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# OSMOREGULATION AND TEMPERATURE EFFECTS ON WATER LOSS AND OXYGEN CONSUMPTION IN TWO SPECIES OF AFRICAN SCORPION

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**Abstract**—1. Aspects of the physiology of two southern African scorpions have been examined. The scorpions are the large desert species *Parabuthus villosus* (Peters) (Buthidae) and the more mesic, burrowing species *Opisthophthalmus capensis* (Herbst) (Scorpionidae).

2. Evaporative water losses were higher in *Opisthophthalmus* at all temperatures.

3. Analysis of haemolymph during prolonged desiccation showed good osmotic and ionic regulation in *Parabuthus* but no regulation in *Opisthophthalmus*.

4. Oxygen consumption of *Parabuthus* was measured after acclimation to 10 and 30°C. Metabolic rates were extremely low but there was no metabolic compensation to increased temperatures.

## INTRODUCTION

Scorpions are a conspicuous feature of desert environments and their adaptations to desert life have been reviewed by Hadley (1974) and Cloudsley-Thompson (1975). Behavioural adaptations are particularly important: scorpions generally avoid high temperatures and low humidities by retreating into burrows during the day and appearing on the surface only at night. As a result of impermeable cuticles and low metabolic rates, evaporative water losses from scorpions are much lower than those from other desert arthropods. Excretory water losses are minimized by the elimination of nitrogenous wastes in insoluble form. Very little, however, is known about the osmoregulatory abilities of scorpions. The limited information available suggests that they simply tolerate increased haemolymph osmolarities during periods of desiccation (Hadley, 1974; Riddle *et al.*, 1976).

Much of the recent research on the environmental physiology of scorpions has concerned North American species. Comparatively little work has been carried out on African scorpions, in particular those of southern Africa. The family Scorpionidae, well represented in Africa, is totally absent from the New World, except for a single genus thought to have been carried across the Atlantic on driftwood (Newlands, 1973). The rich scorpion fauna of Namibia and South Africa belongs entirely to the families Scorpionidae and Buthidae. In the present study we have examined the physiology of two scorpion species against the background of their respective environments. Emphasis has been placed on their evaporative water losses in relation to temperature and their osmotic and ionic regulation.

The two species belong to separate families and both genera are endemic to the Ethiopian faunal region (Lamoral, 1979). *Parabuthus villosus* (Peters) (Buthidae) is a large black scorpion which occurs in extremely arid areas and is widely distributed in Namibia. It does not burrow, but digs shallow scrapes under rocks. It is not strictly nocturnal and specimens have been found walking on the surface at midday in the Namib Desert Park (Lamoral, 1979). The other species studied was a scorpionid, *Opisthophthalmus capensis* (Herbst), which occurs in far more mesic habitats than *Parabuthus*. Like all *Opisthophthalmus*, it digs extensive burrows in the soil, and is one of the more abundant species on Table Mountain, though extending into semi-arid areas on the west coast of South Africa (Eastwood, 1978).

## MATERIALS AND METHODS

Specimens of *Parabuthus villosus* were obtained from the Namib Desert Research Station at Gobabeb, Namibia (mean annual rainfall 18 mm). *Opisthophthalmus capensis* was collected during late summer and autumn from under stones on Table Mountain (mean annual rainfall for collection area is 890 mm). Scorpions were maintained in individual containers partly filled with sand at room temperature (21 ± 2°C) and regularly provided with water and food (immature cockroaches). To minimize errors due to faecal water loss during experiments, food and water were withheld for 3 days beforehand.

Evaporative water loss at various temperatures was determined in a closed, flow-through apparatus similar to that described by Ahearn & Hadley (1969). Relative humidity was controlled by silica gel and Drierite and ranged from 4 to 8% (when measured with a Shaw hygrometer 30 min after the start of an experiment). Temperatures were controlled to ±0.5°C by placing the apparatus in a constant-temperature cell. Weight changes were measured to the nearest 0.1 mg with a Mettler balance. Scorpions were exposed for 6 hr to each of 4 temperatures (25, 30, 35, 40°C) after a pre-desiccation period of 6 hr at 25°C. The latter was necessary because the loss of water loosely bound to the cuticle caused initial transpiration rates to be abnormally high (Hadley, 1970).

