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Sexual dimorphism in desiccation responses of the sand scorpion *Smeringurus mesaensis* (Vaejovidae)

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Abstract

The osmoregulatory and respiratory responses of male and female *Smeringurus mesaensis* (Vaejovidae) to prolonged desiccation were measured. No significant effect of sex on mass-loss rates (MLRs) was found. Still, females maintained their haemolymph osmolality when desiccated to 10% mass loss, whereas that of males increased significantly after loss of as little as 5% of initial mass. Females had a 3-fold larger hepatopancreas, significantly higher hepatopancreas water content and higher metabolic rates when adjusted to hepatopancreas-free dry mass. Thus, females not only store more water in the hepatopancreas but also mobilise it to the haemolymph at a higher rate during desiccation, thus maintaining haemolymph osmolality.

Gas exchange rates of both males and females decrease as desiccation progresses. An initial respiratory exchange ratio (RER) of ~0.9 is followed by a significant increase at mass loss levels of 7.5% and higher. RER values greater than 1.0 may result from partial shift to anaerobic catabolism, which allows closure of the book lung spiracles for longer duration, thus minimising respiratory water loss.

The effects of gas exchange rates on rates of water mobilisation between body compartments and water loss to the environment suggest a trade-off between maintaining osmotic stability and conserving body water stores under stressful conditions.

Keywords: Scorpions; Sexual dimorphism; Water; Osmoregulation; Metabolic rate; Desiccation

1. Introduction

Terrestrial arthropods face a challenge to maintain body water balance in what is often a desiccating environment. Their small body size results in a high body surface area relative to volume. Nevertheless, scorpions, among other arthropods, have successfully colonised habitats of extremely high temperatures and low humidity.

Some arthropods survive extreme environmental conditions by burrowing, thus creating a microhabitat buffered from the harsh surface conditions. Many species have also adopted a nocturnal activity pattern, spending the hot daytime hours in their shelters. These avoidance strategies are complemented by physiological mechanisms that help maintain homeostasis despite constant loss of body water to the surrounding environment. Scorpions exhibit some of the lowest water-loss rates recorded for arthropods (reviewed in Edney, 1977; Hadley, 1994).

Scorpions are generally considered as poor osmoregulators, responding to water loss by simply tolerating increased haemolymph osmolarity until body water stores are replenished (Hadley, 1994). The ability of the South African species, *Parabuthus villosus* (Buthidae), to regulate its haemolymph during desiccation was reported as an exception to this pattern (Robertson et al., 1982). Its enhanced osmoregulatory capability in comparison with a mesic species was interpreted as an adaptation of *P. villosus* to conditions in its xeric habitat. However, similar osmoregulatory capacities have since been reported for two other Buthidae species, *Leiurus quinquestriatus* and *Buthotus judaicus*, suggesting a phylogenetic component to interspecific variation (Gefen and Ar, 2004).

When desiccated, scorpions regulate both haemolymph volume and its osmotic concentration by mobilising water from the hepatopancreas to the haemolymph. These regulatory capacities depend on the metabolic rate, water-loss rate,

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composition of the catabolised metabolites and their long-term availability (Gefen and Ar, 2005). This role of the hepatopancreas as a water reservoir, and the significantly larger organ size of females (Warburg et al., 2002), suggest possible sexual dimorphism in response to desiccation stress. The contribution of metabolic water production and water mobilisation rates to the total water budget during desiccation has so far been estimated by using water-loss rates measured during short exposure to experimental conditions (Robertson et al., 1982) or metabolic rates of hydrated scorpions (Gefen and Ar, 2004). Nevertheless, there is evidence that both water-loss rates (Gefen and Ar, 2004) and metabolic rates (Riddle, 1978) of scorpions decrease during prolonged desiccation. Additionally, previous studies were not controlled for possible effects of sex on desiccation resistance performance in scorpions despite the evident difference in the size of female and male hepatopancreas.

The aim of this study was to test whether the suggested role of the hepatopancreas in water management is reflected in differences in the osmotic response to desiccation between male and female *Smeringurus mesaensis* (Vaejovidae). Metabolic rates of male and female sand scorpions, as well as haemolymph osmolality and hydration state were determined at various experimental mass loss levels. It was tested whether (i) the larger female hepatopancreas contributes to better osmoregulatory capacity compared with that of males, and (ii) osmoregulatory capacities are correlated with metabolic rates, which affect rates of hepatopancreas water mobilisation to the haemolymph.

