The effect of desiccation on water management and compartmentalisation in scorpions: the hepatopancreas as a water reservoir

Eran Gefen* and Amos Ar

Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

*Author for correspondence (e-mail: gefene@unlv.nevada.edu)

Summary

Scorpions of the Family Buthidae have lower water loss rates (WLR) and enhanced osmoregulatory capacities in comparison with sympatric species of F. Scorpionidae. In this study we followed changes in water content of different body compartments in four scorpion species under prolonged desiccation conditions. The high initial WLR previously reported for Scorpionidae result in rapid depletion of body water stores. A significant decrease in total body water content of Scorpionidae was recorded following loss of only 5% of initial mass, whereas no such decrease was recorded for Buthidae following severe desiccation. When desiccated, scorpions lose water primarily from the hepatopancreas, while haemolymph volume is more tightly regulated. However, the haemolymph volume of Scorpionidae decreases as a result of depletion of hepatopancreas water stores following severe desiccation. The increasing lipid fraction in the hepatopancreas of Scorpionidae during desiccation suggests that depletion of body water stores may induce enhanced catabolism of carbohydrates, which may contribute to volume regulation by making initially glycogen-bound water available to the desiccating scorpion.

Key words: scorpion, water, desiccation, hepatopancreas, haemolymph, adaptation, osmoregulation.

Introduction

Terrestrial arthropods are relatively small organisms and, therefore, their surface area to body mass ratio is relatively high. As a result they lose water through the integument by evaporation at a high rate relative to their total body water reserves. Nevertheless, arthropods have successfully occupied terrestrial habitats of extreme environmental conditions, including arid deserts.

Both the behavioural (e.g. burrowing, nocturnal activity) and physiological (e.g. highly impermeable integument) adaptations of scorpions to dry conditions have been extensively studied (reviewed in Hadley, 1990). Generally, arthropods of xeric distribution have integuments more resistant to water loss in comparison with more mesic species (Edney, 1977). In a number of studies scorpions have been shown to follow this general rule (Toye, 1970; Warburg et al., 1980; Robertson et al., 1982), but recent evidence suggests that previously reported interspecific differences in water relations of scorpions may in fact be phylogenetically related (Gefen and Ar, 2004).

Rates of water loss to the environment have been recorded for a number of species, but little is known about water compartmentalisation in scorpions. Seasonal fluctuations of water distribution in several body compartments have been reported (Warburg, 1986; Warburg et al., 2002), but these are of limited contribution to the understanding of water management during prolonged desiccation under controlled laboratory conditions. Moreover, to the best of our knowledge, there are no reported measurements of haemolymph volume for any scorpion species.

The haemolymph is the largest extracellular water reservoir of all land arthropods, and often reduces in volume during dehydration. Insects lose water primarily from the haemolymph during dehydration (Edney, 1977; Hyatt and Marshall, 1985; Naidu and Hattingh, 1986; Albaghdadi, 1987; Zachariassen and Pedersen, 2002). By contrast, Greenaway and MacMillen (1978) reported that the terrestrial crab *Holthuisana transversa* (Martens) loses water proportionately from the haemolymph and a second compartment. Two other species of land crabs *Gecarcoidea lalandii* and *Cardisoma carnifex* maintained their haemolymph volume at the expense of tissue water while dehydrated (Harris and Kormanik, 1981). Muscle tissue (Horowitz, 1970) and the gut and hepatopancreas (Lindqvist and Fitzgerald, 1976) have been suggested as possible sources of water in desiccating isopods. Hadley (1994) suggested that while the developed tracheal system of insects allows them to tolerate large decreases in haemolymph volume during dehydration, some spiders and crustaceans maintain haemolymph volume in order to preserve its respiratory role.

Scorpions of the Family Buthidae were shown to be better

haemolymph osmoregulators and more desiccation resistant than their respective Scorpionidae sympatric species (Gefen and Ar, 2004). In addition, the haemolymph osmotic concentrations of the buthids decreased following mild desiccation, suggesting water mobilization from another body compartment to the haemolymph. We used the same experimental design that was employed in the previous study (Gefen and Ar, 2004), where Buthidae and Scorpionidae were each represented by both xeric and mesic species/subspecies, in order to elucidate the physiological mechanisms involved in the observed between-family differences in water relation traits. We monitored body water management by following changes in the haemolymph, hepatopancreas and total body water stores through a range of mass loss levels under controlled desiccating conditions, in order to determine the role played by the hepatopancreas in the water budget of desiccating scorpions. We also measured the lipid content of the hepatopancreas in an attempt to examine the contribution of oxidized metabolic fuels to the overall water budget of scorpions under prolonged desiccation conditions.

