



## Participation of book lungs in evaporative water loss in *Paraphysa parvula*, a migalomorph spider from Chilean Andes

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### ARTICLE INFO

#### Article history:

Received 16 November 2009

Received in revised form 31 December 2009

Accepted 1 January 2010

#### Keywords:

Evaporative water loss

Metabolism

Theraphosidae

### ABSTRACT

Small animals need efficient water conservation mechanisms for survival and reproduction, which is relevant for the spiders that have large book lungs with large respiratory surface. If lung evaporation is relevant to limit water loss, adjustments of the spiracle opening to metabolic demands should be expected. In this study, we measured the metabolic rate and total evaporative water loss mediated by the opening of the spiracles in the migalomorph spider *Paraphysa parvula*, a resident of fluctuating Mediterranean environments of the mountains of central Chile. We found that the metabolism of *P. parvula* was similar to other Theraphosidae and low compared to other arthropods. Carbon dioxide production and evaporative water loss increased with temperature, particularly at 40 °C. The total evaporative water loss at 40 °C increased dramatically to about 10 times that found with the lower temperatures. Thus, 40 °C will be the limit temperature for this species after which evaporative water loss starts to become damaging, so it has to avoid it. The exposition to hypercapnic environments had as a consequence an increase in evaporative water loss and the involvement of the book lungs in this loss was about 60%. The possibility of losing water could condition this species to seek temperate and oxygenated shelters under rocks.

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### 1. Introduction

Despite possessing an exoskeleton, arthropods have high surface area to volume ratio, making them very susceptible to water loss, which is especially relevant in the hot and dry conditions typical of xeric environments (Gibbs et al., 2003). In spiders, water regulation is provided by specialized organs such as coxal glands, Malpighian tubules and nephrocytes, and other organs that have a secondary role as hypodermic cells and superficial cells of the intestinal diverticulae (Foelix, 1996). In these animals the evaporation occurs in a large proportion by the body surface, and according to Vollmer and MacMahon (1974), to a lesser extent in the book lungs. However, Davies and Edney (1952) in a classic study showed that total evaporation in a spider, estimated by weight loss, increased by about 50% when the opening of the spiracles was induced by exposure to CO<sub>2</sub>, suggesting a significant participation of the book lungs in evaporative water loss. The same authors showed that the rate of water evaporated per unit area (mg/cm<sup>2</sup> h) increased signifi-

cantly at 40 °C, when metabolism is high and the opening of the spiracles is common. It has been proposed that their meticulous control helps prevent water loss and that carbon dioxide is the main stimulus for their opening (Davies and Edney, 1952). The spiracles would open more frequently and more extensively at high temperatures and with increased activity due to the increased need for oxygen (Schmidt-Nielsen, 1998).

Studies of evaporative water loss of spiders after Davies and Edney (1952) are scarce, and have focused almost exclusively on the relationship between evaporation and environment in several species (Vollmer and MacMahon, 1974; Hadley et al., 1981; Hadley and Quinlan, 1989). For example, Hadley et al. (1981) showed that lycosid species from xeric environments have lower evaporation rates than species that live in caves. Later, Hadley and Quinlan (1989) suggested that the low rate of evaporation in the “widow” spider *Latrodectus hesperus* allows successful colonization of desert habitats in southwestern North America. In all ectotherms metabolism is temperature-dependent, which means that oxygen consumption and CO<sub>2</sub> production increase following a power law with increasing temperature, which could cause conflicts with the conservation of water as a result of high rates of water exchange associated with high metabolic rates and increased evaporation at these temperatures. Thus, in some invertebrates there is total

