

# Chapter 1

## Introduction

### 1.1 General Information

The order Scorpiones is ancient and dates back to the Silurian Period some 430 million years ago (Jeram 1990a). Scorpions were one of the first groups of arthropods to become terrestrial and, as testimony to the fact that they were very successful by occupying all ecosystems bar the polar circles, their basic structure and form has remained unchanged. They are characterised by having a large pair of pedipalps, medial and lateral eyes, pectines and an elongated metasoma terminating in a telson comprised of a vesicle bearing a poison gland and aculeus (sting). Scorpions have played an important part in human history appearing in the religious beliefs and myths of many cultures; such veneration reflecting the medical importance of the few deadly venomous species that coexisted with early civilisations. But they are also biologically fascinating in their own right and have received a lot of attention in the scientific community in the past decades. Among their more interesting traits are their success in all nonaboreal habitats, viviparity, ecology (cannibalism, aggregation), sophisticated sensory capabilities, long lives and mode of predation, all of which are touched upon in this thesis.

The early scorpions of the Silurian Period belonged to the suborder Branchioscorpionina and were aquatic, obtaining oxygen via book-gills (Kjellesvig-Waering 1986). Recent evidence suggests that the transition from water to air respiration occurred during the Lower Carboniferous Period (345-321 million years ago), the book-gills evolving directly into the book-lungs (Jeram 1990b). All terrestrial scorpions (both extant and fossil) are placed within the suborder Neoscorpionina (Kjellesvig-Waering 1986).

Scorpions have four pairs of internal mesosomal book lungs which are the sites of gas exchange between the haemolymph and the air. Air enters the book lungs via spiracles at the surface of the mesosomal sterna. Each spiracle expands antero dorsally into an atrial chamber connected to which is the pulmonary chamber housing 140 to 150 lamellae. Air passes through slit like openings in the roof of the atrial chamber and into the interlamellar spaces. Gas exchange occurs by diffusion across the lamellae surface between the haemolymph within the lumen of the lamellae and the interlamellar spaces. Air is drawn into the book lung by expansion of the atrial chamber and opening of the spiracle, both actions effected by the contraction of the ventralis poststigmaticus muscle in *Heterometrus fulvipes* (Vyas and Laliwala 1972). Air is forced out by contraction of the dorsoventral muscles which compress both chambers. When these muscles relax air pressure is reduced in the pulmonary chamber thus drawing air in from the atrial chamber.

The oxygen-carrying pigment in scorpion haemolymph is the copper-containing protein haemocyanin (Padmanabhanaidu 1966b). As with all arachnids the circulatory system is open because the blood circulates through both vessels and sinuses. The heart lies dorsally in the mesosoma and has a pair of ostia arising from each of its seven chambers that lead to systemic arteries supplying the viscera. The metasoma is supplied by the anterior aorta which also gives rise to the pectinal and pulmonary arteries. Blood flow is predominantly directed forward because the posterior end of the heart reaches maximum systole slightly before the anterior end (Farley 1987). From the arterial system the blood enters the ventral sinus where the blood is oxygenated by the book lungs and is then conveyed to the pericardial sinus surrounding the heart via pulmonary veins where the blood enters the heart for recirculation.

Despite their notoriety, the order is a relatively small one consisting of nine extant families and some 1,400 species and subspecies (Sissom 1990). Families and general distributions are as follows (Sissom 1990):

1. Buthidae Simon, 1880

The largest (48 genera) and most widespread family occupying all six faunal regions with greatest diversity in the Old World.

2. Chaerilidae Pocock, 1893

A monotypic family of the genus *Chaerilus* confined to the Oriental Region.

3. Ischnuridae Pocock, 1893

Contains 8 genera of Gondwanaland distribution.

4. Bothriuridae Simon, 1880

A small family of 10 or 12 genera of Gondwanaland distribution.

5. Chactidae Laurie, 1896 (18 genera), Vaejovidae Thorell, 1876 (12 genera) and Iuridae Thorell, 1876 (5 genera). These families are referred to as being "chactoid" and their taxonomy remains uncertain.

6. Diplocentridae Peters, 1861

A small family (7 genera) of the New World and also the Middle East.

7. Scorpionidae Pocock, 1893

Contains 7 genera of Old World originating in eastern Gondwanaland.

Four of the families occur in Australia: Scorpionidae, Ischnuridae (formally scorpionid subfamily Ischnurinae), Bothriuridae and Buthidae.

The success of the Scorpiones has been due to the following combination of characters unique to the order. Scorpions have extremely low metabolic and water loss rates and are thus enabled to inhabit dry habitats of low prey numbers. The evolution of a terrestrial existence resulted in relatively smaller sizes than their aquatic ancestors (Jeram 1990a), enabling them to utilise a large number of microenvironments within the larger, more perceptible macroenvironments. The combination of pedipalps and a venomous sting made them very effective predators capable of subduing prey considerably larger than themselves. All extant scorpions are viviparous (Francke 1982) which is the condition where the eggs are fertilised internally, are retained within the body of the mother for a significant time before deposition and the embryos derive nutrients from the mother (Hogarth 1976). This method of reproduction enabled mothers to ensure the survival and growth of the embryos without restricting her normal activities. Many complex behaviours including courtship (incorporating the *promenade à deux*), postpartum maternal care, prey detection, burrow construction and sponging have been observed and described in detail for many species of scorpions (see McCormick and Polis (1990), Polis (1990), Polis and Sissom (1990), Warburg and Polis (1990)).

## 1.2 Abiotic influences on scorpions

Scorpions are ectotherms, that is, the regulation of body temperature is dependent upon the flow of heat between the animal and its immediate environment or microhabitat (Casey 1988, Pough and Gans 1982), rather than on internal heat production. Furthermore, most scorpions may be classed as thigmothermic, that is, body temperature is dependent on heat transfer from surfaces of the microhabitat and not, for example, solar radiation (heliothermy) (Heatwole 1976). Being ectothermic, a scorpion's metabolic rate and ability to perform essential activities are largely dependent upon the temperatures encountered by the animals. In general it is the microclimate that influences the animal's physiological capacities and ultimately its ecological performance (Huey 1991). The interactions can be quite complex. The manner in which the microclimate influences a scorpion's body temperature is dependent on the animal's physiology, behaviour and morphology. In turn, body temperature interacts with the physiology and morphology of the animal in determining its ability to perform important behaviours (obtaining food, avoidance of predation, sociality). These short-term interactions ultimately affect the growth, survival and reproductive capabilities of the individual and ultimately population and community dynamics (Dunham et al. 1989).

The avoidance of extremes in microclimate is also important. High temperatures can ultimately result in death by causing proteins to denature (McLennan and Miller 1990) and metabolic functions to become uncoordinated (Somero and Hochachka 1976) resulting in tissue degradation and organ failure. Low temperatures can cause cell membranes to become less fluid thus disrupting normal transport processes (Hazel 1988) and affect nerve and muscle tissue resulting in a lack of coordination such that normal activities cannot be maintained (May 1985). Prolonged freezing of tissues disrupts cell membranes thus causing excessive tissue damage (Franks et al. 1990, Storey and Storey 1989).

Temperature also has profound effects on water balance, water being critical for all biological processes. There exists a critical high temperature where the normal water retention properties of the cuticle break down resulting in excessive water loss (Beament 1958). Water lost via respiration also becomes increasingly important at high temperatures. The rate of normal water loss across the cuticle is dependent on the combined effects of air and cuticle temperatures

with relative humidity known as the saturation deficit. Prolonged exposure to a microclimate with a high saturation deficit without the uptake of water can result in excessive water loss. The maintenance of a body water volume is essential along with the regulation of concentrations of electrolytes and organic compounds in the haemolymph and tissue fluids (Withers 1992). This regulation is referred to as osmoregulation. Many cellular functions are dependent on the passive diffusion of materials across the semi-permeable cell membranes and this process is dependent on the relative concentrations either side of the membrane. If too much water is lost or gained by the animal then the differences in concentrations will change thus affecting biochemical processes within the tissues. Excessive water loss can also affect haemolymph fluidity and its oxygen and carbon dioxide carrying capacity (Hillman 1980).

It is obvious then that the control of water gain and loss in scorpions is of paramount importance to their survival. There are three major pathways of water loss: 1) water vapour movement from the tissues and haemolymph through the cuticle to the air referred to as transcuticular water loss, 2) the excretion of waste products and 3), because the surfaces of the respiratory organs must be kept wet yet exposed to air, there is respiratory water loss (Hadley 1990). Transcuticular water loss is compounded by the small size of scorpions and their consequent large surface to volume ratio. Thus evaporation of water across the cuticle is usually the principle source of body water loss under normal conditions (Crawford and Wooten 1973, Hadley 1970b, Robertson et al. 1982, Warburg et al. 1980b).

