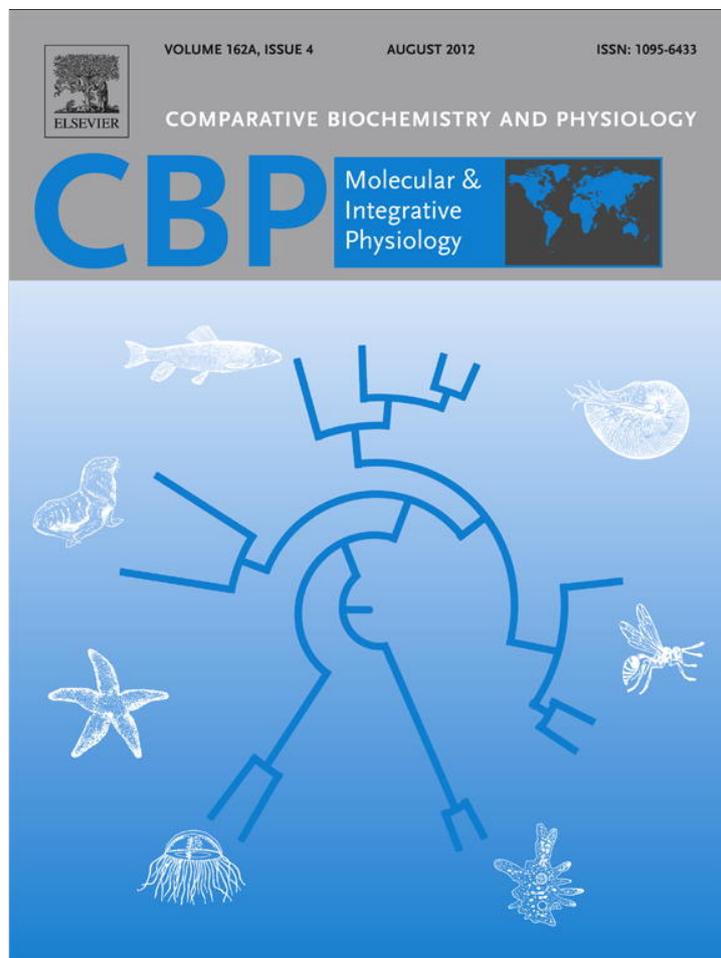


Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>

Contents lists available at [SciVerse ScienceDirect](http://www.elsevier.com/locate/cbpa)

## Comparative Biochemistry and Physiology, Part A

journal homepage: [www.elsevier.com/locate/cbpa](http://www.elsevier.com/locate/cbpa)

## Scorpions regulate their energy metabolism towards increased carbohydrate oxidation in response to dehydration

Bhawna Kalra, Eran Gefen\*

Department of Biology and Environment, Faculty of Natural Sciences, University of Haifa-Oranim, Tivon 36006, Israel

### ARTICLE INFO

#### Article history:

Received 26 February 2012

Received in revised form 9 April 2012

Accepted 10 April 2012

Available online 23 April 2012

#### Keywords:

Scorpions

Desiccation resistance

Carbohydrates

Energy metabolism

Water

### ABSTRACT

Scorpions successfully inhabit some of the most arid habitats on earth. During exposure to desiccating stress water is mobilized from the scorpion hepatopancreas to replenish the hemolymph and retain hydration and osmotic stability. Carbohydrate catabolism is advantageous under these conditions as it results in high metabolic water production rate, as well as the release of glycogen-bound water. Hypothesizing that metabolic fuel utilization in scorpions is regulated in order to boost body water management under stressful conditions we used a comparative approach, studying energy metabolism during prolonged desiccation in four species varying in resistance performance. We used respirometry for calculating respiratory gas exchange ratios, indicative of metabolic fuel utilization, and measured metabolic fuel contents in the scorpion hepatopancreas. We found that hydrated scorpions used a mixture of metabolic fuels (respiratory exchange rates, RER~0.9), but a shift towards carbohydrate catabolism was common during prolonged desiccation stress. Furthermore, the timing of metabolic shift to exclusive carbohydrate oxidation (RER not different from 1.0) was correlated with desiccation resistance of the respective studied species, suggesting triggering by alterations to hemolymph homeostasis.

© 2012 Elsevier Inc. All rights reserved.

### 1. Introduction

Food is the most important source of water for scorpions, meaning that water availability in the wild is largely correlated with fluctuating prey density. Although dehydrated scorpions will often drink in a laboratory setup, in the wild reduced prey abundance in the summer typically coincides with low bulk water availability. There is no current evidence for the ability of any scorpion species to absorb water vapor from subsaturated atmospheres (Hadley, 1990). Therefore the ability of scorpions to maintain their hydration state and osmotic stability during desiccation stress depends on rates of water loss and metabolic water production and the management of existing internal water stores (Gefen and Ar, 2004, 2005).