Osmotic and ionic regulation were studied by analysing haemolymph samples from scorpions dehydrated to varying degrees at 25–30°C. Haemolymph was collected by puncturing an intersegmental membrane. Its osmolarity was determined immediately on a Wescor Vapour Pressure Osmometer. Sodium and potassium concentrations were measured on an IL 243 Flame Photometer (Instrumentation Laboratory), and chloride concentrations were determined on 10 $\mu$l haemolymph samples by a modification of the mercuric nitrate titration method of Schales & Schales (1941).

Experiments on water gain were confined to *Parabuthus*. The possibility of water uptake from subsaturated atmospheres was investigated by pre-desiccating 10 scorpions in the flow-through apparatus at 25°C (mean weight loss after 12 days was 9.4% of initial weight, range 4.3–15.6%). The scorpions were then exposed for 24 hr to 25°C and 96% r.h. (obtained with a saturated solution of $KH_2PO_4$; Winston & Bates, 1960). Since this did not result in any weight increase, these scorpions, along with another pre-desiccated group, were then permitted to drink from cotton wool soaked with distilled water. After drinking had ceased, each scorpion was reweighed, then killed by freezing and its dry weight determined gravimetrically, so that the water content before drinking could be calculated.

In order to examine possible temperature compensation, the oxygen consumption of *Parabuthus* was measured with a Gilson differential respirometer equipped with special wide-mouthed 130 ml flasks. The water bath maintained temperatures to the nearest 0.5°C. Scorpions were equilibrated for 1 hr at each temperature before measurement of their oxygen consumption for a second hour. A solution of 10% KOH was used as $CO_2$ absorbent. Under the constant light conditions used, the scorpions remained inactive at all temperatures. After a preliminary experiment showed that weight-specific respiration rates were independent of body weight, the scorpions were divided into two groups and acclimated for 10 days at 10°C and 30°C. The acclimation chambers were maintained on a 12:12 light:dark cycle and during acclimation the animals were supplied with water but no food. After acclimation the oxygen consumption of each scorpion was measured at 5, 15, 25 and 35°C. All oxygen volumes were corrected to standard conditions (STPD) and expressed as $\mu$l oxygen consumed per gram live weight per hour.

## RESULTS

### *Effect of size on evaporative water loss*

Because of the marked size difference between the two species (*Parabuthus* weighed 1–10 g; *Opisthophthalmus*, 0.1–3.0 g), it was first necessary to determine the relationship between body size and water loss rates. When water losses (measured for 6 hr at 30°C) were expressed as percentage of original weight lost per hour, there was a highly significant negative correlation with body weight for both species (*Parabuthus*, $r = -0.771$, *Opisthophthalmus*, $r = -0.561$; $P < 0.005$). Similar results were obtained for other scorpion species by Hadley (1970) and by Crawford & Wooten (1973).

Compensation for the size effect was achieved by expressing water loss rates in terms of the surface area of the scorpions. Surface areas were derived from the formula $SA = 15(W)^{0.68}$, where SA is the surface area in $cm^2$ of a scorpion weighing W grams (Toolson & Hadley, 1977). After water losses had been expressed as mg per $cm^2$ surface area per hour, the correlations with body weight were insignificant for both species ($P > 0.1$).

### *Effect of temperature on evaporative water loss*

Water loss rates in dry air are plotted as a function of temperature in Fig. 1. *Opisthophthalmus* weighing less than 1 g were not included in this experiment, as they might have been stressed by the total 24 hr exposure to progressively higher temperatures. Weight losses in experiments of this duration are assumed to represent water losses (see Discussion). In both species total water loss increased steadily with increasing temperature, and at all temperatures water loss rates of *Opisthophthalmus* were significantly higher than those of *Parabuthus* (*t*-test, $P < 0.01$).

