

Chapter 5

Water Availability Studies

5.1 Introduction

The lack of seasonal change in permeability suggests that water is not a limiting factor that needs to be conserved except during summer. The difference between the sites may be due to water availability or temperature differences that may result in a greater rate of water loss. The effect of water availability on water balance is examined in terms of external water assuming that the effects are not dependent on whether water comes from prey or free sources.

Scorpions rarely drink from free water sources but obtain most of their water from prey (Yokota 1984) which can be irregular and seasonally dependent in the field. In captivity, however, scorpions can obtain water from soaked cotton wool pledgets or chew or stand on wet sand depending on their state of hydration (Bub and Bowerman 1979, Riddle et al. 1976, Rosin and Shulov 1963, Willmer 1967). No species have been observed to obtain water from saturated atmospheres and they continue to lose water even at 95% RH. Most scorpions are osmoconformers, but the extent of the changes in haemolymph osmolality are dependent on whether the species is mesic or xeric adapted (Robertson et al. 1982, Warburg et al. 1980b). Excessive water loss, however, can affect the fitness of an animal if locomotion is impaired (Wilson and Havel 1989).

Acclimation temperature did not affect the rate of evaporative water loss in *Urodacus manicatus* (Chapter 4). Water gain rather than the conservation of water may be more important in *U. manicatus* that inhabit cool temperate regions, in which prey abundance is variable.

The effects of water availability on *Urodacus manicatus* were examined in this chapter by:

1. comparing metabolic rates before and after excessive hydration and dehydration.
2. comparing temperature selection and activity before and after excessive hydration and dehydration.
3. determining the change in haemolymph osmolality after excessive hydration and dehydration.
4. determining if permeability to evaporative water loss changed with high and low water availability.

5.2 Materials and Methods

The effect of water availability on the rate of water loss in adult females was examined as follows. Low water stress was achieved by placing scorpions that were dehydrated in the RMR experiment described below, at 25°C, 0% RH as described in section 2.2 for 7 days. High water stressed animals were maintained at 25°C for the same period in plastic drinking cups with continuously moist cotton wool as the substrate from which they could not remove themselves. Intermediate water stress was achieved by placing individuals in plastic cups with cotton wool under the same conditions as the high water stress but only two waterings over the 7 days of the experiment. The effects of water availability on the second day permeability was analysed in a one-way ANOVA with the amount of water given (continuous watering, periodic watering and dehydrated) as the independent variable.

The effects of high and low water stress on oxygen consumption, temperature selection and haemolymph osmotic concentration were examined as follows. Adult female and male scorpions were collected from Black Mountain and maintained at 20°C for two weeks with food and water

ad libitum. Food was withheld 48 hours prior to the first measurements and throughout the course of all experiments. High and low water stress were achieved as for the effects on permeability above. Oxygen consumption rates and temperature selection were measured as described in sections 2.3 and 2.4 respectively. Haemolymph osmolality was determined as described in section 3.2.

For the experiments on RMR and osmolality, 8 females were placed in each high and low stress group. Fewer males were collected from the field than females so I decided to examine the effect of sex in the temperature selection trials with all the males available rather than divide them up between those trials and the RMR trials. Thus 8 females and 8 males were placed in each high and low stress group to test the effects on temperature selection.

The effect of hydration state on oxygen consumption ($\dot{V}O_2$) was initially analysed utilising the allometric relationship between $\dot{V}O_2$ and mass (Section 3.3.1). The change in mass with treatment between the hydrated and dehydrated groups, however, was significantly different, $F(1, 14) = 16.30$, $P < 0.001$, thus confounding the use of wet mass as a covariate. Therefore, the suitability of dry mass as the covariate was tested assuming that any changes in mass between the two groups was solely due to water loss or gain. However, the adjustment made by log transformed mass on oxygen consumption was not significant, $F(1, 12) = 1.40$, $P > 0.05$, owing to the small size range of the adult females used. Instead, two one-way between-subjects MANOVAs were performed on the two dependent variables associated with whole animal and mass-specific oxygen consumption rates before and after treatment. The independent variable in each analysis was treatment (dehydration and watered). 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.

The effect of hydration state on haemolymph osmolality was analysed in a one-way between-subjects multivariate analysis of variance that was performed on the two dependent variables associated with haemolymph osmolality before and after treatment. The independent variable was treatment (dehydration and watered). 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.

The effect of hydration state and sex on temperature selection was examined with three profile analyses which were performed on ten repeated measure dependent variables at two levels associated with initial and final temperature selection in 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 treatment (water loss and water gain) and sex. The three analyses separately examined average, maximum and minimum selected temperature. For each analysis, order of entry of the grouping variables was treatment, then 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.

The effect of hydration state on activity was examined by means of a profile analysis which was performed on ten repeated measure dependent variables at two levels associated with initial and final temperature selection in 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 treatment (water loss and water gain) and sex. For each analysis, order of entry of the grouping variables was treatment, then 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. Activity was calculated for each group after 14 days of acclimation in Figures 5.6 and 5.7 as the proportion of animals that had turned in the gradient every 15 minutes.

The mass specific rates of water loss/gain for each day of dehydration/hydration were obtained from the studies of water availability effects on RMR and temperature selection.

5.3 Results

5.3.1 Evaporative Water Loss

Permeability after two days at 30°C, 0% RH was not affected by the degree of water availability (Figure 5.1). Animals that were continuously watered had a greater permeability of 0.096 (± 0.007) mg H₂O cm⁻² h⁻¹ than those that were periodically watered (0.075 \pm 0.007 mg H₂O cm⁻² h⁻¹) or dehydrated (0.080 \pm 0.006 mg H₂O cm⁻² h⁻¹). However, the permeabilities of the three groups were not significantly different, $F(1, 24) = 3.79$, $P = 0.063$.

Evaporative water loss for females and males at 30°C, 0% RH decreased for the first few days and were then constant until day 7 (Figure 5.2). Animals that were continuously hydrated fluctuated around a uniform mass each day (Figure 5.2).

5.3.2 Oxygen Consumption and Haemolymph Osmotic Concentration

Hydration state did not affect the RMR of *Urodacus manicatus* (Table 5.1). Animals that were dehydrated from 1.22 (± 0.03) to 0.99 (± 0.04) g exhibited a small reduction in whole animal VO_2 from 116.50 (± 6.64) to 107.07 (± 10.39) μ l O₂ h⁻¹ and a small increase in mass-specific VO_2 from 94.96 (± 4.44) to 106.42 (± 5.51) μ l O₂ g⁻¹ h⁻¹. The masses of the continuously watered group did not change and their VO_2 remained constant. The effects of hydration state on whole animal VO_2 , $F(2, 12) = 0.42$ and mass-specific VO_2 , $F(2, 12) = 0.65$ were not significant ($P > 0.05$).

