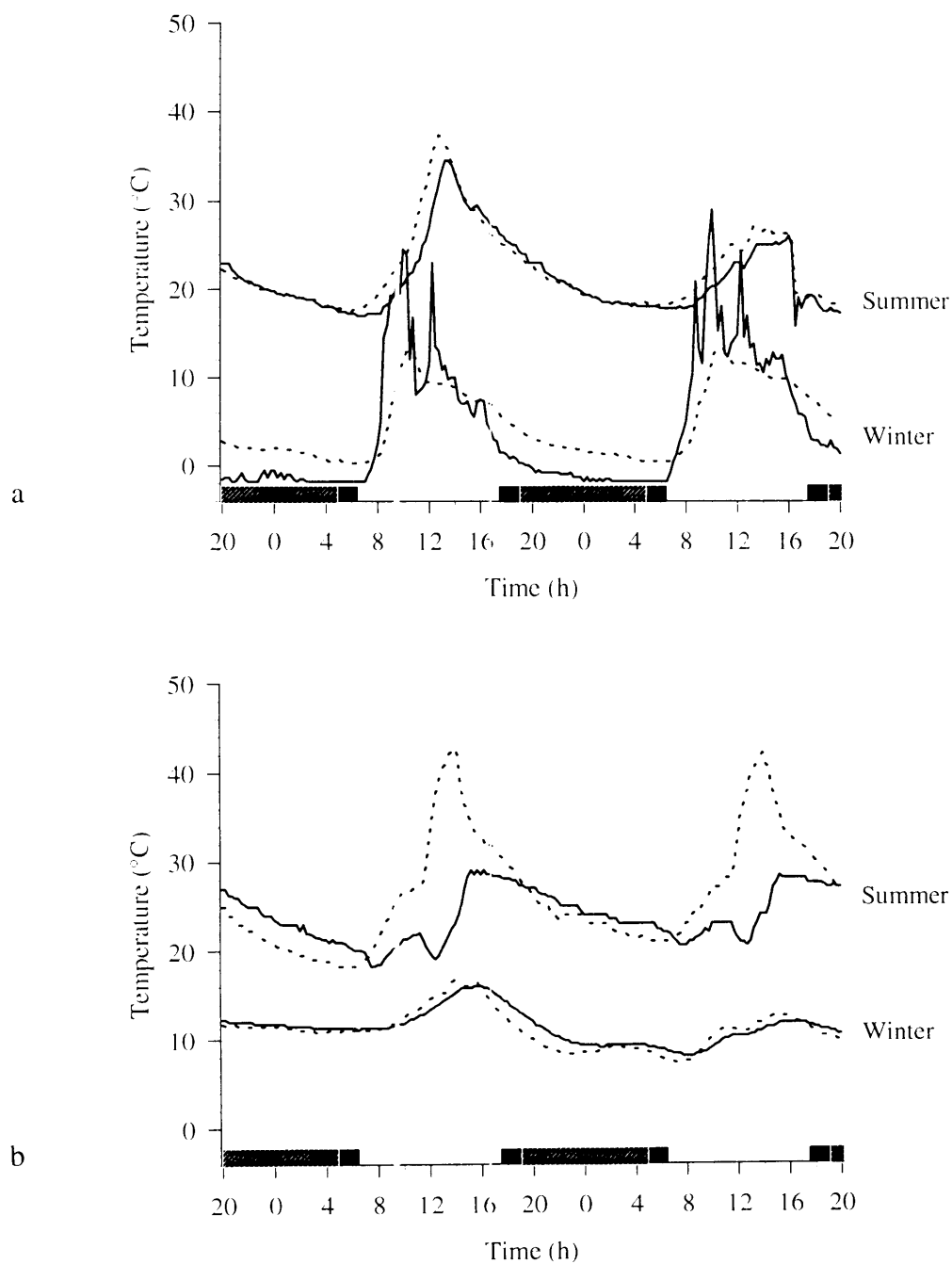
Figure 3.6. Daily temperature changes for typical homesite shelters and adjacent smaller non homesite shelters at (a) Black Mountain and (b) Dubbo. Lines indicate temperatures in living area of homesite shelter (solid) and beneath smaller shelter (dotted) over 24 hours during mid summer and mid winter. Horizontal bars indicate scotophases during mid summer (diagonal) and mid winter (solid) in Eastern Standard Time.
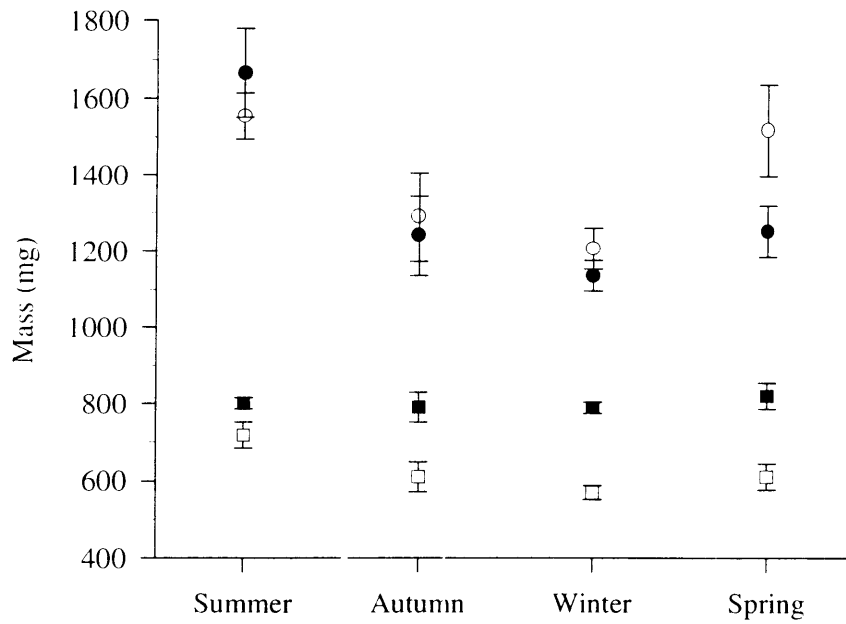
Figure 3.7. Seasonal profiles of the masses of adult scorpions examined in RMR and temperature selection studies, females (circles) and males (squares) collected from Black Mountain (open symbols) and Dubbo (closed symbols). Symbols are mean values ± SE. Seasons were significantly different (P < 0.001) and males from Dubbo were significantly larger than males from Black Mountain (P < 0.001).
