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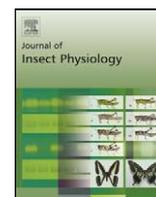
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Partitioning of transpiratory water loss of the desert scorpion, *Hadrurus arizonensis* (Iuridae)

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ABSTRACT

Terrestrial arthropods lose body water to the environment mainly through transpiration. The aim of this study was to determine the fraction of respiratory losses from total transpiratory water loss in scorpions, as relatively high respiratory losses would indicate a fitness benefit from regulation of gas-exchange rate under stressful desiccating conditions. We measured metabolic rates and water-loss rates of *Hadrurus arizonensis* (Iuridae) at a range of ecologically-relevant temperatures. Calculation of respiratory water losses was based on increased metabolic and water-loss rates during nocturnal activity (assuming no change in cuticular resistance at a given constant experimental temperature). Respiratory losses accounted for $9.0 \pm 1.7\%$ of total transpiratory losses at 25 °C, doubled to $17.9 \pm 1.8\%$ at 30 °C and increased to $31.0 \pm 2.0\%$ at 35 °C ($n = 5, 15$ and 15 , respectively). Furthermore, the relative importance of respiratory transpiration is likely to be higher at temperatures above 35 °C, which have been recorded even within the burrows of *H. arizonensis*. Measurements of cuticular lipid melting points do not provide evidence for increased cuticular resistance to water loss at higher temperatures. However, the relatively high fraction of respiratory water losses reported here for *H. arizonensis* supports the notion of respiratory regulation as an evolved mechanism for conserving scorpion body water stores under stressful conditions.

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1. Introduction

One of the major physiological challenges resulting from the transition to terrestrial life is conserving body water in what is a predominantly dry environment. The small size of arthropods, and thus their relatively high body surface area to volume ratio, further compounds the problem. Still, arthropods are the most successful terrestrial animals in both biomass and diversity, and many arthropod species occupy some of the hottest and driest habitats on earth (Hadley, 1994). In some arid areas scorpion biomass exceeds that of all other arthropod taxa except ants and termites, and that of all vertebrates combined (Polis and Yamashita, 1991).

Arthropods have adapted to terrestrial habitats through a range of behavioural, anatomical and physiological adaptations. Most scorpion species avoid exposure to high temperature and low humidity conditions by retreating to their burrows during the hot daytime, with surface activity limited to the early hours of the

evening, soon after sunset (Polis, 1980). In addition, scorpions in general exhibit considerably lower evaporative water-loss rates (WLR) than most other arthropods of comparable body size (Hadley, 1994; Table 3.1 therein).

Despite the relative abundance of data, the contribution of respiratory water loss (RWL) to total water loss in insects is still a matter of debate (Chown, 2002). Recent studies of insect respiratory patterns have contested the idea that respiratory regulation evolved as a mechanism to reduce water loss (Chown, 2002; Chown et al., 2006a; Quinlan and Gibbs, 2006; but see White et al., 2007; Terblanche et al., 2008). Estimates of RWL of up to 30% of total water loss, but usually much lower, have been reported for insects (Chown, 2002, see also Chown et al., 2006b; Gray and Chown, 2008), suggesting that regulation of a rather small portion of the total water loss is unlikely to constitute a fitness benefit (Lighton, 1994, 1998, but see Chown, 2002). Nevertheless, RWL comprises a significant portion of total body water loss in xeric insects with low cuticular permeabilities (Zachariassen, 1996; Addo-Bediako et al., 2001).

Cuticular (CWL) and respiratory water loss are also the two major routes for water loss in scorpions during prolonged desiccation (Hadley, 1970a). However, there are few available data regarding the relative importance of each of the two components to overall water loss in scorpions and non-tracheate arthropods in general. Estimates range from “virtually all water is lost through the cuticle” in *Hadrurus arizonensis* (Iuridae) at

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temperatures of 25–35 °C (Hadley, 1970a) to an estimated RWL of 25–33% of total water loss in *Urodacus armatus* (Scorpionidae) at 20–30 °C (Withers and Smith, 1993). RWL of 15% from total water loss was recently reported for *Urodacus manicatus* at 20 °C (Woodman, 2008).