Hydration state affected the haemolymph osmolality of *Urodacus manicatus* (Table 5.1). Dehydration of scorpions at 30°C, 0% RH for 7 days increased osmolality from 483.56 (± 9.49) to 615.71 \pm 22.06 mOsm kg⁻¹. Continuous watering for 7 days decreased the osmolality from 483.56 (± 9.49) to 447.63 \pm 9.89 mOsm kg⁻¹. The osmolalities of the two groups before treatment were not significantly different, $F(1, 13) = 1.22$, $P > 0.05$, but were significantly different after treatment, $F(1, 13) = 52.91$, $P < 0.001$. Within-subjects analysis indicated that the effect on osmolality over time was different between treatments, $F(1, 13) = 96.45$, $P < 0.001$.

5.3.3 Temperature Selection

Hydration state affected average temperatures selected by adult female (Figure 5.3a) and male (Figure 5.3b) *Urodacus manicatus*. Females and males that were dehydrated for 7 days at 30°C, 0% RH selected lower temperatures between 15:00 and 06:00 h than when hydrated. Selected temperature in the first 3 hours of the scotophase decreased after 7 days from 33.1°C (± 0.9) to 22.4°C (± 1.4) for females (Figure 5.3a) and 32.2°C (± 1.0) to 20.3°C (± 1.9) for males (Figure 5.3b). Females and males that were continuously watered for 7 days selected the same temperatures as when previously periodically watered. Temperatures selected before and after the treatments were significantly different, $F(1, 25) = 33.05$, $P < 0.001$. The difference was influenced by water availability, $F(1, 25) = 26.95$, $P < 0.001$, but not by either sex, $F(1, 25) = 0.15$, $P > 0.05$, or the combined effect of sex, treatment and water availability, $F(1, 25) = 0.78$, $P > 0.05$. The temperatures selected by females and males were the same, $F(1, 25) = 4.50$, $P > 0.05$. The temperatures selected were dependent upon the time of day with an increase in temperature associated with the onset of the scotophase followed by a gradual decrease throughout the night. The three hourly profiles of temperature averaged over all animals deviated significantly from flatness, $F(4, 22) = 31.16$, $P < 0.001$, $\eta^2 = 0.85$. All effects on the temperature profiles were not significant, indicating that the pattern of temperature selection was not affected by water availability or sex.

Maximum temperatures that were recorded for females (Figure 5.4a) and males (Figure 5.4b) within the gradient were affected by hydration state in a similar way to that of average selected temperature. Dehydrated females and males did not travel as far into the hot end of the gradient between 15:00 and 06:00 h as when previously watered and dehydrated males had lower temperatures than dehydrated females. Maximum temperature in the first 3 hours of the scotophase decreased from 36.7°C (± 0.6) to 26.8°C (± 2.2) for females (Figure 5.4a) and 36.9°C (± 0.6) to 24.9°C (± 2.8) for males (Figure 5.4b). Females and males that were continuously watered for 7 days encountered the same maximum temperatures as when previously less watered. Maximum temperatures before and after the treatments were significantly different, $F(1, 25) = 43.57$, $P < 0.001$, and this difference was influenced by water availability, $F(1, 25) = 32.95$, $P < 0.001$, and sex, $F(1, 25) = 4.34$, $P < 0.05$. The combined effect of sex, treatment and

water availability was not significant, $F(1, 25) = 0.98, P > 0.05$. Sex had a significant effect on maximum temperature, $F(1, 25) = 5.24, P < 0.05$. Maximum temperature was dependent upon the time of day with an increase in temperature associated with the onset of the scotophase followed by a gradual decrease throughout the night. The three hourly profiles of temperature averaged over all animals deviated significantly from flatness, $F(4, 22) = 30.79, P < 0.001, \eta^2 = 0.85$. All effects on the maximum temperature profiles were not significant, indicating that the pattern of maximum temperature was not affected by water availability but by sex.

The patterns in the minimum temperatures recorded for females (Figure 5.5a) and males (Figure 5.5b) were similar to that average and maximum temperatures. Females and males that were dehydrated for 7 days at 30°C, 0% RH travelled further into the cool end of the gradient between 15:00 and 06:00 h than when hydrated. Minimum temperature in the first 3 hours of the scotophase decreased after 7 days from 27.7°C (± 1.6) to 19.6°C (± 0.9) for females (Figure 5.5a) and 25.3°C (± 2.1) to 17.0°C (± 1.5) for males (Figure 5.5b). Females and males that were continuously watered for 7 days had the same minimum temperatures as when previously less watered. Minimum temperatures before and after the treatments were significantly different, $F(1, 25) = 17.35, P < 0.001$. This difference was influenced by water availability, $F(1, 25) = 14.33, P < 0.001$, but not by sex, $F(1, 25) = 0.62, P > 0.05$, nor by the combined effect of sex, treatment and water availability, $F(1, 25) = 1.17, P > 0.05$. The difference in maximum temperature between females and males was significant, $F(1, 25) = 7.93, P < 0.01$. The minimum temperatures were dependent upon the time of day, with an increase in temperature associated with the onset of the scotophase followed by a gradual decrease throughout the night. The three hourly profiles of temperature averaged over all animals deviated significantly from flatness, $F(4, 22) = 3.63, P < 0.05, \eta^2 = 0.40$. All effects on the temperature profiles were not significant, indicating that the pattern of temperature selection was not affected by water availability or sex.

5.3.4 Activity

The activity profiles of females (Figure 5.6) and males (Figure 5.7), regardless of their state of hydration, were typical of *Urodacus manicatus*. Activity increased at dusk and then decreased throughout the scotophase. The dependence of the amount activity on the time of day was significant, $F(4, 22) = 21.83$, $P < 0.001$, $\eta^2 = 0.80$. Hydration state affected the level of activity in each 3 hourly period. The activity of dehydrated females (Figure 5.6b) was slightly less than when previously watered (Figure 5.6a). The response in males was greater, with dehydrated animals (Figure 5.7b) much less active than when hydrated (Figure 5.7a). Activity in the first 3 hours of the scotophase decreased with dehydration from $5.00 (\pm 0.63)$ to $4.00 (\pm 1.16)$ turns for females and $5.25 (\pm 0.53)$ to $3.00 (\pm 1.07)$ turns for males. Male activity during the photophase decreased with dehydration from $1.75 (\pm 0.53)$ to $0.57 (\pm 0.30)$ turns. Female activity, after continuous watering, (Figure 5.6d) was not different to when they were less watered (Figure 5.6c). Male activity, after continuous watering, (Figure 5.7d) was more constant throughout the scotophase and greater during the photophase than when they were less watered (Figure 5.7c). Male activity between 03:00 and 06:00 h (pre-dawn) increased with continuous watering from 1.00 ± 0.33 turns to 3.63 ± 0.73 turns. The levels of activity before and after the treatments were significantly different, $F(1, 25) = 9.36$, $P < 0.01$. This difference was influenced by water availability, $F(1, 25) = 8.77$, $P < 0.01$, and the combined effect of sex, treatment and water availability, $F(1, 25) = 5.66$, $P < 0.05$. All effects on the profiles of activity were not significant, indicating that the pattern of activity was not affected by water availability or sex.

