

Comparative water relations of four species of scorpions in Israel: evidence for phylogenetic differences

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Summary

In an attempt to determine the nature of possible interspecific differences in osmotic responses to dehydration, the following species of two scorpion families were examined: *Scorpio maurus fuscus* (Scorpionidae) and *Buthotus judaicus* (Buthidae) from the mesic Lower Galilee (mean annual precipitation ~525 mm); and *Scorpio maurus palmatus* (Scorpionidae) and *Leiurus quinquestriatus* (Buthidae) from the xeric Negev Desert (mean annual precipitation ~100 mm).

When sampled in the laboratory following their capture, *B. judaicus* (548 ± 38 mOsm l⁻¹; mean \pm S.D.) and *L. quinquestriatus* (571 ± 39 mOsm l⁻¹) had higher and less variable haemolymph osmolarities than the scorpionids occupying the same habitats (511 ± 56 and 493 ± 53 mOsm l⁻¹ for *S. m. fuscus* and *S. m. palmatus*, respectively).

In response to 10% mass loss when desiccated at 30°C,

the haemolymph osmolarity of the two buthids increased by 5–9%, compared to ca. 23% in the two scorpionids. Buthids had lower water loss rates than scorpionids. The similar oxygen consumption rates, when converted to metabolic water production, imply a higher relative contribution of metabolic water to the overall water budget of buthids. This could explain why the osmoregulative capabilities exhibited by buthids are better than those of scorpionids.

We conclude that the observed interspecific differences in water and solute budgets are primarily phylogenetically derived, rather than an adaptation of the scorpions to environmental conditions in their natural habitat.

Key words: scorpion, haemolymph, osmolarity, desiccation, water budget, phylogenetic, osmoregulation.

Introduction

The order Scorpiones (Chelicerata; class: Arachnida) consists of nine living families, with about 1400 described species and subspecies. Scorpions have a wide geographic distribution, from tropical to temperate, including deserts, savannas, tropical forests, mountains over 5500 m in altitude, and the intertidal zone (Polis, 1990). Some of these habitats, e.g. arid deserts, are of extreme environmental conditions, and scorpions have a range of adaptations for life in such harsh environments.

Scorpions in general have been reported to show some of the lowest transpiration rates among arthropods (Crawford and Wooten, 1973; Edney, 1977; Hadley, 1990). The nocturnal *Hadrurus arizonensis* (Iuridae) loses water at a rate ten times lower than that of the tenebrionid beetle *Eleodes armata*, which can be active during the hot daytime hours (Hadley, 1970). Edney (1977) found 'good examples of relationships between permeabilities and habitats in all classes of arthropods', and Hadley (1990) pointed out a 'definite trend for lower transpiration rates in the more xeric species' in scorpions.

Interspecific differences in water loss rates of scorpions have been reported in several studies. Relatively high water loss

rates were reported for the tropical *Pandinus imperator* (Scorpionidae) compared with the more xeric *Buthus hottentotta hottentotta* (Buthidae) (Toye, 1970). A comparison of four scorpion species captured in the Mediterranean region of Northern Israel revealed higher water loss rates for *Scorpio maurus fuscus* (Scorpionidae) and *Nebo hierichonticus* (Diplocentridae) than those of *Leiurus quinquestriatus* and *Buthotus judaicus* (both Buthidae) (Warburg et al., 1980). The xeric *Parabuthus villosus* (Buthidae) had significantly lower water loss rates in comparison with the mesic *Opisthophthalmus capensis* (Scorpionidae) (Robertson et al., 1982).

Most scorpion species have been reported to simply tolerate increased haemolymph osmotic and ionic concentrations as a result of dehydration (Hadley, 1974; Riddle et al., 1976; Warburg et al., 1980; Punzo, 1991). The xeric South African buthid, *P. villosus*, was reported as an exception to this trend, showing good osmoregulative capacity in comparison with the mesic scorpionid *O. capensis* (Robertson et al., 1982) and other previously studied species, and comparable to that of tenebrionid beetles, successful desert-inhabiting insects. They view these capabilities, together with the scorpion's large body

size and low metabolic and water loss rates, as a ‘...very useful adaptation to a desert existence’.

Metabolic water, produced during food oxidation and entering the body general reserves, is essential to the water budget of dry-living arthropods. Generally, metabolic water constitutes a small portion of the arthropods’ total water needs (Hadley, 1994). However, the importance of metabolic water increases when other water sources are not available, e.g. in pupae or during long flights, or in animals feeding on dry food (flour moth larvae) (Edney, 1977). Robertson et al. (1982) calculated metabolic water production for *P. villosus*, based on oxygen consumption rates. They concluded, assuming oxidation of lipids, that metabolic water production rate accounts for only ~5% of the total water loss rate (WLR). However, this ratio was based on short-term water loss, and the authors suggest that the fraction of metabolic water from WLR could be higher if transpiration rates decrease significantly during prolonged desiccation.