### *Osmotic and ionic regulation*

Before haemolymph sampling, *Opisthophthalmus* were desiccated until a maximum of 25% of the original weight had been lost (although a few died after weight losses of 20%) and *Parabuthus* specimens were desiccated up to 30% weight loss. Figure 2 illustrates the effect of prolonged desiccation on haemolymph osmolarities and the dramatic difference between the two species in this respect. Regression analysis (equations are given in Table 1) showed a highly significant correlation between weight loss and haemolymph osmolarity for *Opisthophthalmus* ($P < 0.001$), whereas in *Parabuthus* there was no correlation between desiccation and osmolarity ($P > 0.1$). *Opisthophthalmus* thus resembles other scorpions in showing little evidence of osmoregulation. In contrast, the desert species *Parabuthus* possesses considerable osmoregulatory ability.

In normally hydrated *Parabuthus*, haemolymph sodium concentrations of $286 \pm 2\,mM\,l^{-1}$ (mean ± SE) and chloride concentrations of $265 \pm 3\,mM\,l^{-1}$ accounted for most of the measured osmolarity of $575 \pm 17\,mOsm\,l^{-1}$. The corresponding figures for control *Opisthophthalmus* were $258 \pm 5\,mM\,l^{-1}$ sodium, $255 \pm 7\,mM\,l^{-1}$ chloride and an osmolarity of $519 \pm 14\,mOsm\,l^{-1}$. It was therefore to be expected that the effects of desiccation on haemolymph ion levels would closely resemble the effects on total osmolarity. The appropriate regression equations are given in Table 1. *Opisthophthalmus* sodium, potassium and chloride concentrations all show highly significant correlations with extent of desiccation, while in *Parabuthus* the correlation is significant only in the case of haemolymph chloride levels.

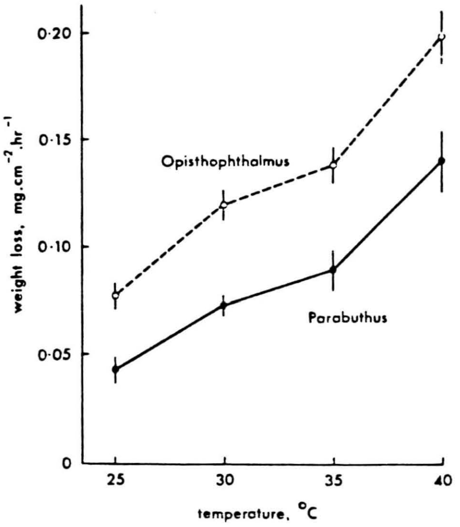

Fig. 1. The effect of temperature on total water loss from *Opisthophthalmus* ($N = 21$) and *Parabuthus* ($N = 13$). Vertical lines represent standard errors.

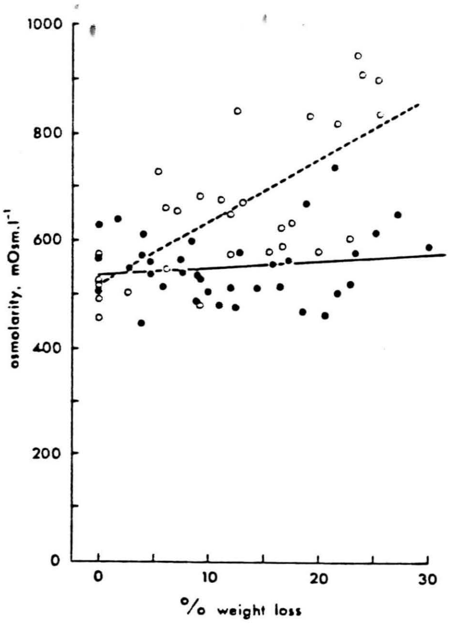

Fig. 2. Effect of prolonged desiccation on the haemolymph osmolarity of *Opisthophthalmus* (○) and *Parabuthus* (●). Equations for the regression lines (*Opisthophthalmus* ---, *Parabuthus* ——) appear in Table 1.