5.4 Discussion

The state of hydration had a profound effect on the haemolymph osmotic concentration and the temperature selection of *Urodacus manicatus*. Dehydration to 81% of original mass resulted in an increase in haemolymph osmolality and a reduction in selected temperatures, but had no effect on metabolic rate. Excessive hydration did not significantly increase mass but did lower haemolymph osmolality. The availability of water had no effect on the rate of evaporative water loss when presented as permeability of the cuticle.

The temperatures selected by *Urodacus manicatus* when water stressed remained more than 10°C below that of scorpions with access to water. A lower temperature and/or higher humidity (lower saturation deficit) facilitates a lower rate of evaporative water loss and it is likely that this was the reason for the low body temperatures of *U. manicatus*. This is supported by the findings for insects, anurans and lizards. The adults of three species *Drosophila* selected lower temperatures when placed at lower humidities to inhibit water loss (Prince and Parsons 1977). Desiccated insects often select higher humidities and hydrated insects select lower humidities (Barton-Browne 1964, Coenen-Staß 1976, Roth and Willis 1951, Willis and Roth 1950). Lizards that had lost more than 20% of their body mass in water selected a lower active body temperature of 31.3°C compared to that of 34.7°C for hydrated lizards (Crowley 1987). Toads dehydrated to 82% of their body mass exhibited a greater reduction in selected body temperature, dehydrated toads selected 16°C while hydrated toads selected 21.9°C. However, observations on scorpions appear to contradict the findings of these studies and this study. Desiccation of the desert scorpion *Leiurus quinquestriatus* for 24 hours (presumably at room temperature) did not affect humidity selection nor did the placement of scorpions in a saturated atmosphere for 24 hours (Abushama 1964). However, the time period before the determination of the response of *L. quinquestriatus* to humidity was shorter than in this study and may not have been enough time to induce behavioural changes in *L. quinquestriatus*.

The metabolic rate of *Urodacus manicatus* was not affected by dehydration to 81% of their original body mass. This suggests that metabolic adjustment was not a means for reducing respiratory water loss in *U. manicatus*. For scorpions at normal temperatures, water loss via respiratory evaporation is negligible compared to transcuticular evaporation (Hadley 1970b, Robertson et al. 1982). The reason for this may lie in the high resistance to water across the respiratory surfaces of scorpions (Dejours and Ar 1991). The desert scorpion *Leiurus quinquestriatus* had a high haemolymph P_{CO_2} and a correspondingly low pH compared to those of other arthropods (Dejours and Ar 1991). This suggested that the resistance of the scorpion's respiratory surfaces to gas and thus water exchange was high (Dejours and Ar 1991). Therefore, the saving of water by means of a lowering of metabolism would be unimportant in the overall water balance of scorpions including *U. manicatus*.

As with *Urodacus manicatus*, *Leiurus quinquestriatus* did not decrease its metabolism with water loss, although its haemolymph P_{CO_2} increased and its pH decreased (Dejours and Ar 1991). The consequence of these changes in the haemolymph with regard to respiratory water conservation was unclear (Dejours and Ar 1991), but may have been due to other changes in the haemolymph after dehydration. Dehydration in anurans resulted in an increase in the viscosity of the haemolymph such that the circulatory system had to do more work (Hillman 1980). The RMR of dehydrated anurans remained the same as when hydrated, but a reduction in their aerobic scope occurred (Gatten 1987, Hillman 1987). To my knowledge, haemolymph viscosity and its relationship to water loss and gas transport has not been examined in scorpions. Dehydration to 82% of initial body mass did not affect the mass-specific VO_2 of the desert scorpion *Hadrurus arizonensis* (Hadley 1970b). However, a lack of change in RMR after a decrease in body water content does not necessarily mean that tissue metabolic rate remained constant. What needs to be shown is that either none of the water lost had come out of respiring cells or that a considerable loss of water from the cytoplasm was sustained without altering the metabolic rate (Buck 1965).

The conditions under which low water stress was achieved here were highly artificial and it is unlikely that a scorpion will frequently experience such a low body water content in the field. Water conservation, however, is still important to *U. manicatus* especially during late autumn and throughout winter when prey availability and rainfall are low. Water availability was further

reduced at Black Mountain during the cooler months as compared to the warmer months, because water did not condense on the underside of the rock shelter or on the scorpions' surfaces with lower temperatures (Chapter 3). Polis and Farley (1979b) found that *Paruroctonus mesaensis* continued to feed into autumn when the incidences of cannibalism and intraguild predation increased as other prey species decreased in abundance, and thus were able to maintain high growth rates and presumably a high water turnover. For *U. manicatus*, however, there was no evidence of cannibalism in the field thus their need for water conservation during autumn was increased.

Similar to temperature selection, water loss greatly reduced the level of activity in male *Urodacus manicatus* (Figure 5.7b). The decrease in activity within the gradient was not due to impaired locomotion as a result of dehydration because these animals moved and responded to stimuli as much as hydrated scorpions (pers. obs.). This suggests that muscle function and coordination were not affected by water loss. Since water from prey is the major source of water for most scorpions (Yokota 1984), the predatory activity of *U. manicatus* during the first hours of the scotophase was not expected to have decreased as observed. When a water source was not available to *U. manicatus*, water conservation strategies became more important than water regaining ones. In association with a lower temperature which reduced the rate of evaporative water loss, a decrease in activity may have further reduced water turnover by decreasing aerobic metabolism.

The osmotic concentration of scorpion and spider haemolymph is much higher than in many other terrestrial animals and may have the advantage in matching juice of partly digested prey (Burton 1984). Approximately half of the free water of the prey is ingested by scorpions during a meal (Yokota 1984). Like most scorpion species that have been studied, *Urodacus manicatus* did not regulate its haemolymph osmotic concentration with its decreasing haemolymph volume, but instead it tolerated a large increase (Dejours and Ar 1991, Punzo 1991, Riddle et al. 1976, Robertson et al. 1982, Warburg et al. 1980b) as the haemolymph became more concentrated (Padmanabhanaidu 1966a). So far, osmoregulation has only been demonstrated in the large desert scorpion *Parabuthus villosus* (Robertson et al. 1982). However, Warburg et al. (1980b) found that mesic species experienced a greater increase in haemolymph osmolality than the more