It is the immediate climatic conditions of small terrestrial animals that must be known if the effects of the abiotic factors of temperature and saturation deficit on an animal's success are to be investigated. This microclimate differs from that of the gross atmospheric conditions (Cloudsley-Thompson 1967a). The climate near the ground becomes more extreme and more continental in its characteristics with increasing radiation (Geiger 1965). It is therefore inadequate to measure or obtain from records meteorological data in an attempt to categorise changes that occur in an animal's microhabitat although such data are valuable in the selection of populations for study. The term "microclimate" has been defined in several ways from a gradation of conditions from areas several kilometres square, to the air above the ground and to that immediate to small

animals referred to by Smith (1954) as the "insect climate". It is the small end of the spectrum that all discussion of microenvironment, microhabitat and microclimate shall be referred to in this thesis.

### **1.3 Habitat modification**

The microclimatic conditions at the ground level can fluctuate enormously, both diurnally (due to heat gain by radiation during the day and heat lost due to its absence at the night) and seasonally (the change in the amount of radiation received during the day). These fluctuations are strongly influenced by altitude and latitude. If these fluctuations are to be tempered, then scorpions must be able to modify or select already modified parts of the ground surface in order to function within tolerable limits.

All species of scorpions are cryptozoic, that is, they lead concealed lives beneath rocks, fallen timber, leaf litter, bark, within burrows, even within the brickwork of dock walls (Benton 1992). The influence of burrows and shelters on the microclimate encountered by scorpions has been examined in a number of studies on different species. Burrows that open directly to the surface of desert-inhabiting species are usually deeply constructed into the sand or soft soil. Soil temperatures and moisture content become fairly constant at a depth depending on the heat conductivity of the soil. According to Geiger (1965), soils with good conductivity give off less heat during the day to the adjacent air, and more of the heat received in radiation is retained within the soil thus the average temperatures of well conducting soils are higher. Diurnal temperature fluctuations therefore will penetrate to depths depending on the conductivity of the soil. The depth at which fluctuations no longer occur is the maximum depth of the burrow at which an inactive animal may experience no or little change in temperature over 24 hours (Hadley 1970a, Kalarani et al. 1991b, Shorthouse and Marples 1980). Relative humidities here are higher and also more seasonally constant deep within the burrow (55-80% RH) compared to conditions outside which may get as low as 5% during the day and near saturation at night (Hadley 1970a, Shorthouse and Marples 1980). Burrowing also provides constant conditions of wind and light (Williams 1966).

Although scorpions essentially inhabit warm climates, they are by no means restricted to desert regions with sand for deep burrows. Many species inhabit rain forest, temperate woodland, dried water courses and even littoral zones (Polis 1990). Those of temperate regions can potentially experience seasonally or diurnally fluctuating conditions that approach those of desert and polar regions.

Ground surface shelters such as rocks, logs and leaf litter provide suitable home sites. Burrows may or may not be constructed below shelters depending on the species and the microhabitat conditions. For the Australian genus *Urodacus*, (Koch 1978) recognised that shallow burrows beneath surface shelters were typical of more temperate habitats, while open ground deep spiralling burrows occurred in the more arid regions.

Enough of the burrow or most often the whole of the burrow can be constructed in a single night to avoid daytime surface temperatures of 50 to 60°C and relative humidities as low as 10% (Shorthouse 1971). Once a burrow is made, most species will maintain and continue to expand it as the individual grows, thus spending a large proportion of its life within the same burrow. Selectively then, relatively more energy can be partitioned to other purposes with less energy being expended on adapting to fluctuating abiotic environments (Polis 1980b). Also, because the stable environment of the burrow is readily available, the limiting factors are usually biological resources (Shachak and Brand 1983). The burrow also allows a rapid retreat from predators as surface foragers tend to stay close to the burrow entrance (Hadley 1970a).

A cryptic existence conveys obvious benefits of both a physiological and behavioural nature. Temperature extremes may be buffered to within more tolerable limits or create a more restricted temperature range. Predation by other animals is reduced by being undetected or difficult to get at. Protection is afforded to non-motile young and also to scorpions during ecdysis when locomotion is not possible and then afterwards when the integument is soft and the animal is more vulnerable and unable to defend itself.

A cryptic existence can also have its disadvantages if conditions within the microhabitat become unfavourable and the scorpion cannot move to or exist as well in a different microhabitat. As examples, during times of unusually heavy rainfall, burrows may become flooded during which

scorpions may either potentially drown or become exposed on the surface during the day. In cool temperate climates, unseasonably heavy snowfalls may leave snow settled on the ground thus reducing scorpion activity and feeding.

## 1.4 Adaptations to fluctuating abiotic factors

While the burrow is important, scorpions by no means spend their entire lives within the confines of a more or less constant microclimate at depth in a burrow. A greater range of environmental factors can be potentially encountered when scorpions leave the burrow to prey on the surface at night or rove the surface in search of mates. Different microclimates can be experienced when moving within the burrow and shallow or non burrowing species may not have home sites which provide the same constancy of conditions as deep burrows do. The potential to encounter extremes or spend time under slightly unfavourable conditions is therefore still great and scorpions have developed a range of anatomical, behavioural and physiological adaptations to deal with this.

### 1.4.1 Anatomical advantages

The retention of a relatively small size in the order has implications regarding body temperature. Nocturnalism in scorpions means that solar radiation and long wave radiation are not important sources of heat, thus heat flow is mostly dependent on conduction and convection though convection is probably more important at night outside the burrow than during the day. As such the heat exchange between a scorpion and its microenvironment may be simply described as:

$$\delta H/\delta t = C(T_b - T_a)$$

where  $T_b$  is body temperature,  $T_a$  is ambient temperature and parameter  $C$  is thermal conductance which is dependent on a number of variables including size, shape, physiological state and the nature of the radiant environment (May 1985). The smaller the terrestrial ectotherm, the higher the value of  $C$  will be and thus will experience greater rates of heat exchange with its



microenvironment. This can be advantageous if a range of ambient temperatures are available to the animal. As ambient temperature increases, the animal will experience high rates of heat flow from the microenvironment to itself thus rapidly obtaining a range of body temperatures conducive to normal activity (Stevenson 1985) referred to as their active temperature range (Pough and Gans 1982). By moving between parts of the microhabitat of different ambient temperatures, small ectotherms can maintain this range by rapidly losing to or gaining heat from the microenvironment (Stevenson 1985). As well, when an animal's body temperature moves towards being lethal, it can be rapidly regulated to a more conducive temperature by moving to a cooler or warmer part of the microhabitat.

The physical means by which scorpions limit water loss is the thickened waxy cuticle and the internalised respiratory organs. The scorpion cuticle is marked by its waterproofing capabilities and is mostly attributable to lipids (waxes) of the epicuticle (Hadley 1990, Hadley and Jackson 1977). Presence of these lipids as free molecules, protein-bound or as deposits on the cuticle surface provide scorpions with some of the lowest rates of water loss of all arthropods. (Filshie and Hadley 1979) gives an account of the structure of the cuticle of the desert iurid *Hadrurus arizonensis* as follows. The sclerite cuticle consists of four layers of varying thickness: the epicuticle (0.3  $\mu\text{m}$ ), hyaline exocuticle (7.5  $\mu\text{m}$ ), inner exocuticle (5  $\mu\text{m}$ ) and endocuticle (65-85  $\mu\text{m}$ ). The intersegmental membrane lacks the hyaline and inner exocuticles and the epicuticle is much thicker in a dense homogenous layer. This dense layer is rich in lipids in insects so it is assumed that the increased thickness in the intersegmental membranes lengthens the diffusion pathway for water through a hydrophobic region (Hadley and Filshie 1979). The importance of the intersegmental membrane in limiting water loss increases when the scorpions mesosoma becomes extended after large feeds or during late pregnancy. The lipids are either synthesised or stored unchanged from the prey in the hepatopancreas from where they are gradually moved to the cuticle (Hall and Hadley 1982).

In addition to a serviceable anatomical design, individual scorpions, like most animals, can improve their survival in the face of changing or extreme environments both physiologically and behaviourally. Physiological changes may include metabolic compensation, supercooling point adjustment and changes of cuticle lipid composition to increase or decrease water loss (see below). Behaviourally in the broadest terms, a scorpion can select a suitable part of the

environment to inhabit, modify the habitat, lead a nocturnal and cryptozoic existence or alter its orientation with respects to the habitat. None of these adaptations are mutually exclusive and even less so for scorpions because biological rates are dependent on the abiotic factors of the microclimate. For example, behavioural changes can alter both ambient and body temperatures which have obvious effects on temperature-dependent rates. Conversely, certain functions may operate optimally within a range of body temperatures (the normal activity range (Pough and Gans 1982)) thus influencing temperature selection behaviour. It is apparent then that there exists complex interactions between the biotic traits of behaviour, physiology and anatomy.