2. Materials and methods

2.1. Scorpions

Adult *S. mesaensis* (Vaejovidae) were collected during summers (July–September) of 2006 and 2007 in the Mojave Desert, south of Zzyzx, California (35.05°N, 116.07°W). Scorpions fluoresce under ultraviolet (UV) light and were located on the sand dunes surface at night using UV flashlights. The scorpions were placed in round plastic containers (~9 cm in diameter) with a layer of sand ~5 cm deep, and brought back to the lab within 24 h of capture. In the lab, the scorpions were kept at 30 °C for 2 weeks, during that time they were provided with live crickets for every 2–3 days.

2.2. Mass loss levels

After the 2-week feeding period, the scorpions were denied food for 24 h prior to initial haemolymph sampling (see below). After haemolymph sample withdrawal, the scorpions were allowed to recover for 24 h, before they were weighed for their initial mass (±0.1 mg) (AG285, Mettler Toledo, Switzerland). The scorpions were then assigned to the experimental groups of 2.5%, 5.0%, 7.5%, and 10% loss from initial body mass, and placed in identical plastic containers without sand until reaching the desired mass loss level. The containers were kept at 30 °C and 15–30% relative humidity (Environmental Growth Chambers, Chagrin Falls, OH, USA).

Mass-loss rates (MLRs) during the desiccation period were determined in scorpions from the first collected sample (summer 2006), which were weighed daily until reaching their respective assigned mass loss levels. MLRs were calculated as the difference in mass between two successive measurements, excluding mass of dry excretions and dividing by the time interval between measurements.

2.3. Haemolymph osmolality

Initial haemolymph samples were taken from the scorpions at least 24 h following removal of any live cricket from their containers. The scorpions were restrained to a wooden plate with two rubber bands placed over their pedipalps and metasoma. A sharpened 25 µl glass micropipette was inserted into the pericardial sinus, and <20 µl haemolymph sample was withdrawn by capillary force. Haemolymph osmolality was measured in 10 µl samples using a VAPRO vapour pressure osmometer (Wescor, Logan, Utah, USA). A second haemolymph sample was collected and measured when the scorpions reached their respective experimental mass loss level, following measurement of gas exchange rates.

A sample of 16 scorpions was used for measuring haemolymph osmolality within 24 h of capture, thus determining 'field' haemolymph osmolality.

2.4. Respirometry

Oxygen consumption ($\dot{M}O_2$) and carbon dioxide emission ($\dot{M}CO_2$) rates were measured in a flow-through respirometry system. The scorpions were placed in glass metabolic chambers (4 cm length; 1.7 cm diameter). Air supply to the chambers was set to 5 ml min^{-1} with FMA-2616A mass flow controllers (Omega, Stamford, Connecticut, USA). Water vapour and CO_2 were removed from the air supply with a silica gel and Ascarite column. The excurrent air from the chambers was dried by passing it through a 1 ml Drierite column before being analysed for gas concentrations in Oxzilla O_2 and CA-2A CO_2 analyzers (Sable Systems International, Las Vegas, Nevada, USA). The metabolic chambers and their respective Drierite columns were flushed at 5 ml min^{-1} for 45–60 min before connecting them to the analyzers. Each chamber was then measured for additional 45–60 min. Metabolic rates were usually determined during the last 15 min of recording, when scorpions had been in their chambers for ~1 h 45 min Datacan V software (Sable Systems International) was used for data acquisition and analysis. Calculation of $\dot{M}O_2$ and $\dot{M}CO_2$ was done using standard equations (Withers, 2001).

2.5. Hepatopancreas and total body water contents

Following respirometry and haemolymph osmolality measurements the scorpions were killed by decapitation, and their hepatopancreas was dissected out. The hepatopancreas and the rest of the animal were dried to constant mass at 55 °C for determination of hepatopancreas and body water contents.

Hepatopancreas water content was determined in females collected in late summer (late September). Female *S. mesaensis* give birth between July and September (Polis and Farley, 1979), and therefore almost all captured females were non-gravid, possibly shortly after giving birth. The few gravid females that were captured were not included in the analysis, and therefore loss of fluid from the ovariuterus during dissection of the hepatopancreas (Warburg et al., 2002) is unlikely to have affected the observed results.

2.6. Hepatopancreas lipid content

Following weighing, the dry hepatopancreas tissue samples were stapled-shut in pre-weighed filter paper bags, weighed again and kept immersed in ether overnight in capped glass bottles. The bags were then dried at 55 °C, and weighed for their lipid-free dry mass. A second overnight ether treatment confirmed the complete extraction of the lipids. Lipid content was calculated as the difference in dry mass before and after extraction.