Species vary in the degree to which mobilization of water from hepatopancreas stores compensates for lost hemolymph volume (Gefen and Ar, 2005). The compensatory capacity of this mechanism depends on oxidation rate and the metabolic fuels being catabolized, and the extent to which oxidation water yield meets water loss to the environment. Compared with lipids, carbohydrate catabolism results in higher metabolic water production rate per unit of ATP formed (Loveridge and Bursell, 1975), and therefore would be favored as far as water budget considerations are concerned. Furthermore, catabolism of stored glycogen results in the release of bound water 3–5 times its

own mass (Schmidt-Nielsen, 1997). Upon glycogen breakdown, this osmotically-inactive volume of bulk water is released and becomes available for replenishing depleting hemolymph volume.

Data on metabolic regulation in stressed insects are conflicting (Edney, 1977). For example, dehydrated grasshoppers shift towards higher use of lipids (Loveridge and Bursell, 1975; Hadley and Quinlan, 1993; Quinlan and Hadley, 1993), counter-productive for water management, whereas drosophilids increase carbohydrate catabolism (Marron et al., 2003). The role of carbohydrate use during desiccation in insects is also implied by increased storage levels in resistant populations (reviewed in Gibbs and Gefen, 2009). Carbohydrate use (e.g. Sinha and Kanungo, 1967; Sinha, 1982), increased hepatopancreas lipid fraction (Gefen and Ar, 2005) and increasing respiratory exchange ratio during desiccation (Gefen, 2008) suggest that water balance constraints under stressful conditions may trigger regulation of energy metabolism in scorpions towards increased carbohydrate catabolism. Respiratory exchange ratios (RER; ratio of CO<sub>2</sub> emission to O<sub>2</sub> consumption rates) are typically a good estimate of RQ (respiratory quotient) which ranges from 0.7 to 1.0 for exclusive catabolism of lipid and carbohydrates, respectively.

We therefore hypothesized a general pattern of increased carbohydrate catabolism in dehydrating scorpions. Furthermore, we compared metabolic fuel utilization in scorpion species for which water relation traits have already been established in order to test whether triggering of metabolic fuel regulation is correlated with desiccation-resistance performance. We combined respiratory data with measurements of metabolite content in the scorpion hepatopancreas, a major site for

\* Corresponding author. Tel.: +972 4 9838837; fax: +972 4 9539608.  
E-mail addresses: [gefene@research.haifa.ac.il](mailto:gefene@research.haifa.ac.il), [gefene@gmail.com](mailto:gefene@gmail.com) (E. Gefen).

metabolic fuel storage, at different levels of exposure to experimental desiccating conditions.

## 2. Material and methods

### 2.1. Choice of species

The employed experimental design follows Gefen and Ar (2004, 2005) by using both xeric and mesic species from the Buthidae and Scorpionidae families. Buthidae were represented by the mesic *Hottentotta judaicus* (formerly referred to as *Buthotus judaicus*) and xeric *Leiurus quinquestriatus*. Scorpionidae included the mesic *Scorpio maurus fuscus* and the xeric *Scorpio maurus palmatus*. *Scorpio maurus* is currently considered a monotypic genus with 17 accepted subspecies, but distinct geographic distribution (Levy and Amitai, 1980) and high genetic divergence (Froufe et al., 2008) suggest a species complex.

### 2.2. Scorpion collection and laboratory maintenance

Mixed-sex samples of the scorpions were collected between January and June 2011. *S. m. fuscus* and *H. judaicus* were collected near Nofit (32°45'N, 35°09'E) in the mesic lower Galilee (mean annual precipitation, ~540 mm), Israel. *L. quinquestriatus* and *S. m. palmatus* were collected in the Negev desert, Israel, near Sede Boqer (30°52'N, 34°46'E). The scorpions were placed in 500 mL plastic containers with soil from the collection site, and were brought to the laboratory within 24 h of capture. In the laboratory the scorpions were initially kept at room temperature (23–25 °C) for 2 weeks during which they were provided with an adult cricket as prey twice a week. The scorpions were then transferred to empty plastic containers and placed in an incubator (Friocell 222, MMM, Munich, Germany) at 30.0 ± 0.2 °C, ambient humidity (~40–60%) and a photoperiod of 14L:8D for a 48 h acclimation to experimental conditions, during which access to food was prevented. The initial mass of the scorpions was determined to the nearest 0.1 mg (CPA224S, Sartorius, Goettingen, Germany), and the scorpions were then assigned to experimental groups that were assayed following 0 (control), 1, 2, 3, 4 or 5-week exposure to experimental conditions.

### 2.3. Respirometry

Closed-system respirometry (Lighton, 2008) was carried out during the 14 h light (L) period, using relatively small metabolic chambers (effective volume of ~15 mL), in order to minimize variation resulting from interspecific differences in activity under these experimental conditions (Gefen, 2011). Dry CO<sub>2</sub>-free air was supplied at a rate of 100 mL·min<sup>-1</sup> simultaneously to the seven chambers containing scorpions and an additional empty chamber used for baselining (MUX 8-channel flow multiplexer, Sable Systems International, Las Vegas, NV, USA). The measuring chambers were then sealed at 15 min intervals for 105 min, before being opened again at 15 min intervals and their respective excurrent flow passed through a pre-calibrated LI-7000 CO<sub>2</sub>/H<sub>2</sub>O analyzer (LI-COR Biosciences, Lincoln, NE, USA), an ascarite column and an Oxzilla-II O<sub>2</sub> analyzer (Sable Systems International). Data were recorded every 5 s and analyzed using Expedata acquisition and analysis software (Sable Systems International). The 120-minute respirometry cycle was repeated 3 times, and analysis was based on data recorded after 6 h acclimation to the metabolic chamber (third cycle). However, on occasions when scorpion activity was detected during chamber washout an earlier trace was analyzed (following 4 h acclimation).