### 1.4.2 Microclimate selection

The range of microclimates available to a scorpion within a day are not simply confined to night time surface conditions and those at depth in the burrow where there is some consistency. Within the burrow itself a range of temperatures and humidities to select from are available to scorpions depending on the length and type of burrow (Hadley 1970a, Hadley 1974, Shorthouse and Marples 1980). Diurnally then, there is effectively a wide choice of microenvironments open to the scorpion that influence water loss and metabolic energy utilisation. The lag in the heat penetration of the soil will result in burrow temperatures at some depth not to peak until well after sundown (Hadley 1970a). Although temperatures may be higher in the upper part of burrow at night, they can be lower at the burrow entrance where some species predate by sitting and waiting with the pedipalps just outside the entrance (Shorthouse and Marples 1980). Foraging species may spend time at the warmer parts of the burrow away from the entrance to raise their body temperature to facilitate locomotor and digestive functions before exiting onto the surface (Hadley 1970a). The scorpionid *Opisthophthalmus ecristatus* ensures the constancy of the burrow conditions during cold months by sealing the entrance of the burrow (Newlands 1972). The type and placement of a burrow may be seasonal within a species. Where the normally mesic diplocentrid *Nebo heirochonticus* (Warburg and Ben-Horin 1979) occurs in the desert, the burrows are deep under rocks during warmer months but mere shallow scrapes during winter (Rosin and Shulov 1963). The buthid *Centruroides exilicauda* normally inhabits under bark but may be found beneath rocks in warmer months (Crawford and Krehoff 1975). The burrow

temperatures of xeric species that construct burrows below rocks drop less rapidly than the outside air temperature but do tend to remain lower during day (Abushama 1962).

While the home sites of xeric species enable the avoidance of high temperatures, the home sites of more temperate distributed species buffer the inhabitant from extreme cold. The burrow (crack) temperatures of *Euscorpius flavicaudis* on frosty nights never past below 3°C (Benton 1992) and the use of rocks as shelters can enable burrow temperatures to remain above that of the surrounding surface in winter (Crawford and Riddle 1974).

### 1.4.3 Cuticular permeability

The degree to which scorpions may be physiologically adapted to mesic and xeric environments is not fully resolved despite the number of studies conducted. Early work on rates of water loss and upper lethal temperatures by Cloudsley-Thompson (1959 and 1962a) showed that differences between species reflected adaptation to different habitats. Species with high rates of water loss and low upper lethal temperatures inhabited more mesic environments. Warburg (1978, 1979 and 1981) demonstrated that temperature selection within a gradient and the rates of water loss of different species were related to habitat. Punzo (1991), however, found that the mesic species *Centruroides hentzi* had rates of water loss in general agreement with previously reported rates of mesic and xeric species, supporting the point of Hadley (1990) that xeric species minimise water loss largely through behavioural (fossorial and nocturnal) mechanisms. The picture is complicated by those species that are usually mesic that have distributions extending into arid and semi-arid zones (Robertson et al. 1982, Rosin and Shulov 1963, Warburg et al. 1980a). This reinforces the fact that it is important to examine the microclimatic conditions more thoroughly and the relationship to home site selection behaviour. *Nebo heirochonticus* maintains a mesic environment where its distribution extends into arid regions by burrowing deep below rocks along water courses (Warburg et al. 1980a). The buthid *Leiurus quinquestriatus* is a desert inhabiting species that has been found to have moderate water conservation where it burrows into the soil below large rocks along water courses (Abushama 1962) yet has high powers of water conservation where it occurs in desert grassland (Warburg and Ben-Horin 1978, Warburg et al. 1980a).

#### 1.4.4 Temperature acclimatisation

The effect of increasing temperature on scorpion metabolic rates is not as pronounced as in many other animals, vertebrate and invertebrate alike. Thus metabolic rate is largely independent of temperature for normal active temperatures (Riddle 1978). Behavioural thermoregulation though, may be facilitated by metabolic compensation (acclimatisation) if there are long-term (for example seasonal) temperature changes in the animal's microhabitat. If a terrestrial ectotherm is unable to obtain temperatures within its activity temperature range (Pough and Gans 1982) due to colder conditions or it regularly exceeds the activity temperature range, then its resting metabolic rate (RMR) across a range of temperatures may shift up or down respectively (Bullock 1955, Prosser 1973) thus lengthening the time that the animal may spend in an active mode. Therefore, the RMR of an organism moved to a higher temperature will decrease with time and vice versa when moved to a lower temperature. In the field we would expect populations that experience different seasonal climates to differ in their patterns of acclimatisation. The standard metabolic rate (SMR) of the lizard *Sceloporus occidentalis* from high latitudes was lowest in winter when they hibernated and highest in spring during reproduction (Tsuji 1988). Animals from a population living at a lower latitude experienced seasonally warmer temperatures and had its lowest SMR in summer, an intermediate SMR in winter when the animals were active on warm days and SMR was highest in spring during reproduction. Such seasonal and population effects on the SMR of scorpions have not been previously measured. Estimates on seasonal energetics have been deduced from laboratory measurements of RMR at different temperatures and the temperatures encountered by the scorpion in the field (Polis and Farley 1979b). The effect of season on the RMR of animals collected from the field is not clear. *Diplocentrus peloncillensis* females maintained a consistent RMR but males increased RMR during the warmer months possibly linked to mating activity (Crawford and Riddle 1975). The RMR of the *Paruroctonus utahensis* males decreased towards the end of summer and increased again into autumn (Riddle 1978).

Metabolic compensation of whole animals to higher or lower temperatures has been demonstrated in some species of scorpion in the laboratory. *Heterometrus fulvipes* exhibited a type 3 response (Precht 1958) when acclimated to either winter or summer temperatures after 11 and 8 days respectively (Kalarani et al. 1991b). *Paruroctonus utahensis* acclimated to 10°C

exhibited higher metabolic rates than those acclimated to 24°C after 13 to 20 days (Riddle 1978). Significant differences were usually only apparent at test temperatures above 19°C. In another study on *P. utahensis*, metabolic compensation occurred after a rise from 14 to 34°C but RMR also decreased after a drop in acclimation temperature from 34 to 14°C (inverse compensation) (Riddle 1979). Inverse compensation was seen as adaptive during times of less feeding due to low surface temperatures. Acclimation responses including metabolic compensation have also been shown at the biochemical and tissue levels. As with whole *H. fulvipes* (Kalarani et al. 1991b), time-course acclimation has been demonstrated in the metabolic rates of pedipalp and heart muscle (Kalarani et al. 1991b), in enzymatic activities and metabolite concentrations of these muscles (Kalarani et al. 1991a) and in the metabolic rate and enzymatic activities of the hepatopancreas (Kalarani et al. 1992). The ATPase activity of cephalothoracic tissue from *Centruroides hentzi* increased with acclimation to 35°C but did not change with acclimation to 15°C (Punzo 1991). Other species have failed to exhibit any long term metabolic compensation (Crawford and Riddle 1975, Dresco-Derouet 1967, Robertson et al. 1982) to temperature. The absence of metabolic compensation has been attributed to the already low energy requirements of maintenance in arachnids in general (Anderson 1970, Robertson et al. 1982).

Thermal acclimation may also affect cuticular permeability. *Centruroides exilicauda* collected in winter had higher rates of water loss attributed to shorter hydrocarbon chains in the epicuticle compared to summer-collected specimens (Toolson and Hadley 1979). The change in composition may also be energetically adaptive during dormancy when lipids continue to be turned over because the shorter hydrocarbon chains require less energy in their formation from acetate than longer chains (Toolson and Hadley 1979).

### 1.4.5 Supercooling

Some temperate scorpions cannot avoid sub zero temperatures within their home sites in winter. This can result in the loss of up to 50% of the population especially in montane areas (Crawford and Riddle 1974). Some mesic species are more cold tolerant combining supercooling and survival of freezing to survive minimum burrow temperatures during winter (Whitmore et al. 1985). The supercooling point is the temperature at which body water spontaneously freezes.

Supercooling points were not clearly related to season for *Diplocentrus peloncillensis* (Crawford and Riddle 1975), but were for *Paruroctonus aquilonalis* (Riddle and Pugach 1976) and may have been related to different feeding rates. *P. aquilonalis* did not survive freezing of the tissues (Riddle and Pugach 1976). The supercooling mechanisms are not entirely known for scorpions but do not seem to involve low-molecular-weight antifreezes such as glycerol or sorbitol (Riddle and Pugach 1976).

## 1.5 Adaptations to fluctuating biotic factors

The survival problems faced by scorpions, as with all animals, can depend on other factors apart from the microclimatic conditions immediate to the animals. Ecological and biotic factors, although inextricably linked to climatic change, impact greatly on scorpions. Cloudsley-Thompson (1956 and 1962c) was curious that the degree of water-proofing in the cuticle was exceptionally high for animals that emerged from their burrows at night when the saturation deficit was at its lowest and concluded that nocturnalism was probably correlated with ecological rather than physiological requirements. This was supported by Toye (1970) who found that day-active scorpions had higher rates of water loss than nocturnal scorpions. Benton (1992) suggested that survival in a new habitat may possibly depend more on a lack of predators and competitors than on abiotic conditions. The sit-and-wait method of predation (which is exhibited by most species of scorpion) can eventuate in there being potentially long periods between meals (Benton 1992). Their small energy expenditure associated with general inactivity and lack of homeostatic regulation, however, enables them to withstand large fluctuations in temperature, water content, metabolic stores etc that are associated with irregular feeds or water uptake and, as such, they are pre-adapted to live in many environments.

