Chapter 7

Reproduction Studies

7.1 Introduction

Urodacus manicatus are typical of scorpionids in that they have a katoikogenic mode of reproduction and produce large litters of offspring. The size increase in the females with embryonic development is great and, together with a lengthy gestation period, may result in large reproductive and somatic "costs" to the gravid females.

In this study, some of the "costs' in late gestation are examined by:

1. comparing metabolic rates of females and offspring before and after parturition.

2. comparing temperature selection and activity of the females before and after parturition.

3. determining the changes in the mass of hepatopancreas, the somatic mass of the females and embryonic mass.

4. relating mean offspring mass, litter mass, litter size and female somatic mass to one another.

5. determining the effect of gravidity on evaporative water loss.

7.2 Materials and Methods

Water loss (Section 2.2) was measured for 8 gravid and 7 non-gravid adult females that had been maintained at 25°C with food and water *ad libitum* for six weeks. The effect of gravidity on evaporative water loss was initially conducted utilising the allometric relationship between water loss and mass. The ability of mass to adjust rate of water loss was first tested in covariate analysis. A one-way univariate ANCOVA was performed on second day rate of water loss (mg H_2O h^{-1}) with mass at the end of the second day as the covariate (g) and gravidity as the independent variable. Mass failed to adjust water loss, $F(1, 12) = 2.23$, $P > 0.05$. Subsequent analyses were, therefore, performed on second day water loss as both permeability (mg H_2O cm^{-1} h^{-1}) and the mass-specific rate (mg H_2O g^{-1} h^{-1}). The value of $k = 12.47$ cm^2 g^{-1} was assumed for both gravid and non-gravid females. Values were log transformed to improve normality in the data. A one-way MANOVA was performed on the three dependent variables with the independent variable of gravidity. Results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory.

The effects of reproductive status on the RMRs of females and their offspring were determined by measuring the VO_2 at 30°C (Section 2.3) of 20 gravid females in late January. Of these females, 8 were allowed to give birth and postpartum VO_2s at 30°C were measured of the mother alone, the mother with her litter on her dorsal surface, and the litter without the mother. The following experiment was performed on the remaining 12 gravid females. Immediately after their VO_2 was measured, each gravid female was decapitated behind the carapace and the entire ovariuterus containing the embryos was removed and separated from the hepatopancreas. The ovariuterus was then immediately placed in the following scorpion perfusion fluid at 30°C (Kalarani et al. 1991b): 8.596 g NaCl, 0.144 g K_2SO_4, 0.532 g $CaCl_2$, 0.464 g $MgCl_2$, 0.500 g glucose in 1 l of water with the pH adjusted to 7.3 by adding 10-15 ml of pH 7.3 tris buffer. The solution lasted several days with refrigeration.

The VO_2 of the embryos was measured using a Hansatech D.W. Oxygen Electrode Unit, which is a Clark type electrode (Plate 7.1). The unit consisted of a platinum cathode mounted in the dome of the electrode disc covered with a fine paper spacer (cigarette paper) atop of a Teflon

membrane with a silver anode mounted in the well of the disc. Three drops of 10% KCl were placed on the paper to act as a bridge between the electrodes. The dome of the electrode disc was inserted into the base of the glass reaction vessel surrounded by a water-jacket. The water-jacket was connected to a temperature-controlled waterbath to maintain a constant temperature in the reaction vessel. A plunger with a capillary through it was placed into the top of the vessel and its length adjusted to minimise contact of the contents of the vessel with the air. The unit was secured to the plate of a magnetic stirrer, the speed of which was kept high and constant for all measurements. To prevent contact between the embryos and the magnetic stirrer a thin length of wire was passed through the capillary of the plunger and coiled in the horizontal plane to provide support for the embryos, but still allow thorough circulation of the fluid (Plate 7.1). The reaction vessel was rinsed five times with distilled water between measurements.

A stable slope of the oxygen saturation baseline was obtained by placing 1.5 ml of aerated perfusion fluid into the reaction vessel and allowing 10 minutes to equilibrate. Excess fluid was removed from the embryos by dabbing them gently with absorbent paper before placing them into the reaction vessel. There was an immediate drop of oxygen in the fluid and the chart was run until a stable slope was obtained. At the end of each measurement excess fluid was removed from the embryos by gently dabbing them with paper towelling before weighing. The number of embryos was recorded. Since oxygen-saturated water at 30°C contains 5.26 $\mu l\ O_2\ ml^{-1}$ (equivalent to the full scale deflection of the chart which was 10 units), the $\dot{V}O_2$ of the embryos was calculated as follows:

(slope of embryo) - (slope of baseline)

units min^{-1}

10 units = 5.26 $\mu l\ O_2\ ml^{-1}$, therefore

units $min^{-1} \Rightarrow \mu l\ O_2\ ml^{-1}\ min^{-1}$

Volume of fluid is 1.5 ml, therefore

($\mu l\ O_2\ ml^{-1}\ min^{-1}$) x (1.5 ml) x (60) = $\mu l\ O_2\ h^{-1}$

The effectiveness of the perfusion fluid to maintain the embryos over the period of the $\dot{V}O_2$ measurements was tested by remeasuring one of the litters after 30 minutes at which time their $\dot{V}O_2$ remained unchanged.

Analysis compared the $\dot{V}O_2$ of seven combinations of the females and their offspring. These groupings were: the two groups of females when gravid, the dissected embryos within the ovariuterus, the postpartum mother without her litter, the litter without the mother, the postpartum mothers with their litter on their dorsal surfaces and the total $\dot{V}O_2$ of the mother and litter when their $\dot{V}O_2$ was measured separately. Utilising the allometric relationship between mass and overall $\dot{V}O_2$ (Section 3.3.4 and Figure 7.1), a one-way ANCOVA was performed on the dependent variable of log $\dot{V}O_2$ at 30°C for the seven groups with log mass as the covariate. 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 in each analysis and log mass was judged to be adequately reliable for covariance analysis. The logarithmic $\dot{V}O_2$-wet mass regressions were determined for each of the seven measurements of females and young, from which the representative $\dot{V}O_2$'s were calculated (Table 7.1). To eliminate size bias from the distributions of each experimental group, the $\dot{V}O_2$'s were calculated from hypothetical wet masses (Cairns 1978). The masses chosen were the median values of each group.

A one-way ANOVA was performed to compare the masses of the gravid females and postpartum females with their litters. To enable comparison of the masses of the embryos and the first instar offspring, it was necessary to assume that the ovariuterus in the two groups of females had the same mass so that the mean mass of the ovariuterus of the postpartum females (49.4 ± 2.53 mg) could be subtracted from the total ovariuterus-embryo mass. Comparisons of total litter mass, litter size and mean offspring mass were made between the embryos and first instar offspring using independent samples *t*-tests.