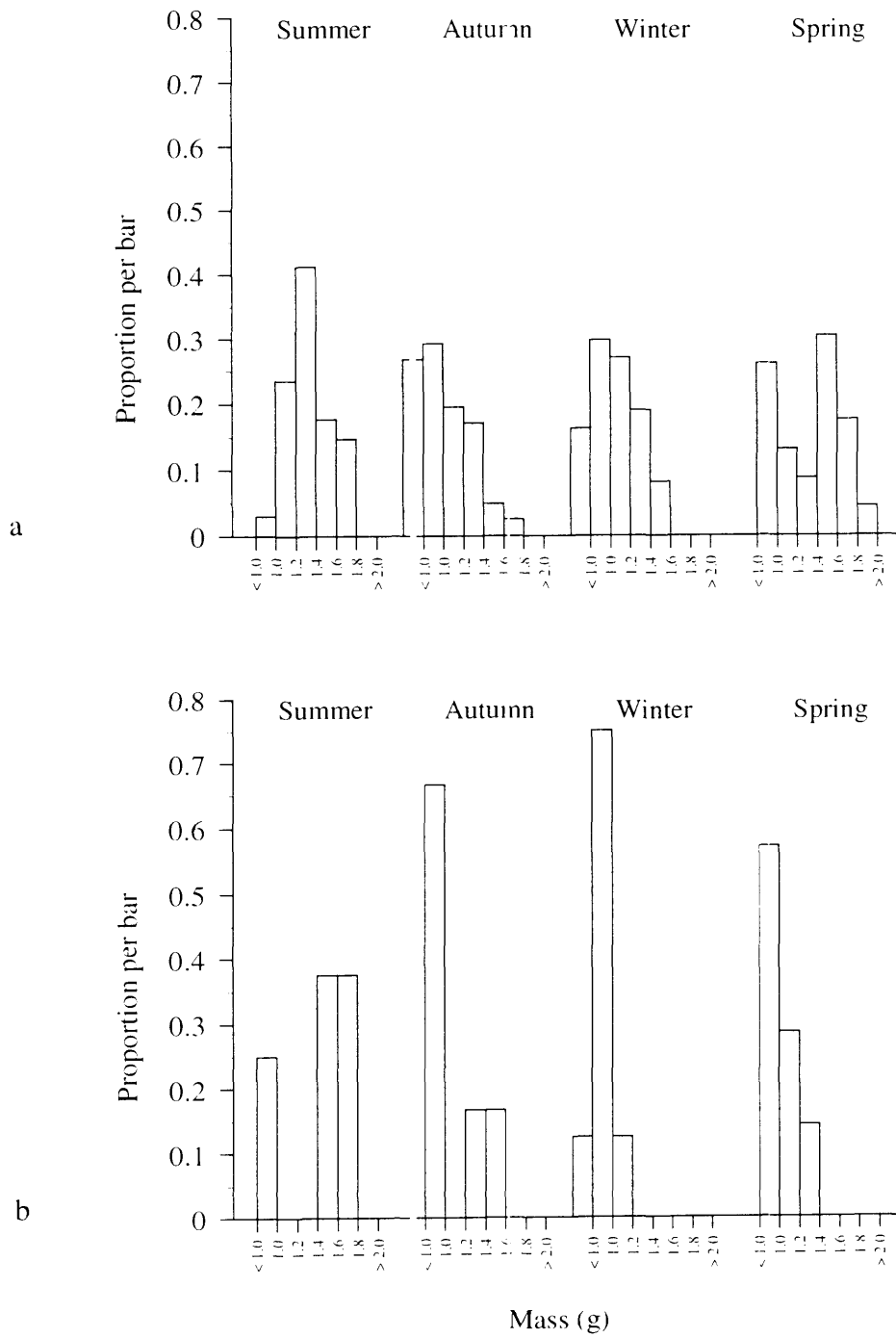
Figure 3.8. Histograms showing seasonal changes in distribution of adult females within each mass class collected from (a) Black Mountain and (b) Dubbo.
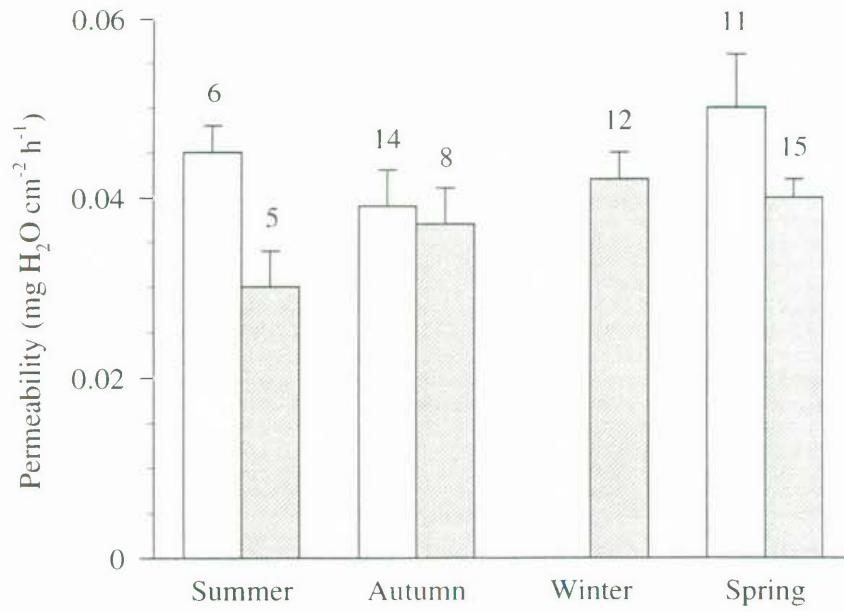
Figure 3.9. Seasonal effect on second day permeability (mg H_2O cm^{-2} h^{-1}) for mixed instar and sexes measured at 30°C, 0% RH collected from Black Mountain (open bars) and Dubbo (striped bars). Seasons were not significantly but populations were (P < 0.05). Bars represent mean values ± SE. Numbers are sample sizes (*n*).