2.7. Statistics

Statistical analyses were performed using Statistica for Windows Version 7.0 (Statsoft, Tulsa, Oklahoma, USA). Tukey HSD tests were carried out for post hoc comparisons whenever ANOVA/ANCOVA yielded significant differences.

3. Results

3.1. Scorpions

A total of 157 scorpions were included in the study. Mean body mass of female scorpions (1.6303 ± 0.0365 g; mean $\pm$ S.E.; $n = 100$) was significantly higher than that of males (1.1836 ± 0.0218 g; $n = 59$) after 2 weeks in the lab ($t_{157} = 8.85$, $p < 0.001$). Dry body mass of fed females (0.5698 ± 0.0461; $n = 8$) was also significantly higher ($t_{15} = 4.55$, $p < 0.001$) than that of males (0.3596 ± 0.0152; $n = 9$).

3.2. Mass-loss rates

Averaged MLRs for the second week of exposure to experimental conditions were not different between males and females (ANCOVA, initial mass as covariate; $t_{25} = 0.90$, $p = 0.77$), and therefore MLRs data in Fig. 1 are pooled for both sexes. Initial MLR averaged > 0.5 mg g^{-1} h^{-1}, but gradually decreased thereafter before reaching steady state at a rate of ~ 0.22 mg g^{-1} h^{-1} during the second week of measurement (Fig. 1). Note the decreasing sample sizes in Fig. 1 as individual scorpions were withdrawn from the sample upon reaching their respective experimental mass loss levels.

3.3. Haemolymph osmolality

No difference was found in field haemolymph osmolality (measured within 24 h of capture) of males (554 ± 13 mOsm kg^{-1}; $n = 6$) and females (542 ± 10 mOsm kg^{-1}; $n = 10$) ($t_{14} = 0.69$, $p = 0.50$). Similarly, haemolymph osmolality of males 606 ± 4 mOsm kg^{-1}; $n = 46$) and females (602 ± 4 mOsm kg^{-1}; $n = 56$) were not statistically different following the 2-week feeding period in the lab ($t_{100} = 0.60$, $p = 0.55$). However, the osmotic response to

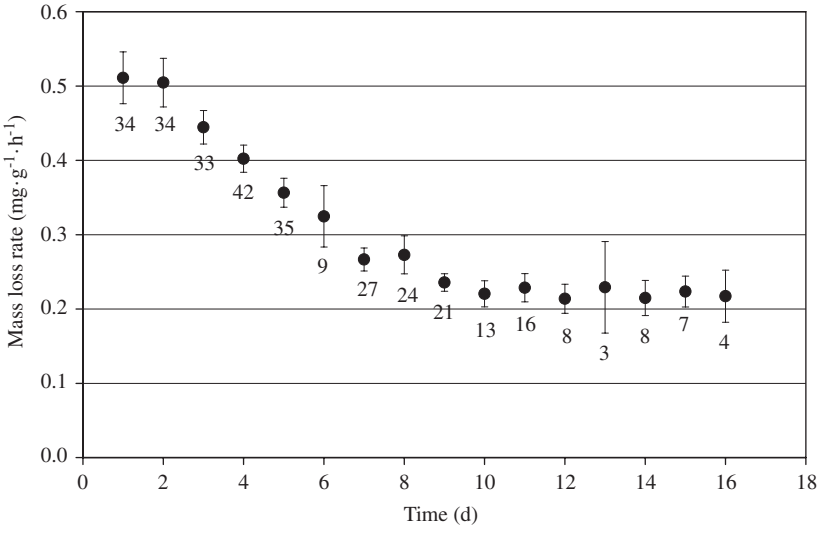

Fig. 1. Mean ($\pm$S.E.) mass-loss rates (mg g^{-1} h^{-1}) of *S. mesaensis* during prolonged desiccation at 30 °C and 15–30% relative humidity. Sample sizes are indicated below error bars.