The somatic body masses (the mass after subtraction of the hepatopancreas and ovariuterus masses from whole wet body mass) of the dissected gravid females and the postpartum mothers were compared to examine any physical costs of reproduction on the females during the end of gestation. The carapace widths (CW) of the females were measured to confirm that the two

groups were equivalent in size. Independent samples *t*-tests were used to compare the two groups of females for somatic mass and CW.

Relative Clutch Mass (RCM) is defined here as the ratio of the wet litter mass to the female's own mass and reflects the degree of physical burdening of the gravid female (Shine 1980). RCM was measured for both groups of females and compared using an independent samples *t*-test. The mass of the hepatopancreas and the hepatosomatic index were measured in the gravid and postpartum females and compared with independent samples *t*-tests. The hepatosomatic index (Subburam and Gopalakrishna Reddy 1980) was calculated as the percentage mass of the hepatopancreas to the masses of the females.

The postpartum female masses, total litter masses, litter sizes and mean offspring masses of 11 females that had produced young in captivity the previous year were combined with the same birth parameters of the 8 females used in the $\dot{V}O_2$ study above. The following relationships were investigated with linear regression: female mass and litter size; female mass and total litter mass; litter size and total litter mass; and total litter mass and mean offspring mass. Three cases were dropped as outliers from the analyses involving female mass because they were found to either exert too much influence on the regressions or because their studentised residuals were greater than -2 (SYSTAT for Windows: Statistics 1992). Female postpartum mass was log transformed and the square root of total litter mass was taken to achieve normality in both. All births observed in captivity during this thesis were combined and the mean litter size was determined. The ratio of females to males was determined for a subset of all births.

Three trials measuring temperature selection (Section 2.4) in six gravid females were conducted. The first trial was conducted three days following collection of the animals from Black Mountain. They were maintained at 25°C with food and water *ad libitum* for a total of eight weeks. Since the date of birth could not be predicted, the second trials of prepartum temperature selection were made in late February by which time other females in captivity had already given birth. Postpartum temperature selection was measured from the subsequent morning to when the young were first noted, the last female giving birth 5 days after the measurements on the gravid females.

The effect of reproductive state on temperature selection was examined by means of three profile analyses which were performed on five repeated measure dependent variables associated

with the time periods: 15-18:00 h, 18-21:00 h, 21-24:00 h, 24-03:00 h and 03-06:00 h. The grouping variable was with respect to parturition: 7 to 8 weeks before parturition, 3 to 5 days before parturition and 1 day postpartum. The three analyses separately examined average, maximum and minimum selected temperature. 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 source of variation in temperature profiles over time of day was examined with a *post hoc* test that used single ANOVAs to examine the mean differences between adjacent time periods.

The effect of reproductive state on activity was examined using profile analysis performed on five repeated measure dependent variables associated with the time periods: 15-18:00 h, 18-21:00 h, 21-24:00 h, 24-03:00 h and 03-06:00 h. The grouping variable was with respect to parturition: 7 to 8 weeks before parturition, 3 to 5 days before parturition and 1 day postpartum. 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 gravid and postpartum group in Figure 7.9 as the proportion of animals that had turned in the gradient every 15 minutes.

7.3 Results

7.3.1 Evaporative Water Loss

Gravidity did not affect evaporative water loss determined as either permeability or mass-specific rates. Permeabilities for gravid and non-gravid animals, respectively, were 0.058 ± 0.007 and 0.051 ± 0.004 mg H_2O cm^{-1} h^{-1} and mass-specific rates were 0.592 ± 0.077 and 0.613 ± 0.055 mg H_2O g^{-1} h^{-1}. The permeabilities of the females were not significantly different, $F(1, 13) = 0.36$, $P > 0.05$, and neither were the mass-specific rates, $F(1, 13) = 1.66$, $P > 0.05$.

7.3.2 Reproductive Investment

The mass-adjusted RMR of gravid female *Urodacus manicatus* did not change during the last two months of gestation and was the same as the total of the females and their litters of first instar young after parturition. The mass-adjusted RMR of the whole litters, however, did increase with an increase in total litter mass during the last two months of gestation. The calculated RMR of the 5 to 8 week premature embryos was 52.59 µl O_2 h^{-1} and that of the first instar offspring was 61.94 µl O_2 h^{-1}. The calculated RMR of postpartum females with litters on their backs was the same as the summed RMRs of females and litters when measured separately as well as the RMR of the females when gravid (Figure 7.2). The combined masses of the females and their litters did not change in the last two months of gestation, $F(3, 32) = 0.13$, $P > 0.05$.

Total litter mass increased during the last two months of gestation. The litter mass of the embryos (minus the mass of the ovarian terus) was 549.09 ± 45.25 mg and that of the first instar offspring was 622.38 ± 71.69 mg. However, the masses were not significantly different, $t(18) = 0.91$, $P > 0.05$. The litter sizes of the two groups of offspring were the same. The litter size of the embryos was 19.5 ± 1.1 and that of the first instar offspring was 16.5 ± 1.7. The litter sizes were not significantly different, $t(18) = 1.60$, $P > 0.05$. However, the mean offspring masses of the first instar offspring were greater than that of the embryos. The first instars averaged 37 ± 3 mg per individual and the embryos averaged 29 ± 2 mg per individual. The difference in mean offspring mass was significant, $t(18) = 2.36$, $P < 0.05$.

The somatic masses of the gravid females were not affected by their reproductive state in the last two months of gestation. The somatic mass of the gravid females was 0.99 ± 0.05 g and that of the postpartum females was 1.00 ± 0.08 g. The means were not significantly different, $t(18) = 0.02$, $P > 0.05$. The carapace widths of the females were the same. The CW of the gravid females was 8.06 ± 0.20 mm, and that of the postpartum females was 7.97 ± 0.17 mm. The CW's were not significantly different, $t(18) = 0.21$, $P > 0.05$.

RCM was greater after birth than two months earlier during gestation. RCM before parturition was 0.429 ± 0.05 and after parturition was 0.535 ± 0.06. However, the means were

not significantly different, $t(18) = 1.34$, $P > 0.05$. Hepatopancreas mass and the hepatosomatic index decreased during the last two months of gestation. The respective masses of the hepatopancreas before and after parturition were 0.30 ± 0.02 g and 0.16 ± 0.03 g, and the equivalent hepatosomatic indices were 15.68 ± 0.98 and 8.86 ± 1.20. The decrease in hepatopancreas mass was significant, $t(18) = 3.89$, $P < 0.001$, as was the decrease in the hepatosomatic index, $t(18) = 4.40$, $P < 0.001$. The relationship between the hepatosomatic index and the mean offspring mass before and after birth is presented in Figure 7.3.