### Rehydration

There was no water uptake by pre-desiccated *Parabuthus* in a 96% r.h. atmosphere. All 10 scorpions lost weight slowly (mean weight loss after 24 hr was 28 ± 4 mg).

Drinking behaviour of the desiccated scorpions was extremely variable. Some individuals drank readily when offered water; others, although quite severely desiccated, did not drink at all. The 17 scorpions, which had lost 3.9–23.0% of their initial weights during dehydration, regained through drinking 75.9 ± 32.7% (mean ± SE) of the weight lost. The extent of drinking (expressed as % of weight loss regained) was not correlated with body size, weight loss or water content after desiccation ($P > 0.05$). The lack of correlation with water content was expected, because water content did not decrease significantly during desiccation.

In arthropods as large as *Parabuthus*, it is possible to take successive haemolymph samples from an individual. One 8.3 g specimen lost 23% of its initial weight during desiccation, then drank 1.9 g water in 18 min, replacing all the weight lost. When sampled immediately before drinking and 44 hr later, its haemolymph showed a decrease in osmolarity from 577 to 424 mOsm $l^{-1}$, and the ion levels decreased proportionately. Regulation of haemolymph composition during rehydration may not be as efficient as in dehydration, but *Parabuthus* is unlikely to drink large volumes of water under natural conditions.

### *Effect of thermal acclimation on oxygen consumption of* Parabuthus

The mean oxygen consumption of 16 *Parabuthus* ranging in weight from 1 to 15 g was 27.5 ± 2.7 $\mu l\ g^{-1}\ hr^{-1}$ at 20°C. There was no correlation between metabolic rate and body weight ($r = -0.13$, $P > 0.1$). Riddle (1978) found metabolic rates of the much smaller *Paruroctonus utahensis* to be inversely proportional to body weight at high temperatures only.

The metabolic rate–temperature curves of scorpions acclimated to 10 and 30°C are shown in Fig. 3.

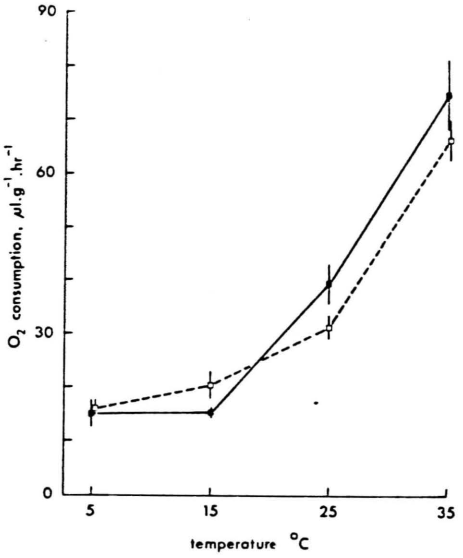

Fig. 3. The effect of thermal acclimation on the oxygen consumption of *Parabuthus*. Metabolic rate–temperature curves are shown for scorpions previously acclimated to 10°C (■——■) or 30°C (□---□). $N = 8$ for each group. Vertical lines represent standard errors.

Table 1. Regression equations describing the effect of desiccation (% weight loss, $x$) on haemolymph osmolarity and ion concentrations ($y$)

| | | Equation | $N$ | $r$ | $P$ |
|---|---|---|---|---|---|
| *Parabuthus* | Osmolarity | $y = 536 + 1.26x$ | 37 | 0.16 | NS |
| | $Na^+$ | $y = 283 + 0.78x$ | 20 | 0.38 | NS |
| | $K^+$ | $y = 9.9 - 0.01x$ | 20 | 0.05 | NS |
| | $Cl^-$ | $y = 253 + 1.16x$ | 19 | 0.46 | <0.05 |
| *Opisthophthalmus* | Osmolarity | $y = 518 + 11.49x$ | 32 | 0.74 | <0.001 |
| | $Na^+$ | $y = 267 + 4.35x$ | 32 | 0.76 | <0.001 |
| | $K^+$ | $y = 7.9 + 0.54x$ | 30 | 0.69 | <0.001 |
| | $Cl^-$ | $y = 258 + 2.88x$ | 30 | 0.53 | <0.01 |

Na, K and Cl concentrations in mM $l^{-1}$.