### 1.5.1 Prey availability

Predatory behaviour opportunities can be highly seasonal due to prey availability and temperature. Spring feeding rates of surface foragers are the highest (Polis 1979) due to high insect abundance (Polis and Farley 1979b). Winter rates are the lowest when surface temperatures are 0 to 15°C

(Polis 1980b). Scorpions rarely seek out prey actively, but remain motionless while waiting for prey to come into their vicinity. It has also been generally assumed that they are not scavengers although Krapf (1986) observed an increase in the incidence of the uptake of dead prey or prey remains in starved and field collected *Androctonus australis* and *Buthus occitanus*. Surface foragers can home in on passing prey from quite a distance (Brownell 1984) whereas those that wait within the burrow entrance require prey to come considerably closer for capture. Food supply can therefore be unpredictable (Riddle 1978) and scorpions may go without feeding for long periods (Benton 1992). Even when a continuous source of food is supplied for them in captivity, scorpions rarely feed (Bender 1959) suggesting they are adapted to infrequent feeding. Although already possessing low RMRs, scorpions may require a means of minimising energy expenditure to conserve metabolic reserves depending on how great the period between meals is and how much time will be spent at higher temperatures. A scorpion can achieve this by a number of means by either lowering its metabolic rate over its activity temperature range (Riddle 1978), increasing the length and/or number of periods of inactivity (Bradley 1982, Sinha 1982, Sinha and Kanungo 1967) or selection of lower temperatures in its microhabitat if available. The lowering of the metabolic rate will be low RMR (metabolic inhibition, Storey and Storey 1990) in times of stress has not been observed for any species of scorpion.

Insect abundance is also related to rainfall and feeding rates for scorpions are high during seasonal rains but low and sporadic during drier months (King and Hadley 1979). A loss of mass may then be correlated with seasons of low rainfall and a gain in mass with high rainfall. Populations that experience differences in seasonal abundance of potential prey result in different adult sizes and seasonal feeding rates (McCormick and Polis 1986).

As well as having a major role as a predator, scorpions also constitute an important part of the diet of many other animals both vertebrate and invertebrate (McCormick and Polis 1990). Both cannibalism and intraguild predation are common in the dynamics of some scorpion populations (Polis 1979, Polis 1980a, Polis and McCormick 1987) and can result in increased growth rates in autumn when other prey become less abundant. Predation by non scorpion species would be highest when surface activities are highest due to feeding activity or male roving activity. Predation of "door-keeping" species, which forage from within the burrow entrance

(Alexander and Ewer 1958), would be expected to be less than that of surface foragers due to less exposure and possibly less seasonal. Species utilising rock shelters would be harder to obtain than open surface sand species that can be caught by digging.

### **1.5.2 Water availability**

Scorpions as a group rarely drink from free water and are not capable of taking up water from the atmosphere even at saturation levels (Crawford and Wooten 1973, Hadley 1970b, Robertson et al. 1982). The contribution made by metabolic water production is very small and of no significance in the overall water budget of a scorpion (Robertson et al. 1982). Some species can obtain water by chewing wet sand (Gaffin et al. 1992) but most gain the majority of their water from prey items. Depending on the prevailing conditions, approximately half of the prey's water content is ingested during feeding (Yokota 1984). Seasonality of insect abundance, therefore, impacts directly on the water availability to scorpions. The usually more mesic conditions of winter assist in physically reducing water loss and this may be facilitated by changes in cuticular permeability previously mentioned.

During times of water and food stress water uptake from the ilium may be increased (Ahearn and Hadley 1977). Scorpions excrete very little water because insoluble purines are the major excretory products (Horne 1965, Kanungo et al. 1962, Yokota 1984). Regulation of the excretion of water may play an important in osmoregulation and water balance (Yokota 1984), but seasonal changes have not been examined.

Scorpions must also avoid gaining and retaining too much water. The rate of water loss increases with feeding (Cloudsley-Thompson 1961, King and Hadley 1979) and decreases with starvation (King and Hadley 1979). Animals not under water stress do not behaviourally nor physiologically decrease water loss (King and Hadley 1979).



### 1.5.3 Reproduction

Males actively seek out females during the mating season (Benton 1992), the timing of which is mostly species-dependent but occurs during the warmer months. Mortality of males can be high at this time due to predation, mate cannibalism or failure to construct a burrow or seek suitable shelter by dawn to escape the adverse daytime conditions (Polis and Farley 1979a). Male feeding rates drop (Polis and Farley 1979a) and the increase in metabolic rate associated with roving activity (Crawford and Riddle 1975, Fiddle 1978) can lead to the depletion of metabolic stores. A further disadvantage is that males often have higher mass-specific RMRs than females due to their smaller size where sexual dimorphism exists (Hadley and Hill 1969, Padmanabhanaidu et al. 1984). The smaller size may also result in a greater rate of water loss. Males must therefore ensure a successful mating to offset their potential removal from the population.

Reproduction in females can have profound influences on their behaviour and physiology especially when the time interval between fertilisation and parturition (birth) can be large, depending on the species. Female mass increases dramatically as they store reserves in their hepatopancreas, the main source of nutrition for the developing young. Scorpions are divided into two groups based on the process by which the young receive nutrients. The families Bothriuridae, Buthidae, Chactidae, Chaerilidae, Iuridae and Vaejovidae are apoikogenic because some yolk is produced with the ova but the young still acquire some nutrients through embryonic membranes (Francke 1982). The families Diplocentridae, Ischnuridae and Scorpionidae are katoikogenic, the ova having no yolk and the young obtaining all their nutrients from the mother (Francke 1982).

Pre-mating females may increase their feeding activity above that of males (Benton 1992) but may seek to reduce energy expenditure at the same time to maximise their reserves for embryonic development. The greatest increase in size of the embryos occurs when temperatures are high during spring and summer (Bradley 1984), although most of the size increase immediately preceding birth is due to water uptake (Toolson 1985). Mothers that feed more during the months before birth have larger and further developed embryos but the same brood size than otherwise (Polis and McCormick 1987), although this is not always the case especially when there exists a large variation in embryo brood mass and size between females (Bradley 1984).

The time of birth is largely species dependent, but most frequently occurs during warmer months. The rate of embryonic development in ectotherms is dependent upon temperature and gravid females may seek warmer parts of the microhabitat to speed development. Oocyte growth in the apoikogenic *L. quinquestriatus* was accelerated by higher temperatures. Gravid females have greater mass-specific RMR than males (Dresco-Derouet 1964) and the increase in the rates of *D. peloncillensis* females from mid to late winter may be due to increasing embryonic development (Crawford and Riddle 1975).

An increase in female mass would be accompanied by a decrease in her surface area to volume ratio and an increase in the exposure of the intersegmental membrane. If the intersegmental membrane has the same permeability as the sclerite cuticle as for the desert scorpion *H. arizonensis* (Hadley and Quinlan 1987), then the mass-specific rate of water loss would decrease with the increase in size (Toolson 1985). However, if the membrane is more permeable as was the case for the mesic *P. imperator*, then the rate of water loss may stay the same or actually be greater than a non-gravid female. If the latter were the case, then a gravid female may have to adjust her behaviour to reduce transcuticular evaporation by selecting more mesic parts of her microhabitat.

Parturition results in a decrease in female surface and feeding activities as the mothers keep the young in the less fluctuating and more mesic conditions of the burrow and away from predators (Benton 1991, Torres and Heatwole 1967a, Ugolini et al. 1986, Vannini et al. 1978). Her inactivity also lessens the chances of losing young off her back (Vannini et al. 1978). The timing of the first moult and the dispersal of the young is dependent on temperature and the seasonal timing of birth (Smith 1966). Second instar young may remain with the mother over the cold months and in some species the young may stay with the mother for much longer as she actively hunts for them (Mahsberg 1990). The hepatopancreas of a post-partum female is often much reduced in mass and the female needs to feed to increase her reserves especially under stressful conditions. If the young also need to feed, they may do so from her captured prey thus reducing her intake. A female then needs to be able to conserve her meagre metabolic stores by either metabolically compensating or selecting cooler parts of the microhabitat.

### 1.5.4 Growth

Size can greatly influence the life of a scorpion. The selection of prey items may depend on the instar stage, generally the earlier the instar the smaller the prey although there is considerable overlap (Polis 1979, Polis 1984). If there are seasonal or diurnal shifts in the general size of prey available (instars or small prey species) then the feeding activities of smaller instars may be different from older instars. Where cannibalism and intraguild predation occur, the smaller individuals are most often preyed upon by larger scorpions (Polis 1980a, Polis 1981, Polis and McCormick 1987). Cannibalism can be avoided by different instars being active at different times of the night (Polis 1980b). Predatory behaviour may change with growth, for example, the early instars of *Pandinus imperator* most often sting their prey while adults will only subdue prey with the pedipalps (Casper 1985). Often there is sexual dimorphism in size, most commonly the male is smaller than the female of same age. Males, however, usually feed upon the same prey species as females and have the same feeding rates (except during times of roving or birth) although they are more general in their selection (Polis 1986). Such sexual dimorphism can result in mating cannibalism though (Polis and Farley 1979a).