Postpartum female mass was positively correlated with both total litter size (Figure 7.4) and litter mass (Figure 7.5) and litter size was positively correlated with total litter mass (Figure 7.6). The results of the regressions are presented in Table 7.2. Mean offspring mass was not correlated with either female mass, $F(1, 20) = 0.16$, $P > 0.05$, or total litter mass, $F(1, 20) = 4.43$, $P > 0.05$.

The total number of births observed in captivity was 55 and the mean litter size was 16.02 ± 0.72, ranging from 3 to 29. For 21 of the 55 births, the ratio of females to males was even. The number of females born per litter in captivity was 6.71 ± 0.97 and the number of males was 7.67 ± 0.84. There was no significant difference between the sexes, $t(20) = 0.778$, $P > 0.05$.

7.3.3 Temperature Selection

The reproductive state of a female did not affect average selected temperature within the temperature gradient; however, the profiles of temperature over the 3 hourly time periods were different around the time of parturition compared to 7 to 8 weeks before (Figure 7.7a). With the onset of the scotophase, average temperature just prior to parturition increased from 24.6°C ($\pm$ 2.2) to 34.7°C ($\pm$ 1.0). One day after birth, an increase from 27.8°C ($\pm$ 2.1) to 33.3°C ($\pm$ 0.5) was recorded, whereas 7 to 8 weeks earlier at the time of capture the temperature decreased from 32.5°C ($\pm$ 1.1) to 31.3°C ($\pm$ 0.8). Average selected temperature decreased throughout the remaining scotophase in all three cases (Figure 7.7a). The non-parallelism in the temperature profiles was significant, $F(8, 24) = 2.95$, $P < 0.05$, $\eta^2 = 0.74$, and *post hoc* tests revealed the

differences were due to the changes in temperature from the photophase to the scotophase, $F(2, 215) = 11.13$, $P < 0.001$. Parturition did not have significant effect on temperature selection, $F(2, 15) = 0.21$, $P > 0.05$.

The reproductive state of a female did not affect the maximum temperatures recorded within the temperature gradient or the profiles of maximum temperature every 3 hours between 15:00 and 06:00 h (Figure 7.7b). Maximum temperatures did not change with the onset of the scotophase at the time of capture or around parturition. Temperatures decreased throughout the scotophase in all cases. The profiles deviated significantly from flatness, $F(4, 12) = 8.90$, $P < 0.001$, $\eta^2 = 0.75$, but were not significantly different, $F(8, 24) = 0.52$, $P > 0.05$. Parturition did not have significant effect on maximum temperature, $F(2, 15) = 0.84$, $P > 0.05$.

The reproductive state of a female did not affect the minimum temperatures recorded within the temperature gradient; however, the profiles of temperature over the 3 hourly time periods were different around the time of parturition compared to 7 to 8 weeks before this (Figure 7.7c). With the onset of the scotophase, average temperature just prior to parturition increased from 21.9°C (± 2.2) to 31.3°C (± 2.0). One day after birth, an increase from 25.7°C (± 2.7) to 31.4°C (± 0.6) was recorded, whereas 7 to 8 weeks earlier at the time of capture the temperature decreased from 30.6°C (± 1.6) to 27.2°C (± 1.5). The decrease of minimum temperature into the second scotophase period was greater and the rest of the profiles were flatter around parturition than at the time of capture (Figure 7.7c). Although the minimum temperature profiles did not significantly deviate from flatness, $F(4, 12) = 2.63$, $P > 0.05$, the profiles at different reproductive states were significantly dissimilar, $F(8, 24) = 2.92$, $P < 0.05$, $\eta^2 = 0.74$. *Post hoc* tests revealed the differences were due to the changes in temperature from the photophase to the scotophase, $F(2, 215) = 8.88$, $P < 0.01$. Parturition did not have significant effect on temperature selection, $F(2, 15) = 2.11$, $P > 0.05$.

7.3.4 Activity

Reproductive state affected activity within the gradient. Females just prior to parturition were less active at the start of the scotophase (Figure 7.8b) than at the time of capture 7 to 8 weeks earlier (Figure 7.8a) or 1 day after parturition (Figure 7.8c). Females after parturition were active for longer into the scotophase. Gravid females decreased in activity from the photophase (2.50 ± 0.76 turns) to the scotophase (1.50 ± 0.34 turns). Activity near the time of capture increased with the onset of the scotophase from 1.33 (± 0.62) to 5.33 (± 0.21) turns and then decreased to 2.83 (± 0.60) turns. Postpartum activity increased from 2.50 (± 0.67) to 3.83 (± 0.95) turns with a further increase to 4.33 (± 0.72) turns in the second scotophase period. The profiles deviated from parallelism mostly due to the difference between the first two periods during which the scotophase commenced, $F(2, 15) = 6.76$, $P < 0.01$. When activity was averaged over all periods, parturition did not have significant effect, $F(2, 15) = 1.52$, $P < 0.05$.

7.4 Discussion

The cost of viviparity in *Urodacus manicatus* is very low. The total RMR of the gravid females was the sum of their maintenance RMR and that of the young indicating that the metabolic cost of reproduction to the female was low at the end of gestation. The large capacity of the hepatopancreas and the low metabolic rates of the developing embryos together with a long gestation period and the sedentary nature of *Urodacus manicatus* ensure that the somatic cost of reproduction to the gravid female was low. An increase in the water content of embryos in the last weeks of gestation may arise from the hepatopancreas. The metabolic reserves of the hepatopancreas after parturition must be large enough to enable the mother to survive decreased feeding rates over autumn and winter. The increased size of the gravid females did not result in greater rates of evaporative water loss compared to non-gravid females.

In the last two to three months of gestation of *Urodacus manicatus*, the total RMR of the gravid females and embryos did not change. Crawford and Riddle (1975) attributed an increase in the metabolic rate of female *Diplocentrus peloncillensis* from mid to late winter to embryonic development. However, the lack of change in female *U. manicatus* RMR with embryonic development together with the constancy of seasonal RMR found in Chapter 3 suggests that the cost of reproduction in *U. manicatus* is too low to be measured by $\dot{V}O_2$. The long gestation period of 16 months together with the low metabolic rate of *U. manicatus* decreases the cost of provision of nutrients to the embryos. The sedentary nature of *U. manicatus* further reduces the extra work done by the heart and leg muscles of the gravid female which are associated with an increase in mass.

There was no increase in the total mass of the gravid females and their embryos and there was no decrease in total mother and litter mass associated with parturition. Most growth of the embryos occurred during the spring before parturition and by early summer the embryos were visible through the distended intersegmental membranes of the females' mesosoma. Embryos continued to increase in mass until parturition although the increase in the RCM was not significant. The increase in mass of the offspring without a discernible increase in their metabolic rate in the last two months of gestation suggests that most of the size increase was due an increase in non-metabolic mass rather than growth. In *Centruroides exilicauda*, Toolson (1985) found that during the last stage of development, the embryos had an increase in water content from 53% of body mass to more than 80% at birth with little growth. The body water contents of the embryos and first instar young were not determined in this study, but an increase in embryonic body water content in the last months of gestation seems likely for *U. manicatus*. In this study, the hepatosomatic index of female *Urodacus manicatus* decreased by 43% during the last 7 to 8 weeks of gestation (Figure 7.3). Females can maintain approximately the same mass when starved for 42 days after a single meal (Chapter 6), so it seems likely that the decrease in the mass of the hepatopancreas was principally due to the movement of water from the hepatopancreas to the embryos and not due to the energy demands of the mother and embryos.