Scorpions are represented in Israel by three families, Buthidae, Scorpionidae and Diplocentridae, consisting of 19 species and subspecies from 9 genera (Levy and Amitai, 1980). Buthidae and Scorpionidae are represented in Israel by more than one species (or subspecies). Within each of the two families there are species/subspecies of distinct geographical distribution, which are thus faced with different environmental conditions. *L. quinquestriatus* (Buthidae) and *Scorpio maurus palmatus* (Scorpionidae) are predominantly xeric species, whereas *B. judaicus* (Buthidae) (previously named *Hottentotta judaica*) and *S. m. fuscus* (Scorpionidae) mainly occupy mesic environments. *B. judaicus* occurs in areas where annual rainfall is at least 350–400 mm, while the Judea mountains constitute the northern and southern distribution borders of *S. m. palmatus* and *S. m. fuscus*, respectively (Levy and Amitai, 1980).

Previous studies referred to *B. judaicus* as a xeric species, which correlated well with the species’ low transpiration rates and fitted the habitat–permeability accepted relationship. However, the actual mesic distribution of *B. judaicus* led us to hypothesize that the observed interspecific differences could be phylogenetically derived. The above four species were used in an attempt to determine whether interspecific differences in water budgets and osmotic responses to desiccation can be viewed as physiological adaptations to environmental conditions, or stem from phylogenetic constraints.

Materials and methods

Scorpion collection

Specimens of *S. m. fuscus* and *B. judaicus* were collected from the same area in the mesic lower Galilee, in pine woodlands near Migdal Ha’emek (mean annual precipitation ~525 mm). *S. m. palmatus* were collected from sandy loess plains near Sde Boker (mean annual precipitation ~100 mm) in the Negev desert, and *L. quinquestriatus* were collected from the stony desert areas of nearby Yerucham.

The two buthids were found under stones, whereas the two scorpionids were captured mostly by digging their burrows. Of the two *Scorpio maurus* subspecies, the mesic *S. m. fuscus* was often captured at its burrow entrance, under stones.

Haemolymph osmolarity

Haemolymph samples were taken from the scorpions within 48 h of collection. The osmolarity values recorded from scorpions within 24 h and 48 h of collection were similar. After weighing the scorpions (to ± 0.1 mg), a sharpened glass capillary was inserted into the pericardial sinus by puncturing the dorsal intersegmental membrane. The tapered tip of the glass capillary enabled an immediate closure of the wound. The haemolymph volume withdrawn was usually 10–15 μ l, of which 8 μ l were required for measuring osmolarity (5100C Vapour Pressure Osmometer, Wescor, Logan, USA).

The scorpions were then held at room temperature (~25°C) in round (9 cm diameter) transparent plastic boxes with soil from their respective collection sites. Food (adult crickets) was supplied *ad libitum* for 14 days in order to monitor the effect of feeding on haemolymph osmolarity, and to minimise variation between individuals that could have resulted from their energetic or hydration status upon capture.

A second haemolymph sample was taken following feeding, when the mean initial masses of the four species were 2.164 g (range 1.028–3.623 g), 2.294 g (1.023–4.404 g), 1.745 g (1.100–2.603 g) and 2.023 g (1.136–3.284 g) for *B. judaicus*, *L. quinquestriatus*, *S. m. fuscus* and *S. m. palmatus*, respectively. Preliminary measurements of haemolymph volume revealed a ~20–30% volume:body mass ratio (E. Gefen and A. Ar, unpublished data), thus a 10 μ l sample from a 1 g scorpion did not constitute more than 5% of the total haemolymph volume. In case of persistent bleeding as a result of haemolymph withdrawal, scorpions were discarded from further investigations.

After allowing 24 h for recovery the scorpions were weighed again, and transferred to identical empty plastic boxes. The boxes were placed in a controlled temperature chamber (30.0 \pm 0.5°C, ambient humidity 40–60%). The scorpions were weighed daily, and following losses of up to 26% of initial mass haemolymph osmolarity was measured again.

A separate group of scorpions was sampled for both total haemolymph osmolarity and ion concentrations. The scorpions were maintained as described above, and up to 30 μ l of haemolymph were extracted following feeding and again after prolonged desiccation. 8 μ l samples of haemolymph were used for osmolarity measurements and determination of chloride (CMT10 chloride titrator; Radiometer, Copenhagen, Denmark) and sodium/potassium content (Model 480 Flame Photometer, Corning, Medfield, USA).

Mass loss rate

Mass loss rates (MLR) were measured at 30°C and 50% relative humidity (RH). The scorpions were fed *ad libitum* until 48 h before the initiation of the measurements. After allowing

Table 1. *Haemolymph osmolarity values of the four scorpion species in the various sampling seasons, measured within 48 h of capture*

Sampling month	Haemolymph osmolarity (mOsm l ⁻¹)			
	<i>B. judaicus</i>	<i>L. quinquestriatus</i>	<i>S. m. fuscus</i>	<i>S. m. palmatus</i>
February	562±36 ^a (24)	573±34 ^a (22)	492±33 ^b (21)	470±42 ^b (30)
April	519±23 ^a (13)	–	491±47 ^a (22)	–
June	524±25 ^b (27)	607±20 ^a (8)	508±52 ^{b,c} (13)	485±40 ^c (28)
August	549±31 ^{a,b} (7)	584±32 ^a (22)	510±46 ^{b,c} (16)	503±61 ^c (79)
October	574±57 ^a (11)	567±43 ^a (49)	530±48 ^{a,b} (5)	491±43 ^b (31)
December	566±28 ^a (17)	556±36 ^{a,b} (28)	527±63 ^{b,c} (30)	503±48 ^c (21)
Mean	548±38 (99)	571±39 (129)	511±56 (107)	493±53 (189)

Values are means ± s.d. (sample sizes in brackets).