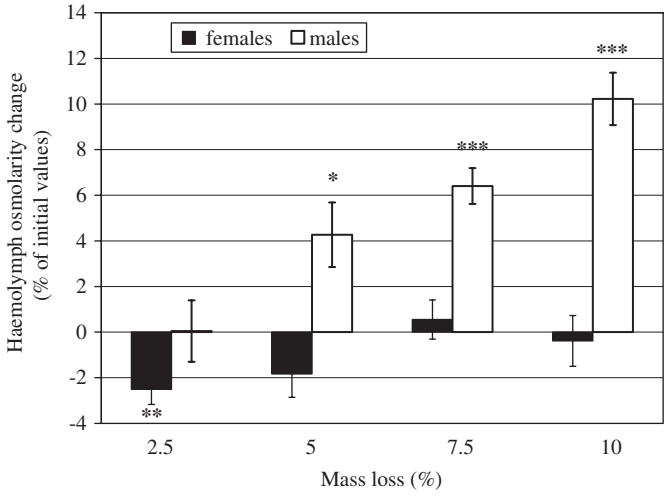

Fig. 2. Mean (±S.E.) changes (%) in haemolymph osmolality of male and female *S. mesaensis* following desiccation at 30 °C and 15–30% relative humidity to 2.5%, 5.0%, 7.5% and 10% loss of initial mass ($n = 10$, 10, 14, and 12 for females, and 9, 9, 8, 9 for males). Paired *t*-test of final and initial osmolality values; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

desiccation differed markedly between the two sexes (Fig. 2). Haemolymph osmolality of females desiccated to 2.5% mass loss showed a significant drop from the initial value, decreasing from 603 ± 7 to $588 \pm 6\,\mathrm{mOsm\,kg^{-1}}$ (paired *t*-test; $t_9 = 3.65$, $p<0.01$). Haemolymph osmolarities of females from 5%, 7.5%, and 10% mass loss groups were not significantly different from initial values (paired *t*-tests, $\alpha = 0.05$; $n = 12$, 14 and 12, respectively). In contrast, the haemolymph osmolality of males after 2.5% loss of initial mass was not different from initial values ($t_8 = 0.03$, $p = 0.97$). This was followed by a significant osmolality increase at 5% ($t_8 = 2.96$, $p = 0.018$), 7.5% ($t_7 = 8.09$, $p<0.001$) and 10% mass loss ($t_{10} = 8.78$, $p<0.001$) (Fig. 2).

3.4. Respirometry

Gas exchange rates of males from the control group were highly variable, and therefore 6 males from the 7.5% and 10%ML groups were measured for gas exchange rates 1 day after the onset of desiccation. Their mean $\dot{M}O_2$ and $\dot{M}CO_2$ values were 68.9 ± 5.8 and $61.1 \pm 5.1\,\mu l\,g^{-1}\,h^{-1}$, respectively. These values were not significantly different from those recorded for males following loss of 2.5% from their initial mass (ANCOVA; $F_{1,10} = 1.38$, $p = 0.27$ and $F_{1,10} = 0.05$, $p = 0.97$). Similarly, no significant differences in $\dot{M}O_2$ ($F_{1,21} = 0.001$, $p = 0.97$) and $\dot{M}CO_2$ ($F_{1,21} = 0.28$, $p = 0.60$) were found between females from the 0% and 2.5%ML groups. Therefore, metabolic rates of the latter group were used to represent scorpions in their fed/hydrated state. These values are within the range of previous metabolic rates for other scorpion species (Withers and Smith, 1993).

Male and female $\dot{M}O_2$ (ANCOVA; $F_{1,92} = 1.43$, $p = 0.23$) and $\dot{M}CO_2$ ($F = 3.21$, $p = 0.08$) did not differ significantly. A significant effect of desiccation level was recorded for both $\dot{M}O_2$ ($F_{3,92} = 12.45$, $p<0.001$) and $\dot{M}CO_2$ ($F = 10.14$, $p<0.001$), with values at 7.5% and 10% mass loss levels significantly lower in comparison with those measured at milder levels of desiccation stress (Table 1).

When gas exchange rates were adjusted to dry mass (ANCOVA, dry mass as covariate), the overall pattern of desiccation stress on metabolic rates was maintained. However, this also yielded significantly higher $\dot{M}CO_2$ (but not $\dot{M}O_2$) for females compared to males ($F_{1,55} = 6.54$, $p = 0.013$). Adjustment to hepatopancreas-free dry mass resulted in both higher $\dot{M}CO_2$ ($F_{1,54} = 9.95$, $p = 0.003$) and $\dot{M}O_2$ ($F_{1,55} = 16.04$, $p<0.001$) for females.

Calculated respiratory exchange ratios (RER) of males and females were not significantly different from each other in any of the mass loss levels (Mann–Whitney test, $p>0.05$). Nevertheless, RER showed a gradual increase as desiccation prolonged, with calculated values at 7.5% (1.62 ± 0.17; $n = 23$) and 10% (1.71 ± 0.26; $n = 26$) mass loss significantly higher than those at 0% (0.88 ± 0.05; $n = 9$) and 2.5% (0.94 ± 0.05; $n = 23$) (Kruskal–Wallis test; $H_{4,108} = 32.8$, $p<0.001$).