The abundance of non scorpion prey species of *Paruroctonus mesaensis* were highest in spring and then decreased over summer (Polis 1980b). A rapid gain in mass was apparent in *Urodacus manicatus* females from Black Mountain after winter (Chapter 3) which was mostly

due to increased foraging rates (Chapter 6) and the concomitant growth of the embryos up until mid summer. Two sources of nutrients are available to the growing embryos: dietary nutrients from the mother or stored nutrients from the hepatopancreas (Subburam and Gopalakrishna Reddy 1980). However, the relative amounts of nutrients supplied to the embryos from each source are unknown in *U. manicatus*. *Paruroctonus utahensis* collected in early spring showed no weight gain associated with pregnancy, but did from then on (Bradley 1982). Females took advantage of the greater abundance of prey items to increase their metabolic reserves to a level that provided enough nutrients for further development of the young from mid summer to autumn when foraging rates decreased. Postpartum female mass was about 1.2 g which was the same as that of females collected from Black Mountain during winter (Chapter 3). Thus, the females had to also ensure that metabolic reserves were large enough after parturition to survive winter.

The level of foraging activity of female *Urodacus manicatus* was lowest just prior to parturition (Figure 7.8b), although active selected temperatures increased over the last weeks of gestation (Figure 7.7a). The increase in active temperatures may have been an acclimatory response to maintenance at a constant 25°C because the selected, maximum and minimum temperatures were similar to the temperatures recorded after acclimation to 30°C (Chapter 5). The decrease in the pre-scotophase temperatures to below active temperatures over the last weeks of gestation appears to be an aberration due to the maintenance of the animals in captivity (Chapter 5). Two of the females had a meal of a single *Tenebrio* larva within a week of capture, but none of the females fed between then and parturition. Thus, even when prey was available, the females did not seek to continuously increase their reserves. As to whether this was due to the conservation of energy by remaining inactive or due to a limitation on the maximum size of the females could not be determined. The amount of food consumed by *Paruroctonus utahensis* from spring until parturition did not affect litter size nor the size of the offspring (Bradley 1982). This suggests a threshold of nutritional status exists in scorpions above which extra feeding did not affect the young and thus female *U. manicatus* did not need to forage during the last stage of gestation. However, the size and rate of development of *Paruroctonus mesaensis* embryos were increased by increased feeding rates of the females towards the end of gestation (Polis and McCormick 1987). The absence/presence of an effect of female nutritional status on their young may depend on the ecology of the scorpions and whether or not larger offspring are selected for.

The increase in activity of the postpartum females may have been due to the removal of the first instars from the females' dorsal surfaces before placement into the gradient. Although scorpion mothers often behave indifferently to their offspring (Torres and Heatwole 1967a), the behaviour of female *U. manicatus* within the gradient may have been influenced by the absence of the offspring. Foraging behaviour in female scorpions normally decreases with parturition (Polis 1980b), the reduction in activity avoiding dislodgment of the young from her back (Vannini et al. 1978). Benton (1992) argued that the greatest benefit of maternal care was to protect the young within the burrow from predation. However, there are other benefits such as the mother can easily regulate the microenvirоment for the young (Torres and Heatwole 1967a, Ugolini et al. 1986, Vannini et al. 1978), aggregation on her back may reduce evaporative water loss (Ugolini et al. 1986) and it has been proposed that there may be a transfer of water or provide epicuticular waxes from the mother to the young (Vannini et al. 1985). Observations on *Centruroides vittatus*, however, revealed that mothers of this species continued to forage and feed on the surface while carrying her young (Brown and Formanowicz 1995).

Heavily gravid animals can incur a reproductive "cost" of survival (Shine 1980). Gravid skinks, for example, were more prone to predation since they could not run as fast and they basked more often. Basking time increased because a greater body mass resulted in a slower heating rate and selection of a greater body temperature may speed embryonic development and compensate for decreased mobility (Shine 1980). Many other viviparous animals must spend more time feeding and often deplete energy reserves during reproduction. Female *Urodacus manicatus* were not likely to incur any of these "costs". Adult female *U. manicatus* rarely left their home sites (Smith 1966) and were well protected beneath the shelter even when foraging.

In this study, the litter size of *Urodacus manicatus* ranged from 3 to 29 with a mean of 16 young, similar to the finding of Willmer (1967). Litter size can sometimes be an underestimate because of the potential for cannibalism of the young by the mother during parturition (Warburg and Rosenberg 1994), although this has never been directly observed for *U. manicatus*. Its use as a birth parameter in this study was valid because: 1) there was no significant difference between the number of young born and the number of embryos in gravid females in the metabolic study (Section 7.3.2, and 2) the average fell between the fecundity estimates of Smith (1966) (15.7) and

Warburg and Rosenberg (1994) (16.8) Unfortunately, no statistical comparison could be made between the different estimates of litter size.

Larger females produced larger litter sizes of greater mass, but not larger offspring, and were thus investing more into reproduction than smaller females to produce more, but no larger offspring, a similar finding to that of Formanowicz and Shaffer (1993) for the buthid *Centruroides vittatus*. The existence of an optimal offspring size was supported by the lack of correlation between offspring mass and total litter mass. No comparisons of reproductive investment were made between the populations at Black Mountain and Dubbo, but Brown and Formanowicz (1995) has shown that populations of *C. vittatus* can differ in their pattern of reproductive investment. *Paruroctonus utahensis* may produce optimally sized offspring (Brown and Formanowicz 1995), but female mass was not correlated with litter size and was negatively correlated with total litter mass (Bradley 1984). In diplocentrid scorpions, female mass was positively correlated with both litter size and offspring size; however, litter size and offspring size were negatively correlated (Francke 1981). The mean mass of second instar *Paruroctonus mesaensis* similarly decreased with litter size (Polis and Farley 1979b).