Oxygen depletion in the sealed metabolic chamber was rarely >2% (5 out of 193 measurements), and CO<sub>2</sub> emission rates in this study are in agreement with recently reported resting metabolic rates for the same species determined by flow-through respirometry (Gefen, 2011).

### 2.4. Metabolic fuel stores

Following respirometry the scorpions were weighed again (for calculation of mass loss), decapitated and their hepatopancreas was dissected out. After determination of hepatopancreas wet mass (±0.1 mg) subsamples (~50 mg) were placed in microfuge tubes and stored at -20 °C for subsequent analysis of metabolic fuel contents. The organ was weighed again, and dried at 60 °C to constant mass for determination of water content and tissue dry mass.

For determination of hepatopancreas metabolic fuel contents frozen subsamples were thawed, homogenized twice in 375 µL 0.05% Tween in water, and centrifuged at 16,000 g for 1 min. The supernatant was used for enzymatic assays for determination of triglycerides, carbohydrates (following additional ×2 dilution) and protein (×20 dilution) as detailed by Gefen and Brendzel (2011).

### 2.5. Statistics

Statistical analysis was carried out using Statistica 8.0 (Tulsa, OK, USA). Analyses of variance and covariance were followed by Tukey's HSD tests for posthoc comparisons of means of unequal sample sizes ( $\alpha = 0.05$ ).

## 3. Results

Interspecific differences in desiccation resistance reported here agree with earlier reports (Gefen and Ar, 2004, 2005; Gefen, 2011). The relatively high water loss rates of Scorpionidae underlie their rapid mass losses (Fig. 1), and quick depletion rates of their hepatopancreas water stores (Fig. 2) during exposure to experimental desiccation. Their relative susceptibility was also reflected in high mortality rates after four (*S. m. fuscus*) to five (*S. m. palmatus*) weeks, hence the shorter exposure to experimental desiccation conditions. In contrast, buthids maintained their hepatopancreas water stores throughout the 5-week duration of the measurements (Fig. 2).

Initial oxygen consumption rates (VO<sub>2</sub>) showed significant interspecific variation ( $F_{3,35} = 4.49$ ,  $p < 0.01$ ), with VO<sub>2</sub> for the two Buthidae species significantly higher than those of *S. m. palmatus* (and *H. judaicus* > *S. m. fuscus*) (ANCOVA, with initial body mass as covariate, followed by HSD test for unequal N) (Fig. 3a), but no interspecific differences in VO<sub>2</sub> were observed thereafter ( $p = 0.33$ – $0.55$ ). Variation in CO<sub>2</sub> emission rates (VCO<sub>2</sub>) was limited to higher values for *H. judaicus* compared with *S. m. palmatus* when unstressed (Fig. 3b).

No interspecific variation in calculated RERs was found for hydrated scorpions (Table 1; ANOVA;  $F_{3,35} = 1.29$ ,  $p = 0.29$ ), with mean values ranging from 0.84 to 0.89 suggesting oxidation of a mixture of metabolic