3.5. Hepatopancreas and total body water contents

Hepatopancreas wet mass of control females (0.5499 ± 0.0427 g; $n = 8$) was significantly higher than that of males (0.1921 ± 0.0145 g; $n = 9$) (ANCOVA, non-hepatopancreas mass as covariate; $F_{1,14} = 46.68$, $p<0.001$). Similarly, differences in dry mass were also highly significant ($F_{1,14} = 81.38$, $p<0.001$) with mean hepatopancreas dry mass of females (0.2706 ± 0.0229 g) more than 3-fold higher than that of males (0.0843 ± 0.0044 g).

Total body water content of the scorpions decreased significantly with increasing desiccation stress levels (ANCOVA, dry mass as covariate; $F_{4,82} = 4.01$, $p<0.01$), with no significant sex or interaction effect ($p>0.05$).

No significant effect was found for sex or desiccation level on the calculated non-hepatopancreas water content (ANCOVA, dry mass as covariate, $p>0.05$). In contrast, water content of the female hepatopancreas was significantly higher than that found in males ($F_{1,81} = 106.17$, $p<0.001$). Hepatopancreas water content was also significantly affected by the experimental desiccation levels, and in the same order of changes in whole body water content ($\alpha = 0.05$; 0^{a}, 2.5^{ab}, 5^{ab}, 7.5^{b}, 10^{c}). Higher hepatopancreas water content in females ($F_{1,81} = 7.55$, $p<0.01$), and decreasing water content with increasing desiccation stress ($F_{4,81} = 8.59$, $p<0.001$) were also found when adjusted for hepatopancreas dry mass (ANCOVA, hepatopancreas dry mass as covariate).

Fig. 3 summarises the relative contribution of hepatopancreas in body water storage, and changes in these stores during desiccation. Hepatopancreas water stores in females account for ~50% of body water in the hydrated state, compared to ~30% in males. Arcsine transformation of

Table 1
Mean (±S.E) mass-specific O_2 consumption and CO_2 emission rates ($\mu l\, g^{-1}\, h^{-1}$), of *S. mesaensis* at 30 °C

Mass loss (%)	0	2.5	5	7.5	10
Females					
${}_{sp}\dot{M}CO_2$	51.4±3.6 (8)	50.3±2.5^{a} (16)	47.8±2.4^{a} (18)	32.8±2.6^{b} (15)	41.7±2.5^{b} (16)
${}_{sp}\dot{M}O_2$	58.0±5.6	57.3±3.9^{a}	50.6±3.7^{a}	21.3±4.1^{b}	32.5±3.9^{b}
Males					
${}_{sp}\dot{M}CO_2$	61.1±5.1* (6)	60.0±4.4^{a} (7)	52.9±3.8^{ab} (9)	49.1±3.8^{b} (9)	44.6±3.5^{b} (11)
${}_{sp}\dot{M}O_2$	68.9±5.8*	62.4±7.8^{a}	45.4±6.8^{ab}	44.4±6.8^{ab}	34.5±6.2^{b}

Superscript letters denote statistically significant differences between means at the different desiccation levels (ANCOVA, body mass as covariate; $\alpha = 0.05$). Sample sizes given in brackets.

*Initial gas exchange rates for males were measured using 3 individuals from each of the 7.5% and 10%ML groups, which were then measured again at their respective mass loss levels (see text).

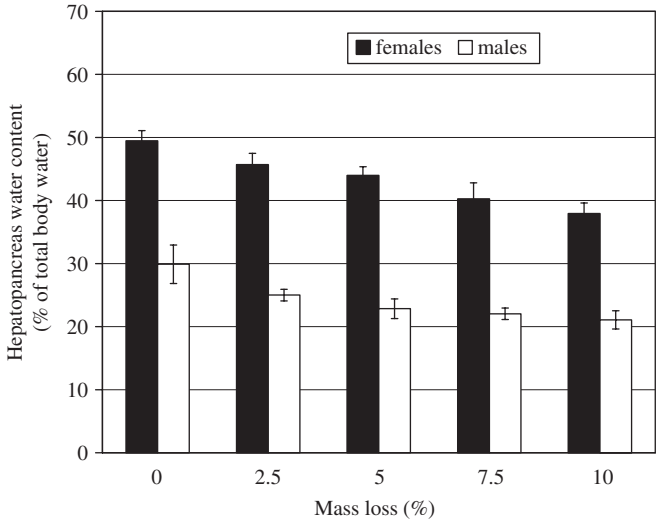

Fig. 3. Mean (±S.E.) hepatopancreas water content (expressed as % of total body water content) of male and female *S. mesaensis*, as a function of experimental mass loss level. Relative hepatopancreas water content of females was significantly higher than that of males (ANOVA of arcsine-transformed %, $p<0.001$). Relative hepatopancreas water content in both males and females decreases during prolonged desiccation ($p<0.01$).