The metabolic rates of scorpions acclimated to 30°C were not significantly lower than those of the scorpions acclimated to 10°C ($t$-tests, $P > 0.1$ at 5, 15 and 35°C, $P > 0.05$ at 25°C). Metabolic rates at all temperatures were extremely low, and the oxygen consumption of 70.8 $\mu l\ g^{-1}\ hr^{-1}$ at 35°C reflects the inactivity of the scorpions even at this temperature.

## DISCUSSION

Edney (1977) has summarized some of the available data on transpiration rates in relation to habitats of terrestrial arthropods, showing that the two are closely related in all arthropod classes. The southern African scorpions investigated here are no exception in this regard, the more xeric-adapted *Parabuthus* displaying water loss rates considerably lower than those of *Opisthophthalmus* at all temperatures. The very low permeability of *Parabuthus* is also important in the absence of burrowing behaviour in this species, and during its occasional diurnal activity on the surface of the gravel plains of the Namib Desert, where surface temperatures can be very high. In contrast, burrowing behaviour is characteristic of the genus *Opisthophthalmus*, even second instar *O. capensis* constructing small burrows (Eastwood, 1978). Burrows protect this species from high temperatures and desiccation, especially in the drier parts of its range, but they also offer protection from the cold, wet Cape winters. Eastwood (1978) found that *O. capensis* remained deep in their burrows on cold days and that on mountain slopes the burrow entrances beneath stones always faced downhill, so that the burrows could not be flooded with rainwater.

Evaporative water losses of *Opisthophthalmus*, though significantly higher than those of *Parabuthus*, are still remarkably low, as in most scorpions. Warburg *et al.* (1980) have compiled a table comparing rates of water loss in various scorpions, and the values obtained in the present study are well within the range for both New and Old World scorpions from xeric and mesic habitats.

Few studies have attempted to assess the cuticular and respiratory components of total transpiration in scorpions. In *Hadrurus* and *Diplocentrus* respiration was apparently a major avenue of water loss only at temperatures above about 40°C (Hadley, 1970; Crawford & Wooten, 1973). On the other hand, Yokota (1978) found that respiratory water loss in *Paruroctonus* accounted for 40% of the total loss from living animals at 24°C. The spiracles played an important role in regulating respiratory water loss, with the result that the ratio of respiratory water loss to oxygen consumption decreased with increasing temperature. The fact that both evaporative water loss and oxygen consumption of *Parabuthus* doubled between 25 and 35°C does not imply that most of the water loss at these temperatures was via the respiratory system.

From the oxygen consumption data, we can calculate metabolic water production during desiccation in *Parabuthus*. Assuming that lipid is metabolized and that the metabolism of 1 mg lipid requires 2.02 ml $O_2$ and produces 1.07 mg water (Edney, 1977), metabolic water at 25°C amounts to 0.45 $mg\ g^{-1}$ body weight $day^{-1}$, and at 35°C it is 0.90 $mg\ g^{-1}\ day^{-1}$. Therefore during 6 hr exposure to dry air at 25°C, scorpions weighing on average 6.16 g lost 13.2 mg body weight and produced 0.69 mg metabolic water, metabolizing 0.64 mg lipid in the process. At 35°C these figures are approximately doubled. Metabolic water production is thus insignificant during short-term measurements of water loss, representing only about 5% of the transpiratory water loss. Only if the transpiration rate declined markedly during prolonged desiccation would the contribution of metabolic water increase in importance.