Materials and methods

Scorpion collection

Scorpio maurus fuscus (Scorpionidae; Ehrenberg 1829) and *Buthotus judaicus* (Buthidae; Simon 1872) were collected from the same area in the mesic Lower Galilee, in pine woodlands near Migdal Ha'emek (mean annual precipitation ~525 mm). *Scorpio maurus palmatus* (Scorpionidae; Ehrenberg 1828) were collected from sandy loess plains near Sde Boker (mean annual precipitation ~100 mm) in the Negev desert, while *L. quinquestriatus* (Buthidae; Ehrenberg 1828) were collected from the stony desert areas of nearby Yerucham.

Haemolymph osmolarity

Following capture, the scorpions were held in the laboratory at room temperature in round (9 cm diameter) plastic boxes with soil from the collection site, and fed adult crickets *ad libitum* for 14 days. The boxes were perforated in order to allow gas exchange with ambient atmosphere.

The scorpions were then weighed to the nearest 0.1 mg, and a sharpened glass capillary was inserted into the pericardial sinus by puncturing the dorsal intersegmental membrane. The haemolymph (~10 μl) withdrawn was used for determining its initial osmolarity (5100C Vapour Pressure Osmometer, Wescor, Logan UT, USA). The tapered tip of the glass capillary enabled immediate closure of the wound. Nevertheless, occasionally specimens had to be excluded from further investigations when haemolymph withdrawal resulted in persistent bleeding.

After allowing 24 h for recovery, the scorpions were weighed again, transferred to identical empty plastic boxes and randomly assigned to one of the experimental mass loss groups. The boxes were placed in a controlled temperature chamber (30±0.2°C, 40–60% RH). The animals and their excretions were weighed daily, and following losses of 5, 10,

15 or 20% of initial mass (excluding dry excretions) the haemolymph osmolarity of the scorpions was measured again.

Body water distribution
(1) Total body and hepatopancreas water contents

Following haemolymph sampling the scorpions were decapitated, and their hepatopancreas was removed. Fresh mass of the hepatopancreas was measured to the nearest 0.1 mg. Then the hepatopancreas and rest of the body were dried separately at 60°C to constant mass. Whole body and hepatopancreas water contents were calculated using the differences between wet and dry masses, and expressed as a percentage of total body and hepatopancreas masses respectively.

(2) Haemolymph volume

Additional scorpions were sampled for haemolymph volume, which was determined in control (following 14 days of *ad libitum* feeding), and in 10% and 20% mass loss experimental groups. Haemolymph volume was measured using isotope dilution of [methoxy-^{3}H]-inulin (specific activity: 380 mCi g^{-1}; Perkin Elmer, Boston MA, USA). 5 μl (10±0.1 μl syringe, Hamilton, Bonaduz, Switzerland) of ^{3}H-inulin solution (30,000 cpm μl^{-1}) were injected into the pericardial sinus through the dorsal intersegmental membrane using a micromanipulator. Dilution time was set to 75 min since preliminary experiments had shown no changes in inulin concentrations between 60–90 min following injection. Haemolymph was sampled after 75 min by withdrawing the syringe and collecting haemolymph with a glass capillary. Its osmolarity was measured, and 8 μl aliquots were placed in scintillation vials with 5 ml Optifluor liquid scintillation fluid (Packard, Meriden CT, USA). Radioactivity was counted in a liquid scintillation analyzer (Tri Carb 2100TR; Packard, Meriden, CT, USA). Volume determination was carried out by comparing haemolymph radioactivity levels to standards of known water volume injected with 5 μl of the [methoxy-^{3}H]-inulin solution.

Hepatopancreas lipid content

Dried hepatopancreas was used for determination of hepatopancreas lipid content. The tissue was placed in emptied tea-bags, which were then stapled shut, weighed to the nearest 0.1 mg and put in the extractor of a Soxhlet apparatus, with petroleum ether as the extraction solvent. Samples were extracted for 24 h, and then dried at 60°C to constant weight. Hepatopancreas lipid content was determined by the difference in dry mass of the samples before and after the lipid extraction, and expressed as a percentage of the total hepatopancreas dry mass. No significant difference was found between pairs of samples from the same hepatopancreas (Wilcoxon matched pairs test, P=0.33; N=15).

Statistics

Statistical analysis was carried out using STATISTICA© 6.0 for Windows software. All data expressed as a percentage were arcsine transformed prior to further statistical analysis.