the fraction of hepatopancreas water from total body water content yields significantly higher values for females compared to males (ANOVA, $F_{1,82} = 265.68$, $p<0.001$). A gradual and continuous decrease ($F_{4,82} = 4.40$, $p<0.01$) in the relative contribution of hepatopancreas stores to overall water stores is clear in both males and females throughout the range of experimental desiccation levels. Initial values were significantly higher than those calculated for the 10% mass loss group.

3.6. Hepatopancreas lipid content

Lipids accounted for 31.3±1.3% and 33.6±3.2% of the hepatopancreas dry mass in females and males, respectively ($n = 8$ each). These values increased gradually with desiccation, and reached significantly higher values of *ca.* 36% in females and 40% in males after losses of 7.5–10% from initial mass (ANOVA of arcsine-transformed percentages, $F_{4,82} = 3.21$, $p = 0.02$).

No significant change in hepatopancreas lipid content was found among the different desiccation levels (ANCOVA, non-hepatopancreas dry mass as covariate, $F_{4,80} = 0.77$, $p = 0.55$). In contrast, hepatopancreas lipid-free dry mass at 10% mass loss level was significantly lower than initial values (ANCOVA, non-hepatopancreas dry mass as covariate, $F_{4,80} = 2.80$, $p = 0.03$).

4. Discussion

Scorpions in general exhibit some of the lowest recorded water-loss rates among all arthropods (Hadley, 1994). Among scorpions, interspecific differences in water-loss rates have been attributed to both environmental adaptations (reviewed by Hadley, 1990) and phylogeny (Gefen and Ar, 2004). MLRs of *S. mesaensis* (Vaejovidae) measured in this study (Fig. 1) are within the range of previously reported values for other scorpion species (Hadley, 1990). During exposure to experimental desiccation stress, high initial MLRs were followed by a gradual decrease before reaching steady state during the second week of measurement. Decreasing MLRs during the first 1–2 days of exposure have been reported for other arthropods, with water loss from the cuticle and higher initial activity levels suggested as possible explanations (see Hadley, 1994). Nevertheless, the MLR pattern reported here shows a gradual decrease which extends to the second week of exposure (Fig. 1), similar to reported data for two *Scorpio maurus* (Scorpionidae) subspecies (but not Buthidae; Gefen and Ar, 2004). This indicates an ability of some scorpions to reduce body water loss in response to desiccation stress.

Larger body size has been suggested as an explanation for to the higher desiccation resistance of female tenebrionid beetles compared with that of males (Renault and Coray, 2004). The role of the hepatopancreas in water management during desiccation stress (Gefen and Ar, 2005), and the significantly larger size of this organ in females compared to males (Warburg et al., 2002) suggest

that female scorpions are potentially better osmoregulators in comparison with males. Both wet and dry hepatopancreas mass of female *S. mesaensis* were significantly higher than those of males ($p<0.001$). In fact, these explained most of the observed sexually dimorphic body size, as dry body mass excluding the hepatopancreas was not significantly different between females (0.2992 ± 0.0267 g; $n=8$) and males (0.2753 ± 0.0162 g; $n=9$) ($t_{15}=0.79$, $p=0.44$).

Results in Fig. 2 show different osmotic responses of male and female *S. mesaensis* to desiccation, which correlate with differences in hepatopancreas size. Males maintained initial haemolymph osmolarities during the first 2–4 days of desiccation (2.5% mass loss), but a significant increase in osmolality was observed after loss of 5% of initial body mass. Haemolymph osmolality continued to increase thereafter, and was 10% higher than initial values at the 10% mass loss level (increasing from 594 ± 5 to 654 ± 8 mOsm kg^{-1}; $n=11$). This change in haemolymph osmolality was similar in magnitude to that reported for the Scorpionidae species *Opistophthalmus capensis* (Robertson et al., 1982) and *S. maurus* (Gefen and Ar, 2004). As predicted by differences in hepatopancreas size, osmoregulatory capacities of female *S. mesaensis* were considerably higher, with haemolymph osmolality maintained at its initial level throughout the range of experimental desiccation levels (Fig. 2). The osmoregulatory response of female *S. mesaensis* is comparable to that previously reported only for the Buthidae species *P. villosus* (Robertson et al., 1982), *B. judaicus* and *L. quinquestriatus* (Gefen and Ar, 2004). The osmotic response of females included a significant decrease in haemolymph osmolality after loss of 2.5% of initial body mass. Interestingly, such an initial decrease in haemolymph osmolality was observed for all three Buthidae species. Gefen and Ar (2004) calculated that release of metabolic and bound water from the hepatopancreas might exceed transpiration rates when glycogen is exclusively catabolised at the relatively high initial values of $\dot{M}O_2$ and water-loss rates (WLR).