In addition to a low energetic cost, evaporative water loss in *U. manicatus* was not significantly affected by reproduction. The mesosomas of gravid female *Urodacus manicatus* were more rounded than those of non-gravid females such that the gravid females had a smaller surface area to volume ratio. Assuming no change in cuticle permeability, gravid females would be expected to have lower rates of transcuticular water loss per surface area. Although the permeabilities of the sclerite cuticle and intersegmental membrane were not measured *per se*, the similar rates of water loss between gravid and non-gravid *U. manicatus* suggest that the intersegmental membrane may be more permeable to water. The permeability of the intersegmental membrane of the mesic scorpionid *Pandinus imperator*, was 2.5 times greater than the sclerotised sternite cuticle (Hadley and Quinlan 1987). In the desert species *Hadrurus arizonensis*, water loss was the same as the sclerotised sternite cuticle, the thickness of the membrane being 2.3 to 3 times greater and the barrier to water movement in the membrane was of the same lipid composition.

This study has shown that viviparity is an inexpensive mode of reproduction for *Urodacus manicatus* despite the large number of offspring produced. The long gestation period and low metabolic rates result in a gradual provision of nutrients to the embryos such that the cost to the female remains low. Growth of the embryos is then maximised when foraging rates are high during spring. Continual development of the embryos occurs over late summer without further growth such that the energy demands of the embryos have not increased at a time when foraging activity by the mothers has decreased. Females can retain body water to the same extent as non-gravid females, and water moves from the hepatopancreas of the mother to the embryos prior to parturition.

The increase in the water content of the embryos may prevent the postpartum first instar young from losing too much water due to their small size and the greater permeabilities of their untanned cuticles (Toolson 1985). Recently moulted scorpions and small instars after the first instar may encounter similar problems with increased evaporative water loss. The effects of ecdysis and size on the rate of evaporative water loss are examined in the next chapter.

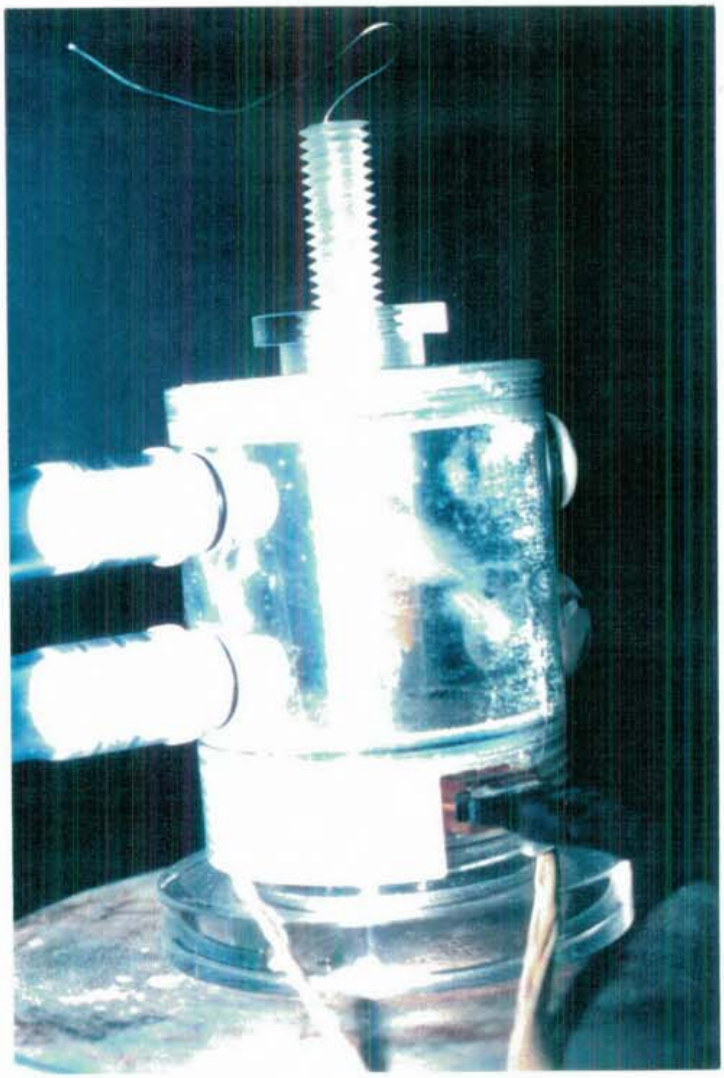

Plate 7.1. Hansatech D.W. Oxygen Electrode Unit for measuring embryo and ovariuterus VO_2. A thin length of wire was passed through the capillary of the plunger and coiled in the horizontal plane to provide support for the embryos but still allow thorough circulation of the fluid.

Table 7.1. Results of linear regressions of log transformed $\dot{V}O_2$ ($\mu l\ O_2\ h^{-1}$) on log transformed mass (g) to indicate adjustment made by mass for covariance analysis with sample sizes *n*. Regression coefficients of intercept *a* and slope *b* are given. The $\dot{V}O_2$s are plotted against mass in Figure 7.2.

	n	*a*	*b*	r^2
Pre parturient 'dissection' female	10	1.906 ± 0.137	1.192 ± 0.502	0.413*
Pre parturient 'parturition' female	8	1.873 ± 0.074	1.140 ± 0.283	0.730**
Post parturient 'parturition' female	8	1.888 ± 0.018	0.967 ± 0.147	0.878***
Post parturient female with litter	8	1.972 ± 0.054	0.810 ± 0.201	0.729**
Post parturient female plus litter	8	2.002 ± 0.035	0.730 ± 0.129	0.842***
'Parturition' litter	8	1.976 ± 0.061	0.862 ± 0.110	0.912***
'Dissection' litter	22	1.868 ± 0.035	0.642 ± 0.133	0.700***

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

Table 7.2. Results of linear regressions of birth parameters with sample sizes *n* and coefficients of intercept *a* and slope *b* given. Postpartu m female mass was log transformed and total litter mass was transformed by taking the square roots.

	n	*a*	*b*	r^2	
Postpartum female mass and litter size	19	3.520 ± 0.173	6.912 ± 1.935	0.429**	Figure 7.4
Post partum female mass and total litter mass	19	0.420 ± 0.044	2.219 ± 0.497	0.540***	Figure 7.5
Litter size and total litter mass	22	-0.443 ± 0.122	0.253 ± 0.031	0.772***	Figure 7.6