Different superscript letters indicate significant differences between species within a sampling month (one-way ANOVA followed by Tukey's HSD test, $\alpha=0.05$).

24 h for acclimation in the temperature chamber (30.0±0.5°C, ambient humidity 40–60%), the scorpions were placed in individual plastic boxes with perforated bottoms, which were put on a raised plastic grid in a sealed tank. Air supply (100 ml min⁻¹) at 50%RH through the boxes was achieved by mixing dry air with air saturated with water vapour at the experimental temperature. Flow rates were controlled by flow controllers (5800 Series, Brooks, Veenendaal, Holland), and the humidity level was validated using a humidity sensor (±2%RH; Almemo, Holzkirchen, Germany). Animal weighing was performed every second day, and MLR calculated by the difference in mass between successive measurements (excluding dry mass of excretions), divided by the elapsed time between measurements. Mass-specific MLR were calculated by dividing MLR by the mass recorded in the previous weighing.

Gas exchange mass loss, based on measured oxygen consumption rates and RQ values, was used for estimation of water loss rates (WLR). The maximal mass loss rate (assuming carbohydrate catabolism) that may have resulted from gas exchange was subtracted from the total MLR, and the remainder taken as water loss rate (WLR).

Oxygen consumption

Oxygen consumption rates (\dot{M}_{O_2}) were calculated from a pressure drop in a closed system, comprising two 100 ml glass syringes (cell volume 40 ml), which contained ascarite for absorption of CO₂ and water vapour. The tips of the two syringes were connected to the two sides of a differential pressure transducer (model DP15TL, Validyne, Northridge, USA). The scorpion was held in one syringe, while an identical empty syringe was used as a reference cell. Both syringes were placed in a temperature-controlled water bath (30±0.2°C). The voltage output recorded from the pressure transducer was converted to oxygen consumption rate by injecting known volumes of oxygen to the measurement cell in order to restore initial pressure. Cell pressure was not allowed to drop by more than 0.25%.

Results

Haemolymph osmolarity

Table 1 summarises the field haemolymph osmolarity values of the scorpions throughout the study. Significant within-species seasonal differences occurred in all four species examined [one-way analysis of variance (ANOVA), $P<0.05$]. These differences were not consistent across species, as *L. quinquestriatus* values were higher in the summer (June–August), while the other species had higher haemolymph osmolarities in October–December. Table 1 shows a consistent pattern of higher haemolymph osmolarities for bothids throughout the sampling months. In addition, haemolymph osmotic concentration of scorpionids was generally more variable than that of bothids. No significant differences in mean haemolymph osmolarities within a family were found, despite the consistently higher mean haemolymph osmolarities of the mesic *S. m. fuscus* in comparison with the xeric *S. m. palmatus*.

Fig. 1 presents the haemolymph osmotic change after 14 days of *ad libitum* feeding, as a function of the deviation in haemolymph osmolarity of the individual scorpion from its sample mean upon capture. In all species variability decreased following feeding; haemolymph osmolarity increased when initial values were lower than the mean, whereas relatively high initial osmolarities resulted in moderate increase or even decrease in osmolarities following feeding (Fig. 1). However, the results suggested that Buthidae and Scorpionidae differ in their osmotic response to *ad libitum* feeding following capture (Table 2). Within-family slopes did not differ significantly for either Scorpionidae ($F_{1,262}=0.585$, $P=0.45$) or Buthidae ($F_{1,186}=0.013$, $P=0.91$), but the between-families difference in slopes was significant ($F_{1,452}=8.687$, $P=0.003$). Feeding resulted in significantly increased haemolymph osmotic concentrations in all species (t -test for dependent samples, $P<0.01$), but a milder increase was observed for *B. judaicus* (Fig. 1; Table 2).

Fig. 2 shows the effect of desiccation at 30°C and 40–60% RH (expressed as % mass loss, excluding dry excretions) on