Transpiration rates in scorpions are some of the lowest recorded for any group of arthropods, often an order of magnitude lower than those of xeric species of various insect orders (Hadley, 1994). Therefore, despite their low MR compared to insects of comparable body size (~24% at 25 °C; Lighton et al., 2001), RWL could constitute a significant component of total water loss in scorpions. A relatively high fraction of respiratory transpiration from total water loss may increase the adaptive value of respiratory regulation when water supply is scarce. Two vaejovid species, *Paruroctonus utahensis* and *Smeringurus mesaensis*, exhibited a significant decrease in gas-exchange rates in response to food and water deprivation (Riddle, 1978; Gefen, 2008). Metabolic downregulation during prolonged desiccation may indicate a mechanism for conserving body water, by means of tighter control of spiracles opening to the book lungs. This study uses direct measurements of both water-loss rates and metabolic rates (expressed as CO₂ output) in scorpions using a flow-through measuring system, in order to determine the relative importance of RWL. By calculating RWL of *H. arizonensis* across a range of temperatures experienced by the scorpion in its natural habitat, we were able to test potential supporting evidence for the hypothesis that respiratory regulation may have evolved as a mechanism for conserving water in scorpions under stressful environmental conditions (Gefen, 2008).

Interspecific and seasonal variation in cuticular lipid composition has been reported in scorpions. Lower WLR measured in *H. arizonensis* compared to *Uroctonus apacheanus* (Vaejovidae) were associated with higher proportion of long-chain hydrocarbons (HC) (Toolson and Hadley, 1977). Similarly, epicuticular lipids of *Centruroides sculpturatus* (Buthidae) from summer collections contained a larger fraction of longer-chain HC (Toolson and Hadley, 1979). In this study we also measured cuticular lipid melting points after acclimation to the experimental temperatures in order to test whether regulation of cuticle waterproofing ability affects variation in CWL and RWL proportions with temperature.

2. Materials and methods

2.1. Scorpions

Fifteen adult *H. arizonensis* were used for measurements at all three experimental temperatures. The scorpions were collected from sand dunes in the Mojave Desert, near Zzyzx, California (35.05°N, 116.07°W). They were placed in round plastic containers (9 cm diameter) with sand from the collection site, and brought to the lab within 24 h. In the lab, the scorpions were kept in the dark at room temperature (23–25 °C), and were fed 1–2 adult crickets per week. The scorpions were then acclimated to experimental temperatures (± 1 °C) in a PTC-1 portable incubator (Sable Systems International, Las Vegas, NV, USA) for at least 2 weeks prior to measurements, at a photoperiod of 22D:2L. We chose this photoperiod because in nature the burrowing *H. arizonensis* is only exposed to light for a short period during evening surface activity. Feeding dates of scorpions were recorded, and all measurements were carried out 3–8 days after feeding. Maintaining similar non-feeding intervals of 6.2 ± 0.5 , 5.3 ± 0.5 and 5.7 ± 0.4 days before measurement (mean \pm S.E.; for 25, 30 and 35 °C, respectively) meant that differences in starvation/desiccation status were unlikely to affect results (see Section 3). All the scorpions were acclimated to experimental temperatures in the order 25–30–35–25 °C. Respirometry measurements at 25 °C were carried out either prior to ($n = 4$)

or after ($n = 11$) acclimation and measurements at higher temperatures. No significant effect of measurement order was found for either CO₂ ($F_{1,12} = 0.911$; $p = 0.36$) or H₂O ($F_{1,12} = 0.496$; $p = 0.50$) output at 25 °C (ANCOVA; body mass as covariate).