Results in this study indicate that high osmoregulatory capability of scorpions is even more prevalent than previously thought. The osmotic response of female *S. mesaensis* to desiccation (Fig. 2) is comparable to those reported for surface-dwelling Buthidae, despite being an obligate fossorial species which spends 92–97% of the time in its burrow (Polis, 1990). The haemolymph osmolality of another burrowing Vaejovid, *Paruroctonus aquilonalis*, was reported to increase steadily with desiccation of up to 40% loss of initial mass (Riddle et al., 1976). However, haemolymph osmolarities of males desiccated to 10% of their initial mass were not different from initial values (Fig. 3 and Table 1 therein).

To the best of my knowledge, none of the studies published to date included a comparison between the osmotic responses of adult male and female scorpions to desiccation. Moreover, while some included one of the sexes exclusively, other reports include both and therefore results could be skewed depending on the actual sex ratios. The sexually dimorphic osmoregulatory responses to desiccation reported here highlight the need to account for possible sex effects in future studies of water balance in scorpions.

Mobilisation of water from hepatopancreas stores to the haemolymph during desiccation results in a decrease in the fraction of hepatopancreas water from total water content (Fig. 3). Female *S. mesaensis* have significantly higher hepatopancreas water content compared to males (Fig. 3), but this role of hepatopancreas-stored water in replenishing haemolymph volume depends on the rate of mobilisation. Scorpions catabolise carbohydrates from hepatopancreas stores during food deprivation (Sinha and Kanungo, 1967; Sinha, 1982). Although carbohydrate levels were not measured directly in this study, similar patterns of decrease in the non-lipid fraction of the hepatopancreas and that of hepatopancreas water, while lipid content remained unchanged, provide further support for carbohydrate catabolism in scorpions under stressful conditions. In addition, initial RER of ~0.9 (Table 1) indicate the use of carbohydrates as a major energy source. Hence, as *S. mesaensis* catabolise carbohydrates during the early stages of exposure to experimental conditions, 0.56 mg metabolic water is produced for every 1 mg of carbohydrate catabolised. Furthermore, glycogen binds water 3–5 times its own mass, which is released upon glycogen catabolism (Schmidt-Nielsen, 1997; Gibbs et al., 1997).

The consistent lower lipid fraction from female hepatopancreas dry mass in *S. mesaensis* was just short of the usually accepted statistical significance level (ANOVA on arcsine-transformed percentages, $F_{1,82}=3.67$, $p=0.059$), but may suggest that sexual dimorphism occurs not only in hepatopancreas size but also in its composition. This is correlated with and may explain the significant higher water content in females' hepatopancreas adjusted to the organ's dry mass. It has been previously reported that hepatopancreas mass-specific glycogen content in female *H. fulvipes* (Scorpionidae) was 23% higher in comparison with males (Raju et al., 1978), suggesting that female scorpions generally have higher hepatopancreas water content than males.

However, availability of water from either source for replenishing haemolymph losses depends on the rate of glycogen breakdown. Thus, assuming carbohydrate catabolism by both male and female *S. mesaensis*, it is the metabolic rate that determines the availability of water stored in the hepatopancreas and thus the ability to regulate haemolymph volume and osmotic concentration. No significant effect of sex on metabolic rate was found when gas exchange rates were expressed in a wet mass-specific manner. However, expressing metabolic rates in a mass-specific manner does not take into account that water and stored metabolites do not contribute to the metabolic rate (Djawdan et al., 1997). The highly significant effect of sex on the size of the hepatopancreas, the main organ for water and nutrient storage in scorpions, suggests that

differences in total body size could result from differences in the content of non-metabolising mass. Both $\dot{M}O_2$ and $\dot{M}CO_2$ of females were significantly higher than those of males when adjusted to hepatopancreas-free dry mass manner.