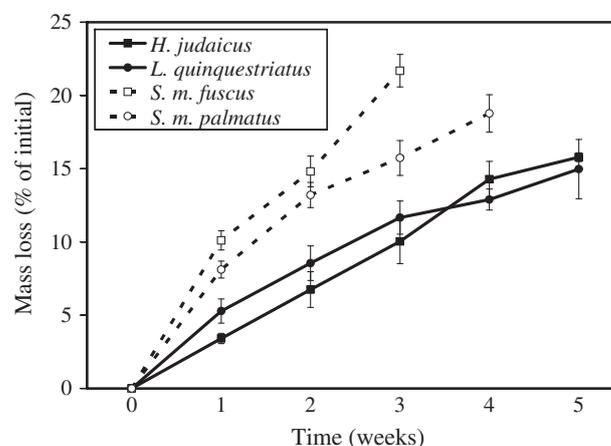
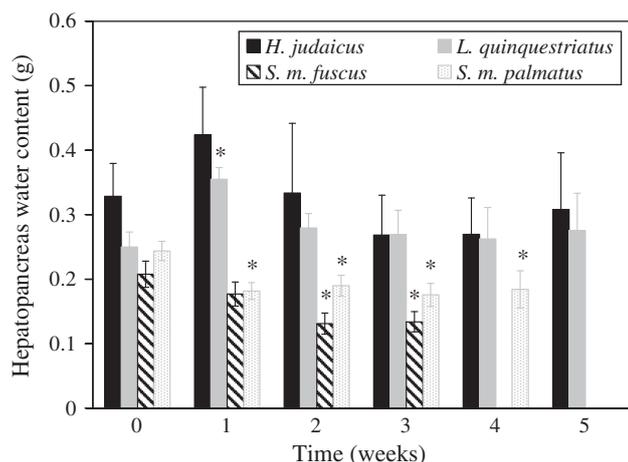
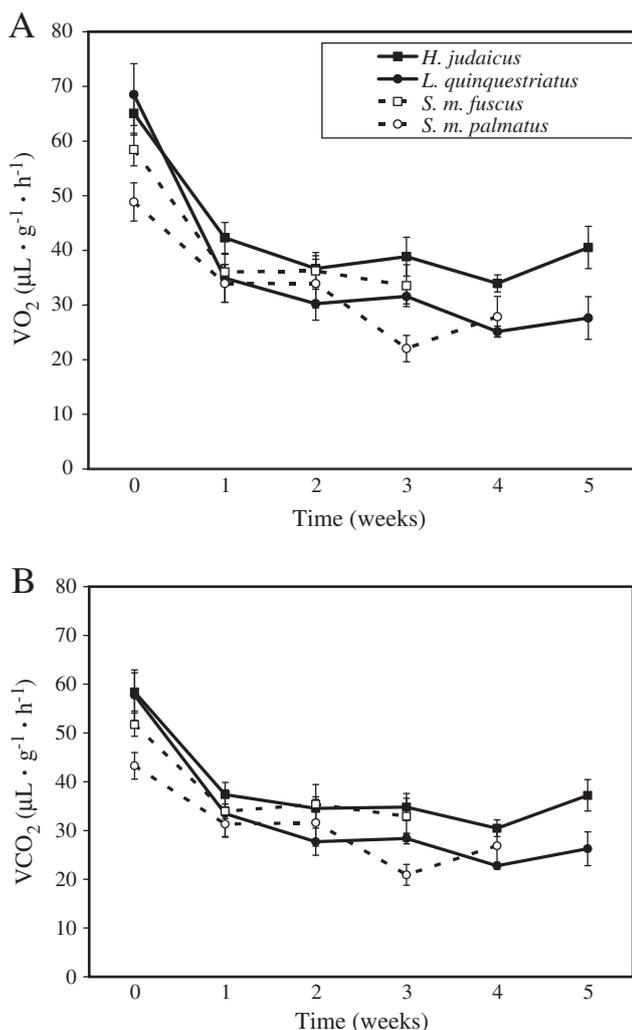


Fig. 1. Mass loss (% of initial; mean ± S.E.) as a function of exposure time to desiccation at 30 °C.



**Fig. 2.** Hepatopancreas water content (mean ± S.E.) as a function of desiccation duration at 30 °C. Asterisks denote significant differences from initial control values (ANCOVA, with hepatopancreas dry mass as covariate, followed by Tukey's HSD test for unequal N;  $\alpha = 0.05$ ).

fuels. RERs increased with exposure to desiccation, indicative of increased carbohydrate catabolism at the expense of lipid and/or protein oxidation. Three of the four species reached RER values not



**Fig. 3.** Mass-specific oxygen consumption (A) and CO<sub>2</sub> emission (B) rates (mean ± S.E.) measured at 30 °C.

significantly different from 1.0 ( $t$ -test for one sample mean;  $\alpha = 0.05$ ), suggesting exclusive catabolism of carbohydrates. The timing of the shift to exclusive carbohydrate catabolism appeared to be correlated with the respective species' desiccation resistance performance. Among Scorpionidae, the more susceptible *S. m. fuscus* (Fig. 1, 2) exhibited RER not different from 1.0 following 2 weeks of desiccation. Similar RER values were reached only after 4 weeks of desiccation in the xeric *S. m. palmatus* (Table 1). Interestingly, a transient increase to RER of 1.0 was recorded in *L. quinquestriatus* following a 1-week exposure to desiccation, before returning to control values for 3 additional weeks under stressful conditions and eventually rising again (week 5, Table 1). Calculated mean RERs for *H. judaicus* did not reach 1.0 during the 5-week duration of the measurements. The use of mixed-sex samples required ruling out the possible effect of sex on metabolic fuel utilization patterns. Indeed, no significant effect of sex on gas exchange ratio was found for *H. judaicus* ( $F_{1,45} = 0.21$ ,  $p = 0.65$ ) or *S. m. fuscus* ( $F_{1,50} = 0.004$ ,  $p = 0.95$ ; ANCOVA on  $VCO_2$ , with  $VO_2$  as covariate). Only four of 49 sampled *L. quinquestriatus* were males, whereas *S. m. palmatus* included in this study were all females.