2.2. Water and CO₂ output

During the 2 h daily light period the scorpions were transferred to a plastic metabolic chamber (5.5 cm diameter \times 22.5 cm length), which was placed in a controlled temperature cabinet (TriTech Research, Los Angeles, CA, USA) under an identical diurnal cycle. The chamber was supplied with dry CO₂-free air (room air passed through a silica gel/Ascarite column) through a mass flow controller (Sierra Instruments, Monterey, CA, USA) at a flow rate of 100 ml min⁻¹. Temperature within the metabolic chamber remained within ± 0.2 °C of the respective experimental temperatures. The scorpions were acclimated to the measuring chamber and measurement system for 24 h prior to measurement. After the acclimation period, excurrent air was passed through a LI-6262 CO₂/H₂O analyzer (Li-Cor, Lincoln, NE, USA), and voltage output was recorded every 7.5 s and analyzed using Datacan V data acquisition and analysis software (Sable Systems International, Las Vegas, NV, USA). Total recording time was typically ~ 20 h per scorpion, depending on the activity pattern exhibited by individual scorpions. Following successful recording the scorpions were transferred to a controlled temperature cabinet for acclimation to the subsequent experimental temperature.

2.3. Partitioning of WLR

Recording started typically at 16:30 to 17:00, during the light period, and continued overnight. The nocturnal activity pattern of scorpions allows calculation of RWL based on the differences in CO₂ and H₂O output between (daily) resting values and those recorded during (nocturnal) activity. Calculation of RWL is also based on the assumption that the increase in water loss during activity results only from an increase in RWL, and that CWL remains unchanged under each given constant experimental temperature (Withers and Smith, 1993). Integration of the CO₂ and H₂O output component resulting from activity (Fig. 1) was used to calculate the amount of water lost per unit of CO₂ output. We assumed a similar relationship between RWL and CO₂ output in resting scorpions, and used these ratios to estimate RWL and

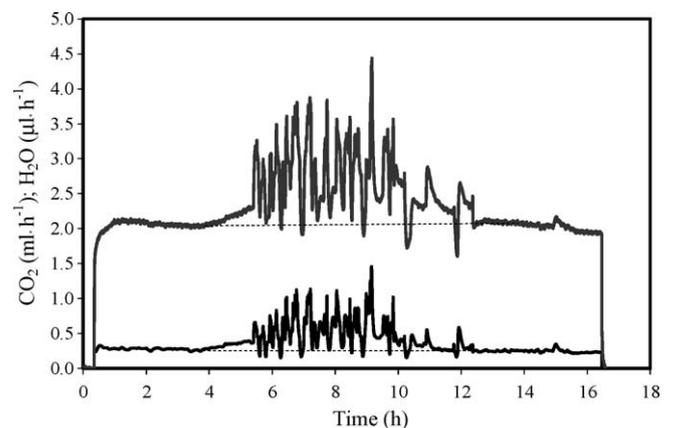


Fig. 1. Typical recording of CO₂ (bottom) and H₂O (top) output of a 7.65 g *Hadruus arizonensis* at 35 °C. The ~ 0.5 l cylindrical metabolic chamber allowed the scorpion free movement. Resting rates can be calculated at 2–4 and 13–15 h of measurement. The activity bout (dotted area; 4–13 h) allows calculation of RWL by integrating the area above the dashed lines, which represent increased gas exchange and RWL. Dry CO₂-free air flow directly to the analyzer at 0–0.5 and 16.5–17 h allowed baselining for drift correction.

partition total water loss between RWL and CWL in resting scorpions.

2.4. Cuticular lipid melting points

Following respirometry measurements the scorpions were kept at room temperature (~ 23 – 25 °C) for several weeks before being tested for thermal acclimation of cuticular lipid melting points (T_m). The scorpions were assigned to two experimental groups, which were acclimated to 25 and 35 °C, respectively for 30 days, then frozen at -20 °C. Cuticular hydrocarbons (HC) were extracted and isolated by submerging the thawed scorpions in hexane for 10 min. The solvent was then evaporated, and the extracts were frozen until analysis.