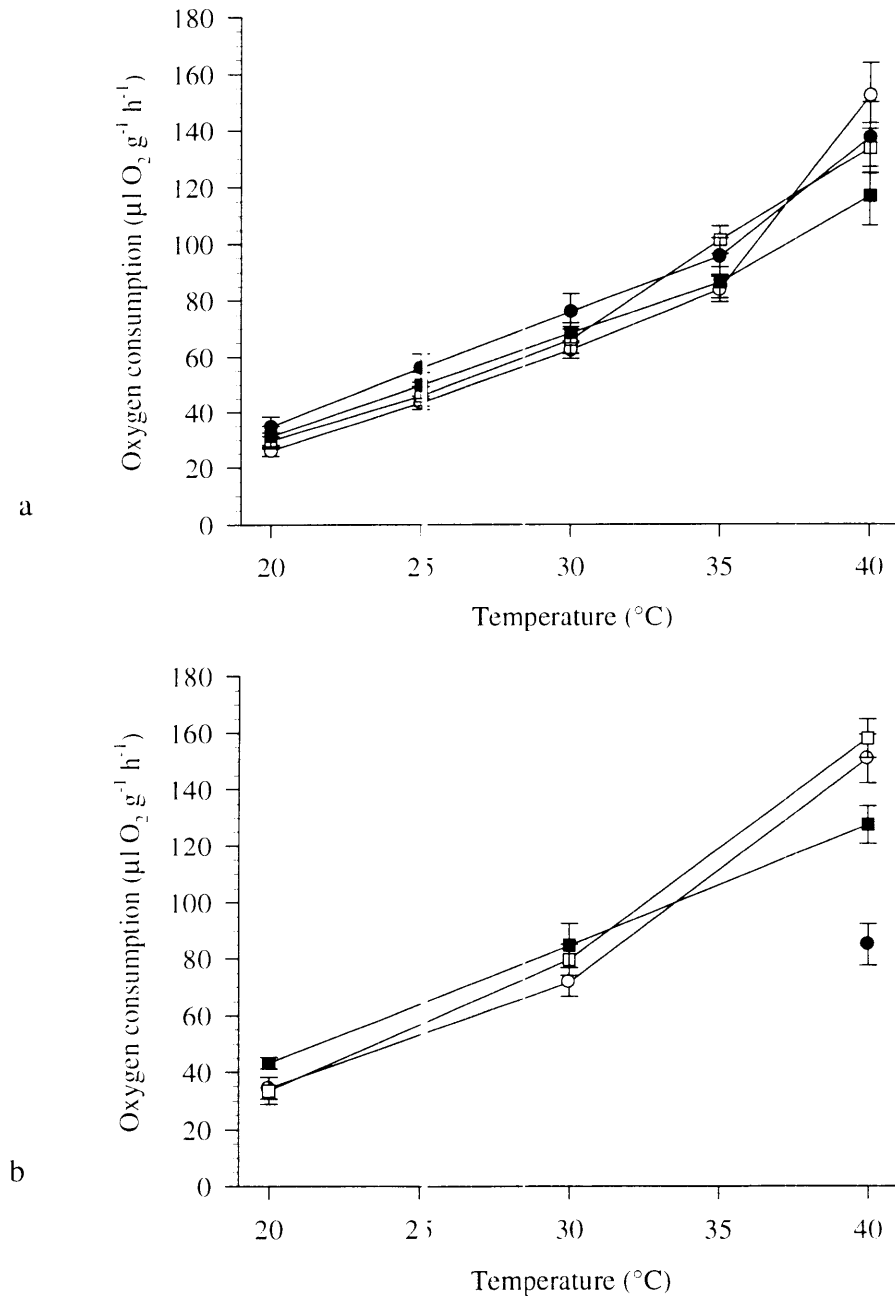
Figure 3.10. Seasonal mass-specific oxygen consumption rate (μl O_2 g^{-1} h^{-1}) as a function of temperature (°C) for (a) adult females and (b) adult males collected from both populations. Symbols with sample sizes for females and males respectively are: open circles, summer $n = 16$ and 10; open squares, autumn $n = 13$ and 7; closed circles, winter $n = 14$ and 8; and closed squares, spring $n = 16$ and 10). Points represent mean values ± SE.