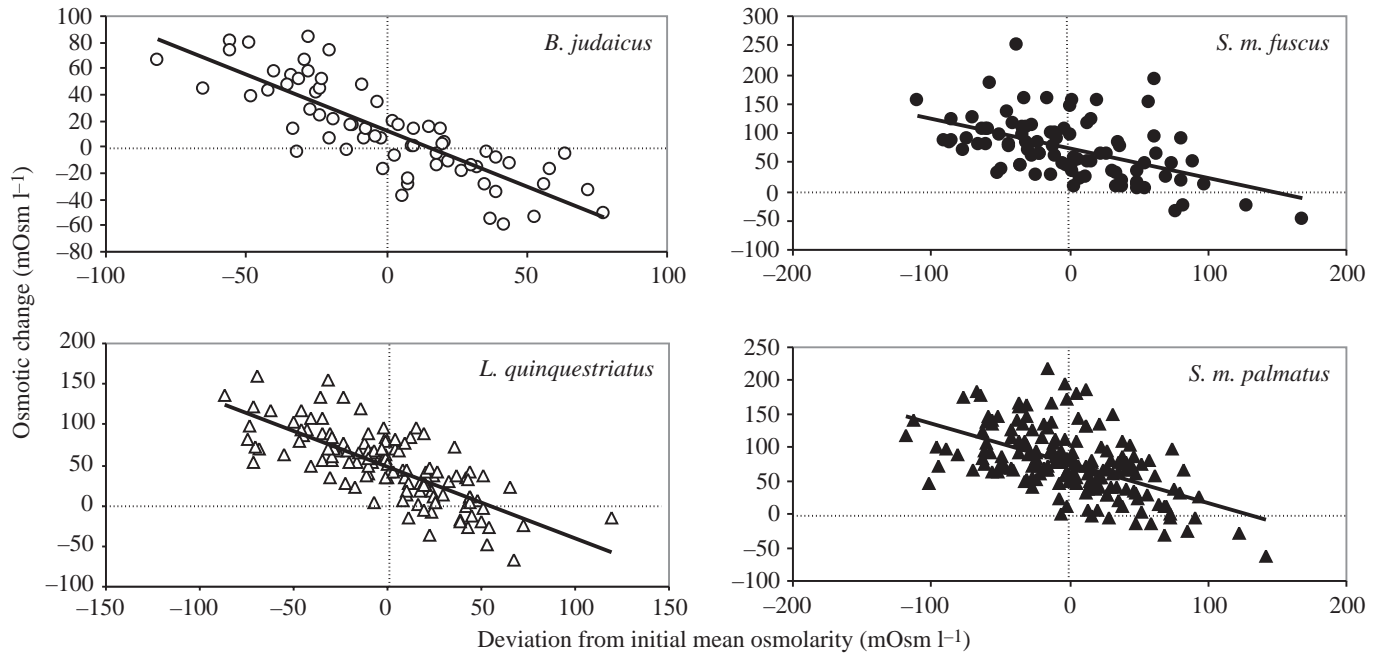


Fig. 1. Haemolymph osmolarity change (mOsm l^{-1}) after a 14-day period of *ad libitum* feeding, as a function of the deviation of haemolymph osmolarity of the individual scorpion from the initial sample mean (before feeding; mOsm l^{-1}). For significance and regression equations, see text and Table 2.

haemolymph osmolarity. *S. m. palmatus* and *S. m. fuscus* show a sharp increase in haemolymph osmolarity even at moderate desiccation levels (5–10% mass loss, excluding dry excretions). The two Buthidae, *L. quinquestratus* and *B. judaicus*, show a certain capability to withstand higher water losses while maintaining relatively stable haemolymph osmotic concentrations. Moreover, buthids often displayed a decrease in osmotic concentration of the haemolymph with the onset of desiccation (Fig. 2). Such a decrease was not observed for either of the two scorpionid subspecies.

Most of the measured osmolarity values, before and after feeding, were accounted for by sodium and chloride ions (>93% of total osmolarity). These remained the main ions contributing

to the total haemolymph osmolarity following desiccation in all four species. However, at 570 mOsm l^{-1} sodium and chloride ions accounted for more than 90% of the total osmotic concentration of the haemolymph, but their combined contribution decreased to ~80% of the $>700 \text{ mOsm l}^{-1}$ of desiccated scorpions (Fig. 3). The concentration of potassium ions appeared to increase with desiccation, but never exceeded 2% of the total haemolymph osmolarity.

Table 3 presents the results of haemolymph osmotic change (% of initial), after desiccating scorpions of the four species to 10% loss of their initial mass under similar experimental conditions. Interspecific comparison (ANCOVA, initial mass as covariate) of arcsine-transformed % osmolarity changes (decreased osmolarity was designated as 0% change) matched the overall picture reflected in Fig. 2. The haemolymph osmolarities of both *L. quinquestratus* and *B. judaicus* were significantly ($P < 0.05$) less affected by a 10% body mass loss, than were those of the two *Scorpio maurus* subspecies. The moderate increase in haemolymph osmolarity of *B. judaicus* is further highlighted by the observation that one specimen had an uncharacteristic increase of 27% in haemolymph osmolarity, while the other 11 showed an increase of $3.05 \pm 2.53\%$ following a 10% mass loss. Furthermore, of all scorpions desiccated to 10% mass loss, only five *B. judaicus* specimens exhibited a decreased haemolymph osmolarity compared to initial values. These results are in agreement with the general osmotic pattern presented in Fig. 2, and confirm that buthids show a relatively moderate osmotic concentration increase in response to desiccation, regardless of their geographic distribution.

Table 2. Linear regression equations describing the effect of initial haemolymph osmotic status of the scorpion (x), expressed as deviation from the sample mean, on the osmotic change as a result of feeding (y)

Species	Initial osmolarity (mOsm l^{-1})	Equation	N	r^2
<i>B. judaicus</i>	553 ± 36	$y = -0.86x + 12.03$	66	0.68
<i>L. quinquestratus</i>	573 ± 36	$y = -0.88x + 48.49$	124	0.56
<i>S. m. fuscus</i>	505 ± 52	$y = -0.50x + 72.36$	90	0.26
<i>S. m. palmatus</i>	488 ± 45	$y = -0.60x + 77.05$	176	0.31

Initial haemolymph osmolarity values are means \pm S.D.

For all species, $P \leq 0.001$.

Fig. 2. Haemolymph osmolarity change (% of initial osmolarity) following desiccation, as a function of mass loss (% of initial mass, excluding dry excretions). (1) *Buthotus judaicus*: $y=0.10x^2-1.05x$, $r^2=0.61$; (2) *Leiurus quinquestriatus*, $y=0.09x^2-0.76x$, $r^2=0.64$; (3) *Scorpio maurus fuscus*: $y=0.10x^2+0.49x$, $r^2=0.73$; (4) *Scorpio maurus palmatus*: $y=0.06x^2+0.67x$, $r^2=0.54$.