Melting points of cuticular lipids were measured using Fourier transform infrared (FTIR) spectroscopy as described by Gibbs et al. (2003). Lipid extracts were dissolved in hexane, and samples were deposited on CaF₂ windows. After the solvent evaporated the samples were placed in a temperature-controlled spectrometer cell. The sample temperature was then increased from 25 to 75 °C in 2.5 °C increments. As lipids melt, the frequency of $-\text{CH}_2-$ symmetric stretching vibrations increases (Gibbs and Crowe, 1991). Melting points (T_m ; the midpoint of the phase transition) were determined by logistic regression. Scorpion mortality during the acclimation period resulted in smaller sample sizes compared to respirometry measurements, with $n = 4$ and $n = 7$ for 25 and 35 °C, respectively.

2.5. Statistics

Statistical analyses were performed using Statistica for Windows Version 7.0 (Statsoft, Tulsa, Oklahoma, USA). Temperature effects on body mass, metabolic rates and water-loss rates were analyzed by repeated measures ANOVA, followed by Tukey HSD tests for post-hoc comparison of means.

3. Results

Mean (\pm S.E.) body mass of the 15 scorpions included in this study was 6.470 ± 0.282 g. Only large adults (body mass range of 4.649–8.543 g) were included in the measurements, as no significant correlation was found between body mass and mass-specific water loss rates (WLR) in *H. arizonensis* 4 g or larger (Hadley, 1970a). The scorpions were offered *ad lib* feeding between experiments, and therefore recorded values represent their hydrated state despite varying times between measurements and the previous last meals of individual scorpions. Although body mass of individual scorpions varied between measurements at different temperatures as a result of feeding during the acclimation periods, mean body mass did not differ among the three experimental temperatures (ANOVA; $F_{2,28} = 1.26$, $p = 0.30$). Furthermore, neither mass-specific WLR nor CO₂ output rate were significantly correlated with non-feeding period prior to measurements at 25 °C (Pearson correlation; $p = 0.29$ and $p = 0.29$, respectively), 30 °C ($p = 0.96$ and $p = 0.78$) and 35 °C ($p = 0.61$ and $p = 0.77$) (see Gefen, 2008).

Resting mass-specific metabolic rates (expressed as CO₂ output) and WLR at the different temperatures are presented in Table 1. Significant increases in metabolic rates were recorded with temperature increases from 25 to 30 °C and 30 to 35 °C (repeated measures ANOVA; $F_{2,28} = 54.3$, $p < 0.001$). As expected, increasing temperature also resulted in a significant increase in WLR (repeated measures ANOVA; $F_{2,28} = 15.6$, $p < 0.001$). However, post-hoc comparison of means showed a significant increase in WLR only for the 30–35 °C temperature range. Q_{10} values for resting metabolic rate were 2.92 (25–30 °C) and 3.13 (30–35 °C), and 1.33 (25–30 °C) and 1.88 (30–35 °C) for WLR.

Table 1

Mean (\pm S.E.; $n = 15$) resting CO₂ output and water-loss rates at the different experimental temperatures. Values with different superscript letters are significantly different from each other ($\alpha = 0.05$).

Temperature (°C)	CO ₂ output rate ($\mu\text{l g}^{-1} \text{h}^{-1}$)	Water loss rate ($\mu\text{l g}^{-1} \text{h}^{-1}$)
25	16.9 ± 1.3^a	0.217 ± 0.017^a
30	28.9 ± 2.1^b	0.250 ± 0.022^a
35	51.1 ± 4.6^c	0.343 ± 0.020^b

Table 2

Calculated RWL at 25 °C ($n = 5$), 30 °C ($n = 15$) and 35 °C ($n = 15$). Values expressed as water loss per unit of CO₂ output, and as % of total body water loss. Different superscript letters denote statistically significant effect of temperature ($\alpha = 0.05$).