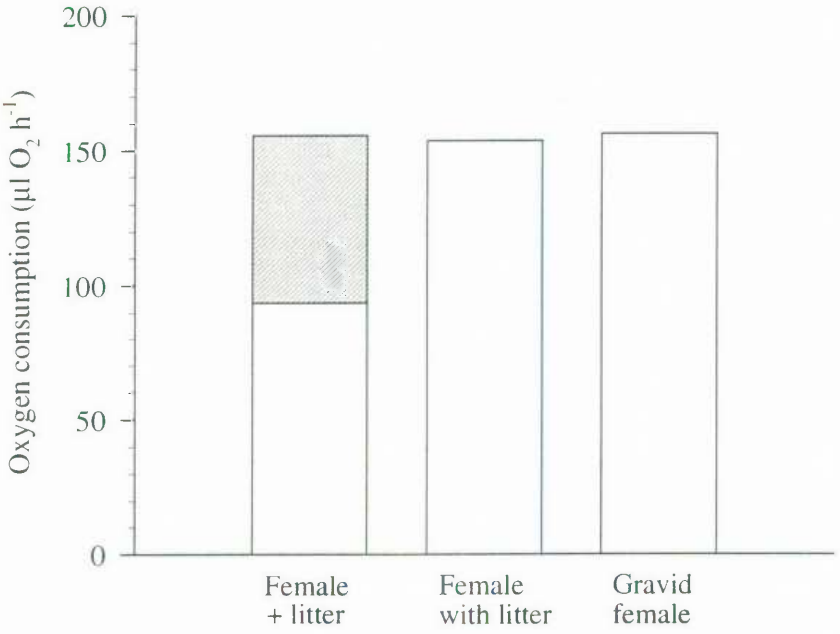

Figure 7.1. Effect of parturition on oxygen consumption rate (µl O_2 h^{-1}) at 30°C as a function of mass (g). Gravid females 5 to 7 weeks before parturition (open and closed circles), post-partum females with first instar young (open triangles), embryonic litter 5 to 7 weeks before parturition (closed circles) and first instar litter (open circles). The significant regression results are presented in Table 7.1.

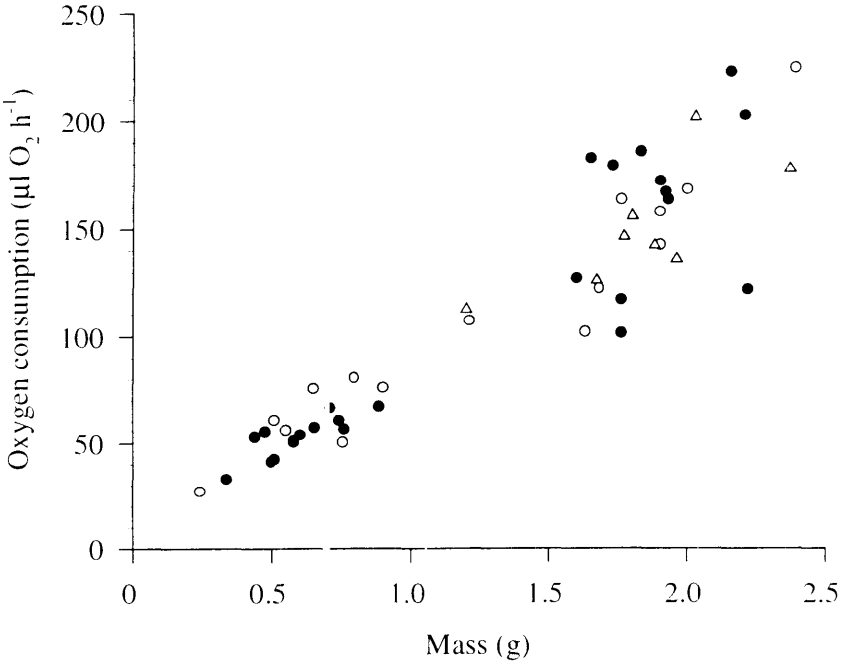

Figure 7.2. Oxygen consumption (µl O_2 h^{-1}) measured at 30°C calculated from the logarithmic mass-VO_2 regressions of adult females and first instar litter using median masses. No change occurred in the total RMR of the mothers and their litters with parturition. Shaded area is litter RMR with RMR of post parturient mothers below. Bars represent mean values, n = 8. Error bars are ± SE.

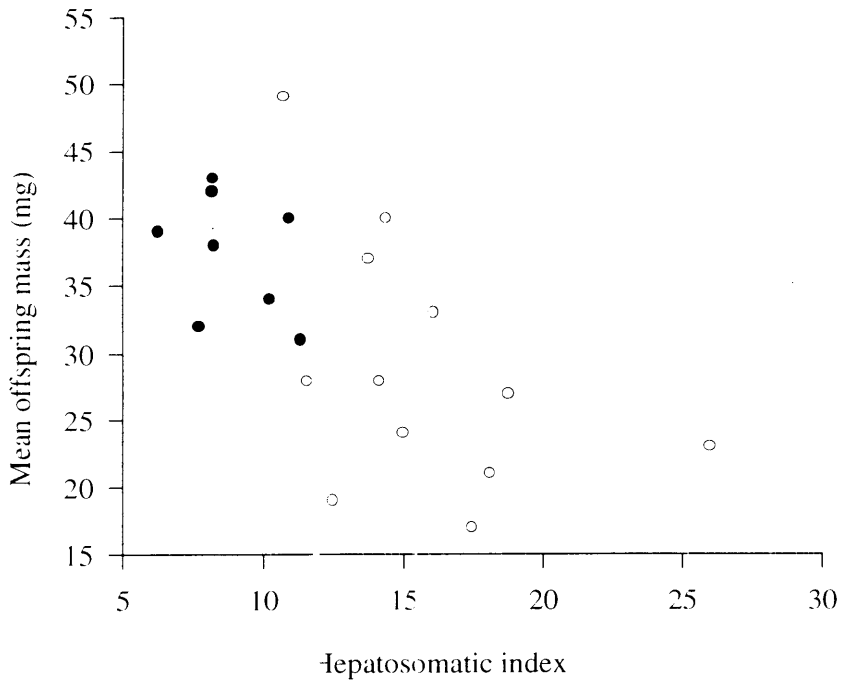

Figure 7.3. Relationship between hepatosomatic index and mean offspring mass for post-partum (closed circles) and 5 to 7 weeks pre-partum (open circles) scorpions.

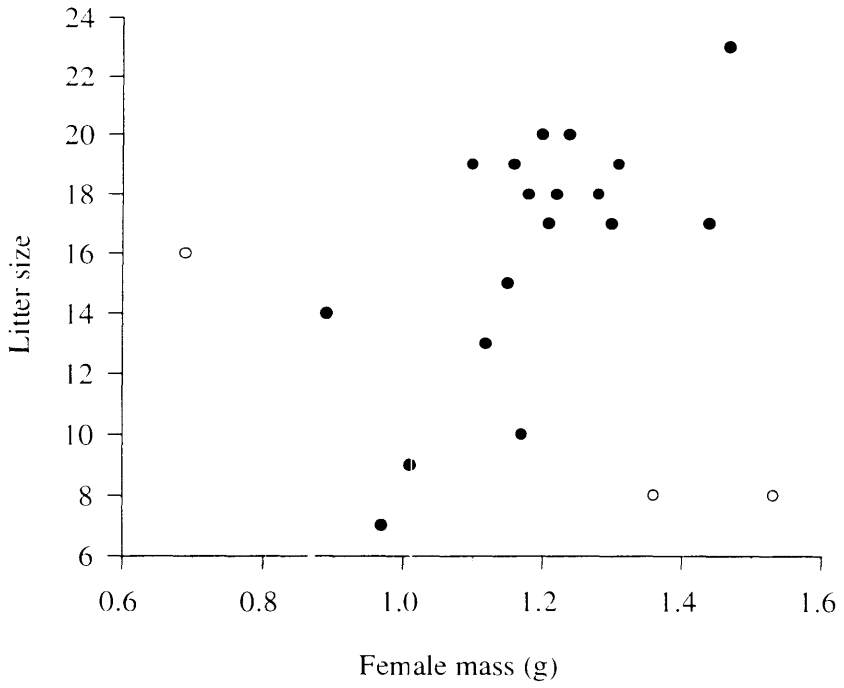

Figure 7.4. The relationship between post-partum female mass and litter size. Regression of black points is significant and white points are statistical outliers (see text for explanation). Results of the regression are presented in Table 7.2.