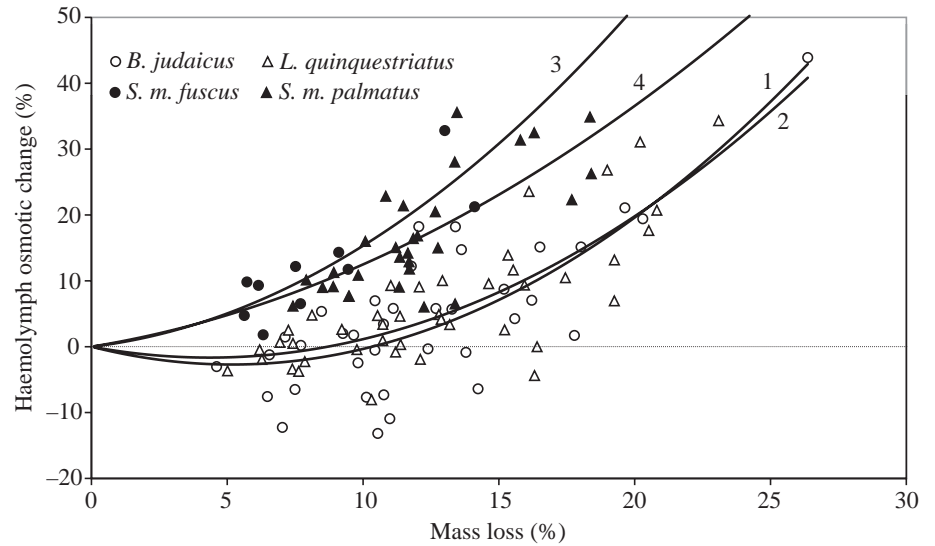


Table 3. Haemolymph osmotic changes following a loss of 10% from initial body mass (excluding dry excretions)

Species	N	Initial mass (g)	Initial osmolarity (mOsm l ⁻¹)	Mass loss (%)	Time (days)	Osmotic change (%)
<i>B. judaicus</i>	12	2.224±0.698	579±24	9.9±0.1	20.7±6.5	5.1±10.9 ^b
<i>L. quinquestriatus</i>	9	2.598±1.113	631±23	9.9±0.2	16.4±4.3	9.1±2.7 ^b
<i>S. m. fuscus</i>	5	1.619±0.495	578±49	9.9±0.4	6.8±1.6	23.6±8.4 ^a
<i>S. m. palmatus</i>	14	2.198±0.814	562±32	10.0±0.2	9.6±2.2	23.1±7.2 ^a

Values are means ± s.d.

Different superscript letters indicate significant differences (ANCOVA of arcsine-transformed % osmolarity changes, with initial mass as covariate, $\alpha=0.05$).

Mass loss rate

Mass-specific mass loss rates (MLR) at 30°C and 50%RH are shown, at 2-day intervals over a 2-week measurement period, in Fig. 4. The two buthids show a steady MLR throughout the measuring period, while those of the

scorpionids decrease during the first week and reach steady-state values towards the second week. While the xeric *S. m. palmatus* had a seemingly lower MLR than its mesic subspecies *S. m. fuscus* (though without statistical significance between steady state values), it was the mesic *B. judaicus* with the lower MLR among the buthids. Interspecific differences in MLR were more evident following a short desiccation period as a result of the decreasing MLR of scorpionids (Fig. 4). Comparison of average steady-state MLR (days 8–14) revealed a significant difference ($P<0.05$) between the two mesic species only, *B. judaicus* and *S. m. fuscus* (one-way ANOVA of values adjusted to initial body mass, followed by Tukey's HSD test).

Lipid catabolism (RQ ~0.7) results in negligible mass change as a result of gas

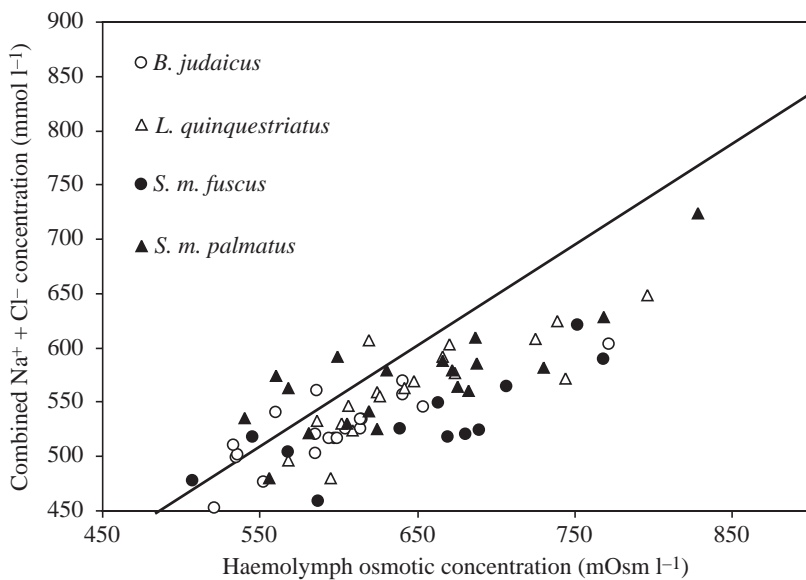


Fig. 3. Sum of sodium and chloride ion concentrations at increasing haemolymph osmotic concentrations following desiccation. The slope (0.95) of the solid line represents the initial fraction of sodium and chloride ions from total haemolymph osmolarity (for individuals included in this sample).