Table 1. *Osmotic increase following desiccation*

Family	Buthidae		Scorpionidae	
Species	*B. judaicus*	*L. quinquestriatus*	*S. m. fuscus*	*S. m. palmatus*
Initial mass (g)	2.009±0.091	2.543±0.191	1.913±0.089	1.951±0.090
Haemolymph osmolarity (mOsm l^{-1})				
Initial	569±3 (47)	605±5 (36)	572±6 (38)	573±6 (37)
Haemolymph osmolarity increase (percentage of initial)				
5% Mass loss	5.1±0.9 (9)	3.8±1.7 (8)	14.8±1.3 (6)	5.2±1.6 (8)
10% Mass loss	6.5±2.0 (8)	8.1±2.4 (8)	22.9±2.6 (7)	11.1±2.9 (8)
15% Mass loss	14.6±2.3 (9)	11.3±3.9 (8)	30.3±5.0 (6)	24.9±4.0 (6)
20% Mass loss	18.8±4.0 (8)	25.9±2.5 (4)	39.1±5.1 (7)	30.1±4.5 (6)

Initial body mass and haemolymph osmolarities, and osmotic increase following desiccation to the various experimental mass loss levels at 30°C and 40–60%RH. Values are means ± S.E.M.; (*N*), number of scorpions.

Newman-Keuls test was used for posthoc comparisons. Total body mass was used as a covariate for ANCOVA. Mass did not have a significant effect on transformed values (*P*>0.25).

Results

Haemolymph osmolarity

Initial values and changes in the haemolymph osmotic concentrations of the four species are summarized in Table 1. *B. judaicus* and *L. quinquestriatus* exhibited better osmoregulatory capacities than the two scorpionid subspecies, particularly at high mass loss levels. The between-family difference in osmotic response to desiccation was most pronounced after loss of 15% from initial mass, where the increase in the haemolymph osmolarity of the two *Scorpio maurus* subspecies was 30.3% and 24.9%, compared with 14.6% and 11.3% for the respective sympatric buthids. No significant difference was found between the osmoregulatory responses of the two buthids, while the xeric *S. m. palmatus* osmoregulated its haemolymph better than the mesic *S. m. fuscus* following mass loss of 5–10% only (ANOVA, performed separately for each sampling month; α=0.05).

Differences in WLR of the four species have been reported previously (Gefen and Ar, 2004), and can also be inferred from the time interval until desiccation to a given level of mass loss. For example, *B. judaicus* and *L. quinquestriatus* (Buthidae) lost 10% of their initial mass after 18.6±5.2 (mean ± S.D.) and 19.3±6.9 days, respectively, while the two scorpionids lost mass twice as fast, losing 10% body mass after 9.4±5.0 and 8.6±2.9 days (for *S. m. fuscus* and *S. m. palmatus*, respectively).

Body water distribution

(1) Total body and hepatopancreas water contents

Scorpion samples included both males and females and it was, therefore, necessary to address possible differences between the sexes. The number of male specimens was relatively low, and a statistical comparison within each species and mass loss level would therefore have been of little
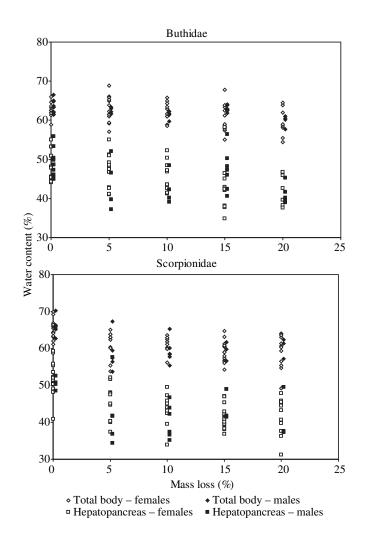

Fig. 1. Total body and hepatopancreas water contents (percent of the respective fresh mass) of male and female buthid (top) and scorpionid (bottom) scorpions following losses of 0, 5, 10, 15 and 20% of their initial mass.

meaning. However, it is evident from Fig. 1 that water content values (percent of total mass) of male and female scorpions are
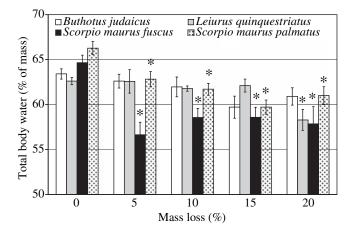
Fig. 2. Total body water contents (percent of the total body fresh mass; mean ± S.E.M.) of the four species following losses of 0, 5, 10, 15 and 20% of initial mass. Asterisks indicate significant differences from initial value (ANCOVA of arcsine transformed percentages, with body mass as covariate, followed by Newman-Keuls test; α=0.05). See Table 1 for sample sizes. Sample sizes of control groups were 13, 8, 10 and 9 for *B. judaicus*, *L. quinquestriatus*, *S. m. fuscus* and *S. m. palmatus*, respectively.