The smaller the animal the greater its surface area to volume ratio and the greater the mass-specific rate of water loss. The body temperature of small animals is more susceptible to ambient temperature changes and mass-specific metabolic rate is greater than that of larger animals. As such it would be expected that the earlier the instar the less the saturation deficit of its selected burrow or shelter would be. However, scorpion home site selection appears to be independent of size with regards to microclimate parameters. Smaller scorpions may actually be disadvantaged by their smaller size and have less favourable home sites than older larger scorpions if competition for home sites exists. Rock size affects the burrow temperature below it, larger rocks buffer against fluctuations in temperature better than small rocks. Most scorpions are anti-social resulting in competition for appropriate home sites. Thus larger scorpions will compete more successfully for rocks due to their size depending on whether rocks are a limiting resource. In addition, early instars of open surface burrowing species have shallower burrows and thus spend more time at higher temperatures than older instars that can burrow down to a more constant and favourable temperature (Shorthouse 1971).

Intraspecific differences in size are reflected in the distribution of species of the genus *Urodacus*, desert species being usually larger than more temperate species. The trend is less distinct across all the world's species with some of the largest species in the world occurring in both rainforest and desert regions.

Scorpions grow by periodic moulting of the cuticle immediately followed by expansion in body size. The number of moults to adulthood is species-dependent, ranging from 4 to 9 with the ages ranging from 6 to 83 months (Poole and Sissom 1990). Moulting disrupts the normal functions of the animal, the new cuticle being without pigment, very soft and easily damaged. Feeding activities are stopped several days before and after ecdysis (Le Berre 1979, Yusubov 1991) and burrow construction by second instars is delayed even after dispersal (Williams 1966). It is a period of high mortality (especially during the first moult) in the laboratory (Francke 1976, Francke and Jones 1982, Sissom and Francke 1983). Shorthouse and Marples (1982), however, suggests that mortality in the field is low due to protection from predation in the burrow. The stable microclimate of the burrow also reduces water loss because the permeability of the new cuticle is higher than intermoult tanned cuticle (Crawford and Wooten 1973, Francke 1976, Riddle 1978).

The increase in size during moulting is not large in scorpions due to the ineffectualness of the book-lungs to volumetrically expand the body after ecdysis (Francke 1976). The number of moults to the adult stage are low in number though to decrease the amount of time the scorpion is susceptible (Francke 1976).

## **1.6 *Urodacus manicatus***

The family Scorpionidae consists of three subfamilies: Urodacinae, Hemiscorpiinae and Scorpioninae. The subfamily Urodacinae is endemic to the Australian mainland and is monotypic, the genus *Urodacus* containing nineteen species (Koch 1977 and 1981). *Urodacus manicatus* is a darkly coloured species (Plate 1.1), the colouration due to both dark cuticle (fingers and aculeus) and epidermal pigmentation (tergites) (Locket 1986). Its distribution covers south-eastern Australia ranging from Yarram, Queensland, to Yorke Peninsula and Kangaroo

Island, South Australia (Figure 1.1a). It is chiefly found along the Great Dividing Range and Mt Lofty Range in areas of winter rainfall and its distribution partly overlaps that of *U. elongatus* and *U. armatus*. Koch (1977), in an attempt to explain the distribution of scorpion species in Australia, compared his distribution maps with those of rainfall and temperature and concluded that *U. manicatus* occurs in regions of 350-1000 mm and 12-18°C. The small size of this species relative to other members of Urodacinae may be a reflection of its temperate distribution, the more xeric species being the largest of scorpions in Australia (Koch 1981)

The construction of burrows is typical of all *Urodacus* species and are usually one of two types: shallow burrows under ground objects or deep spiralling burrows in open ground (Koch 1978). *U. manicatus* builds shallow burrows beneath shelters which in most cases are rocks that are reasonably well embedded and with bare soil below (Koch 1981, Smith 1966, Southcott 1954, Willmer 1967). At Black Mountain, Australian Capital Territory, Smith (1966) reported that the population there constructed burrows under rocks of ground cover 36 to 71 cm<sup>2</sup> in the following pattern: a "runway entrance" from the edge of the rock that opened into an expanded excavation (the "living area") from which ran a short burrow, the size of which was related to body size. Not all scorpions were found with all three components of the home site present. Smith (1966) concluded that those animals with both a living area and a burrow made up the stationary portion of the population. The 20% that lacked a burrow had recently become stationary and the 12% that lacked both a burrow and a living area were considered to indicate that this proportion of the population were mobile at any one time. Smith (1966) measured home site rock sizes and found the majority of rocks were in the size class range of 36-71 cm<sup>2</sup>. The selection of rocks at the lower limit of 23 cm<sup>2</sup> was not related to season or age (Smith 1966) and may have merely reflected availability.

*U. manicatus* is ideal for examining seasonal and habitat effects on terrestrial arthropod physiology and behaviour. Its wide distribution covers a range of habitat and seasonal climatic variety, the populations of which can be expected to exhibit different degrees of acclimatisation and behaviour. This species is easily captured by lifting selected shelters and rarely is digging necessary. Maintenance is simplified by its small size for housing and infrequent and non fastidious feeding. Specimens can be found during all seasons and are abundant in the right localities. Like most scorpions, the sexes are distinct at all instars and thus can be considered

separately for experiments. Southcott (1954) reported that the sexes could be distinguished by the appearance of the dorsal surface of the abdomen, that of males being dull grey and finely granular, whereas females' had darker, more smoothed and polished abdomens. I found, however, that a reasonable initial guess could be made by examining the general shape of the animal (males being slender and more cylindrical than females) and then confirming by examining the pectines (males' are larger and with a greater number of pectinal teeth), the genital operculum (divided in males) and, in mature males only, the presence of a pair of papillae beneath the operculum (Plate 1.2).

## 1.7 Aims

In this study, I examine some of the questions raised previously in this introduction by examining a "door-keeping" scorpionid of wide distribution, *Urodacus manicatus*. The majority of studies on the environmental physiology of scorpions have been centred on those that inhabit desert regions with potentially more severe microhabitats. Few studies have examined scorpions that are more temperately distributed (Hadley 1990, Punzo 1991, Warburg and Ben-Horin 1978, Warburg et al. 1980a). Similarly, "door-keeping" foraging species have received less attention than the surface foragers (Benton 1992) probably because the nocturnal activities of surface foragers are more readily examined under a UV light and are easily trapped by pit-traps outside the burrow or along lines. Studies on the microclimates of scorpion burrows have also been centred on species whose burrows are constructed in open soil, e.g. (Hadley 1970a, Shorthouse 1971). Few studies have been made on the microclimates of shallow burrows beneath shelters. The effect of shelters with different thermal properties on the profiles of burrow temperature may be different to the effect of soil temperature on burrow temperature at different depths.

The major aim of this thesis was to study the physiological and behavioural responses of *Urodacus manicatus* to changing abiotic and biotic factors that impact upon this species. In particular, I wanted to study seasonal changes in the microclimates of the scorpions as well as spatial and temporal differences in scorpions' responses. Manipulative experiments in the laboratory were then performed to determine which factors were responsible for the observed

differences in adaptations in field-collected specimens. I have presented six results chapters and a final discussion chapter in this thesis.

To accomplish the aims of the present study, I examined the following aspects of the environmental physiology of the scorpion *Urodacus manicatus*.

1. I examined the microhabitats and microclimates of two populations from different climatic regions in Chapter 3 and addressed the following questions:
  - (a) Do seasonal changes in microclimate differ between the populations?
  - (b) Do seasonal changes in the rates of evaporative water loss, body water content, haemolymph osmolality differ between the populations?
  - (c) Do seasonal changes in metabolic rate, temperature selection and activity differ between the populations?
  - (d) What is the supercooling point of scorpions from the more xeric parts of the distribution?
  
2. Temperature is an abiotic factor. In Chapter 4, I determined the extent of thermal acclimation in *U. manicatus* by asking:
  - (a) Does *U. manicatus* metabolically compensate to temperature change?
  - (b) Do temperature selection and activity change in response to different acclimation temperatures?
  - (c) Can the rate of evaporative water loss be adjusted in response to different acclimation temperatures?
  
3. Water availability can be treated as either an abiotic (free or substrate water) or, more importantly to scorpions, as a biotic factor (prey water). However, substrate water is more easily controlled than prey water. The effect of water availability on *U. manicatus* was examined by addressing the following questions:
  - (a) Does metabolic rate change in response to different levels of hydration?
  - (b) Do temperature selection and activity change in response to different levels of hydration?

- (c) To what extent can *U. manicatus* regulate their haemolymph osmolality?
  - (d) Can the rate of evaporative water loss be adjusted in response to different levels of hydration?
4. I examined the effects of nutritional state (a biotic factor) on *U. manicatus* in Chapter 7 by asking:
- (a) Does metabolic rate change with feeding and starvation?
  - (b) Do temperature selection and activity change with feeding and starvation?
5. The biotic factor of reproduction was examined in Chapter 7 by addressing the following questions:
- (a) How large is the energetic cost of reproduction in the last months of gestation?
  - (b) Do temperature selection and activity change with gestation and parturition?
  - (c) How large is the somatic cost of reproduction in the last months of gestation?
  - (d) What are the relationships between offspring mass, litter mass, litter size and female somatic mass?
  - (e) Does an increase in size with gravidity affect evaporative water loss?
6. In Chapter 6, I investigated other biotic effects on evaporative water loss by asking:
- (a) Does moulting result in an increase in cuticle permeability?
  - (b) Do diurnal and nocturnal rates of evaporative water loss differ?
  - (c) What are the relationships between size and evaporative water loss for female and male *U. manicatus*?
  - (d) What is the relationship between instar stage and evaporative water loss?