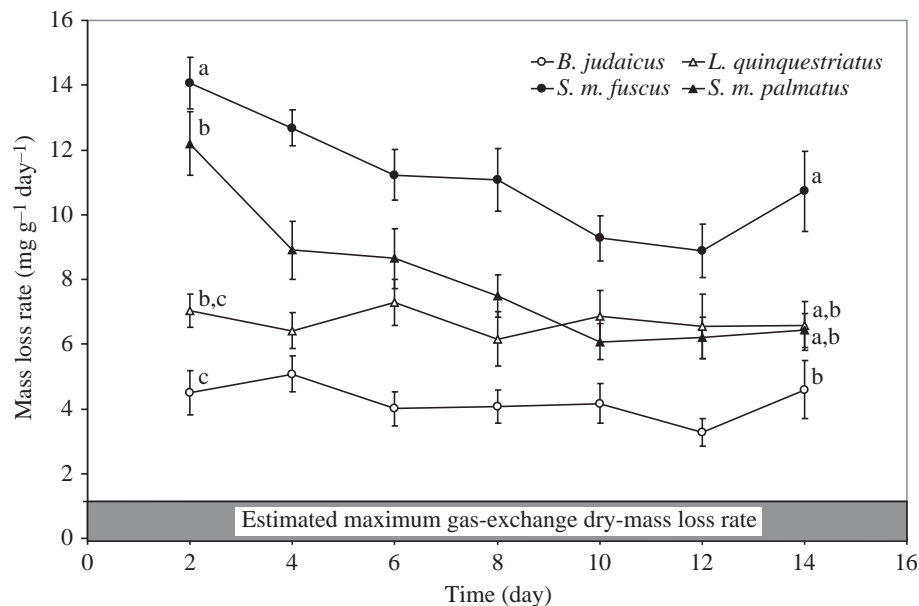


Fig. 4. Daily mass-specific mass loss rates (mean \pm S.E.M.) as a function of time from initiation of measurements at 30°C and 50%RH. Letters represent statistically significant differences (one-way ANOVA on mass-adjusted MLR; $\alpha=0.05$) between day 2 and between averaged days 8–14 MLR values. The filled area represents the estimated maximal dry-mass loss as a result of metabolism, based on carbohydrate catabolism and an oxygen consumption rate of 0.1 ml g⁻¹ h⁻¹ (see text).

exchange, because the molecular weight ratio of consumed O₂ to emitted CO₂ is also ~ 0.7 . However, when carbohydrates are metabolised, and RQ is 1.0, this molecular mass ratio results in net dry mass loss. Dry mass loss is maximal when carbohydrates are the sole metabolic fuel, whereas values are intermediate when both lipids and carbohydrates are catabolised. Therefore, the filled area in Fig. 4 represents the estimated maximal dry-mass loss rate of the scorpions, based on carbohydrate catabolism and an \dot{M}_{O_2} of 0.1 ml g⁻¹ h⁻¹. Assuming the four species included in this study use similar proportions of lipids and carbohydrates as metabolic fuels, the measured differences in mass loss rates represent differences in the water loss rates of the species.

Oxygen consumption rate

Oxygen consumption rates (\dot{M}_{O_2}) of resting scorpions at 30°C (mean \pm S.D.), and their body mass range are given in Table 4. ANCOVA (body mass as covariate) confirmed a lack of any interspecific difference in \dot{M}_{O_2} ($F_{(3,26)}=0.324$, $P=0.81$). The combined effect of body mass on the oxygen consumption of the four scorpion species was:

$$\dot{M}_{O_2} = 0.11M^{0.90},$$

where \dot{M}_{O_2} is in ml O₂ consumed h⁻¹ and M is mass (g) ($N=31$; $r^2=0.68$).

Discussion

The haemolymph osmolarity of scorpions is relatively high in comparison with other terrestrial organisms, including other arthropods (Hadley, 1994). These elevated osmotic concentrations are unlikely to result in a much more favourable water activity gradient between body fluids and the environment. Doubling the haemolymph osmolarity from 300 to 600 mOsm l⁻¹ will only decrease the equilibrium point with

Table 4. Mass, mass range and mass-specific oxygen consumption rates of the four scorpion species at 30°C

Species	<i>N</i>	Mass (g)	Mass range (g)	Mass specific \dot{M}_{O_2} (ml g ⁻¹ h ⁻¹)
<i>B. judaicus</i>	8	2.5211 \pm 1.0725	1.1249–4.0233	0.107 \pm 0.029
<i>L. quinquestriatus</i>	9	3.5461 \pm 1.6283	1.2945–5.8385	0.098 \pm 0.022
<i>S. m. fuscus</i>	7	2.5357 \pm 0.8400	1.6095–3.7735	0.105 \pm 0.034
<i>S. m. palmatus</i>	7	2.2210 \pm 0.6785	1.4202–3.2772	0.105 \pm 0.028

Values are means \pm S.D.

the surrounding atmosphere from 99.5% to 99.0%RH (Willmer, 1980). However, it has been suggested that a high haemolymph osmotic concentration might be advantageous in matching that of the partly ingested prey (Burton, 1984). It has been shown for *H. arizonensis* (Iuridae) that an increased osmotic gradient between the mucosal and serosal sides of the ileal epithelium enhances water flux to the haemolymph (Ahearn and Hadley, 1977). It is evident from results in Table 1 that despite seasonal fluctuations, haemolymph osmolarity of buthids is consistently higher than that of scorpionids. This is in accordance with previously reported values (Robertson et al., 1982), and supports the notion of phylogenetically derived physiological characteristics. As buthids generally do not burrow (Polis, 1990), and are thus more exposed to harsh environmental conditions, they could benefit from the higher haemolymph osmolarity levels in order to maximise retention of prey water.