Temperature (°C)	Saturation deficit (mmHg)	RWL ($\mu\text{lH}_2\text{O mlCO}_2^{-1}$)	RWL at rest (% of total)
25	23.8	1.32 ± 0.24^a	9.0 ± 1.7^a
30	31.8	1.50 ± 0.17^a	17.9 ± 1.8^b
35	42.2	2.20 ± 0.18^b	31.0 ± 2.0^c

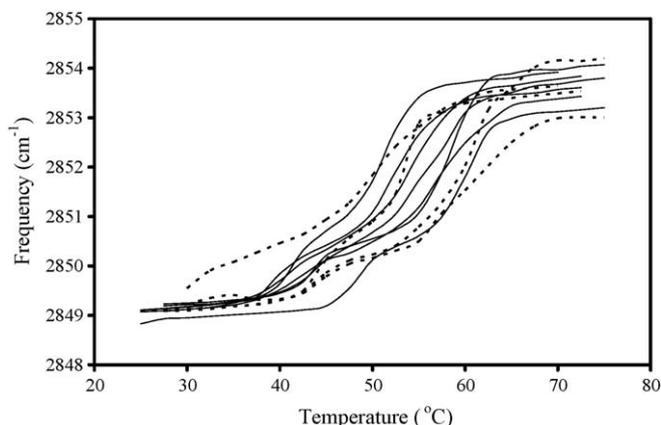


Fig. 2. Frequencies of $-\text{CH}_2-$ symmetric stretching vibrations as a function of temperature for epicuticular lipid samples from scorpions acclimated to 25 °C (dashed lines) and 35 °C (solid lines).

While resting metabolic rates and WLR are available for all three experimental temperatures, the scorpions were rarely active during measurements at 25 °C. Therefore, partitioning of water loss to the cuticular and respiratory components (see Section 2) was possible for only 5 individuals at 25 °C, and all 15 at the higher experimental temperatures.

Calculation of water loss per unit of emitted CO₂ during activity bouts showed a significant increase from $1.50 \pm 0.17 \mu\text{lH}_2\text{O mlCO}_2^{-1}$ at 30 °C to $2.20 \pm 0.18 \mu\text{lH}_2\text{O mlCO}_2^{-1}$ at 35 °C (sign test, $p < 0.01$) (Table 2). Respiratory water loss (RWL) accounted for an increasing fraction of total water loss at rest with increasing temperature. Accounting for $9.0 \pm 1.7\%$ of total water loss at 25 °C, RWL significantly increased to $17.9 \pm 1.8\%$ at 30 °C (paired *t*-test of arcsine-transformed percentages; $t_4 = 4.46$, $p < 0.05$) and then to $31.0 \pm 2.0\%$ at 35 °C ($t_{14} = 5.39$, $p < 0.001$).

Despite the observed increase in WLR with increasing temperature, we did not find a significant effect of acclimation temperature on cuticular lipid melting point (T_m) ($T_9 = 0.48$; $p = 0.64$). Calculated mean (\pm S.E.) lipid T_m were 54.3 ± 2.5 °C ($n = 4$) and 53.1 ± 1.2 °C ($n = 7$) for scorpions acclimated to 25 and 35 °C, respectively (Fig. 2).

4. Discussion

Desiccation resistance performance of terrestrial arthropods could be enhanced by increasing the ability to store body water,

reducing rates of water loss to the environment and/or tolerance of decreasing body water content. Variation in the ability of insects to conserve body water under stressful conditions has been shown to be the major component in interspecific desiccation resistance, and is largely correlated with differences in resistance between xeric and mesic species (Gibbs, 2002a). There are currently no available data on variation in water storage capability or tolerance of low body water contents in scorpions and their possible contribution to desiccation resistance.