xeric species under the same conditions. This suggests that some regulation of osmolality may occur, the degree of which is dependent on the microhabitat of the species. Although *U. manicatus* lost 19% of its original body mass, their increase in haemolymph osmolality was only 132 mOsm kg⁻¹ which is more comparable to that of the xeric species studied by Warburg et al. (1980b). The haemolymph osmolality of the xeric scorpion *Leiurus quinquestriatus* increased by 218 mOsm kg⁻¹ with a loss of 8.40% and that of *Buthotus judaicus* increased by 97 mOsm kg⁻¹ with a loss of 6.47% original body mass. Of the mesic species, *Nebo heirochonticus* increased by 591 mOsm kg⁻¹ after losing 12.45%, and *Scorpio maurus fuscus* increased by 569 mOsm kg⁻¹ with a loss of 17.37% (Warburg et al. 1980b). From the equation of Robertson et al. (1982) describing the effect of water loss on haemolymph osmolality for *Opisthophthalmus capensis*, this mesic species experienced an increase in osmolality of 218 mOsm kg⁻¹ after losing 19% of the original body mass. In the present study, when water was provided in excess to *U. manicatus* at a high humidity, their haemolymph osmolality decreased as the haemolymph became more diluted, although their body mass remained the same as when the animals were at a normal level of hydration. Under a normal laboratory water regime, *U. manicatus* regulated their haemolymph at the same osmolality as animals collected from the field (Chapter 3).

Desert adapted tenebrionid beetles are very similar to scorpions in their environmental physiology. They are cryptic during the day thus avoiding the desiccating conditions at the ground surface (Zachariassen and Einarson 1993), have low rates of transcuticular water loss (Edney 1971, Edney 1977, Zachariassen et al. 1987) and have low metabolic rates which, being a tracheal breather, decreases respiratory water losses (Zachariassen et al. 1987). Most important with regards to the findings of this chapter is that tenebrionids, like scorpions, tolerate a large drop in total body water which can be up to 75% of the original body mass (Zachariassen and Einarson 1993). The mechanism by which tenebrionids tolerate a large decrease in total water content may be similar to that in *Urodacus manicatus* and scorpions in general. The intracellular water of the beetles made up 60% of their total body water and was highly regulated while the haemolymph acted as a reservoir during desiccation. As the haemolymph mass substantially decreased, the cell water content was reduced by only 50% (Zachariassen and Einarson 1993). The mechanism proposed for this was that sodium, which made up 25% of the total extracellular osmotically active particles, was entirely removed from the beetle and water was redistributed

into the cellular compartments during dehydration. The mechanism by which scorpions tolerate low body water contents has not been studied and needs further investigation. (Bricteux-Gregoire et al. 1963) found that the desert scorpion *Androctonus australis* when hydrated had an osmotic concentration of 530 mOsm l⁻¹ that was almost entirely due to sodium and chloride ions. Maintenance of cell water content in scorpions at the expense of the haemolymph would explain why $\dot{V}O_2$ was not affected by a water loss of 19% (see above). The open circulatory system of scorpions may be able to accommodate an increase in haemolymph viscosity such that extra work is not needed to pump the haemolymph through the sinuses. Although the haemolymph must pass through the ostia into the heart, the rate of movement of the haemolymph may not need to be increased as the haemolymph becomes more viscous because of the low metabolic rates of scorpions.

Soil moisture is potentially a very important source of water for *Urodacus manicatus* as indicated by this species' ability to take up water from moistened cotton wool, but its importance in other species is not clear. The soil beneath the rock home sites of *Heterometrus fulvipes* were often wet and in captivity this species took up water to the extent that its mesosoma became extended and the animal had a gravid appearance (Padmanabhanaidu 1966a). However, Hadley (1970b) found that the amount of water taken up by the desert-inhabiting *Hadrurus arizonensis* from strips of moistened sponge was not enough to warrant substrate water as an important source. Gaffin et al. (1992) though, found that another desert species, *Paruroctonus mesaensis*, could detect substrate moisture via chemosensory hairs on the ventral surfaces of the tarsi. Moisture was then extracted from the soil by imbibition (sand chewing) (Gaffin et al. 1992).

The capacity of *Urodacus manicatus* to take up substrate moisture by absorption was not determined, but animals were often observed tearing at the dampened cotton wool and bringing the pieces to their mouthparts as described by Southcott (1954). The dilution of the haemolymph in continuously watered animals (as indicated by the decrease in osmolality) suggests that this species is either very opportunistic in obtaining water or that water uptake cannot be regulated in a wet environment. Excessive water may have entered *U. manicatus* via absorption across the cuticle. Interestingly, the masses of the periodically watered and continuously watered scorpions were the same suggesting that, in lieu of there being no significant difference in cuticle permeability, water loss was increased along another avenue, possibly by excretion.

The excessive uptake of water by *Urodacus manicatus* in the field could possibly occur after substantial rains when the soil of the burrows becomes saturated due to seepage under the edges of the home site shelter (pers. obs.). A day after rain at Dubbo, the relative humidities of the burrows were above 95% indicating that wet soil increased the water vapour pressure density of the air of the burrows. Consequently, the rate of evaporative water loss from the scorpion must have been reduced following rain. Continual water uptake and low water loss rates may present a problem for a species that does not leave its burrow as the volume of the animal will rapidly attain its maximum size.

The lack of a major change in permeability with water loss in captivity was probably due to the short time allowed for change and also the already high rates of permeability compared to field-collected *Urodacus manicatus*. Maintenance conditions within the laboratory provided water in excess to what scorpions normally ingested or absorbed in the field. Even after 7 days of dehydration, the water loss rates of scorpions that had previously been provided with water *ad libitum* were twice that of animals collected from the field (Chapter 3). For *Urodacus manicatus*, excessive water gain may be more of an ecologically important factor than excessive water loss. This contrasts with desert-inhabiting species for which water conservation is more important for than the regaining of water (Hadley 1974). King and Hadley (1979) found that starvation in the desert species *Hadrurus arizonensis* resulted in lower water loss rates than when fed, the water ingested with the prey, together with an increased metabolism, resulted in an increase in the turnover of water. Insects that had previously been partially dehydrated lost less water than when fully hydrated (Ahearn and Hadley 1969, Edney 1971) due to the reduction of available water reserves decreasing the total amount of water subsequently lost (Ahearn and Hadley 1969).

Urodacus manicatus uses behavioural mechanisms to regulate water loss. The lack of a change in mass-specific metabolic rate which suggests that water is not lost from the tissues of the animals, but from the haemolymph that acts as a reservoir. This is further supported by the observations on the lack of impairment of motor functions which suggest that the tissues are protected from water loss. Any reduction in metabolic rate would not result in a saving of water at rest because of the low rates of respiratory water loss, although an avoidance of elevated active metabolic rates may assist in the conservation of water. As with many other scorpions, *U.*

manicatus tolerates an increase of concentration of the haemolymph with water loss. In addition, evidence is provided that a tolerance of the dilution of the haemolymph may be important at times of excessive water gain.