The haemolymph osmolarity of scorpionids appear to be more variable than that of buthids (Table 1; Kimura et al., 1988). The calculated coefficients of variance for haemolymph osmolarities of scorpionids and buthids are 10.8% and 6.8%,

respectively. This may result from the scorpionids' higher WLR (Fig. 4), which is more challenging for maintaining a balanced water budget, reflected in the haemolymph osmotic concentrations measured immediately following capture (Table 1). This osmotic variability decreases in all four species after feeding, but the significantly higher slopes found for buthids (Fig. 1, Table 2) provide further evidence of their better haemolymph osmoregulative capabilities compared with scorpionids. It is interesting to note that following a 14-day *ad libitum* feeding period the haemolymph osmolarity of *B. judaicus* remained fairly stable, while that of the other three species showed an increase of 50–70 mOsm l⁻¹ (Table 3). The 'osmotically favourable' state in which specimens of *B. judaicus* are found in the field could be attributed to their lower WLR.

Both Fig. 2 and Table 3 highlight the fact that the two buthids have a better capability to osmoregulate following water loss, compared to the two *Scorpio maurus* subspecies. It has been suggested that most scorpion species respond to dehydration by '...simply tolerating increases in haemolymph osmolality and ionic concentrations...' (Hadley, 1994). Nevertheless, our results (Fig. 2), together with those from a previous study (Robertson et al., 1982) suggest that scorpions vary in their ability to osmoregulate in response to water loss.

Fig. 3 shows that the contribution of sodium and chloride ions to total haemolymph osmolarity decreases with increasing total osmolarity. The decrease in the relative combined contribution of these two ions to the total haemolymph osmolarity of desiccated scorpions correlates with an observed decrease in excretion rate with time during prolonged desiccation (E. Gefen and A. Ar, unpublished data). Scorpions reaching haemolymph osmolarities of >700 mOsm l⁻¹ (Fig. 3; *N*=11), and averaging 757 mOsm l⁻¹, had an initial mean value of 586 mOsm l⁻¹. The decrease in the relative sodium and chloride ion contribution from the initial 93% (545 mOsm l⁻¹) to 81% following desiccation (613 mOsm l⁻¹) means that these two ions are responsible for ~40% of the total increase in the haemolymph osmolarity of the desiccated scorpions. It has been shown that dehydration results in an increase in solute content in the haemolymph of beetles, probably due to the accumulation of excretory products such as allantoin and urea (Cohen et al., 1986; Naidu, 1998, 2001). It appears possible that at least some of the remaining unknown accumulated solutes found in the haemolymph of the desiccated scorpions are excretory metabolites. These are usually excreted in the hydrated organism, but may be retained during desiccation in an attempt to reduce excretory water loss.

Fig. 2 shows that the osmotic response of scorpions to desiccation is not linear. Polynomial rather than linear regression lines are best fitted to data of all four species. This is particularly evident for *L. quinquestriatus* and *B. judaicus*, with linear regression *r*² values of 0.34 and 0.23, respectively. The non-linear osmotic response is further supported by the decreasing osmolarities recorded for buthids in the early stages of desiccation. A similar pattern is evident in the osmotic response of *P. villosus* to prolonged desiccation (figure 2 in

Robertson et al., 1982). A non-linear osmotic response to desiccation was also reported for the isopod *Porcellio scaber* (Horowitz, 1970). The author suggests movement of water from tissues to the haemolymph, which later increases in osmolarity when the water supply does not meet demand. A decreased haemolymph osmolarity following mild desiccation has also been reported for *P. scaber*, with the hepatopancreas being mentioned as a possible source for water movement to the haemolymph (Lindqvist and Fitzgerald, 1976). It has also been shown (Woodring, 1974) that osmoregulative capacity in the millipede *Pachydesmus crassicutis* depends on desiccation rates.

Buthids lose body water at a relatively low rate (Fig. 4) and thus may allow compensatory mechanisms, in the form of water stores other than the haemolymph, to keep haemolymph osmolarity levels stable during mild dehydration. In comparison, scorpionids lose the first 10% of their initial mass within 6–10 days of high WLR (Fig. 4; Table 3). These rates may be higher than the rate of water movement to the haemolymph, which could explain why scorpionids do not exhibit the initial decrease in haemolymph osmolarity. Following prolonged desiccation water stores may be exhausted, and cannot keep haemolymph osmolarity stable under these experimental conditions.