Expressing metabolic rates per hepatopancreas-free dry mass possibly overlooks differences in the size of metabolising hepatopancreas tissue while tending to account for differential nutrient storage. However, significantly higher hepatopancreas lipid content of females, as well as differences in glycogen storage (Raju et al., 1978) indicates that differences in hepatopancreas size are largely a result of higher nutrient content in the female hepatopancreas. This means that expressing metabolic rates as non-hepatopancreas mass specific may be more relevant than the often used total-mass-specific values. Expressed in this manner, higher metabolic rates found for females mean higher rates of glycogen breakdown and of water mobilisation from hepatopancreas stores to the haemolymph during desiccation stress. This may explain the better osmoregulatory capacity of females despite comparable MLRs to those of males.

Nevertheless, higher gas exchange rates come at the cost of increased rates of respiratory water loss. The contribution of respiratory water loss to total water loss in insects, and its possible effect on evolution of respiratory patterns is the subject of ongoing debate (Chown, 2002; Quinlan and Gibbs, 2006; White et al., 2007). In contrast, very few data are available for the effect of desiccation stress on respiratory regulation in scorpions. The two available reports on the effect of food prevention on gas exchange rates in scorpions have contrasting results. Oxygen consumption rates of control and desiccated *Hadrurus arizonensis* (Iuridae) were not significantly different (Hadley, 1970), but metabolic rates of starved *Parurocto-nus utahensis* (Vaejovidae) females were significantly lower in comparison with controls (Riddle, 1978). Results in this study show a significant decrease in gas exchange rates during desiccation (Table 1), possibly in an attempt to reduce rates of respiratory water loss to the environment. Therefore, regulation of metabolic rates during desiccation could involve a trade-off between water loss to the environment and the ability to mobilise stored water between different body compartments.

Initial RER values of ~0.9 at the onset of desiccation and at 2.5% mass loss are followed by an increase in RER as desiccation progressed (Table 1). Some exceptionally high RER values were associated with low measured $\dot{M}O_2$ values of $\sim 10\,\mu l\,g^{-1}\,h^{-1}$ or less, and could be an overestimation resulting from measurement inaccuracy. However, the use of non-parametric statistics for comparisons between the various experimental groups confirms the significant increase in RER during desiccation. High RER values in dormant pulmonate snails were attributed to bursts of CO_2 release, and were coupled with periods of low RER, resulting in average RER of ~1.0 (Barnhart and McMahon, 1987). However, 4 of the 49 gas exchange measurements for scorpions desiccated to 7.5% or 10% mass loss levels yielded RER values <1.0 (whereas 20 were >1.5), suggesting that the high values recorded for *S. mesaensis* do not result from an intermittent pattern of CO_2 release. The low flow rate used for respirometry measurements ($5\,ml\,min^{-1}$) and the resultant washout times also rule out intermittent gas exchange as a possible cause for recorded high RER values.

RER values higher than 1.0 could be explained by a partial shift to anaerobic catabolism. This may result from tighter control of book lung spiracle opening aimed at reducing respiratory water loss under stressful conditions. Results in this study show that decreasing $\dot{M}O_2$ values (Table 1) are correlated with a decrease in MLRs (Fig. 1) and possibly WLR. Estimated respiratory losses of 30–40% of total WLR (Yokota, 1979; Withers and Smith, 1993; but see Hadley, 1970) mean that reducing gas exchange rates by a partial shift to anaerobic catabolism is advantageous for body water conservation despite the resulting decrease in metabolic water production. There is some supporting evidence for anaerobic metabolism in starved scorpions, as the haemolymph of starved *L. quinquestriatus* (Buthidae) showed increased pCO_2 and decreased pH values compared to controls (Dejours and Ar, 1991). High RER values could also result from lipid synthesis from a carbohydrate source (Kleiber, 1961). Biosynthesis of fatty acids from glucose and anaerobic end-products such as pyruvate and alanine has been shown to occur anaerobically in pulmonate snails (van der Horst et al., 1974). Breakdown of glycogen for lipid synthesis would increase the rate of bound water release, while conserving energy stores, and could potentially increase the availability of extracellular water when body water is lost to the environment. However, results in this study do not support this mechanism, as no significant increase was found in the hepatopancreas lipid content of *S. mesaensis* within the range of experimental desiccation stress levels.

This study shows that the larger hepatopancreas size in female *S. mesaensis* compared to males is correlated with a considerably better osmoregulatory capacity. Females have higher hepatopancreas water content and higher mobilisation rates of water from the hepatopancreas to the haemolymph during desiccation. Still, decreasing gas exchange rates and increased RER during prolonged desiccation suggest that other factors are involved in respiratory response to desiccation stress, as conservation of body water and energy stores is essential. An increase in RER values as desiccation progresses suggests a shift to anaerobic metabolism when desiccation stress dictates tighter control of the book lung spiracles.

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