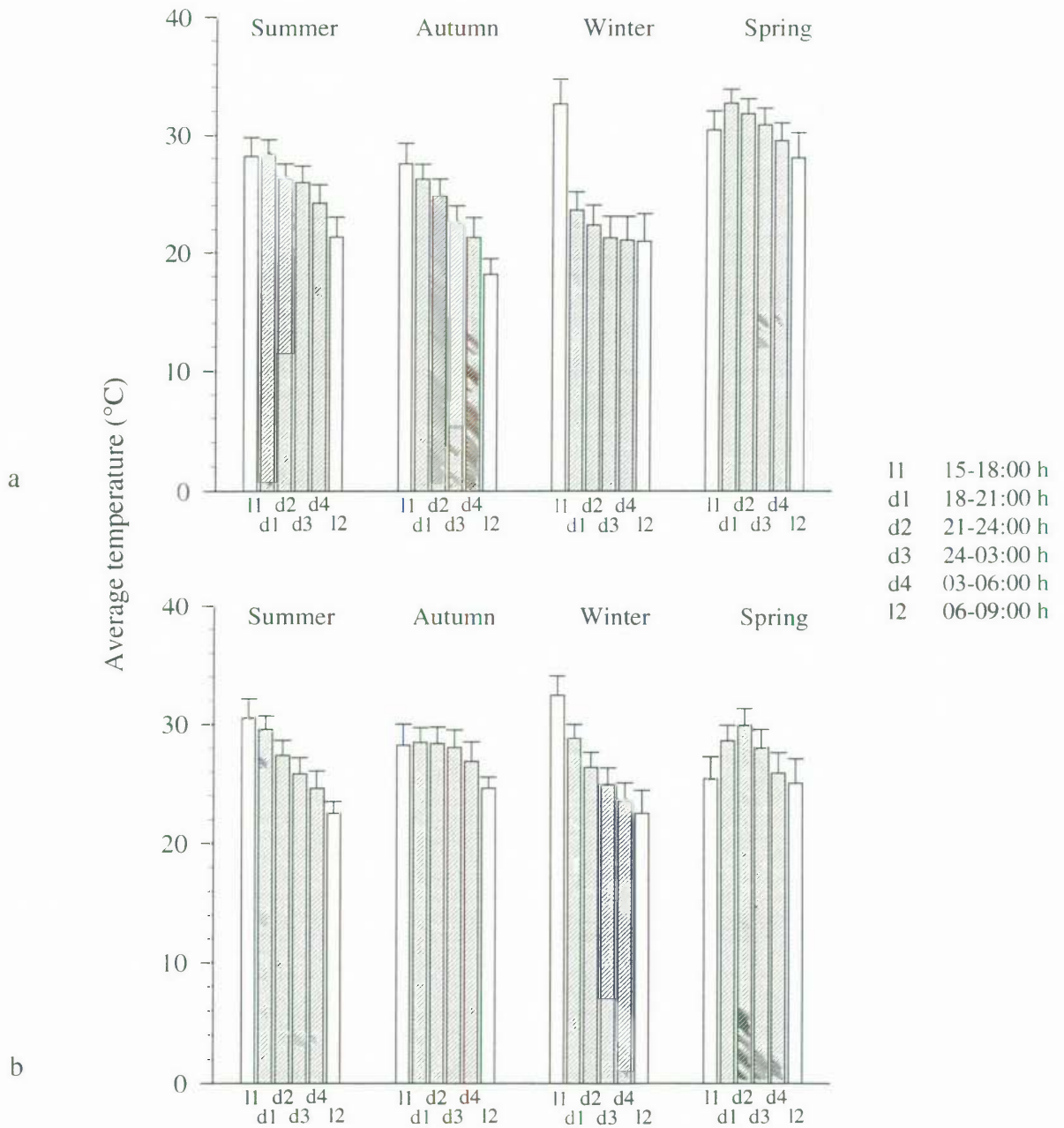
Figure 3.11. Seasonal changes in average selected temperature (°C) of adults collected from (a) Black Mountain and (b) Dubbo. Averages are of 12 measurements per animal within the 3 hour periods of 15-18:00 h (l1), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3), 03-06:00 h (d4) and 06-09:00 h (l2). Bars represent mean values ± SE of each sample. Sample sizes for Black Mountain and Dubbo respectively are: summer, $n = 14$ and 15; autumn, $n = 12$ and 12; winter, $n = 8$ and 14; and spring, $n = 14$ and 11. Shaded bars highlight the scotophase periods.