Robertson et al. (1982) described the osmoregulative capabilities of *P. villosus* (Buthidae), together with its low WLR and large body size, as a possible adaptation to its xeric habitat. This was in contrast to the mesic scorpionid *O. capensis*, which had higher WLR, and did not exhibit similar ability to osmoregulate its haemolymph following desiccation. The difference in WLR of *P. villosus* and *O. capensis* is in agreement with the habitat–water permeability relationship reported for arthropods in general (Edney, 1977), as is that between *B. hottentota* (Buthidae) and *P. imperator* (Scorpionidae) (Toye, 1970). Another study concluded that among four scorpion species found in Northern Israel, those inhabiting mesic habitats lose water at a higher rate than xeric species (Warburg et al., 1980).

However, none of the previous comparative studies (Toye, 1970; Warburg et al., 1980; Robertson et al., 1982) distinguished between the suggested ecologically adaptive nature of the physiological phenomenon and its possible phylogenetic origin. It is therefore important to note that the lower WLR reported by all the above authors are those of Buthidae. Likewise, reference to *B. judaicus* as a xeric species (Warburg et al., 1980), despite its distribution being limited to areas of at least 350–400 mm annual rainfall (Warburg and Ben-Horin, 1978; Levy and Amitai, 1980), could be just as misleading.

The results of the present study, together with previously available comparative data, lead us to suggest that interspecific differences in water relations of scorpions are likely to be largely the result of phylogenetic constraints rather than simply an adaptation of the organism to environmental stress in its natural habitat. The importance of minimising water loss for the overall water budget of terrestrial arthropods, coupled with the low

WLR reported for buthids in general, could explain the better osmoregulative capabilities of scorpions of this family (Robertson et al., 1982; this study). Many buthids have a range of life history characteristics that differ from scorpions of other families, e.g. accelerated life history and developmental plasticity (Polis, 1990; Lourenco et al., 2003). These characteristics, together with lowered integument permeability, correlate well with the extreme and unpredictable environmental conditions encountered by the surface-dwelling buthids.

Robertson et al. (1982) calculated a metabolic water production rate ($\text{met}_{\text{H}_2\text{O}}$) to transpiration rate ratio of 5% for *P. villosus* at 25°C, though they stress that this seemingly small contribution applies to short-term (6 h exposure) desiccation. Scorpions in general have relatively low metabolic rates, compared to other arthropods of similar body size (Lighton et al., 2001), and as a result the total amount of metabolic water produced is low. However, the highly waterproof integument of scorpions contributes to their water budget by lowering WLR, and thus increases the relative contribution of metabolic water to the overall water budget. Lighton et al. (2001) calculated the metabolic rate of scorpions to be 24% that of typical terrestrial arthropods, while the transpiration rates of six buthid species calculated by Hadley (1994) are at least an order of magnitude lower than those of most xeric insects. Therefore, the significance of metabolic water in the water budget of scorpions is expected to be relatively high in comparison with other arthropods. This should also be reflected in osmoregulative capabilities correlated with WLR of the respective species.

The results of our measurements of oxygen consumption rates (\dot{M}_{O_2}) (Table 4) are comparable to previously reported values for scorpions (Withers and Smith, 1993). \dot{M}_{O_2} converted to metabolic water production (1.89 ml O_2 mg^{-1} H_2O for lipid oxidation) yielded higher percentages of the total water loss for all four species included in this study, compared to the value reported by Robertson et al. (1982). The calculated $\text{met}_{\text{H}_2\text{O}}$:WLR ratio for scorpionids was 9–18% in the early stages of desiccation, and up to 22% in the second week. In comparison, WLR values of buthids are lower, and as a result a higher percentage (20–34%) of their WLR is accounted for by $\text{met}_{\text{H}_2\text{O}}$.

Glycogen levels in the hepatopancreas of scorpions have been shown to decrease during starvation (Sinha and Kanungo, 1967). Assuming carbohydrate metabolism, and accounting for the resultant mass loss as a result of gas exchange (Fig. 4), the $\text{met}_{\text{H}_2\text{O}}$:WLR ratios were ~30% for *L. quinquestriatus* throughout the experiment, and as high as 50% for *B. judaicus*. These high ratios for buthids contribute to their better osmoregulative capabilities in response to desiccation. In fact, of the four species in this study, *B. judaicus* appears to be the best osmoregulator (Table 3), which is well reflected in its $\text{met}_{\text{H}_2\text{O}}$:WLR ratio. In comparison, until reaching 10% mass loss, the contribution of $\text{met}_{\text{H}_2\text{O}}$ to the overall water turnover of *S. m. fuscus* and *S. m. palmatus* is 13–17% and 15–27%, respectively, as a result of their high initial WLR (Fig. 4). Furthermore, glycogen binds water, estimated at 3–5 times its

own mass (Schmidt-Nielsen, 1990), which is made available when glycogen is catabolised. Thus, for low WLR, rates of release and transfer of water (bound and metabolic) to the haemolymph may exceed evaporation rate. Therefore, glycogen oxidation and the low WLR recorded for buthids (Fig. 4) could account for the observed initial decrease in the haemolymph osmolarity in species of this family (Fig. 2). However, availability of this water source may diminish with depleting glycogen stores.

In conclusion, interspecific differences in the osmoregulative capabilities of scorpions occur, and we suggest that phylogenetic constraints play a major role in the ability of scorpions to resist desiccation. The enhanced osmoregulative capacity of buthids is likely to have evolved in response to the surface-dwelling existence of scorpions of this family. The results of this study suggest that the contribution of metabolic water to the overall water budget of scorpions is higher than previously thought, and influences their osmoregulative capabilities.

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