During the initial two week exposure to experimental conditions hepatopancreas carbohydrate content decreased significantly, as did values for protein and/or triglycerides for each of the studied species ( $\alpha = 0.05$ ; Fig. 4, Table 2). In contrast, while carbohydrate depletion persisted thereafter, hepatopancreas protein and triglyceride contents following 1-week desiccation were not different compared with values recorded subsequently in all four studied species (Table 2). Interestingly, initial carbohydrate content did not vary among species ( $F_{3,26} = 0.20$ ,  $p = 0.89$ ), but bordered on the accepted significance level following 1-week desiccation ( $F_{3,28} = 2.89$ ,  $p = 0.05$ ) whereas carbohydrate content for the desiccation-resistant *H. judaicus* was significantly higher following an additional week exposure to desiccation ( $F_{3,30} = 19.6$ ,  $p < 0.001$ ) indicating lower depletion rates.

#### 4. Discussion

Interspecific variation in desiccation resistance and depletion pattern of hepatopancreas water stores reported here (Fig. 1, 2) are in agreement with earlier reports (Gefen and Ar, 2004, 2005; Gefen, 2011). The low resistance of *S. m. fuscus* and *S. m. palmatus* resulted in high mortality rates as desiccation exposure progressed, hence their shorter exposure to experimental stress. The rapid loss of body water in *S. m. fuscus* results in a 10% mass loss during the first week of desiccation at 30 °C (Fig. 1). In comparison, similar body mass loss levels in *H. judaicus* were reached only after 3 weeks under the same experimental conditions. In light of these considerable differences in loss rates to the environment it is predicted that maintaining hemolymph homeostasis requires variation in triggering of compensatory mechanisms (Gefen and Ar, 2004, 2005).

Adequate replenishing of lost hemolymph volume from hepatopancreas stores largely depends on the rate of metabolite oxidation and the substrate being oxidized. Similar  $VO_2$  values among the studied species (Fig. 3) do not support increased oxidation rate as means for increasing metabolic water production rates. Although carbohydrate oxidation results in higher metabolic water yield compared with other fuels (per unit of energy output), increasing metabolic rate under the experimental conditions is unlikely to result in net water gain. The oxidation of 1 mg of carbohydrates requires consumption of 829  $\mu LO_2$ , yielding 0.56  $\mu L$  metabolic water (Hadley, 1994), whereas respiratory water losses associated with this level of respiratory gas exchange range from 1.05 to 1.75 mL  $H_2O$  in hydrated scorpions of the same species at 30 °C and <5% RH (Gefen, 2011). Although diffusive respiratory losses are likely to be lower at higher ambient humidity these data indicate that increased metabolism as means for water gain in ecologically-relevant conditions for these species is improbable. Moreover, limited water gains at relatively high humidity would hinge on decoupling oxidation from ATP

**Table 1**

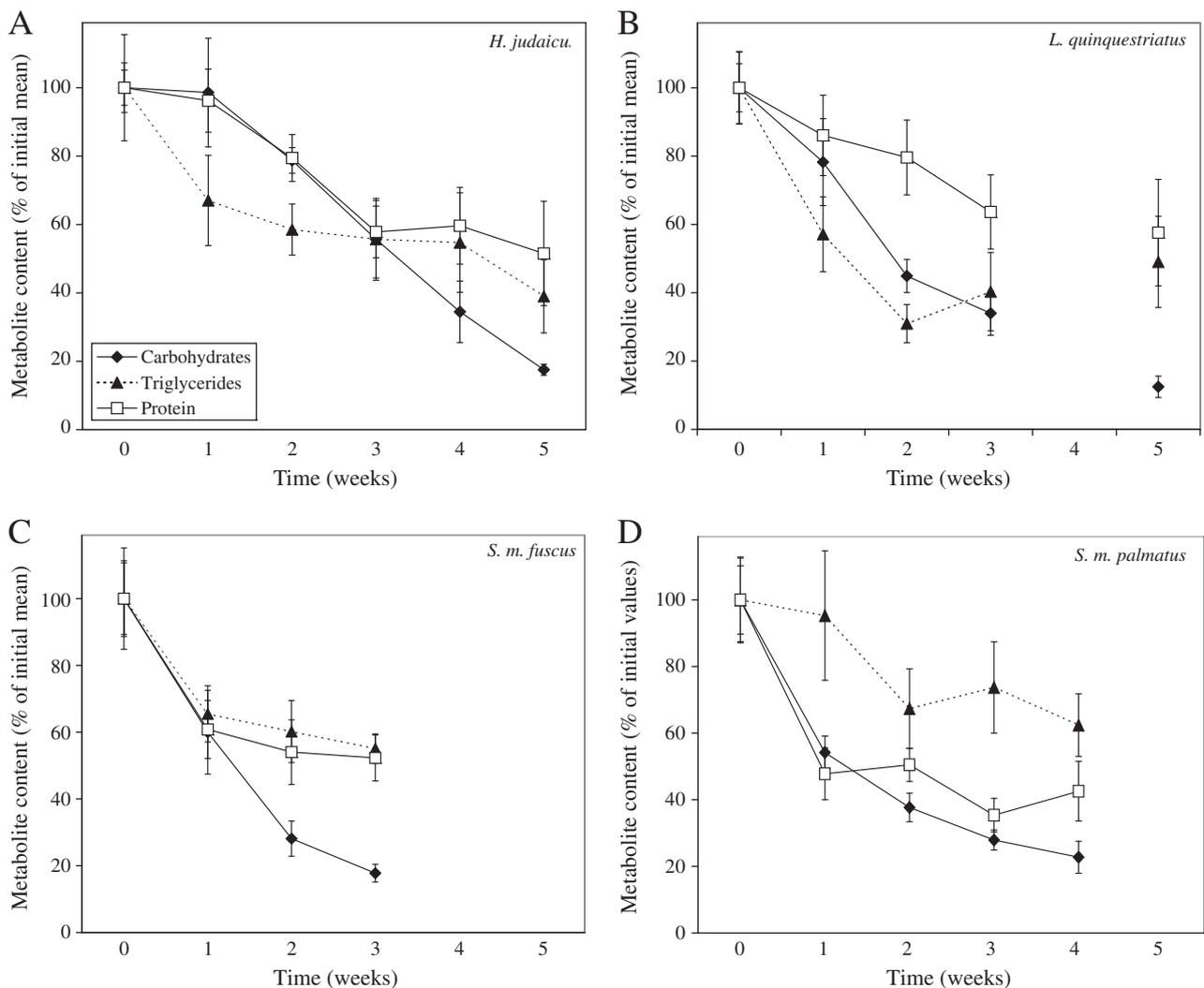
Calculated RERs (mean ± S.E.) as a function of exposure time (weeks) to desiccation at 30 °C. \* denotes values not statistically different from 1.0 (*t*-test for one sample mean). Sample sizes in parentheses.