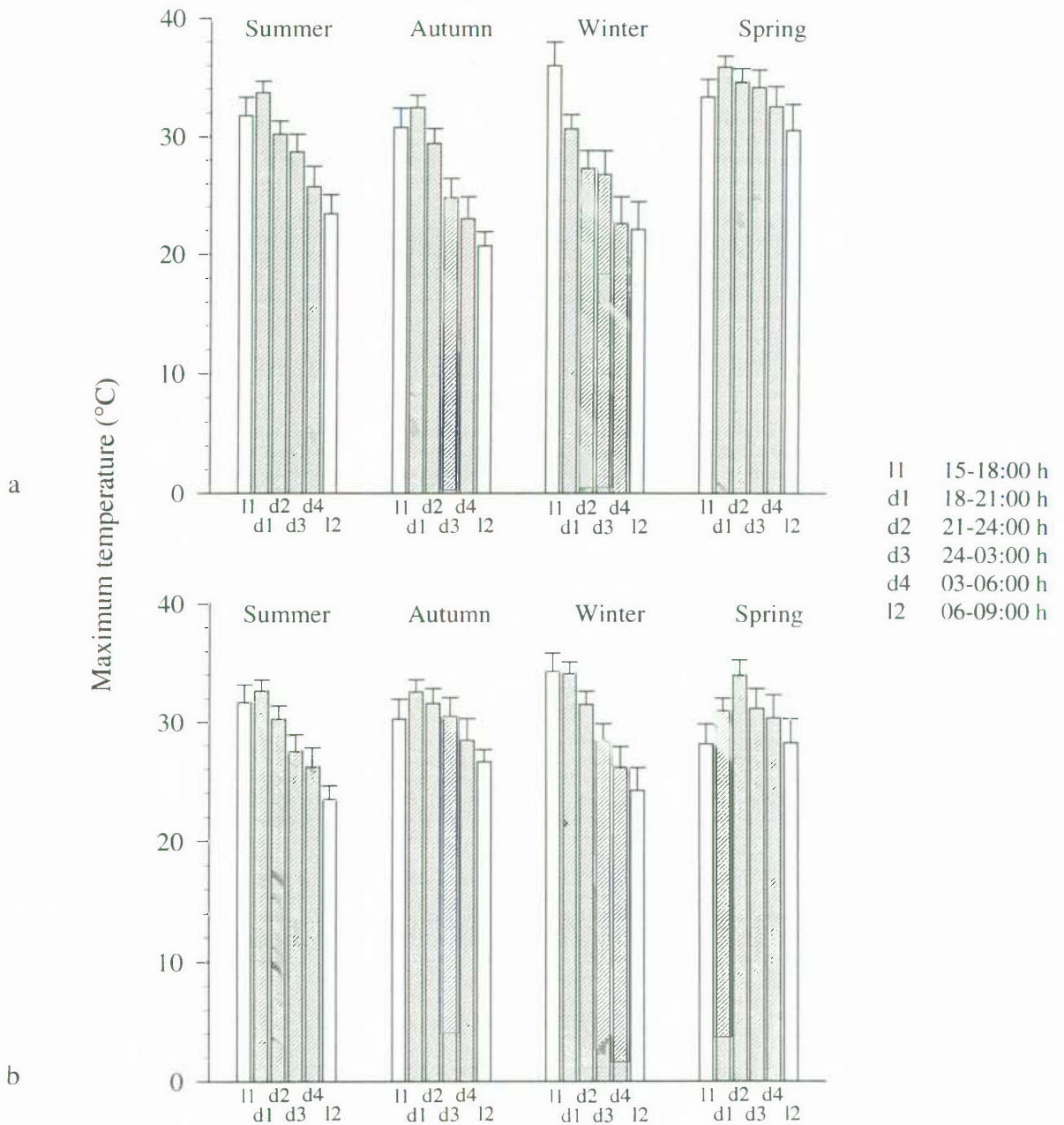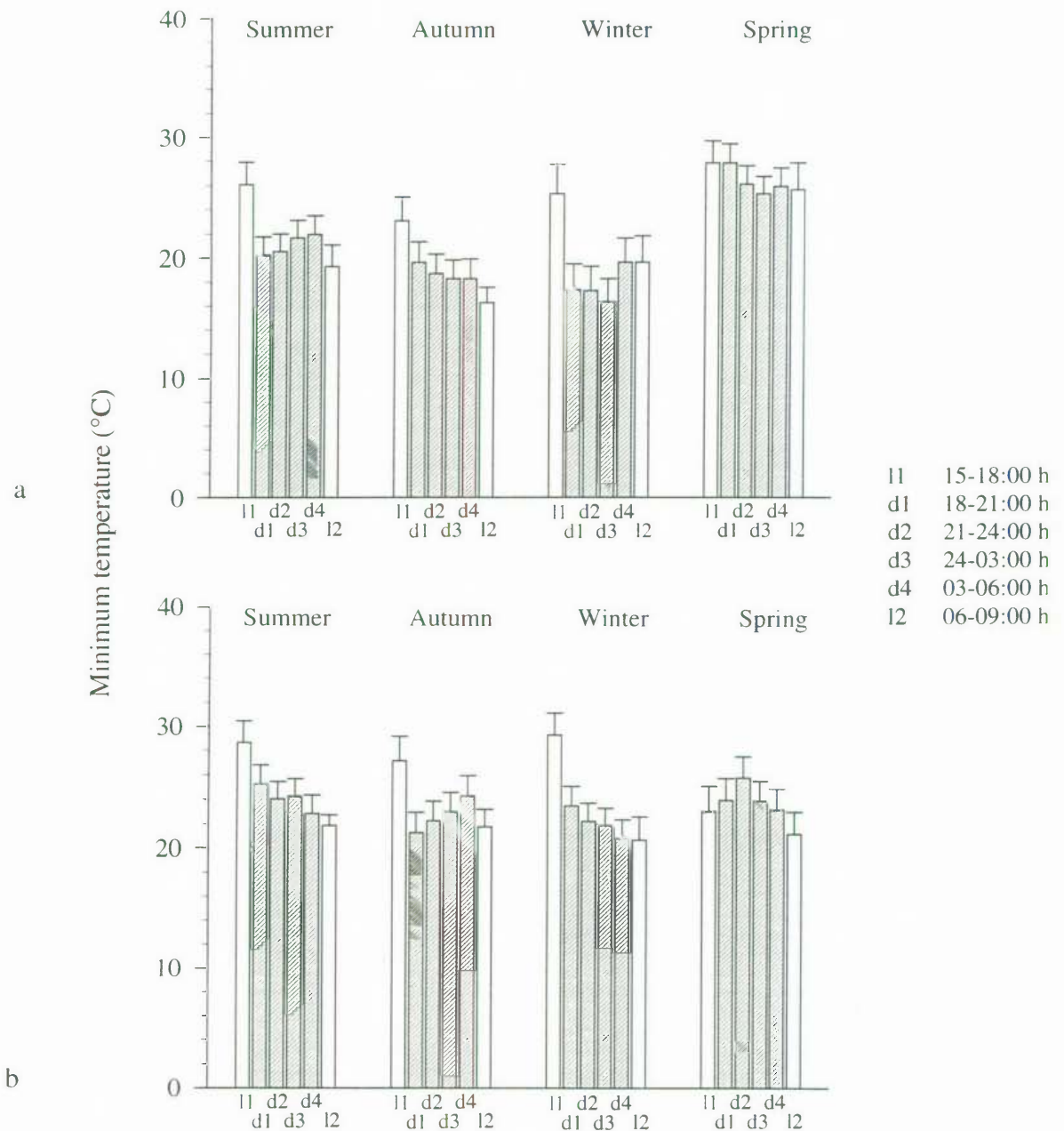
Figure 3.12. Seasonal changes in maximum temperature (°C) recorded in the gradient for adults collected from (a) Black Mountain and (b) Dubbo. Maxima are from 12 measurements per animal within the 3 hour periods of 15-18:00 h (l1), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3), 03-06:00 h (d4) and 06-09:00 h (l2). Bars represent mean values ± SE of each sample. Sample sizes for Black Mountain and Dubbo respectively are: summer, $n = 14$ and 15; autumn, $n = 12$ and 12; winter, $n = 8$ and 14; and spring, $n = 14$ and 11. Shaded bars highlight the scotophase periods.