Fig. 3. Hepatopancreas water content (percent of the hepatopancreas fresh mass; mean ± S.E.M.) of the four species following losses of 0, 5, 10, 15 and 20% of initial mass. Asterisks indicate significant differences from initial value (ANCOVA of arcsine transformed percentages, with body mass as covariate, followed by Newman-Keuls test; α=0.05). For sample sizes see Fig. 2.

similar, allowing pooling male and female values within each species.

Fig. 2 shows the body water contents of the four species, expressed as a percentage of total body fresh mass, over the range of experimental mass loss levels. Initial water stores of the two Scorpionidae are higher, but only that of *S. m. palmatus* is statistically significant, in comparison with those of the Buthidae (ANCOVA, *P*<0.01). However, the body water stores of Buthidae are better maintained when the scorpions are exposed to desiccating experimental conditions. Despite their higher initial values, the water contents of Scorpionidae decrease rapidly, and are significantly lower than initial values after mass loss of only 5% (Fig. 2). By comparison, a statistically significant decrease in water contents of *L. quinquestriatus* is only seen at 20% mass loss, while *B. judaicus* kept body water content stable throughout the range of experimental mass loss levels.

A similar pattern was recorded for depletion of water stored in the hepatopancreas (Fig. 3). As with total body water, both
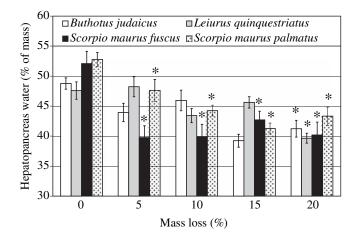
scorpionids appeared to have larger water stores in the hepatopancreas in comparison with buthids, although these differences were marginally short of the generally accepted significance level of 0.05 (e.g. for *S. m. palmatus vs L. quinquestriatus* *P*=0.053). Scorpionids also exhibited a significant decrease in hepatopancreas water stores after mass loss of 5%, while a similarly significant decrease was recorded in buthids only after loss of 20% of initial body mass (Fig. 3).

The role of the hepatopancreas in the water budget of scorpions was further assessed by calculation of the hepatopancreas water content as a fraction of the total water content of the scorpion, following the same experimental desiccation levels (Fig. 4). As females generally have a larger hepatopancreas than males, and because of the smaller number of captured males, this comparison was limited to females only. *L. quinquestriatus* females stored significantly more water in the hepatopancreas (expressed as fraction of total body water) in comparison with the other three studied species [$F_{(3,17)}$=3.52; *P*=0.04]. In all examined species, the proportion of hepatopancreas water to the total body water decreased

Table 2. *Ratio of haemolymph volume to body mass*

Species	Initial mass	Haemolymph volume to body mass ratio (%)		
		0% Mass loss	10% Mass loss	20% Mass loss
Buthotus judaicus	2.139±0.114 (31)	28.5±0.7 (12)	27.2±1.8 (10)	28.1±1.0 (9)
Leiurus quinquestriatus	2.655±0.219 (31)	22.5±0.7[a] (11)	26.6±0.5[b] (10)	28.2±0.6[b] (11)
Scorpio maurus fuscus	1.743±0.116 (12)	31.1±2.4 (7)	29.1±1.2 (5)	–
Scorpio maurus palmatus	2.202±0.184 (22)	33.8±1.6 (11)	35.0±0.9 (11)	–

Different superscript letters represent significant differences between mass-loss treatments (ANCOVA of arcsine transformed percentages, with body mass as covariate, followed by Newman-Keuls test; α=0.05). Ratio (given as percentage) of haemolymph volume (ml) to body mass (g) in control group (0% mass loss), and following desiccation (percent mass loss, excluding dry excretions). Ratios and initial body masses are presented as means ± S.E.M.; (*N*), number of scorpions.

significantly during prolonged desiccation (regression of arcsine transformed percentages, α=0.05), but the steepest slope calculated was also that of the xeric buthid *L. quinquestriatus* (Fig. 4). This slope was not significantly different (*t*-test, *P*=0.21) from that of the other buthid, *B. judaicus*, but was steeper in comparison with the slopes for *S. m. palmatus* and *S. m. fuscus* at significance levels of 0.05<*P*<0.1 and *P*<0.05, respectively.