In the next chapter, the effect of food availability on the temperature selection and metabolic rate of *Urodacus manicatus* is examined. The scorpions were supplied with water throughout the study such that any effects were due to the intake of nutrients and not the water ingested with the prey.

Table 5.1. Wet body mass (g), whole animal oxygen consumption rates (VO_2) at 30°C, mass-specific oxygen consumption rates (VO_2/g) at 30°C and haemolymph osmolality of adult female scorpions watered and dehydrated to 81% original body mass.

	<i>n</i>	Mass (g)	VO_2 ($\mu\text{l O}_2 \text{ h}^{-1}$)	VO_2/g ($\mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$)	Osmolality (mOsm kg^{-1})
Normal feed and water	16	1.22 \pm 0.03	116.50 \pm 6.64	94.96 \pm 4.44	483.56 \pm 9.49
Dehydrated	7	0.99 \pm 0.04	107.07 \pm 10.39	106.42 \pm 5.51	615.71 \pm 22.06
Watered	8	1.22 \pm 0.04	115.89 \pm 10.09	94.98 \pm 8.00	447.63 \pm 9.89

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

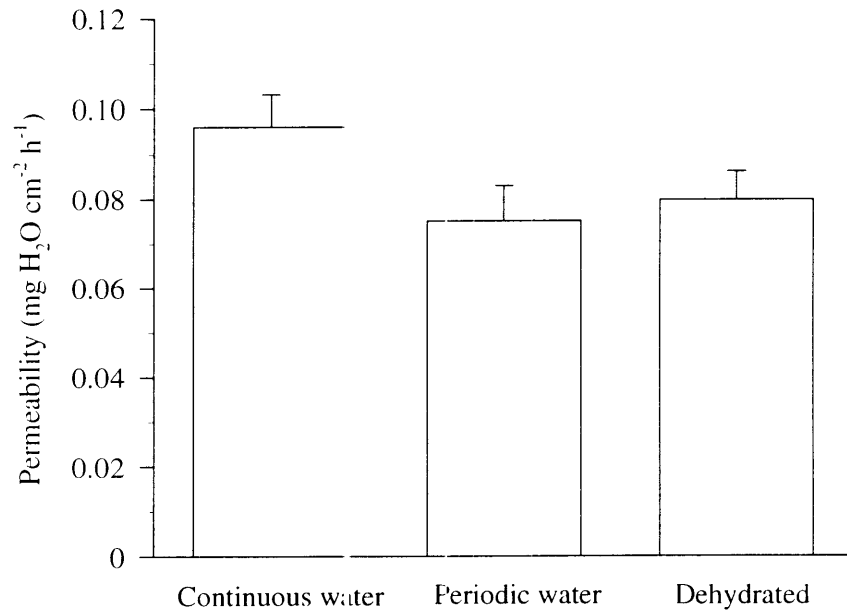


Figure 5.1. Effect of differing water availabilities for 7 days on second day permeability (mg H₂O cm⁻² h⁻¹) measured at 30°C, 0% RH of adult females. Sample sizes are: scorpions continuously watered, $n = 16$; scorpions periodically watered, $n = 10$; and scorpions dehydrated at 30°C, 0% RH, $n = 8$.

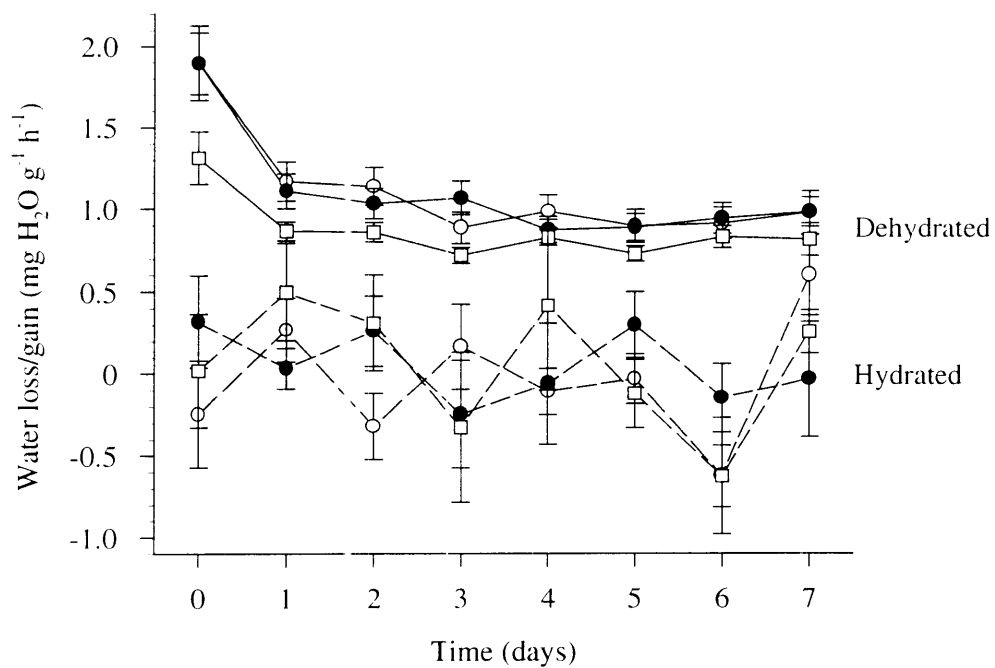


Figure 5.2. Mass specific rates of water loss and gain ($\text{mg H}_2\text{O g}^{-1} \text{h}^{-1}$) for each day of dehydration and watering. Data is from the studies of the effects of water availability on RMR and temperature selection. Symbols are: dehydrated animals (continuous line), watered animals (broken line), RMR females (open circle), temperature selection females (closed circles) and temperature selection males (open square). For all cases $n = 8$.