In Chapter 9, I present the final discussion for the thesis and discuss the conservatism of the physiological traits of scorpions with regard to their environment.





a



b

Plate 1.1. Dorsal views of adult (a) female and (b) male *Urodacus manicatus*. Scorpions shown 2 times actual size.



Plate 1.2. Ventral views of female (left) and male (right) *Urodacus manicatus* of same mass. Male is adult and female is sub adult. Note split genital operculum (go) and larger pectines of male (p). Scorpions shown 1.6 times actual size.



Figure 1.1. Maps showing (a) the distribution of *Urodacus manicatus* in Australia (from Koch 1977) with inset (b) showing locations of a cool temperate population at Black Mountain and a more xeric population in Goonoo State Forest near Dubbo, both in New South Wales.

## Chapter 2

### General Materials and Methods

#### 2.1 Animal Collecting and Maintenance

Scorpions were hand-collected during the day by moving the home site shelters to one side to reveal the burrow and scorpion. Specimens remained placid if handled by grasping the telson with the thumb and forefinger and extending the second finger of the same hand to provide a surface for the animal to stand on. Animals were placed individually into collecting jars of appropriate size. The scorpions were transported back to the Department of Zoology at the University of New England as soon as possible. Respiratory measurements were delayed for at least one day to avoid elevated oxygen consumption rates associated with excitement from transport and handling (Riddle 1978) and to control for any short-term starvation or digestion effects (Riddle 1979). Attempts were made to catch or study movements of *U. manicatus* at night utilising their fluorescence under UV light (Honetschlager 1965, Stahnke 1972). However, due to the grassy and herbaceous habitat, the small size of this species and their infrequent excursions away from the burrow, animals were very rarely found and thus the method proved infeasible.

Early in the research scorpions were kept individually in rectangular white plastic takeaway food containers (16 x 10 x 6 cm) and provided with soil to the depth of 1 cm and with a moistened pledget of cotton wool for water. The cotton wool was regularly replaced to prevent moulding. Thick black card was placed at one end of the container to provide cover. Such preparations were later regarded as being unnecessary and to conserve space and encourage prey location I resorted to the use of clear disposable plastic drink cups with a cover of soil in the bottom and a moistened pledget of cotton wool. The scorpions retained vigour for a long time under these basic conditions. Tenebrionid larvae of an unknown species were frequently found as prey items in the field, thus larvae of the black flour beetle *Tenebrio molitor* (Coleoptera: Tenebrionidae) were provided *ad libitum* in captivity but a feed of one or two sufficed for sometimes up to two months at 20°C. Larvae that had not been killed after a week were replaced

with new larvae. Colonies of the flour beetle were maintained in large plastic food storage containers with bran and pollard as the medium. Vegetable matter (mostly lettuce, potato peelings and sliced apple) were placed upon a mesh on top of the medium. The colonies required very little maintenance. Occasionally other insects such as scarabaeid beetles and cockroaches were provided for scorpions being kept long term. Remains and dead larvae were removed as soon as possible to avoid fungal growth. General cleaning was only required every two months to remove faeces from the sides of the container and to provide fresh soil. The containers were kept in temperature and photoperiod controlled cabinets ( $\pm 0.5^\circ\text{C}$ ).

## 2.2 Evaporative Water Loss

Food was withheld for 48 hours prior to the commencement of measurements to reduce faecal production (Hadley 1970b) except for seasonal studies when weighing commenced immediately once transported back to the laboratory. Daily water loss was measured under 0% relative humidity by placing scorpions individually into plastic jars with diameters accordant to the length of the animal to minimise movement. The jars were then placed into large glass desiccating jars containing silica gel which were kept under constant temperature and 12:12 photoperiod in temperature controlled cabinets ( $\pm 0.5^\circ\text{C}$ ). The animals were weighed at the same time daily 0.1 mg. Scorpions that defecated during the course of the experiment were excluded from analysis. Water loss was expressed as  $\text{mg H}_2\text{O g}^{-1} \text{h}^{-1}$ . Daily weighing ensured that the measurements of early instars were not erratic due to insensitive recordings obtained over small time periods (Crawford and Wooten 1973).

This method has been used in many studies on water loss in scorpions and even where differences in technique exist, comparisons between species are still useful (Hadley 1970b, Hadley 1990). The ease at which the experiments were set up made this method a more viable option especially for examining changes in permeability within the same species. Live animals were used in all experiments to enable comparisons to be made with the literature and with parts of this thesis whereby the animals were needed alive for further manipulation. By placing a thermohygrometer (Hanna instruments model HI 8564) wholly within the desiccator, 0% RH was

found to be rapidly re-obtained within five minutes when resealed after the weighing of animals. Beament (1958) pointed out that the temperature of the cuticle should be determined when considering cuticular permeability. Cuticle temperature is usually lower than that of the air due to the equilibrium set up between heat loss by evaporation and heat gain from the surroundings by conduction and convection. A "typical" insect evaporating water at  $0.1 \text{ mg min}^{-1}$  would have a cuticle-air temperature difference of the order of  $1^\circ\text{C}$ . *Urodacus manicatus* lost water at magnitudes 2 to 3 times smaller, thus air temperature was a good approximation of the cuticle temperature for a scorpion at equilibrium.

Initial measurements indicated that the amount of water lost on the first day greatly exceeded that lost on succeeding days. For this reason, water loss rates were calculated from second day measurements. Hadley (1970b) attributed initial high rates of water loss to water absorbed into the cuticle and water loosely bound to cuticular protein and lipid molecules.

Water loss can also be expressed as the mass of water lost per unit surface area of cuticle per time ( $\text{mg H}_2\text{O cm}^{-2} \text{ h}^{-1}$ ). This measure is assumed to be the permeability of the cuticle as water loss via respiration is negligible at ordinary temperatures (Crawford and Wooten 1973, Hadley 1970b, Robertson et al. 1982, Warburg et al. 1980b). Surface area scales allometrically to wet mass according to the equation

$$\text{SA} = a \text{ mass}^b.$$

When surface area is expressed in  $\text{cm}^2$  and body mass in g, the coefficient  $a$  becomes  $k$ , a species-specific constant ( $\text{cm}^2 \text{ g}^{-1}$ ) (Cloudsley-Thompson 1959). Since the use of planimetry to measure the surface area of a scorpion's cuticle may produce large errors due to the complex shape of the animal and the inability to flatten all parts of the animal out completely, another technique was tried. An attempt was made to determine  $k$  for *U. manicatus* by first deriving the relationship between cuticle mass and cuticle surface area from which the relationship between body mass and surface area could then be determined.

Soft tissue was dissolved by placing freshly killed specimens in boiling 10% KOH until only the cuticle remained. A leather hole punch, 3 mm in diameter, was used to remove discs across

both sclerite and intersegmental membrane cuticle to take into account the variability in cuticle thickness. A sample of 60 discs were collected equally from the dorsal and ventral surfaces of the mesosomas of 15 specimens. Only adults that were not distended by feeding or gravidity were used to ensure the discs of cuticle were as flat as possible. The relationship between cuticle mass and surface area was then determined from the mean mass of the cuticle samples and their calculated surface area; 1 g of cuticle being equivalent to 207.9 cm<sup>2</sup> with the implied assumption that cuticle thickness was a constant.

*U. manicatus* exhibit sexual dimorphism in body shape from the second instar with males having a smaller cross-sectional area than females, therefore I decided to test females and males separately as a check of the capacity of cuticle mass to predict surface area. Logarithmic transformation of the surface area - mass equation results in the following linear relationship:

$$\log SA = \log k + b \log m.$$

Thus gender comparisons could then be made by a single factorial analysis of covariance with log wet mass as the covariate and log SA as the dependent variable. Log wet mass met the criteria as a suitable covariate. After adjustment was made for log mass, males and females differed significantly in predicted surface area,  $F(1, 59) = 9.37, P < 0.01$ . However, the regressions of surface area on mass determined for both sexes yielded low coefficients of regression with concomitant high standard errors. The logarithmic relationship between female SA and mass was:  $\log SA = 1.071 (\pm 0.027) + 0.818 (\pm 0.160) \log m, r^2 = 0.384, F(1, 42) = 183.27, P < 0.001$ . The logarithmic relationship for males was:  $\log SA = 1.203 (\pm 0.045) + 0.710 (\pm 0.287) \log m, r^2 = 0.277, F(1, 16) = 7.81, P < 0.05$ . The allometric relationships were thus

females:  $SA \text{ (cm}^2\text{)} = 11.78 m^{0.818} (\frac{2}{3})$  and

males:  $SA \text{ (cm}^2\text{)} = 15.96 m^{0.710} (\frac{2}{3})$ .

The large standard errors of the estimates made comparisons of the slopes with 0.67 (as predicted by the surface area to mass law) unreliable,  $t(44) = 0.93$  for females and  $t(18) = 0.14$  for males,  $P > 0.05$  for both. The proportionately larger cuticle mass of males was probably responsible for their larger value of  $k$  than that obtained for females.