(2) Haemolymph volume

Another contribution to the higher total water content of scorpionids comes from their high haemolymph volume in comparison with that of buthids (Table 2). The two scorpionids had significantly higher ratios of haemolymph volume to total body mass than *L. quinquestriatus*. The haemolymph volume fraction of *S. m. palmatus* was also significantly higher than that of *B. judaicus* (ANCOVA, *P*=0.05). None of the four species showed a significant decrease in the ratio of haemolymph volume to total body mass following desiccation to 10% loss of initial mass (Table 2). Furthermore, following desiccation of *L. quinquestriatus* to 10% loss from initial body mass, the fraction of haemolymph volume to total body mass increased significantly (Table 2, ANCOVA, *P*<0.001). Haemolymph volume measurements of scorpionids following 20% mass loss was not possible due to the difficulty of withdrawing haemolymph, thus suggesting a severe decrease in haemolymph volume at these desiccation levels.

Hepatopancreas lipid content

Lipid contents of the hepatopancreas, expressed as a percentage of tissue dry mass, are given in Table 3 for the four species over the range of experimental mass loss levels. No
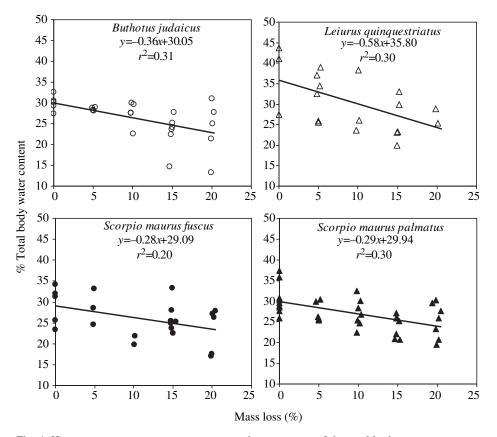

Fig. 4. Hepatopancreas water content, expressed as a percent of the total body water content, as a function of the mass loss of the four species.

significant difference (*P*>0.05) is evident for either of the Buthidae following losses of up to 20% of initial mass. By comparison, for Scorpionidae there was a significant increase in hepatopancreas lipid fraction as a result of desiccation. The most pronounced increase was that of *S. m. fuscus*, which showed a significantly higher hepatopancreas lipid fraction following loss of as little as 5% of its initial mass (Table 3).

Assessment of changes in the total lipid contents was hampered by the variability in body and hepatopancreas masses, together with the limited sample sizes. For example, initial body masses of *S. m. fuscus* were 2.124±0.254 g and 1.625±0.107 g (mean ± S.E.M.) for 5% and control mass loss groups, respectively. Nevertheless, the hepatopancreas lipid

Table 3. *Hepatopancreas lipid content*

| Species | Mass loss | | | | |
	0%	5%	10%	15%	20%
Buthotus judaicus	49.4±2.2 (13)	47.9±3.7 (8)	48.4±2.4 (8)	55.7±2.2 (7)	54.3±4.1 (6)
Leiurus quinquestriatus	40.3±1.4 (8)	43.4±1.5 (8)	47.9±4.3 (7)	50.9±2.6 (8)	49.9±5.7 (4)
Scorpio maurus fuscus	43.5[a]±2.2 (9)	57.3[b]±2.1 (6)	57.1[b]±2.5 (7)	53.7[b]±2.3 (8)	53.3[b]±2.7 (7)
Scorpio maurus palmatus	44.4[a,b]±1.1 (9)	44.6[a]±2.0 (9)	48.7[a,b,c]±1.7 (9)	53.0[b,c]±1.5 (9)	54.3[b,c]±4.3 (8)

Different superscript letters represent significant differences between mass-loss treatments (ANCOVA of arcsine transformed percentages, followed by Newman-Keuls test; α=0.05). Hepatopancreas lipid content (percentage of hepatopancreas dry mass) following losses of 0, 5, 10, 15 and 20% of the scorpions' initial mass. Values are mean ± S.E.M.; (*N*), number of scorpions.

content of the 5% mass loss group was 249.7±38.4 mg (57.3% of total hepatopancreas dry mass of 435.7±63.4 mg; *N*=9, four males and five females), which constituted a significant increase in lipid content from control values (111.4±15.6 mg, 43.5% of 255.8±28.3 mg hepatopancreas dry mass; *N*=6, three males and three females), even when accounting for the evident difference in hepatopancreas dry mass (ANCOVA, *P*<0.05).

Discussion

The osmotic responses to desiccation of the four species (Table 1) are similar to those reported in a previous study, in which buthids were shown to have enhanced osmoregulatory capacities in comparison with scorpionids under the same desiccating conditions (Gefen and Ar, 2004). These differences were seen as an adaptation of Buthidae to their surface-dwelling existence, whereas Scorpionidae like most other scorpion species burrow and, thus avoid harsh environmental conditions. The ability of *S. m. palmatus* to regulate its haemolymph osmolarity following mild desiccation appears to be better than previously reported, but the overall osmotic pattern is maintained (Table 1). The fact that the highest and most consistent differences in osmoregulatory responses are those between the sympatric *B. judaicus* (Buthidae) and *S. m. fuscus* (Scorpionidae) supports the suggestion that these differences are phylogenetically related.