Time (weeks)	0	1	2	3	4	5
<i>H. judaicus</i>	0.89 ± 0.01 (9)	0.88 ± 0.02 (10)	0.94 ± 0.02 (7)	0.90 ± 0.02 (7)	0.90 ± 0.03 (7)	0.93 ± 0.02 (8)
<i>L. quinquestratus</i>	0.84 ± 0.01 (9)	0.96 ± 0.03* (8)	0.92 ± 0.01 (8)	0.90 ± 0.01 (9)	0.92 ± 0.02 (10)	0.96 ± 0.01* (5)
<i>S. m. fuscus</i>	0.89 ± 0.03 (14)	0.94 ± 0.01 (16)	0.98 ± 0.04* (12)	0.97 ± 0.03* (11)	-	-
<i>S. m. palmatus</i>	0.89 ± 0.01 (7)	0.94 ± 0.02 (8)	0.91 ± 0.03 (8)	0.94 ± 0.03 (8)	0.98 ± 0.03* (7)	-

synthesis in organisms whose energy resources are often limited and unpredictable.

Nevertheless, metabolic water may still constitute a significant avenue for water gain in terrestrial invertebrates during dehydration (see discussion in Edney, 1977). In contrast with a shift towards lipid catabolism in dehydrated locusts (Loveridge and Bursell, 1975; Hadley and Quinlan, 1993), unfavorable in terms of metabolic water gain, *Drosophila* have been shown to switch to carbohydrate catabolism during desiccation stress (Marron et al., 2003). Together with metabolic water production glycogen catabolism also results in the release of bound water, and a six-fold higher volume of water available for transport compared with lipid oxidation (Chown and Nicolson, 2004). Results in this study

indicate regulation of metabolic fuel utilization under stressful conditions. Initially, scorpions utilize a combination of metabolic fuels, reflected in RER values of 0.8–0.9 (Table 1) and depleting hepatopancreas contents of at least two metabolic energy sources (Fig. 4, Table 2). RER of 0.9 was also previously reported for hydrated *Smeringurus mesaensis* (Vaejovidae) (Gefen, 2008). Similar to *S. mesaensis*, the four species included in this study exhibit an increase in RER as exposure to desiccating conditions extends (Table 1). Moreover, with the exception of an early transient change in RER for *L. quinquestratus*, early shift to exclusive carbohydrate catabolism (as indicated by RER not different from 1.0; Table 1) occurred earlier in the more desiccation susceptible Scorpionidae (and *S. m. fuscus* in particular) compared



**Fig. 4.** Hepatopancreas relative metabolite content as a function of exposure time to desiccation at 30 °C and ~50%RH. Presented values are percentages (mean ± S.E.) of control treatment mean.

**Table 2**  
Hepatopancreas metabolic fuel content ( $\text{mg} \cdot \text{g dry mass}^{-1}$ ; mean  $\pm$  S.E.) in hydrated scorpions, and following 1 to 5 weeks of desiccation at 30 °C. Mean ( $\pm$  S.E.) initial body mass are given for the four species. Different superscript letters denote significant differences ( $p < 0.05$ ; ANOVA, followed by Tukey's HSD tests for unequal sample size). Sample sizes in parentheses.