The few previous studies of scorpion osmoregulation have concerned desert species. Hadley (1974) suggested on the basis of preliminary results that *Hadrurus* simply tolerated increased osmotic and ionic concentrations until it could replenish lost body water. Data on haemolymph composition of this species presented by Ahearn & Hadley (1977) show that even mild dehydration (2 weeks without food at 35°C) raised haemolymph osmolarity and ion levels. In *Paruroctonus aquilonalis* desiccation caused a steady increase in haemolymph osmolarity, and rehydration on moist soil depressed osmolarity below field levels (Riddle *et al.*, 1976). Similarly, *Paruroctonus mesaensis* did not regulate its haemolymph osmolarity during dehydration (Yokota, 1978). Most recently, Warburg *et al.* (1980) have shown an apparent inability to regulate haemolymph osmolarity during dehydration in four scorpion species from Israel. *Opisthophthalmus* exhibits the same pattern of osmotic tolerance rather than regulation.

The present study provides the first evidence for efficient osmotic and ionic regulation in a scorpion. The osmoregulatory ability of *Parabuthus* compares well with that of other successful desert arthropods, the tenebrionid beetles (Riddle *et al.*, 1976; Nicolson, 1980). This ability, together with the scorpion's large size, low transpiration rate and low metabolic rate, is a very useful adaptation to a desert existence.

Insect prey is probably the main source of ingested water for most scorpions. *Parabuthus* was unable to absorb water vapour from subsaturated air: this inability has been demonstrated in three other species of scorpion (Hadley, 1970; Crawford & Wooten, 1973; Riddle *et al.*, 1976). However, certain scorpions may drink free water or from moist soil. The large desert species *Hadrurus* showed no appreciable weight gain when pre-desiccated and left in contact with a moist substrate (Hadley, 1970) but *Paruroctonus* and second instar *Diplocentrus* were able to replenish lost body water from moist soil (Riddle *et al.*, 1976; Crawford & Wooten, 1973). Water intake in these cases is probably via the mouth. A desert buthid, *Centruroides*, drinks from irrigation canals and in the laboratory regained through drinking most of the weight lost during desiccation (Hadley, 1971), although, as in *Parabuthus*, there was no correlation between weight loss and weight gain. We have not yet observed *Parabuthus* or *Opisthophthalmus* drinking in their natural habitats, but *Opisthophthalmus* drinks readily in a dry laboratory atmosphere (Eastwood, 1978) and occurs in areas of moderate rainfall. Even *Parabuthus* may have drinking water available to it: the regular advective fogs of the Namib Desert are an important water source for many desert animals (Louw, 1972) and *Par-*

*abuthus* may drink fog droplets which have condensed on the stones of the gravel plains.

The low metabolic rates of scorpions reduce respiratory water losses and conserve food reserves during long periods of starvation. In short-term experiments, the oxygen consumption of *Parabuthus* was directly proportional to temperature, with $Q_{10}$ values remaining approximately 2 throughout the range 5–35°C (Fig. 3). The scorpions failed to exhibit acclimation with respect to oxygen consumption. In the desert scorpion *Paruroctonus utahensis*, Riddle (1978, 1979) has demonstrated that acclimation to high temperatures causes a lateral shift in the metabolic rate–temperature curve, with lowered metabolic rates after acclimation. This response is adaptive in that metabolic rate becomes partially independent of seasonal temperature fluctuations, reducing energy expenditure in summer. However, another *Paruroctonus* species, *P. mesaensis*, showed little acclimation of metabolic rate due to season (Yokota, 1978).

Metabolic rates of *Parabuthus* determined in the present study are considerably lower than those of most other scorpions, including another large desert species, *Hadrurus* (Hadley, 1970). *P. mesaensis*, however, has metabolic rates as low as those of *Parabuthus* (Yokota, 1978) and both species fail to show metabolic compensation to increased temperatures. This lends some support to the idea of Anderson (1970) that temperature acclimation ability is associated with standard metabolic rates, being poorly developed in arachnids with very low standard metabolic rates because of the requirement for a certain minimum level of energy expenditure.

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