Initial WLR, measured during the early stages of desiccation, are relatively high (Hadley, 1994; Gefen and Ar, 2004). Therefore, the recorded desiccation time is not an accurate measure of steady-state WLR of the four species, particularly at lower mass loss levels. Nevertheless, it has been previously shown that osmoregulatory capacities of scorpions are negatively correlated with WLR (Gefen and Ar, 2004). The haemolymph osmolarity of the millipede *Pachydesmus crassicutis* was shown to increase more at high dehydration rates (Woodring, 1974). Likewise, interspecific differences in the osmoregulatory capacities of terrestrial isopods were found to correlate well with their position on a 'terrestriality gradient' and their respective WLR (Price and Holdich, 1980).

It has been suggested that isopods mobilise water from a different body compartment to the haemolymph upon desiccation (Horowitz, 1970; Lindqvist and Fitzgerald, 1976). If the same mechanism occurs in scorpions, their ability to osmoregulate their haemolymph may depend on the ratio of water mobilisation rate from another body compartment ($\dot{R}_1$) to the water loss rate (WLR, $\dot{R}_2$). Thus, at a high desiccation rate, when $\dot{R}_2/\dot{R}_1$ is relatively high, water supply cannot meet demand and haemolymph osmotic concentration can not be maintained. By comparison, if $\dot{R}_2/\dot{R}_1$ is lower at lower desiccation rates, the regulation of haemolymph osmolarity may be possible. However, while water vapour deficit across the integument dictates continuous transpiratory water loss ($\dot{R}_2$ remains steady), depletion of body water stores (decreasing $\dot{R}_1$ as desiccation is prolonged) means that osmoregulation may be restricted to a limited range of water losses. Interestingly,

the observed initial decrease in haemolymph osmolarity following the onset of desiccation in buthids (Gefen and Ar, 2004) suggests that water mobilisation from another compartment is not triggered by increased haemolymph osmolarity.

The interrelated water loss and mobilisation rates and osmoregulatory capacities are also consistent with the water depletion pattern shown in Fig. 2. The significant decrease in total body water stores of scorpionids following a mass loss of only 5% from initial body mass is coupled with their relatively high WLR, particularly during the early stages of desiccation. Similarly, the highest drop in total body water stores was recorded for *S. m. fuscus* (Fig. 2), which correlates well with the species' highest WLR and poorest osmoregulatory capacity among the four studied species (Table 1; Gefen and Ar, 2004). By contrast, the reported low WLR of buthids are reflected in the ability to maintain body water and osmotic stability. These lower WLR allow metabolic water production to compensate better for water loss to the environment, thus minimising loss of initially stored bulk water during desiccation (Gefen and Ar, 2004).

The hepatopancreas is a large organ, which fills the entire mesosoma and the first two metasomal somites of scorpions (Warburg et al., 2002). The similar depletion patterns of the hepatopancreas (Fig. 3) and total body water stores (Fig. 2) hint at the important role played by the former in the overall water budget of the desiccating scorpion. Furthermore, initially comprising ~30% of the total body water content of *B. judaicus*, *S. m. fuscus* and *S. m. palmatus*, the fraction of hepatopancreas water from total body water decreases during desiccation (Fig. 4). This suggests that the contribution of hepatopancreatic water stores is higher than expected if water was to be proportionally lost from all body compartments. The contribution of hepatopancreatic water to haemolymph volume regulation (Table 2) is therefore fundamental for maintaining osmotic stability of the haemolymph.

The role of the hepatopancreas in water management during desiccation is even more pronounced in the case of the xeric buthid *L. quinquestriatus*. Among the studied species, female *L. quinquestriatus* store the highest amount of water in the hepatopancreas. Furthermore, their significantly higher slope (Fig. 4) indicates that female *L. quinquestriatus* mobilise more water from the hepatopancreatic stores during prolonged desiccation, with the hepatopancreas water content reaching values similar to those of the other species after 20% loss of initial mass (Fig. 4). The significantly higher contribution of the hepatopancreatic water stores of *L. quinquestriatus* to the total water loss may contribute to its haemolymph osmotic stability. The ability of *L. quinquestriatus* to osmoregulate its haemolymph during prolonged desiccation is similar to that of *B. judaicus*, despite the higher WLR recorded for the former at 30°C (Gefen and Ar, 2004).