Figure 3.13. Seasonal changes in minimum temperature (°C) recorded in the gradient for adults collected from (a) Black Mountain and (b) Dubbo. 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), 03-06:00 h (d4) and 06-09:00 h (l2). Bars represent mean values ± SE of each sample. Sample sizes for Black Mountain and Dubbo respectively are: summer, n = 14 and 15; autumn, n = 12 and 12; winter, n = 8 and 14; and spring, n = 14 and 11. Shaded bars highlight the scotophase periods.
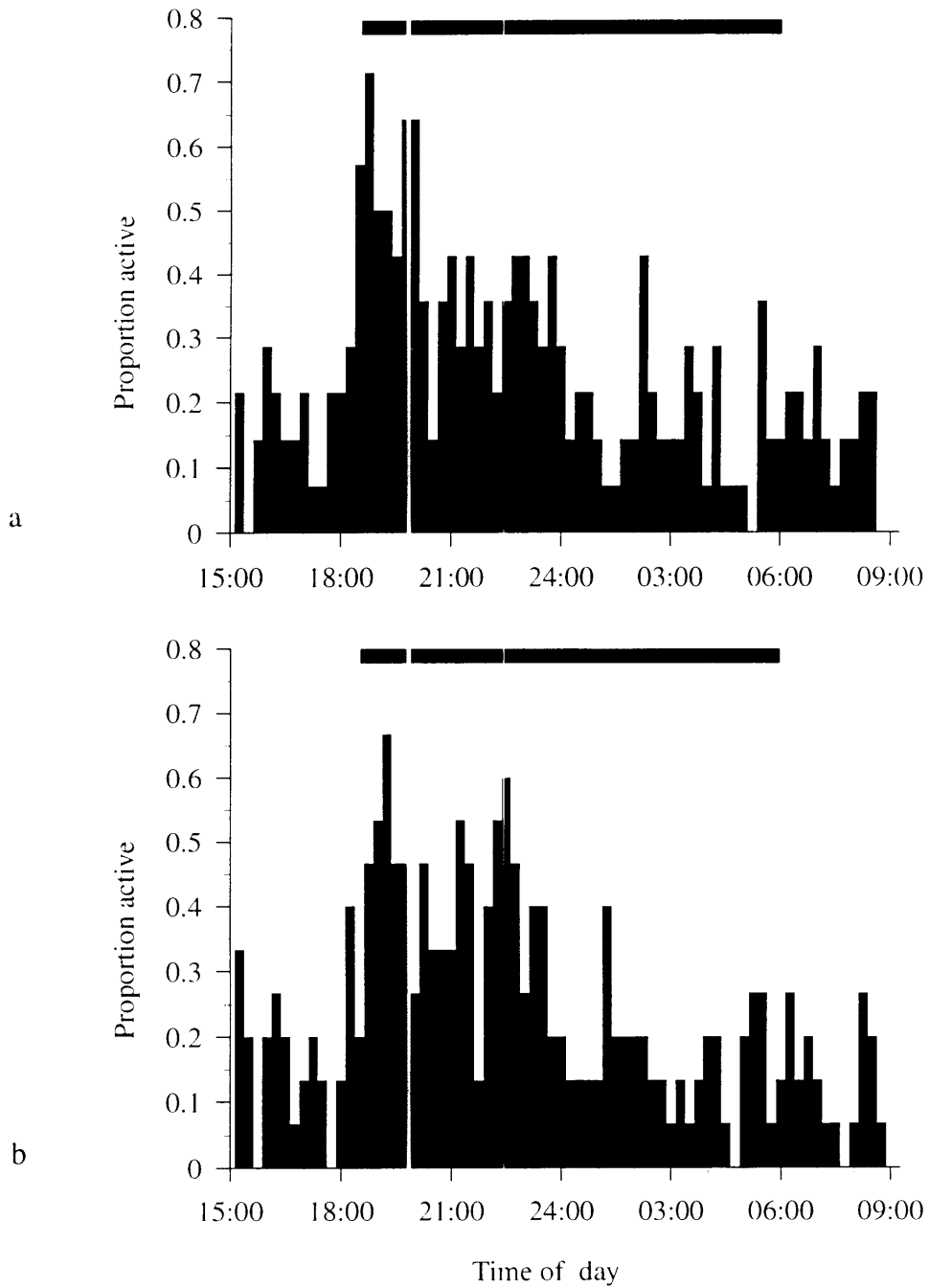
Figure 3.14. Activity profiles of adults collected during mid summer from (a) Black Mountain (*n* = 14) and (b) Dubbo (*n* = 15). Each bar represents the proportion of animals that had turned in the gradient resulting in a 5°C or greater change in selected temperature from the preceding temperature. Temperature was measured every 15 min. Horizontal bar represents scotophase from 18:00 to 06:00 hours.