Time (weeks)	0	1	2	3	4	5
<i>H. judaicus</i> (initial body mass $1.682 \pm 0.109$ g)						
Carbohydrates	$25.9 \pm 2.0^a$ (8)	$25.5 \pm 4.4^a$ (8)	$20.4 \pm 1.8^{ab}$ (8)	$14.4 \pm 3.3^{abc}$ (7)	$8.9 \pm 2.5^{bc}$ (6)	$4.5 \pm 0.5^c$ (5)
Proteins	$251.2 \pm 13.8^a$	$241.7 \pm 24.8^{ab}$	$199.6 \pm 28.9^{ab}$	$145.3 \pm 20.5^b$	$149.8 \pm 30.8^{ab}$	$129.4 \pm 42.9^b$
Triglycerides	$3.5 \pm 0.6^a$	$2.4 \pm 0.5^a$	$2.1 \pm 0.8^a$	$2.0 \pm 0.4^a$	$1.9 \pm 0.6^a$	$1.4 \pm 0.4^a$
<i>L. quinquestriatus</i> (initial body mass $2.374 \pm 0.131$ g)						
Carbohydrates	$26.6 \pm 2.0^a$ (8)	$20.9 \pm 3.6^{ab}$ (8)	$12.0 \pm 1.4^{bc}$ (8)	$9.1 \pm 1.9^c$ (7)		$3.3 \pm 0.9^c$ (5)
Proteins	$209.5 \pm 23.5^a$	$180.3 \pm 26.4^a$	$166.8 \pm 24.5^a$	$133.4 \pm 24.6^a$		$120.7 \pm 36.5^a$
Triglycerides	$3.9 \pm 0.4^a$	$2.2 \pm 0.5^{ab}$	$1.2 \pm 0.2^b$	$1.6 \pm 0.5^b$		$1.9 \pm 0.6^{ab}$
<i>S. m. fuscus</i> (initial body mass $1.574 \pm 0.070$ g)						
Carbohydrates	$24.6 \pm 3.0^a$ (8)	$14.8 \pm 3.3^b$ (8)	$6.9 \pm 1.4^{bc}$ (11)	$4.4 \pm 0.7^c$ (11)		
Proteins	$176.1 \pm 20.2^a$	$107.1 \pm 16.4^b$	$95.2 \pm 17.9^b$	$92.1 \pm 12.8^b$		
Triglycerides	$1.9 \pm 0.3^a$	$1.2 \pm 0.5^{ab}$	$1.1 \pm 0.2^b$	$1.0 \pm 0.1^b$		
<i>S. m. palmatus</i> (initial body mass $1.796 \pm 0.088$ g)						
Carbohydrates	$24.3 \pm 2.8^a$ (6)	$13.1 \pm 1.3^b$ (8)	$9.1 \pm 1.1^{bc}$ (8)	$6.8 \pm 0.8^c$ (8)	$5.5 \pm 1.2^c$ (8)	
Proteins	$175.3 \pm 24.7^a$	$83.8 \pm 14.6^b$	$88.5 \pm 9.4^b$	$62.0 \pm 9.6^b$	$74.6 \pm 16.8^b$	
Triglycerides	$2.2 \pm 0.3^a$	$2.1 \pm 0.5^a$	$1.5 \pm 0.3^a$	$1.6 \pm 0.3^a$	$1.4 \pm 0.2^a$	

with Buthidae. Interestingly, the early transient increase of RER to  $\sim 1.0$  in *L. quinquestriatus* coincides with an early increase in hepatopancreas water content (Fig. 2), and precedes an increase in the relative hemolymph volume during mild desiccation (Gefen and Ar, 2005). The biphasic response in this species could explain the decrease in hemolymph osmolality in mildly dehydrated *L. quinquestriatus* (Gefen and Ar, 2004), although details of a mechanism required for water transport up an osmotic gradient are not yet known.

Despite considerable variation in hepatopancreas metabolic fuels content, changing levels during prolonged exposure to desiccation stress support our findings based on respiratory gas exchanges. Depletion of more than one metabolic fuel characterizes the first 2 weeks of exposure in all four studied species, whereas longer desiccation stress leads to a shift towards exclusive carbohydrate catabolism (Fig. 4). Changes in hepatopancreas metabolic fuel content represent utilization during periods of both resting and activity, whereas gas exchange rates are measured (and RERs calculated) over a 2 h resting period. This suggests that interspecific variation in fuel utilization patterns is unlikely to be a direct result of differences in activity levels among the studied species (Gefen, 2011). Instead, the shift in metabolic fuel utilization appears to reflect, and perhaps result from, changes in the internal environment of the animals. Exclusive carbohydrate catabolism is inferred for *S. m. fuscus* after a two-week exposure to 30 °C and  $< 5\% \text{RH}$  (Table 1), during which  $\sim 15\%$  of initial mass were lost (Fig. 1). Similar mass loss levels are calculated for *S. m. palmatus* and the two buthids following 1 and 3 additional weeks of exposure, respectively (Fig. 1), correlated with their relative delayed switch to exclusive carbohydrate catabolism. The relatively high water loss rates of Scorpionidae underlie their poor osmotic response to dehydration (Gefen and Ar, 2004). It is possible that the rapid increase in hemolymph osmolality provides the trigger for a shift in metabolic fuel utilization. In contrast, exclusive carbohydrate oxidation at  $\text{VO}_2$  of  $0.96 \text{ mL} \cdot \text{d}^{-1}$  (Fig. 3a) means catabolism of 1.16 mg carbohydrates, yielding 0.64 mg metabolic water and the release of 3.5–5.8 mg bound water a day, which more than fully compensates for the total daily water loss of *H. judaicus* (Gefen and Ar, 2004). The highly impermeable integument of *H. judaicus* means that hemolymph volume and osmotic concentration may be maintained in this species at 30 °C without resorting to exclusive carbohydrate catabolism, as manifested in lower carbohydrate depletion rates (Table 2) and RER values significantly lower than 1.0 throughout the experimental exposure to desiccation (Table 1).