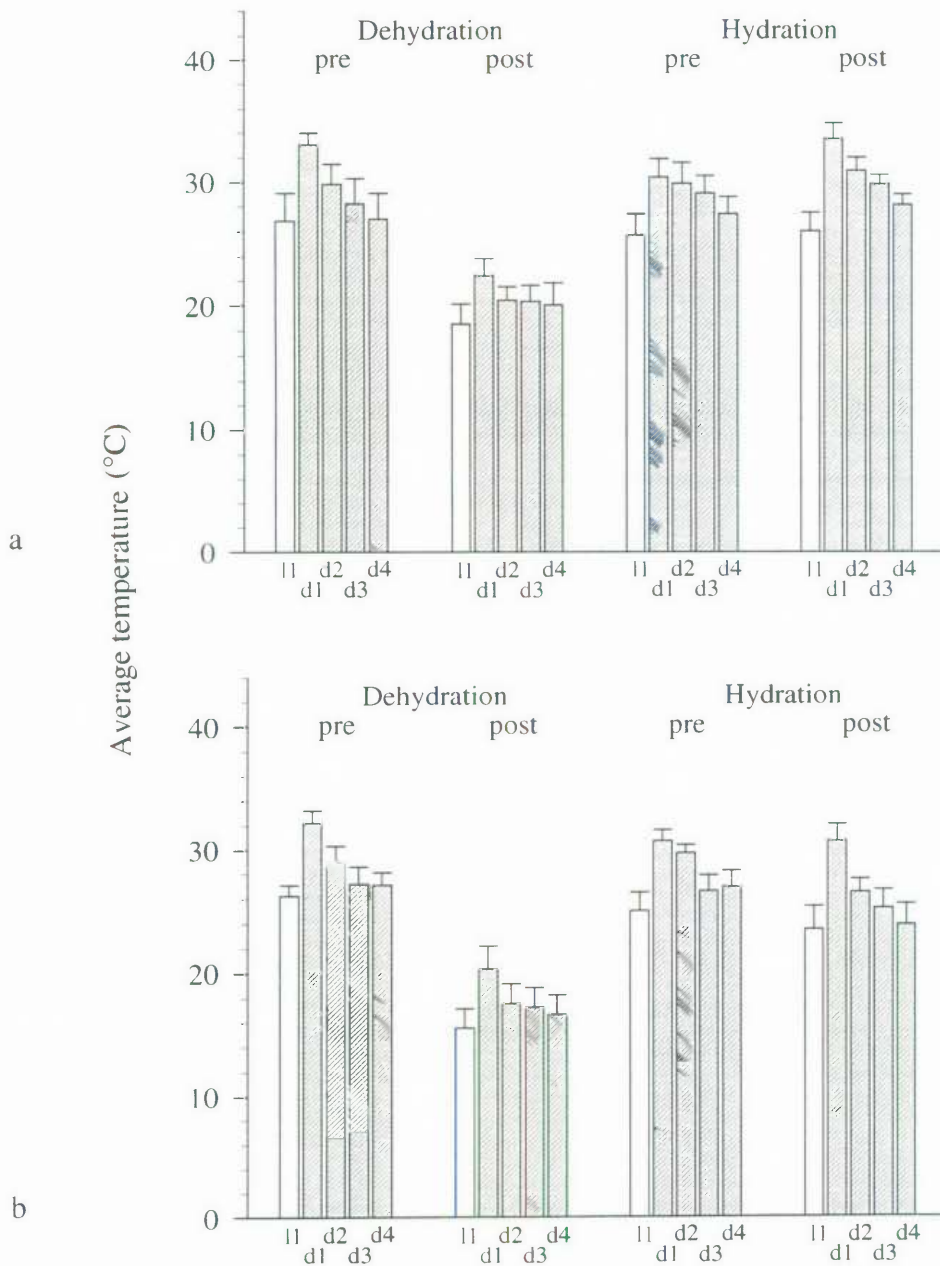


Figure 5.3. Effect of 7 days continuous watering and dehydration at 30°C, 0% RH on average selected temperature (°C) of (a) adult females and (b) adult males. Averages are of 12 measurements per animal within the 3 hour periods of 15-18:00 h (11), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3) and 03-06:00 h (d4). Bars represent mean values \pm SE of each sample. Shaded bars highlight the scotophase periods. For all cases $n = 8$.

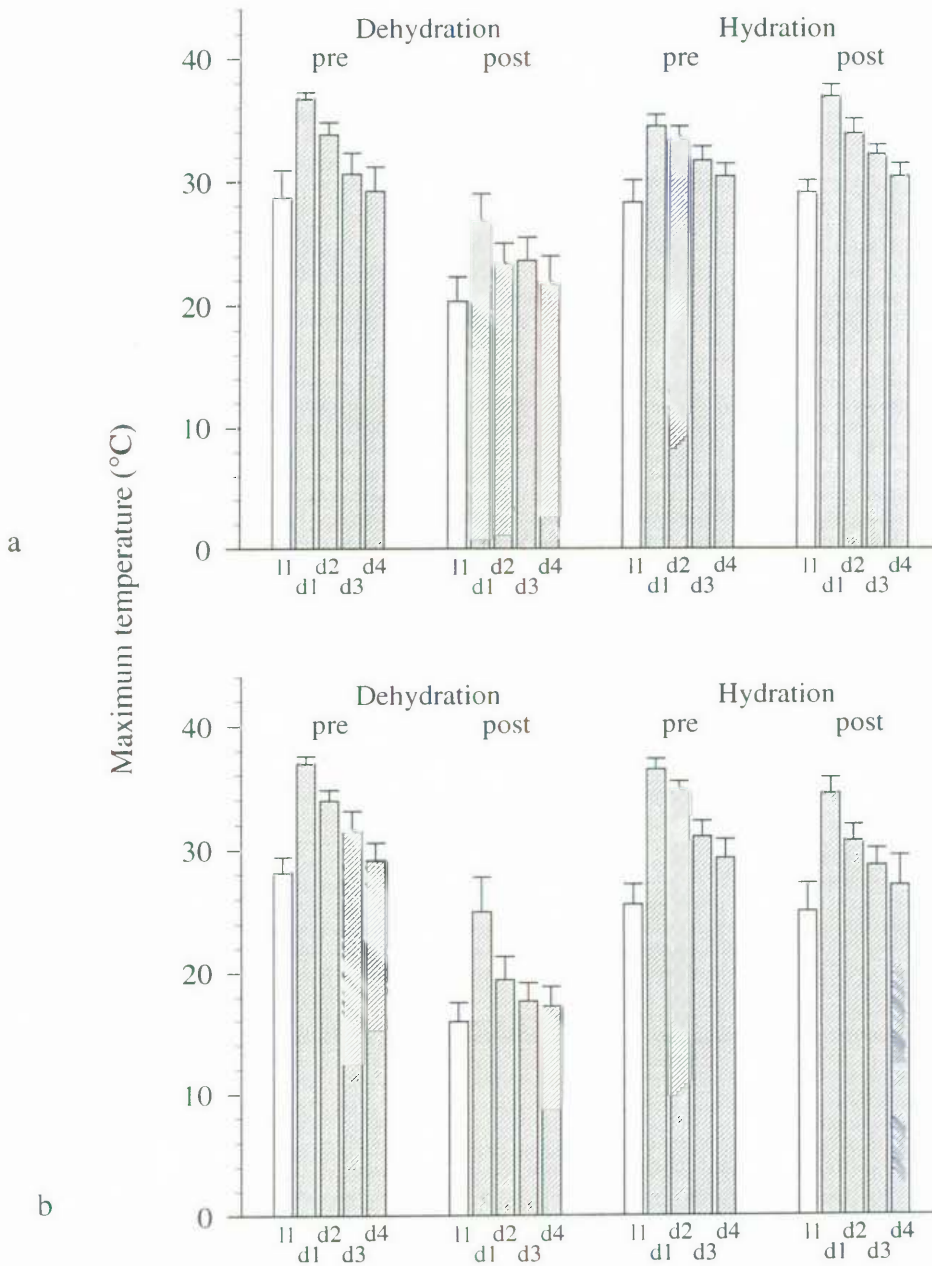


Figure 5.4. Effect of 7 days continuous watering and dehydration at 30°C, 0% RH on maximum temperature (°C) recorded in the gradient for (a) adult females and (b) adult males. Maxima are from 12 measurements per animal within the 3 hour periods of 15-18:00 h (11), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3) and 03-06:00 h (d4). Bars represent mean values \pm SE of each sample. Shaded bars highlight the scotophase periods. For all cases $n = 8$.