Mobilisation of hepatopancreatic water stores may contribute to volume regulation of the haemolymph during desiccation. Unlike insects, scorpions lack a tracheal system, and rely on haemolymph oxygen carriers for respiratory gas exchange

between their tissues and the surrounding environment through their book lungs. It has been suggested that this respiratory role of the haemolymph in some non-insect arthropods may explain the strategy of regulating haemolymph volume at the expense of tissue water stores during desiccation (Hadley, 1994). Measurements of haemolymph volume (Table 2) and hepatopancreas water content (Fig. 3) are in agreement with this suggested pattern. None of the four examined species showed a decrease in the fraction of haemolymph volume to total body mass of the scorpion following desiccation to 10%. Furthermore, as total body water stores diminish during desiccation (Fig. 2), the fraction of haemolymph to total body water stores increases.

Mobilisation of water from hepatopancreatic stores to the haemolymph is best shown for *L. quinquestriatus*, where the significantly highest rate of hepatopancreas water depletion (Fig. 4) is coupled with a significant increase in the water fraction of the haemolymph from total body mass (Table 2). However, we have not managed to determine the origin of this characteristic of the xeric *L. quinquestriatus* (Buthidae). The present study and a previous one (Gefen and Ar, 2004) have shown interspecific differences in water relations of scorpions to be primarily phylogenetically related rather than mechanisms of adaptation to arid habitats. Unfortunately, *B. judaicus* (Buthidae) specimens used for haemolymph volume determination were kept under laboratory conditions longer than the other species before measurements and, therefore, may not have started desiccation at their fully hydrated state. Their initial haemolymph osmolarity of 638 ± 12 mOsm l^{-1} in comparison with $\sim569\pm3$ mOsm l^{-1} of fully hydrated *B. judaicus* (Table 1) indicates that this suggestion can not be ruled out.

The two *Scorpio maurus* subspecies regulated their haemolymph volume during mild desiccation, but their haemolymph volume could not be determined after loss of 20% of initial mass (Table 2). This severe level of desiccation made haemolymph withdrawal impossible, probably as a result of considerable haemolymph depletion. Fig. 3 shows that the water stored in the hepatopancreas of Scorpionidae is rapidly lost up to loss of 10% of initial body mass, whereas the relative volume of their haemolymph is maintained (Table 2). Following further desiccation, haemolymph volume may decline as demand for water cannot be met by the depleting hepatopancreatic stores (Fig. 3). It is worth noting that mortality rates among scorpionids following severe desiccation were much higher in comparison with buthids (E.G. and A.A., unpublished).

Terrestrial arthropods can survive desiccation stress through one or more of three physiological mechanisms. These include (1) storage of large quantities of water as bulk water or metabolic water; (2) reduced rates of water loss to the environment; (3) the ability to tolerate the loss of relatively large fraction of their initial body water stores (Gibbs et al.,
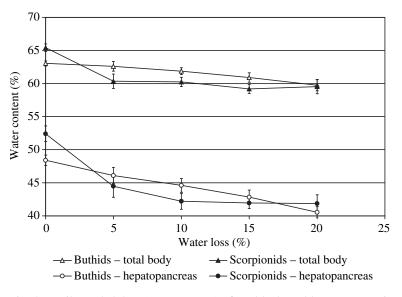

Fig. 5. Family-pooled data (mean ± S.E.M.) of total body and hepatopancreatic water contents (percent of body and hepatopancreas fresh masses, respectively), following losses of 0, 5, 10, 15 and 20% of initial mass. Sample sizes are as in Fig. 2 (pooled within families).

2003). When pooling total body and hepatopancreas water contents of the two studied scorpion families (Fig. 5), it seems that they adopt different strategies in avoiding desiccation. Scorpionids exhibit a rapid depletion of body water stores, which reflects their higher WLR in comparison with those of buthids (Gefen and Ar, 2004), but appear to store more bulk water in their bodies when fully hydrated (Fig. 5). The changes in hepatopancreas lipid content during desiccation also appear to be phylogenetically related, with only the two Scorpionidae exhibiting a significant increase in lipid fraction following desiccation (Table 3). Glycogen water binding capacity is estimated to be 3–5 times its own mass (Schmidt-Nielsen, 1997). However, glycogen-bound water is only available to the organism as glycogen is catabolised. Therefore, high WLR may necessitate glycogen catabolism not only for meeting energetic needs and production of metabolic water, but also for making bulk water available for maintaining homeostasis during prolonged desiccation. This could be accompanied by lipogenesis from a carbohydrate source, as has been suggested for several *Drosophila* species exposed to desiccating conditions (Marron et al., 2003).