Scorpions in general exhibit some of the lowest WLR recorded among all terrestrial arthropods (Hadley, 1994), which are considered as a major adaptation or pre-adaptation contributing to scorpions' successful existence in some of the driest habitats on earth. Still, published data show considerable interspecific variation in WLR of scorpions, which has been attributed to adaptation to xeric environments (reviewed by Hadley, 1990) and phylogenetic constraints (Gefen and Ar, 2004).

Previously reported WLRs for *H. arizonensis* of 0.021, 0.028 and 0.035% body mass h^{-1} (Hadley, 1970a), determined gravimetrically at 25, 30 and 35 °C, respectively, are very similar to directly measured values in this study (Table 1). The low calculated WLR of the burrowing *H. arizonensis*, within the range characteristic of non-burrowing Buthidae species (Hadley, 1990; Gefen and Ar, 2004) could partly result from the body size of this large North American scorpion. Unfortunately, differences in experimental methodology (e.g. flow-through vs. closed system, scorpion hydration state, duration of measurement) prevent meaningful mass-corrected comparisons between data from different studies. Nevertheless, despite the buffering effect of the burrow from surface conditions, temperatures of 35–40 °C were measured inside the burrows of *H. arizonensis* near Guadalupe, Arizona (Hadley, 1970b). In addition, scorpions collected for this study were found foraging on the soil surface at temperatures of >35 °C during early evening hours in the summer. This means that despite their burrowing homing behaviour, *H. arizonensis* are likely to have evolved other desiccation resistance features which allow them to successfully colonize habitats of extremely harsh environmental conditions, such as the Sonora and Mojave Deserts. These may include both a relatively large body size and high resistance to loss of body water.

Terrestrial arthropods lose water through their integument, from respiratory surfaces during gas exchange and by excretion. Mechanisms for minimising water loss could involve reducing rates of water loss from any of these routes. However, excretory water loss is considered a minor component of transpiratory water loss in desiccating insects (Gibbs et al., 1997; Gibbs, 2002a). In scorpions, excretion of nitrogenous waste mainly in the form of insoluble guanine and uric acid contributes to body water conservation (Hadley, 1974). The decrease in excretion rate and regulation of excretions' water content (Ahearn and Hadley, 1977) mean that evaporation is probably the major route for water loss in desiccating scorpions.

Several attempts have been made to partition evaporative water loss in scorpions to the cuticular (CWL) and respiratory (RWL) channels. These were initially based on determination of water loss through the cuticular route only by measuring WLR of freshly killed specimens after sealing the book lung spiracles. However, the validity of the method is questionable, as recorded cuticular water-loss rate values of *H. arizonensis* and *Diplocentrus spitzeri* (Diplocentridae) were higher than total WLR of live specimens (Hadley, 1970a; Crawford and Wooten, 1973). Despite the evident overestimation of CWL, the respiratory component of WLR is still considered a major component of WLR at high temperatures only (Hadley, 1994). Values of 26–33% of WLR were reported for live specimens of *U. armatus* (Scorpionidae) at 20–30 °C (Withers and Smith, 1993). Employing a different approach,

the authors attributed the increase in WLR associated with increased metabolic rate under dark conditions to an increase in RWL. A recent study employing the "hyperoxic switch" method yielded an RWL value of 15% from total water loss at 20 °C for *U. manicatus* (Woodman, 2008). Based on a flow-through modification to the method used by Withers and Smith (1993), RWL values calculated from $\text{CO}_2/\text{H}_2\text{O}$ output rates in this study show a gradual increase with temperature at a range of 25–35 °C (Table 1). The calculated RWL of 31% of total body water lost at 35 °C is similar to values reported for *U. armatus*, and the trend of increasing RWL significance with temperature (Hadley, 1970a; this study) mean that water loss through this route is substantial at ecologically relevant temperatures. Temperature measurements over a 24 h period showed that *H. arizonensis* may experience burrow temperatures of >40 °C for several hours during late afternoon and early evening after soil surface temperature had reached ~60 °C at noon (Hadley, 1970b).