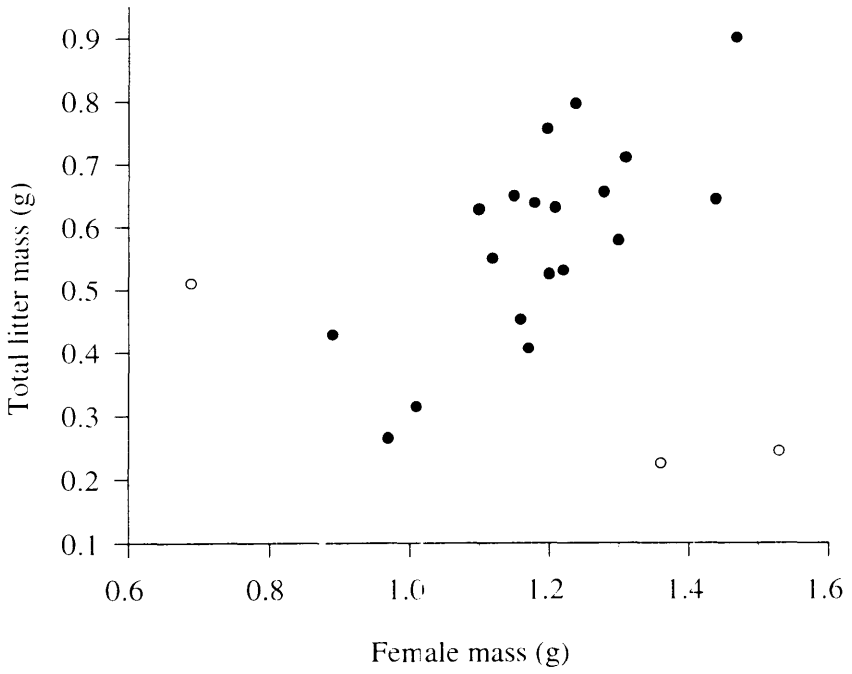

Figure 7.5. The relationship between post-partum female mass and total litter mass. Regression of black points is significant and white points are statistical outliers (see text for explanation). Results of the regression are presented in Table 7.2.

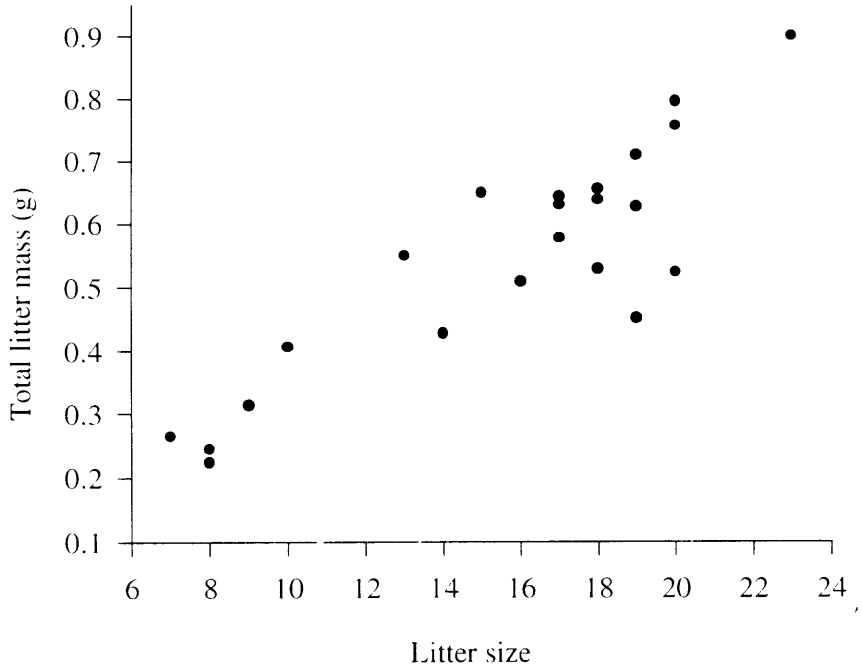

Figure 7.6. The significant relationsh p between litter size and total litter mass. Results of the regression are presented in Table 7.2.