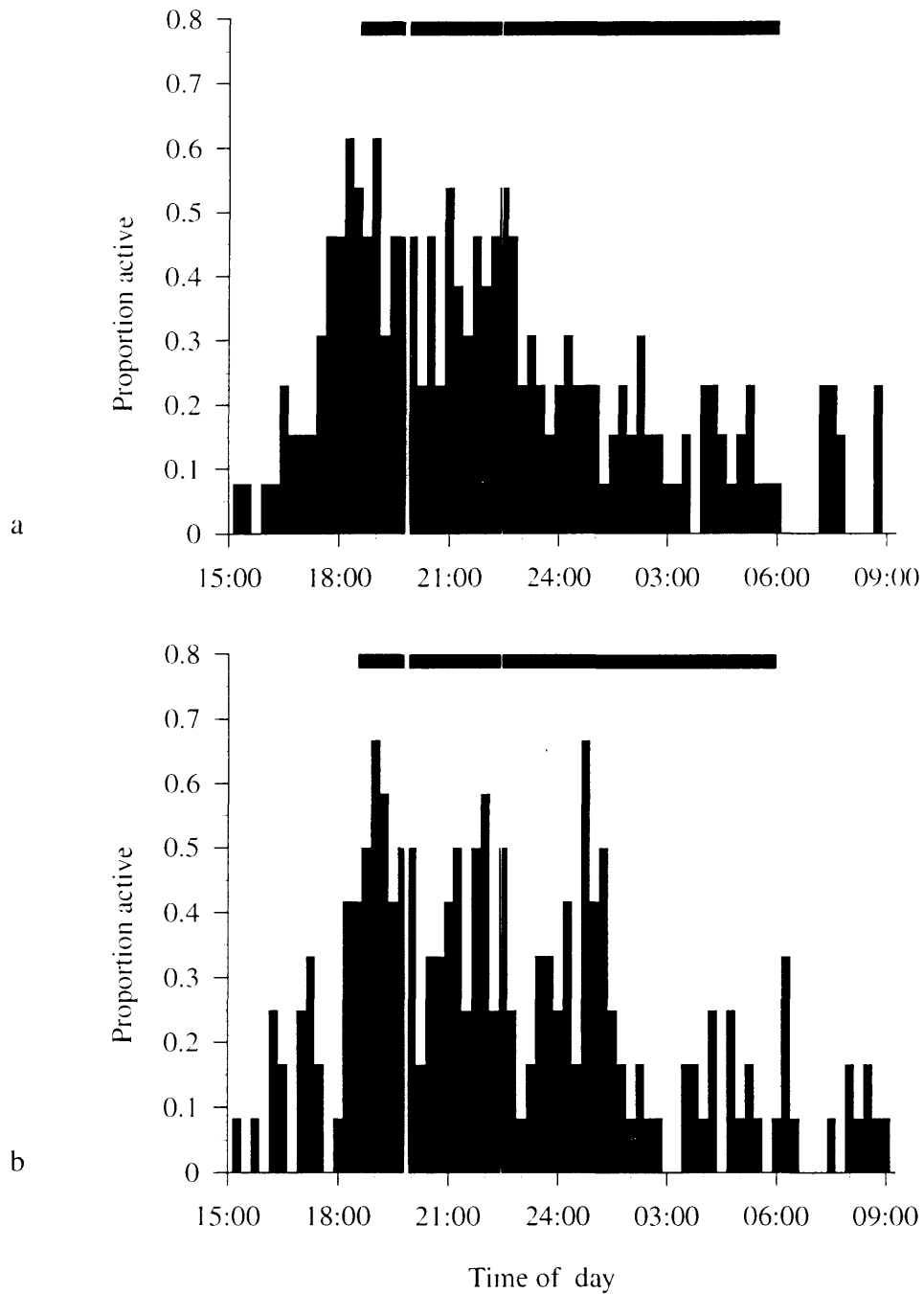
Figure 3.15. Activity profiles of adults collected during mid autumn from (a) Black Mountain (*n* = 13) and (b) Dubbo (*n* = 12). Each bar represents the proportion of animals that had turned in the gradient resulting in a 5°C or greater change in selected temperature from the preceding temperature. Temperature was measured every 15 min. Horizontal bar represents scotophase from 18:00 to 06:00 hours.
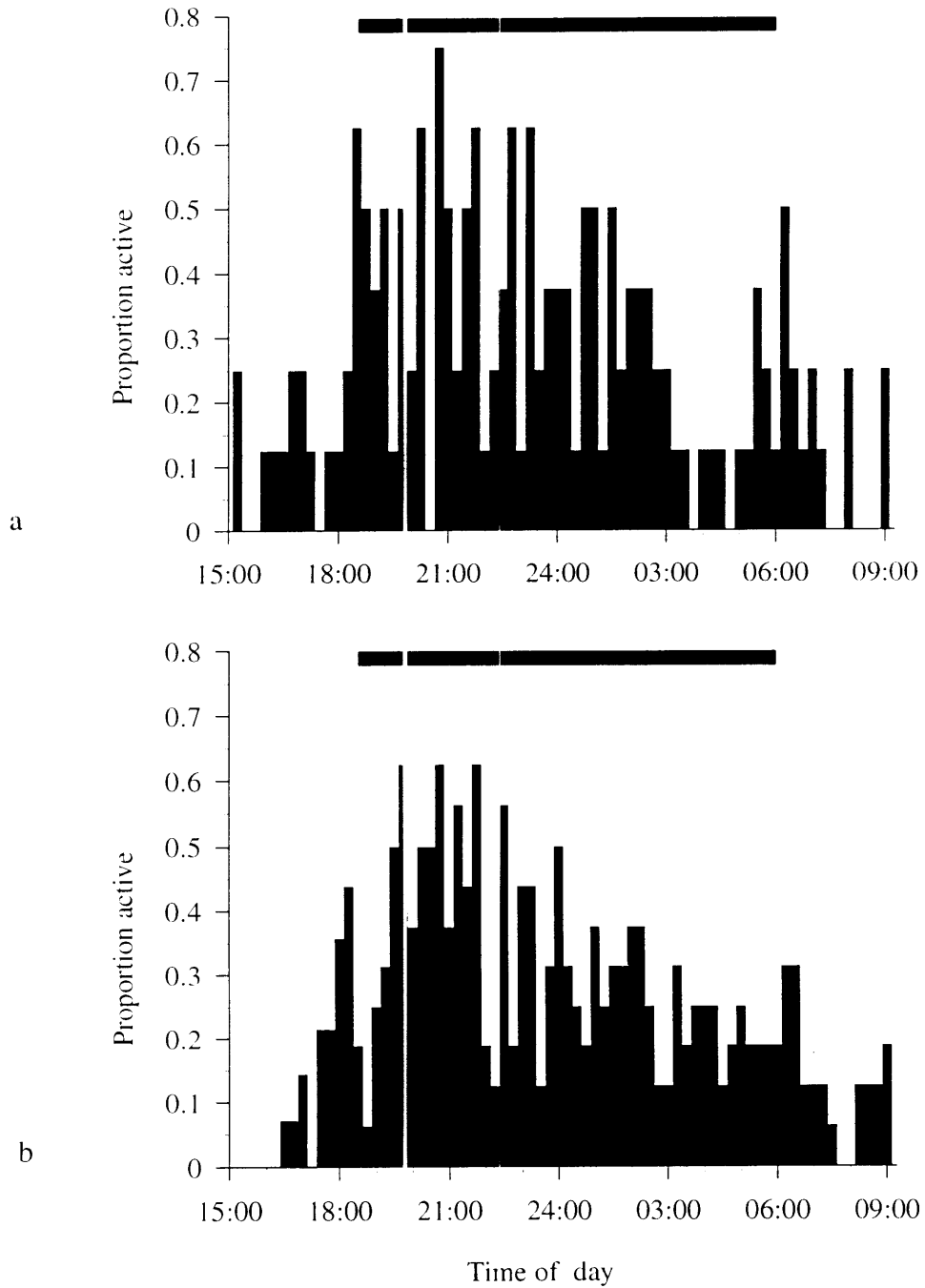
Figure 3.16. Activity profiles of adults collected during mid winter from (a) Black Mountain (*n* = 8) and (b) Dubbo (*n* = 16). Each bar represents the proportion of animals that had turned in the gradient resulting in a 5°C or greater change in selected temperature from the preceding temperature. Temperature was measured every 15 min. Horizontal bar represents scotophase from 18:00 to 06:00 hours.