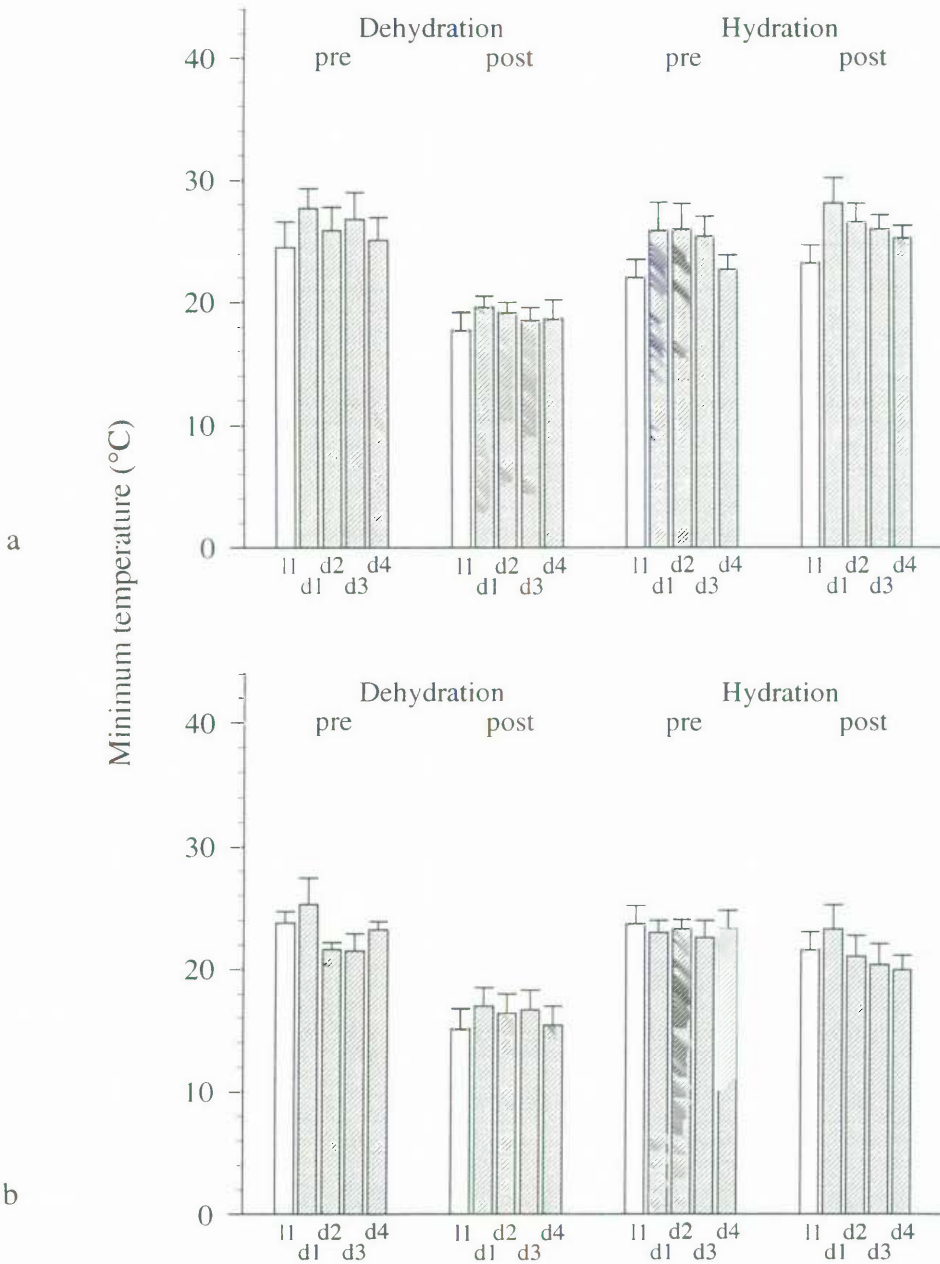


Figure 5.5. Effect of 7 days continuous watering and dehydration at 30°C, 0% RH on minimum temperature (°C) recorded in the gradient for (a) adult females and (b) adult males. Minima are from 12 measurements per animal within the 3 hour periods of 15-18:00 h (11), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3) and 03-06:00 h (d4). Bars represent mean values ± SE of each sample. Shaded bars highlight the scotophase periods. For all cases *n* = 8.