The relative importance of RWL values as reported in this study is positively correlated with increasing temperature and saturation deficit between the dry environment and the saturated respiratory surfaces of the scorpion (Table 2). It is also positively correlated with the increasing gas exchange rates (Table 1) which yield Q_{10} values of ~3.0 at 25–35 °C. Water-loss rates (Table 1) and RWL fractions (Table 2) allow calculation of rates of water loss through either transpiratory route at rest. Calculated RWL values at 25, 30 and 35 °C are 0.020, 0.045 and 0.106 $\mu\text{l g}^{-1} \text{h}^{-1}$, respectively. Relative to this marked increase, changes in calculated CWL are minimal with respective values of 0.198, 0.205 and 0.237 $\mu\text{l g}^{-1} \text{h}^{-1}$.

The measured melting points (T_m) of *H. arizonensis* cuticular lipids (Fig. 2) were well above ambient temperatures encountered by the scorpion, and may explain its unique high resistance to water loss. It should be noted that lipids began melting at ~40 °C, temperatures which may be experienced in burrows (Hadley, 1970b). Partial melting may occur, and indeed may be necessary for surface lipids to spread and cover the cuticle (Gibbs, 2002b). It is intriguing that the beginning and end of phase transitions occurred at ~40 and ~65 °C, respectively. These are the same approximate temperatures at which Hadley (1970a) observed transitions in cuticular permeability in *H. arizonensis*. Hadley's results would seem to suggest a biphasic response of the surface lipids to temperature, which we did not observe. Overall, the lack of thermal acclimation in cuticular lipid properties suggests that thermal acclimation does not affect regulation of epicuticular waterproofing ability. Therefore, the minimal changes in CWL despite the increasing saturation deficit (Table 2), which is the driving force for CWL, indicate a possible active mechanism for resistance to water loss through the integument.

It is widely accepted that the main barrier for transpiration across the scorpion integument are the epicuticular lipids (Hadley, 1990; Gibbs, 2002b). Cuticular water-loss rates of two scorpion species were negatively correlated with amounts of cuticular lipid (Toolson and Hadley, 1977). Rates of CWL were also correlated with seasonal changes in surface hydrocarbon composition (Toolson and Hadley, 1979). Nevertheless, surface hydrocarbon density in the cuticle of *H. arizonensis* is two orders of magnitude lower than that of *Eleodes armata* (Coleoptera; Tenebrionidae) which exhibits much higher WLR (Toolson and Hadley, 1977). This suggests that resistance to CWL in scorpions may include unique characteristics which contribute significantly to their overall almost impermeable integument. The observations that rates of CWL of freshly-killed scorpions (with sealed book lungs) are higher than total WLR of live specimens (Hadley, 1970a; Crawford and Wooten, 1973) may also indicate a role for an active resistance mechanism. Studies on *Periplaneta americana* show that a significant portion of cuticular transpiration occurs through

integumental structures which are regulated by a hydration-dependent mechanism (Machin et al., 1994; Smith et al., 1995). Although the goal of this study was not to identify components contributing to cuticular resistance to water loss, the fairly constant rates of CWL despite increasing temperatures and saturation deficits (Table 2) may hint at regulated CWL in scorpions.

A relatively high fraction of respiratory transpiration from total water loss, as reported here for *H. arizonensis*, may increase the adaptive value of respiratory regulation when water supply is scarce. Downregulation of gas exchange during prolonged desiccation (Riddle, 1978; Gefen, 2008) suggests an evolved mechanism for body water conservation under desiccation stress in scorpions. Further supportive observations are increasing haemolymph acidity (Dejours and Ar, 1991) and RER values of >1.0 (Gefen, 2008) in desiccated scorpions, indicating tighter control of book lung spiracles.

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