In conclusion, we show that scorpions use the hepatopancreas as a water reservoir. Water is stored both as bulk water, and as potential metabolic water source in the form of metabolic fuels. The hepatopancreatic water stores are used to replenish lost haemolymph water upon desiccation, as haemolymph volume is regulated at the expense of other body stores. However, the contribution of hepatopancreatic water under the experimental conditions is sufficient for maintaining osmotic stability only when WLR are relatively low, as in the case of Buthidae. By contrast, the high WLR of Scorpionidae results in rapid depletion of body water stores and poor osmoregulatory capacities during desiccation.

References

Albaghdadi, L. F. (1987). Effects of starvation and dehydration on ionic balance in *Schistocerca gregaria*. *J. Insect. Physiol.* **33**, 269-274.

Edney, E. B. (1977). *Water Balance in Land Arthropods*. Springer-Verlag, Berlin.

Gefen, E. and Ar, A. (2004). Comparative water relations of four species of scorpions in Israel: evidence for phylogenetic differences. *J. Exp. Biol.* **207**, 1017-1025.

Gibbs, A. G., Fukuzato, F. and Matzkin, L. M. (2003). Evolution of water conservation mechanisms in *Drosophila*. *J. Exp. Biol.* **206**, 1183-1192.

Greenaway, P. and MacMillen, R. E. (1978). Salt and water balance in the terrestrial phase of the inland crab *Holthuisana (Austrothelphusa) transversa* Martens (Parathelphusoidea: Sundathelphusidae). *Physiol. Zool.* **51**, 217-229.

Hadley N. F. (1990). Environmental Physiology. In *The Biology of Scorpions* (ed. G. A. Polis), pp. 321-340. Stanford, CA: Stanford University Press.

Hadley N. F. (1994). *Water Relations of Terrestrial Arthropods*. San Diego, CA: Academic Press.

Harris, R. R. and Kormanik, G. A. (1981). Salt and water balance and antennal gland function in three Pacific species of terrestrial crab (*Gecarcoidea lalandii, Cardisoma carnifex, Birgus latro*). II. The effects of desiccation. *J. Exp. Zool.* **218**, 107-116.

Horowitz, M. (1970). The water balance of the terrestrial isopod *Porcellio scaber*. *Ent. Exp. Appl.* **13**, 173-178.

Hyatt, A. D. and Marshall, A. T. (1985). Water and ion balance in the tissues of the dehydrated cockroach *Periplaneta americana*. *J. Insect Physiol.* **31**, 27-34.

Lindqvist, O. V. and Fitzgerald, G. (1976). Osmotic interrelationship between blood and gut fluid in the isopod *Porcellio scaber* Latr. (Crustacea). *Comp. Biochem. Physiol. A* **53**, 57-59.

Marron, M. T., Markow, T. A., Kain, K. J. and Gibbs, A. G. (2003). Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. *J. Insect Physiol.* **49**, 261-270.

Naidu, S. G. and Hattingh, J. (1986). Water balance and osmoregulation in *Stips stali*, a nocturnal tenebrionid from the Namib Desert. *J. Insect Physiol.* **32**, 891-896.

Price, J. B. and Holdich, D. M. (1980). Changes in osmotic pressure and sodium concentration of the haemolymph of woodlice with progressive desiccation. *Comp. Biochem. Physiol. A* **66**, 297-305.

Robertson, H. G., Nicolson, S. W. and Louw, G. N. (1982). Osmoregulation and temperature effects on water loss and oxygen consumption in two species of African scorpion. *Comp. Biochem. Physiol. A* **71**, 605-609.

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment.* Cambridge: Cambridge University Press.

Toye, S. A. (1970). Some aspects of the biology of two common species of Nigerian scorpions. *J. Zool. Lond.* **162**, 1-9.

Warburg, M. R. (1986). The distribution of water in the body compartments of four scorpion species. *Comp. Biochem. Physiol. A* **84**, 637-641.

Warburg, M. R., Goldenberg, S. and Ben-Horin, A. (1980). Thermal effect on evaporative water loss and haemolymph osmolarity in scorpions at low and high humidities. *Comp. Biochem. Physiol. A* **67**, 47-57.

Warburg, M. R., Elias, R. and Rosenberg, M. (2002). The hepatopancreas of *Scorpio maurus fuscus*; seasonal changes in mass and water content as related to gender and oogenesis. *J. Zool. Lond.* **256**, 479-488.

Woodring, J. P. (1974). Effect of rapid and slow dehydration on the hemolymph osmolarity and Na+-K+ concentration in the millipede *Pachydesmus crassicutis*. *Comp. Biochem. Physiol. A* **49**, 115-119.

Zachariassen, K. E. and Pedersen, S. A. (2002). Volume regulation during dehydration of desert beetles. *Comp. Biochem. Physiol. A* **133**, 805-811.