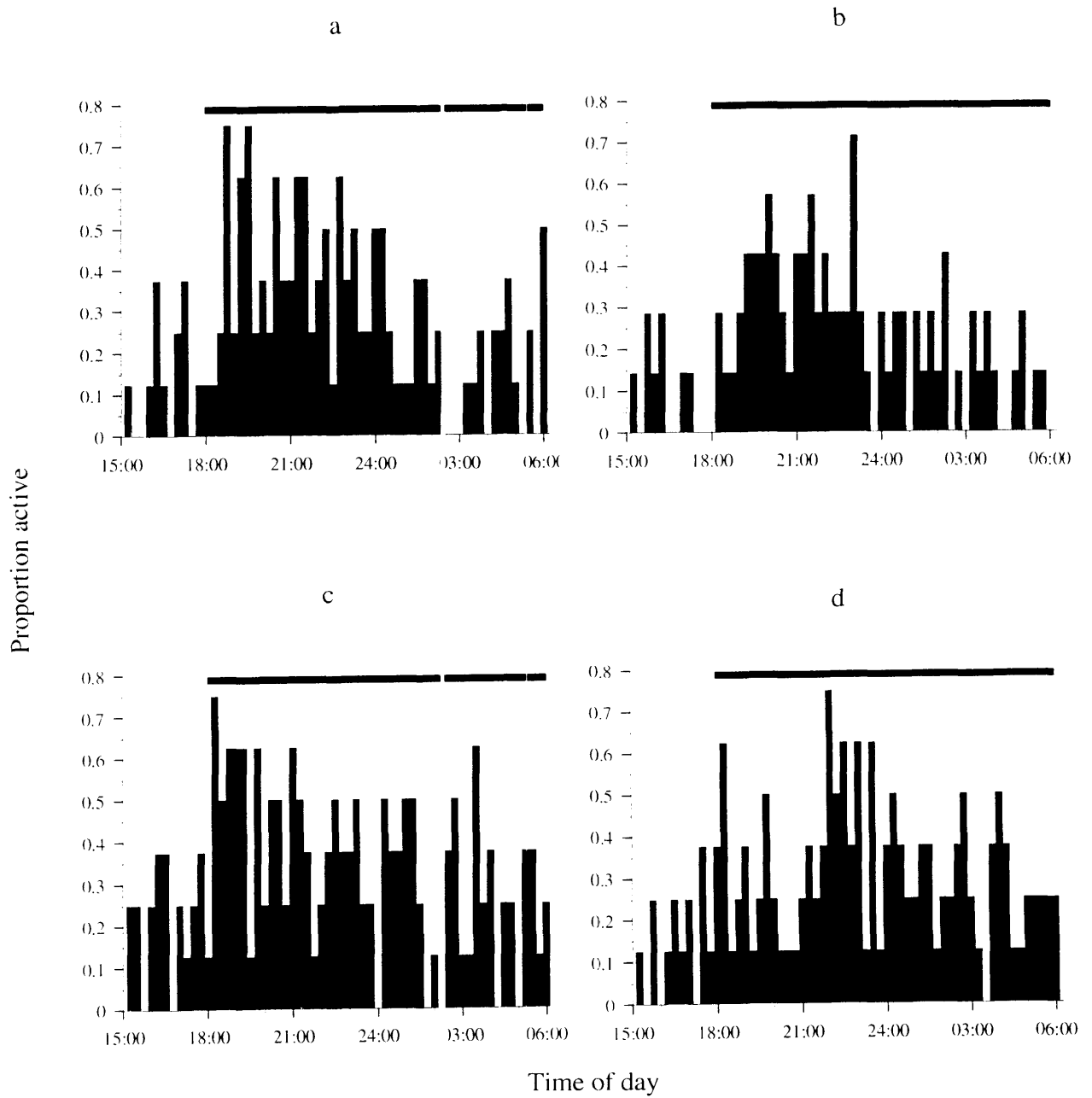


Figure 5.6. Activity profiles of adult females: (a) before and (b) after 7 days of dehydration at 30°C, 0% RH; and (c) before and (d) after 7 days of continuous watering. Each bar represents the proportion of animals that had turred in the gradient resulting in a 5°C or greater change in selected temperature from the preceding temperature. Temperature was measured every 15 min. Horizontal bar represents scotophase from 18:00 to 06:00 hours. For all cases $n = 8$.

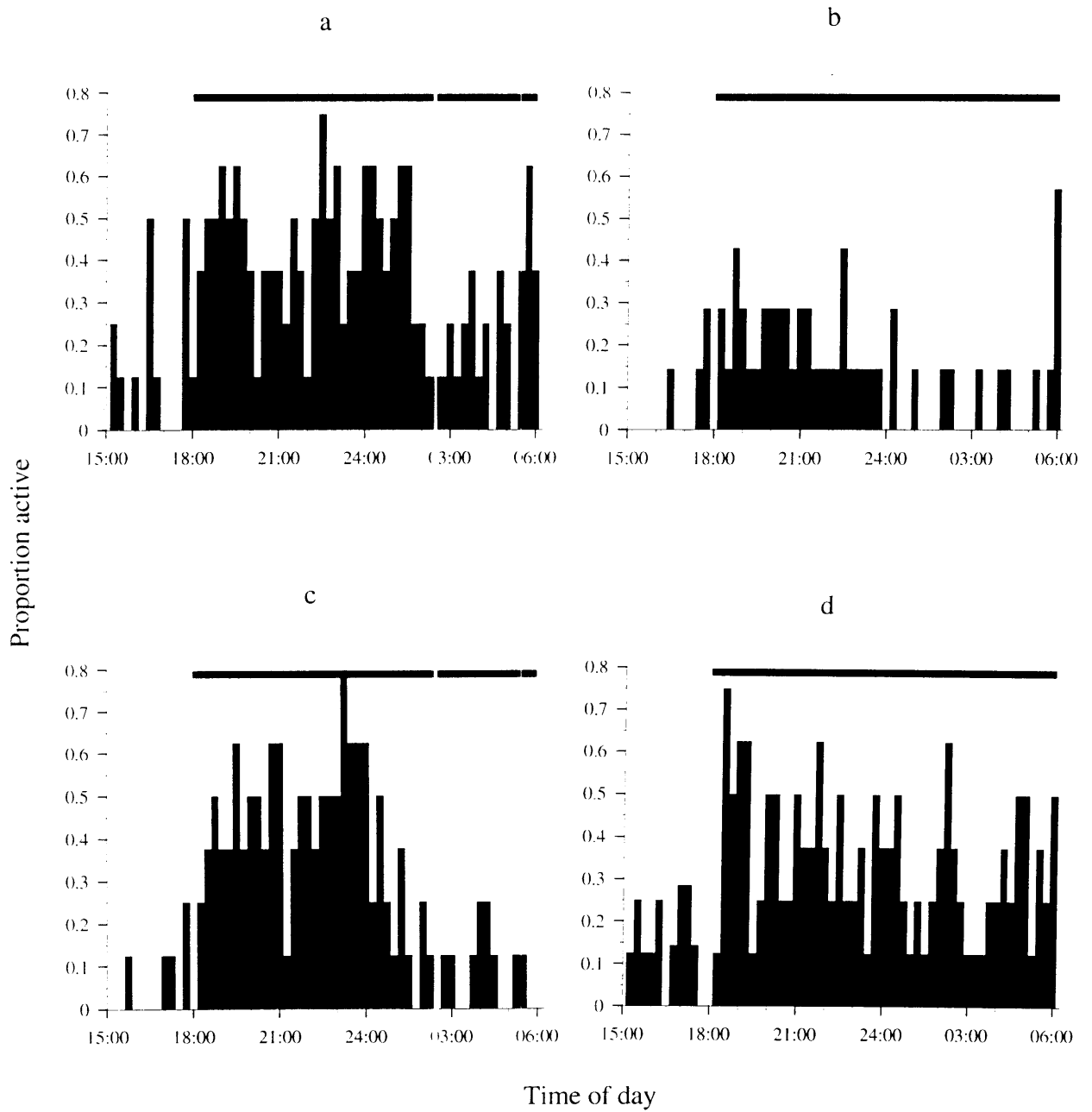


Figure 5.7. Activity profiles of adult males: (a) before and (b) after 7 days of dehydration at 30°C, 0% RH; and (c) before and (d) after 7 days of continuous watering. Each bar represents the proportion of animals that had turred in the gradient resulting in a 5°C or greater change in selected temperature from the preceding temperature. Temperature was measured every 15 min. Horizontal bar represents scotophase from 18:00 to 06:00 hours. For all cases $n = 8$.