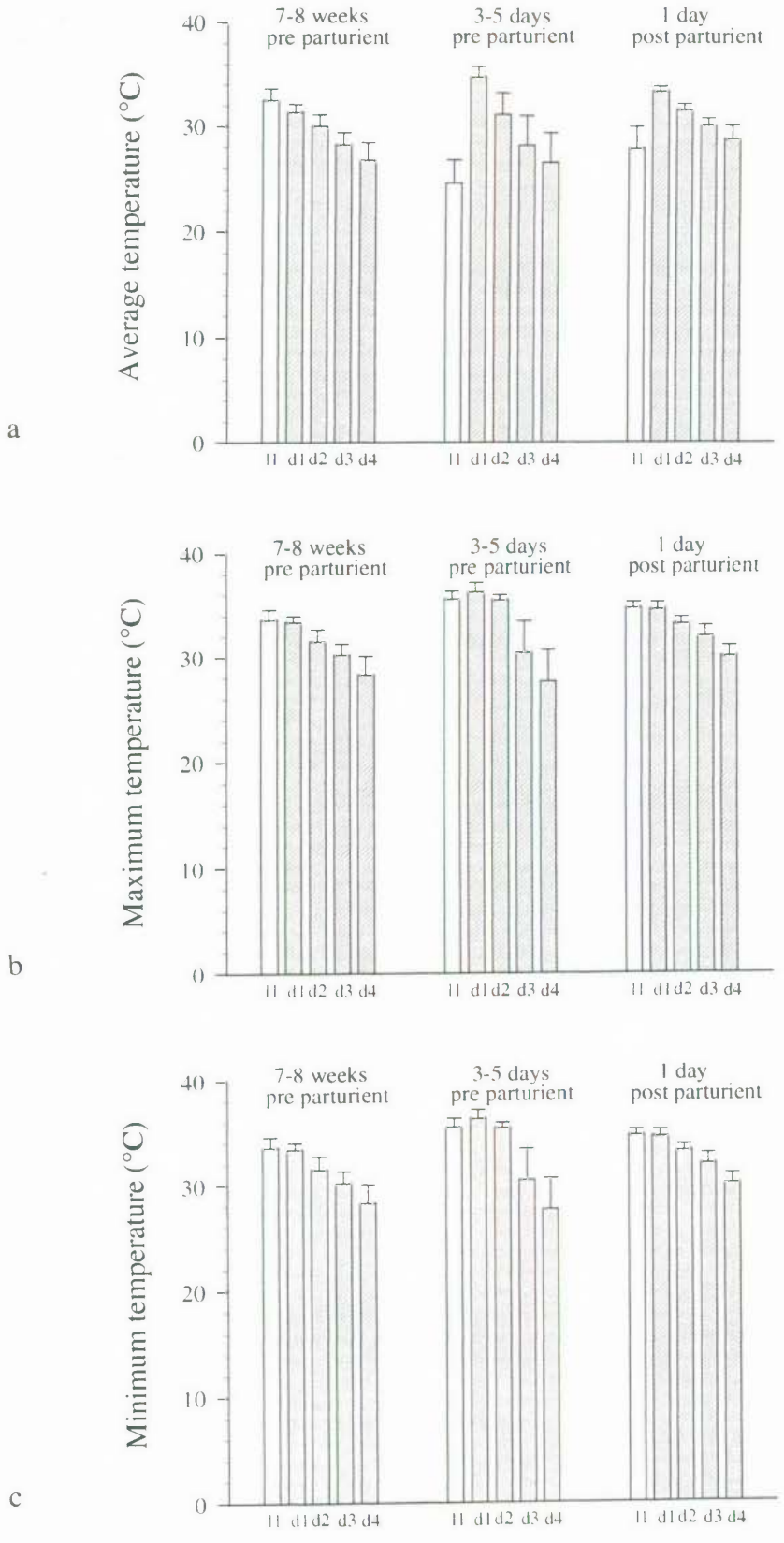

Figure 7.7. Effect of gravidity on adult female profiles of (a) average selected temperature (°C), (b) maximum and (c) minimum temperatures (°C) recorded in the gradient. Values are of 12 measurements per animal within the 3 hour periods of 15-18:00 h (l1), 18-21:00 h (d1), 21-24:00 h (d2), 24-03:00 h (d3) and 03-06:00 h (d4). Bars represent mean values ± SE (n = 8). Shaded bars highlight the scotophase periods.

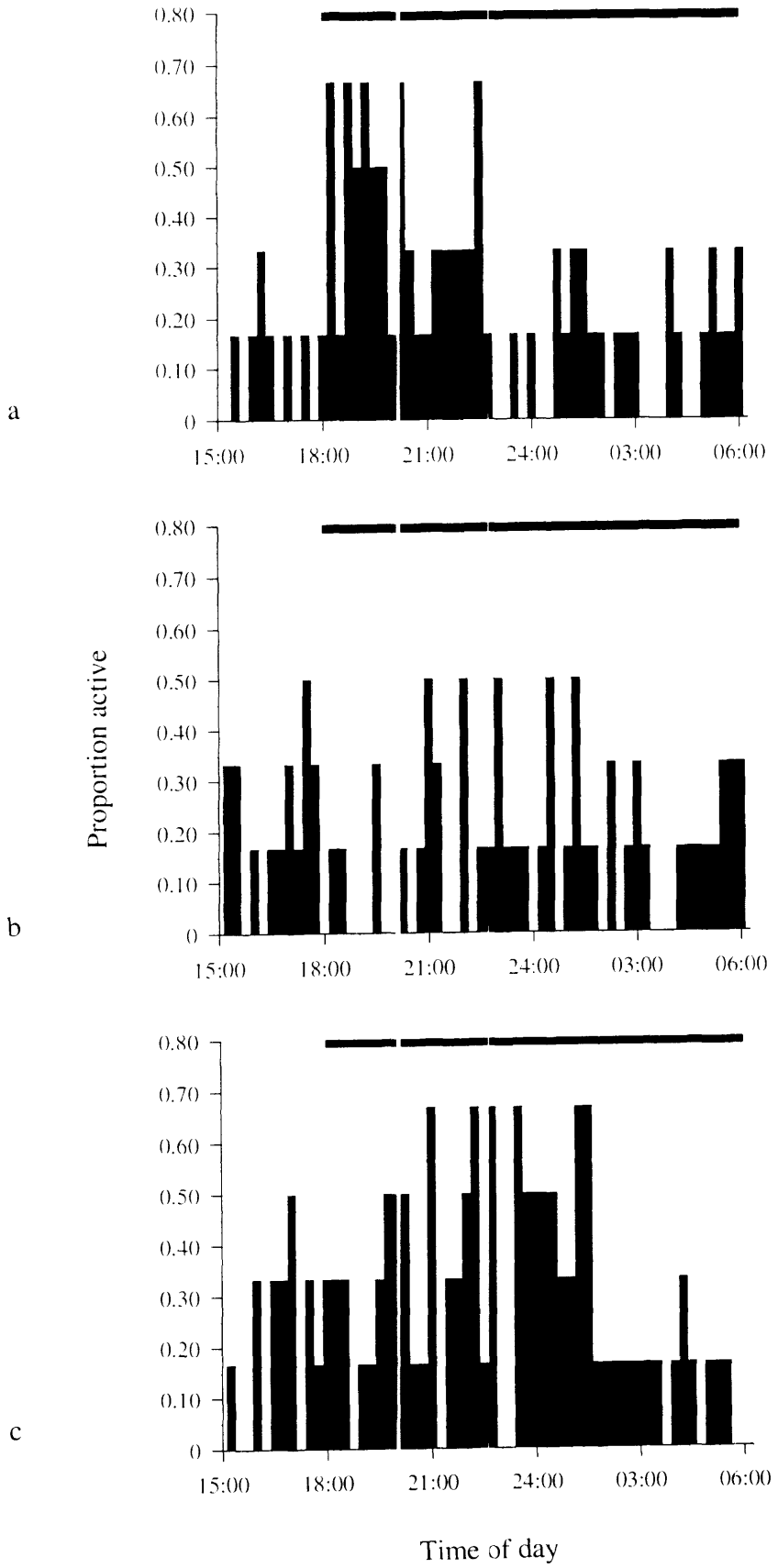

Figure 7.8. Activity profiles of adult females (a) at the time of capture 7 to 8 weeks pre-parturient, (b) 3 to 5 days pre-parturient, and (c) 1 day post-parturient. Each bar represents the proportion of animals that had turned in the gradient resulting in a 5°C or greater change in selected temperature from the preceding temperature. Temperature was measured every 15 min. Horizontal bar represents scotophase from 18:00 to 06:00 hours. For all cases $n = 6$.