The method of estimating surface area directly was resorted to by stretching the cuticles obtained above over fine grid paper. Sex differences between the log surface areas adjusted for mass were again tested by means of a single factor analysis of covariance. The adjustment made to each sex was highly significant, females  $t(44) = 13.36$ ,  $P < 0.001$  and males  $t(18) = 3.26$ ,  $P < 0.01$ . Sex had no significant effect on surface area after adjustment for mass,  $F(1, 59) = 1.71$ ,  $P > 0.05$ . The result of the combined logarithmic regression was:  $\log SA = 1.096 (\pm 0.005) + 0.629 (\pm 0.032) \log m$ ,  $r^2 = 0.863$ ,  $P < 0.001$ ,  $n = 62$ . The slope was not significantly different from 0.67,  $t(62) = 1.28$ ,  $P > 0.05$ . The allometric equation then relating surface area to mass was:

$$SA \text{ (cm}^2\text{)} = 12.47 m^{0.63} \text{ (g)}.$$

This value of  $k$  conforms to that generally obtained for other species.

## 2.3 Oxygen Consumption

Resting metabolic rate (RMR) was measured as the oxygen consumption ( $VO_2$ ) of animals at rest at metabolic chamber temperature  $T_a$ .  $VO_2$  was measured in an open flow through system with an Applied Electrochemistry S-3A/II oxygen analyser and traced by a Lloyd Graphics 2002 recorder. The metabolic chambers were made from 20 ml plastic syringes with a single hole of 1 mm diameter drilled through the plunger of each syringe to provide an inlet for air with the outlet through the syringe nozzle. The twin channel analyser enabled two animals to be measured simultaneously. Two metabolic and two blank chambers were constructed and bundled together within a large clear plastic container which had air pumped into it from outside the building. The chambers were contained within a temperature controlled cabinet. The  $T_a$  of each chamber was measured to 0.1°C by inserting a calibrated thermocouple through the plunger hole of each



metabolic chamber and attaching them to a hand held electronic thermometer (Omega Model HH-71 T). Each metabolic chamber was linked to its corresponding blank chamber by a Y-type connector which then passed the air through a syringe containing silica gel to remove water before the analyser. Switching between the air from the metabolic and blank chambers was achieved by alternately clamping and unclamping aquarium hose clamps placed on each line above the Y-type connector outside of the cabinet. To obtain even flow the air from the analyser was passed through large glass jars before the pump. Low flow rates ( $10\text{-}15\text{ ml min}^{-1}$ ) were required to obtain a measurable change in oxygen content between the metabolic and blank chambers. Once stable zero traces were obtained from the blank chambers, the lines were switched over to the metabolic chambers and new steady traces reflecting a lower oxygen content were allowed to stabilise after peaks resulting from the flushing out of the chambers.  $\dot{V}O_2$  measurements were calculated from the percentage differential of  $O_2$  between the outside air (control) and from air from the animal chambers according to Withers (1977). All measurements were corrected to standard temperature and pressure.

Each scorpion was allowed 45-60 minutes to come to rest after placement in the chamber and to equilibrate its body temperature to that of the chamber. Scorpion body temperature was found to equalise rapidly with  $T_a$  (Section 2.4). A covered hole at the front of the cabinet enabled periodic observations to be made on the animal and times of movement and rest were noted on the chart. Scorpions have a highly characteristic stance when at rest in the relaxed immobility state (Tobler and Stalder 1988); thus frequent observations of the animals assured that RMR was measured. The effect of temperature on  $\dot{V}O_2$  was determined by measuring  $\dot{V}O_2$  at the highest temperature first. Control air was measured during the time  $T_a$  was dropped to the next preset value as a check for any drift in the zero traces.

All measurements of oxygen consumption were conducted during the day when activity was lowest. Some measurements were made at night but there was no evidence of any diurnal fluctuation in RMR as in other scorpions (Hadley 1970b, Hadley and Hill 1969, Riddle 1979).

## 2.4 Temperature Selection

An eight track temperature gradient was used in all experiments (Figure 2.1). This was constructed from aluminium guide tracks for sliding doors measuring 25 mm wide and 1 m in length. The leading edges were pinched towards one another so as to produce a 2 mm gap running the length of the gradient. Strips of sandpaper were placed along the track floor to provide traction for the scorpions. The tracks were secured together and placed upon stands. One end of the apparatus was heated from below by three ceramic heat lamps to maintain a maximum temperature of 70°C within the track. The other end was cooled from below by coiled copper tubing that was connected to a temperature controlled waterbath. A 1:1 mix of deionised water and glycerol was circulated through the tubing. In preliminary trials the temperature of the water was set so that the minimum temperature in the gradient was at 1°C. It became apparent that scorpions that travelled to this end would become too cold so as not to be able to move away again. The temperature was consequently raised to 6°C and this alleviated the problem. The gradient was switched on at least one day prior to measurement to allow the temperature gradient to stabilise. Animals were introduced to the track via the cooled end and encouraged to move midway along the track. Normally it would then move towards the hot end until the high temperature forced the animal to turn back. Up to eight individuals were run at a time, one scorpion per track. Scorpions were usually placed into the gradient in the morning, allowed to run overnight and removed the next morning. The room in which the gradient was contained was photoperiod controlled.

Hadley (1970a) was able to insert a 30 swg thermocouple beneath the dorsal surface of the metasoma into the body cavity of *Hadrurus arizonensis* and sealed it with nail polish. The small size of *U. manicatus* (5-6 cm compared to 7-10 cm for *H. arizonensis*) and the lack of a suitable orifice prevented the insertion of a fine thermocouple to measure true body temperature directly. Insertion beneath the cuticle resulted in the excessive loss of haemolymph and internal damage resulting in a loss of vigour and eventually death. Stabbing and bleeding induces thirst in flies thus altering their behaviour (Barton-Browne 1964). As an alternative, temperature selection was measured by attaching a calibrated vinyl coated copper-constantan thermocouple (42 swg) to the dorsal side of the scorpion's mesosoma which was connected to a eight channel data logger. The

thermocouple was secured to the scorpion by small strips of band-aid. To facilitate this, a thin strip of aluminium foil was glued perpendicular near the junction of the thermocouple to provide anchorage. The junction could then be held flat to the dorsal side of the scorpion and the band-aid strips placed either side and across the thermocouple. This was found to be the most satisfactory means of attachment. Many attempts were made with various waxes, glues and rosin but the thermocouples were either easily removed by the scorpion or simply came loose over the course of a measurement. These methods were also time consuming and awkward. The band-aid strips conformed with the animal's surface which aided adhesion but could easily be peeled off at the end of a run. The thermocouples were suspended 1 m above the gradient with enough slack to allow the animals to move freely but prevent tangling. Temperatures were measured every 15 minutes. The measured temperature data were corrected for each thermocouple by applying regression equations that equated thermocouple temperature with real temperature. To ensure that the scorpions were settled in the gradient, only data from 15:00 h on (i.e. approximately 6 hours after measurements began) were analysed. Data were blocked into 3 hour periods to simplify analysis: 15:00-18:00 (I1), 18:00-21:00 (d1), 21:00-24:00 (d2), 24:00-03:00 (d3), 03:00-06:00 (d4) and 06:00-09:00 h (I2). The photoperiod in all experiments was L12:D12, thus analyses covered a period of photophase before lights out (I1), then four scotophase periods (d1-d4) followed by a single photophase period in the morning (I2).

By tying one thermocouple to the metasoma, inserting another into the mesosoma and releasing the desert species *Hadrurus arizonensis* down its deep burrow, Hadley (1970a) found that body temperature ( $T_b$ ) was equal to or slightly below (less than  $0.5^\circ\text{C}$ ) the burrow air temperature, the greater mass of this species perhaps responsible for the lag in  $T_b$ . Also, Cabanac and Le Guelte (1980) found that the body temperatures of *Buthus occitanus* and *Androctonus australis* (both larger than *U. manicatus*) followed within seconds of the animals' displacements in a temperature gradient. The cuticle surface temperatures of small ectotherms are often the same as core body temperature (Anderson et al. 1979, Casey 1976). A trial was therefore performed to test whether dorsal cuticle temperature ( $T_c$ ) was a valid measure of body temperature while *U. manicatus* was in the temperature gradient. Four animals were placed into the gradient with a thermocouple attached dorsally as described above and another inserted into the mesosoma latero-dorsally to avoid puncturing of the heart. A small drop of haemolymph was always lost

when the thermocouple was inserted, but the placing tape over the site of insertion prevented further body fluid loss during the course of the trial. Temperatures were recorded every 15 minutes.  $T_b$  was always higher than  $T_c$ . The mean temperature difference ( $\pm$  SE) was plotted against time of day (Figure 2.2). The hotter floor surface heated the body by conduction from below while  $T_c$  depended on the heat flow from the body to the slightly cooler air temperature 1 cm above the floor. The maximum mean difference during the period of most activity was  $1.50^\circ\text{C}$  ( $\pm 0.16$  SE) and the minimum was  $0.59^\circ\text{C}$  ( $\pm 0.12$  SE). A maximum individual difference of  $2^\circ\text{C}$  was experienced by a larger specimen (1.60 g) immediately after moving to a warmer region of the gradient thus a lag existed between  $T_b$  and  $T_c$ . Thus the largest differences in temperature occurred when moving between ambient temperatures and the smallest temperature differential was closer to the steady state. It was not deemed necessary to adjust  $T_c$  to gain  $T_b$  because relative changes in selected temperatures were sought after, not absolute values of  $T_b$ . Thus all temperatures from the gradient studies will be referred to as being the "selected temperature".