In conclusion, this study demonstrates a shift in metabolic fuel utilization in scorpions during prolonged desiccation stress. The

species-specific timing of its triggering correlates with interspecific variation in desiccation-resistance suggesting a common mechanism for ameliorating the effects of dehydration on hemolymph stability.

#### Acknowledgments

We would like to thank three anonymous reviewers whose comments helped improve an earlier version of this manuscript. This study was supported by Israel Science Foundation awards 1645/08 and 975/08 to EG, and by the Israeli Ministry of Foreign Affairs Cultural and Scientific Relations Division.

#### References

- Chown, S.L., Nicolson, S.W., 2004. Insect Physiological Ecology: Mechanisms and Patterns. Oxford University Press, New York, NY.
- Edney, E.B., 1977. Water Balance in Land Arthropods. Springer-Verlag, Berlin.
- Froufe, E., Sousa, P., Alves, P.C., Harris, D.J., 2008. Genetic diversity within *Scorpio maurus* (Scorpionidae: Scorpionidae) from Morocco: preliminary evidence based on CO1 mitochondrial DNA sequences. *Biologia* 63, 1157–1160.
- Gefen, E., 2008. Sexual dimorphism in desiccation responses of the sand scorpion *Smeringurus mesaensis* (Vaejovidae). *J. Insect Physiol.* 54, 798–805.
- Gefen, E., 2011. The relative importance of respiratory water loss in scorpions is correlated with species habitat type and activity pattern. *Physiol. Biochem. Zool.* 84, 68–76.
- Gefen, E., Ar, A., 2004. Comparative water relations of four species of scorpions in Israel: evidence for phylogenetic differences. *J. Exp. Biol.* 207, 1017–1025.
- Gefen, E., Ar, A., 2005. The effect of desiccation on water management and compartmentalisation in scorpions: the hepatopancreas as a water reservoir. *J. Exp. Biol.* 208, 1887–1894.
- Gefen, E., Brendzel, O., 2011. Desiccation resistance and mating behaviour in laboratory populations of *Drosophila simulans* originating from the opposing slopes of Lower Nahal Oren (Israel). *J. Evol. Biol.* 24, 2110–2117.
- Gibbs, A.G., Gefen, E., 2009. Physiological adaptations in laboratory environments. In: Garland, T., Rose, M.R. (Eds.), *Experimental Evolution*. University of California Press, Berkeley, CA, pp. 523–550.
- Hadley, N.F., 1990. Environmental physiology. In: Polis, G.A. (Ed.), *The Biology of Scorpions*. Stanford University Press, Stanford, CA, pp. 321–340.
- Hadley, N.F., 1994. *Water Relations of Terrestrial Arthropods*. Academic Press, San Diego, CA.
- Hadley, N.F., Quinlan, M.C., 1993. Discontinuous carbon dioxide release in the eastern lubber grasshopper *Romalea guttata* and its effect on respiratory transpiration. *J. Exp. Biol.* 177, 169–180.
- Levy, G., Amitai, P., 1980. *Fauna Palestina. Arachnida. : Scorpiones*, vol. 1. Israel Academy of Sciences and Humanities, Jerusalem.
- Lighton, J.R.B., 2008. *Measuring Metabolic Rates: A Manual for Scientists*. Oxford University Press, New York, NY.
- Loveridge, J.P., Bursell, E., 1975. Studies on the water relations of adult locusts (Orthoptera, acrididae). I. Respiration and the production of metabolic water. *Bull. Entomol. Res.* 65, 13–20.

- Marron, M.T., Markow, T.A., Kain, K.J., Gibbs, A.G., 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. *J. Insect Physiol.* 49, 261–270.
- Quinlan, M.C., Hadley, N.F., 1993. Gas exchange, ventilatory patterns, and water loss in two lubber grasshoppers: quantifying cuticular and respiratory transpiration. *Physiol. Zool.* 66, 628–642.
- Schmidt-Nielsen, K., 1997. *Animal Physiology: Adaptation and Environment*. Cambridge University Press, USA.
- Sinha, R.C., 1982. Effect of starvation and refeeding on the scorpion *Buthus tamulus*. *Jpn. J. Physiol.* 32, 983–989.
- Sinha, R.C., Kanungo, M.S., 1967. Effect of starvation on the scorpion *Palamnaeus bengalensis*. *Physiol. Zool.* 40, 386–390.