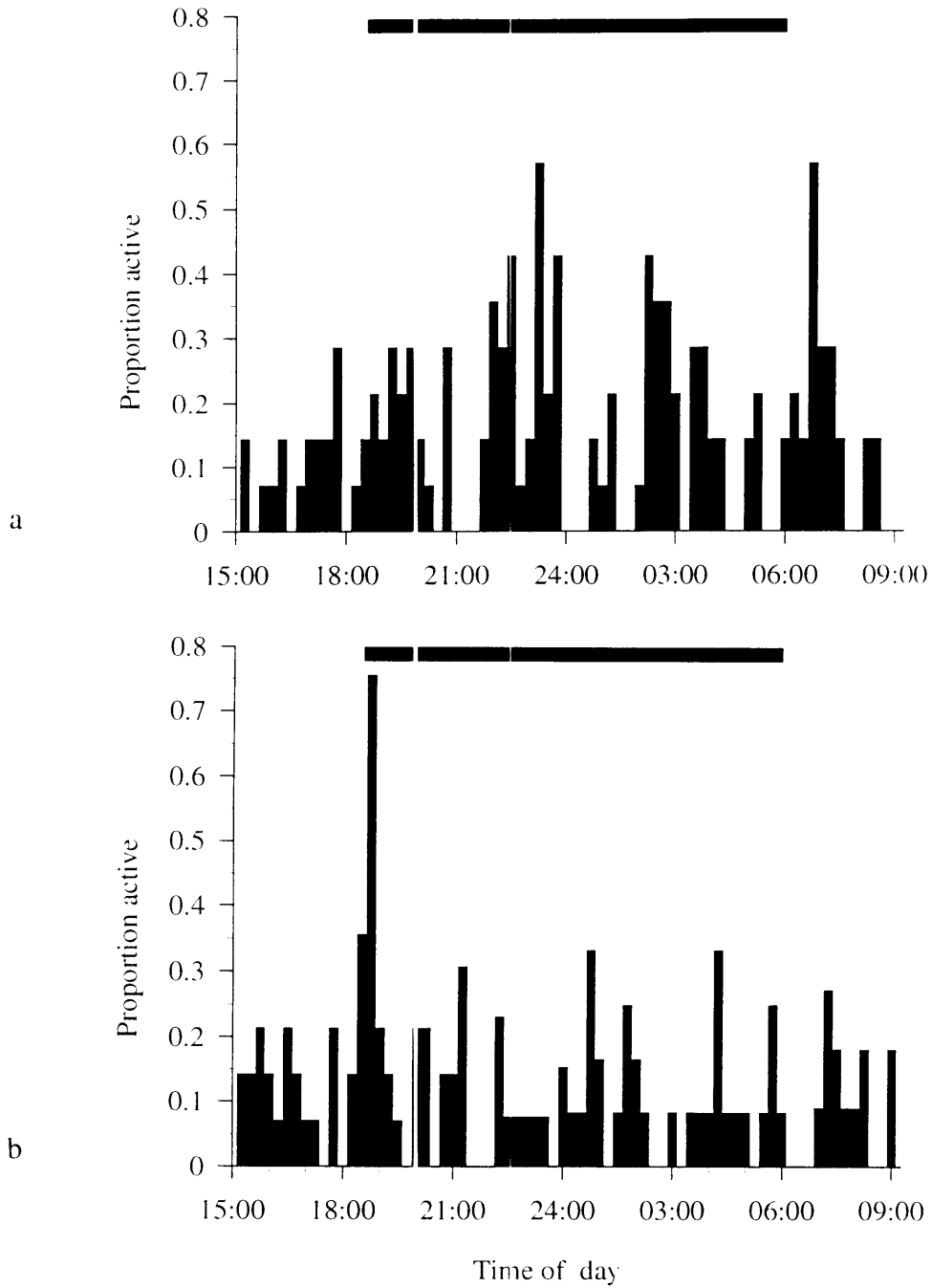
Figure 3.17. Activity profiles of adult; collected during mid spring from (a) Black Mountain (*n* = 14) and (b) Dubbo (*n* = 11). Eac1 bar represents the proportion of animals that had turned in the gradient resulting in a 5°C or greater change in selected temperature from the preceding temperature. Temperature was measured every 15 min. Horizontal bar represents scotophase from 18:00 to 06:00 hours.