Another trial was performed to test whether the attachment of a thermocouple affected movement and temperature selection. Adult females were used. Thermocouples were attached to four individuals which were placed in alternate tracks. Another four were placed in the remaining tracks without thermocouples and allowed to run for 9 hours including 3 hours dark. The data logger was set to measure every 30 minutes at which time the animals were examined and the dorsal temperatures of all scorpions were measured with a thermocouple connected to a hand held electronic thermometer (Omega Model HH-71 T) so that all were equally disturbed. A single factor analysis of variance was performed on two repeated measure dependent variables associated with 3 hours light and 3 hours dark. The independent variable was presence/absence of a thermocouple. Wilk's criterion indicated that the thermocouple did not significantly affect the combined repeated measures,  $F(2, 5) = 0.04$ ,  $P > 0.05$ . A similar analysis on activity revealed no significant effect of the thermocouple on levels of activity,  $F(2, 5) = 0.62$ ,  $P > 0.05$ . It may be concluded then that the attachment of a thermocouple to a scorpion's dorsal surface did not interfere with normal activity within the gradient nor affect the animal's temperature sensory capabilities. This fits the finding of Alexander and Ewer (1958) who found no evidence of temperature receptors on the dorsal surface of *Opisthophthalmus latimanus*.

## 2.5 Activity

Activity was a relative measure obtained from the temperature selection data with 15 minute intervals. A score was given for every reversal in the direction of increasing or decreasing temperature change that was greater than 5°C. That is, for every set of three consecutive temperatures recorded, if the second value was either higher or lower than both the first and third values and the difference from the third value by more than 5°C, then a score was given. The sum of these scores was calculated per animal for each time period. Where activity was plotted every 15 minutes, the sum across the sample was used. The calculation of activity was not intended to reflect the amount of activity of a scorpion in the field. Rather it was used as a means for gauging when the animal's were in an alert immobility state (Tobler and Stalder 1988) and to indicate the relativity of change in activity between subjects. Maximum and minimum temperatures recorded within the gradient reflected the extent to which animals traversed the gradient and their general sensitivity to temperature. Therefore, the maxima and minima were estimates of the high and low avoidance temperatures.

## 2.6 Statistical Analyses

All computer statistical analyses were performed with SYSTAT Version 5.01 copyright, 1990-1992 SYSTAT, Inc. The following abbreviations were used: (M)ANOVA = (multivariate) analysis of variance and (M)ANCOVA = (multivariate) analysis of covariance. All logarithmic transformations are base 10 unless stated otherwise. In the covariate analyses the covariates were tested for their suitability by testing the homogeneity of slopes before analysis. This is the test of non-significance of the constructed between subjects interaction terms of the independent variable(s) and the covariate(s). The term 'repeated measures' is used here to describe subjects that have been measured repeatedly with time on the same dependent variables. Profile analysis refers to the application of MANOVA to the repeated measures (Tabachnick and Fidell 1989). Descriptions of all statistical procedures used in the thesis are given where appropriate within each chapter. All means are presented with standard errors ( $\pm$  SE).

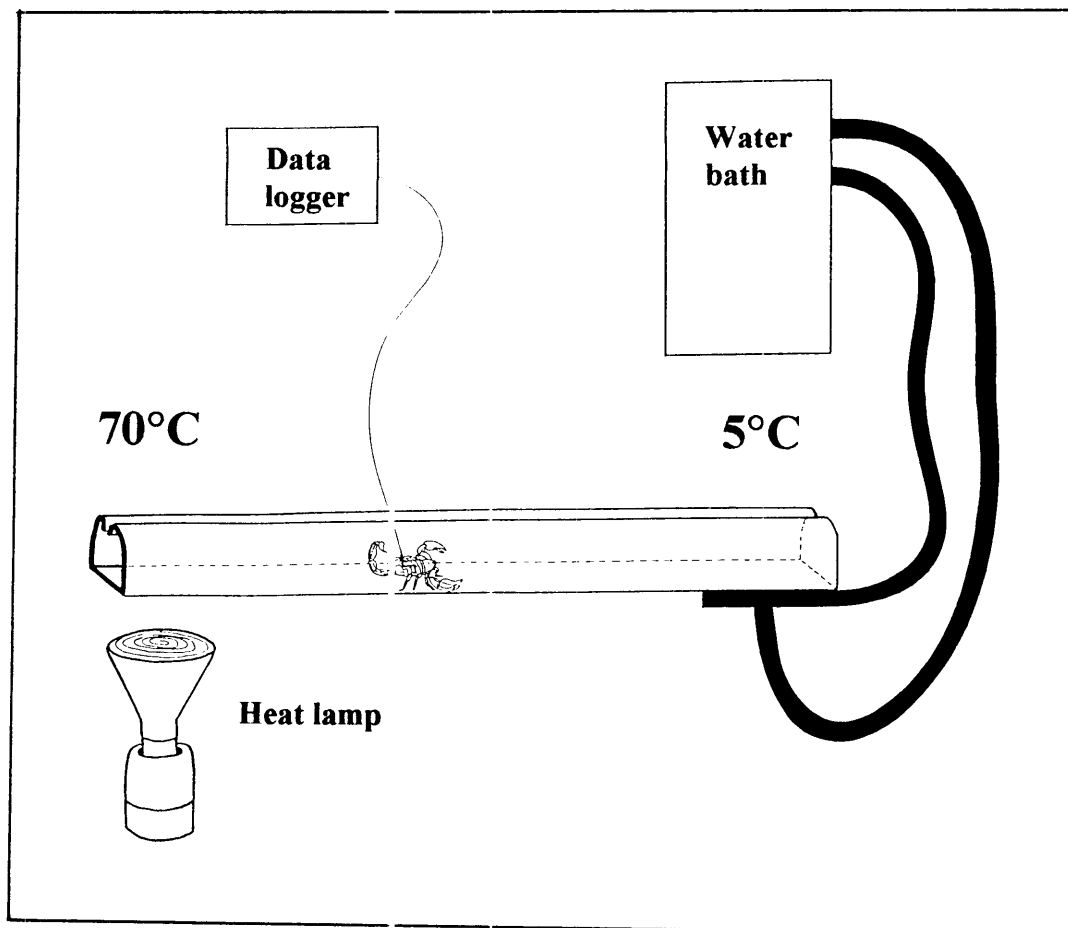


Figure 2.1. Temperature gradient apparatus used for measuring temperature selection in *Urodacus manicatus* showing one track of eight.

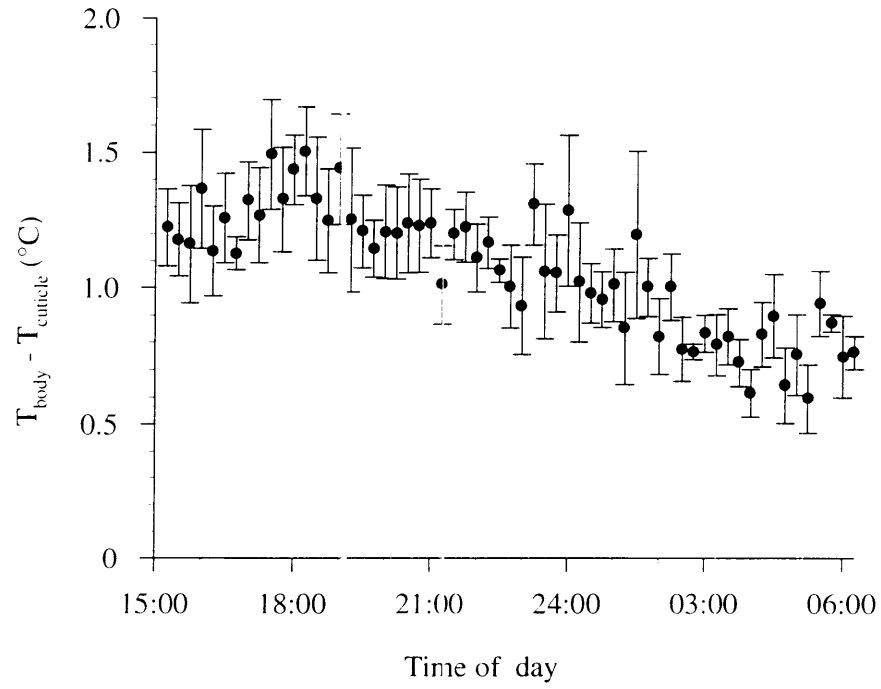


Figure 2.2. Difference between body temperature ( $T_{\text{body}}$ ) and dorsal cuticle temperature ( $T_{\text{cuticle}}$ ) of *Urodacus manicatus* measured in the temperature gradient apparatus over time. Values are means  $\pm$  SE for  $n = 4$ .