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disruption of metabolism during some periods, which has been interpreted by some authors as an adaptation to prevent desiccation (Alpert, 2006). The role played by the book lungs and tracheae as organs of O<sub>2</sub> and CO<sub>2</sub> exchange by diffusion depends on several factors, such as the activity level of the animal (Strazny and Perry, 1984). For example, Schmitz and Perry (2001) used stereological morphometric methods to investigate the morphological diffusion capacity of the lungs and the walls of the secondary tracheae; they found that the tracheal system plays an important role in gas exchange in jumping spiders. Also, the oxygen consumption of migmalomorph spiders is low, about half of that reported for other ectotherms of similar mass (Anderson, 1970, 1974; Greenstone and Bennett, 1980; Canals et al., 2007). However, Lighton and Fielden (1995) examined several hexapod and arachnid orders and concluded that their metabolism scaled identically as a function of body mass. The low metabolism in spiders has been proposed as an energy adaptation to predation in fluctuating environments, and metabolic depression was reported in 3 weeks fasted migmalomorph spiders (Canals et al., 2007). If pulmonary or tracheal evaporation is relevant to the metabolic changes associated with these factors, to limit water loss spiders should adjust their spiracle openings to the metabolic demands (Fincke and Paul, 1989). The Andean environments of Chile are characterized by its great variability in temperature and atmospheric changes in water vapor pressure affecting the energy expenditure and evaporative water loss in small animals. There is also a great seasonal variation in prey availability which also affects the metabolism and predation strategy (Canals et al., 2007). In these conditions, and in order to maintain water balance, migmalomorph spiders oscillate between meeting the challenges of avoiding metabolic depression and preventing dehydration. In those spiders we expect to find a low metabolism and an efficient water loss control at their usual microenvironmental temperatures. We studied the metabolism, spiracle opening and evaporative water loss in the migmalomorph spider *Paraphysa parvula*, a resident of fluctuating Mediterranean environments of Central Andes Range of Chile. Specifically, we measured the metabolic rate at different temperatures and estimated the involvement of the book lungs and the role of spiracle opening in evaporative water loss.

## 2. Materials and methods

### 2.1. Animals and study area

The animal model was *P. parvula* (Araneae, Theraphosidae). Adults females of this species have body mass ranging between 6 and 10 g and it inhabits the central mountains (altitudes above 2000 m). It is a crepuscular and nocturnal spider, although males of this species can be seen occasionally at noon. During the day it can be found in shelters under flat stones. Its reproductive period occurs between December and January.

Sampling was conducted in the mountainous area of Farellones (2200 m a.s.l.) in an area dominated by low shrubs, principally by *Chuquiraga oppositifolia*, *Ephedra chilensis*, *Acaena andina* and *A. splendens* (Rosaceae), where *P. parvula* is usually found. Initially, 15 adult females were captured (Mb = 8.4 ± 1.6 g) between October and December 2008 using manual removal of rocks, and immediately translated to the ecophysiology laboratory of the Faculty of Sciences of the University of Chile in Santiago.

In the laboratory, animals which were kept separate in transparent boxes of 34 cm × 34 cm × 7 cm at 20 °C ± 4 °C and photoperiod of 12 h:12 h L:D. Water was administered *ad libitum* by maintaining a moist cotton ball in the bottom of the box. Two to three larvae of *Tenebrio molitor* were administered daily as a food source *ad libitum*.

### 2.2. Evaporative water loss and CO<sub>2</sub> production at different temperatures with and without spiracular occlusion

After a period of acclimatization of 3 weeks, the total evaporative water loss (TEWL) and CO<sub>2</sub> production were measured at temperatures of 25, 30, 35 and 40 °C. The animals were weighed using an electronic balance (±0.05 g) and then placed in metabolic chambers of 100 cm<sup>3</sup> specially designed for the metabolic trial. All metabolic measurements were made during the day, which corresponds to the resting phase of this species, with the metabolic chamber in the dark. We used a computerized open flow system for determination of CO<sub>2</sub> production (Sable Systems). The equipment was calibrated with a known mixture of oxygen (20%) and nitrogen (80%) which was certified by chromatography (BOC, Chile). The metabolic chamber received dried air at a flow of 50 ml/min. The air passed through columns of Drierite and Baralime to remove water and CO<sub>2</sub>, respectively. We recorded simultaneously the total evaporative water loss with a hygrometer attached to the metabolic chambers (Sable Systems) (Sabat et al., 2006). The outputs of the water and CO<sub>2</sub> analyzers were digitized using a Universal Interface II (Sable Systems) and recorded on a personal computer using EXPEDATA data acquisition software (Sable Systems). Our sampling interval was 1 s. The spiders were kept in chambers for at least 3 h for each metabolic trial.

We calculated metabolic water production (MWP) from the estimated oxygen consumption, considering the expected respiratory quotient (RQ = 0.77) from the carbohydrate (53.97%), lipid (33.87%) and protein composition (3.38%) of the larvae of *T. molitor* (from [www.thelizardlounge.com/content/insect/mealworm.asp](http://www.thelizardlounge.com/content/insect/mealworm.asp)). We considered a production of 0.1107 mg H<sub>2</sub>O/cal and that 1 ml O<sub>2</sub> yields 5 cal (Schmidt-Nielsen, 1998).

To estimate the participation of the book lungs and the role of the opening of the spiracles in evaporative water loss in this species, in the same animals TMS and TEWL were measured as before at temperatures of 25, 30, 35 and 40 °C, but with occlusion of two of the spiracles of a randomly selected animal's side. The occlusion of the spiracles was performed with polyvinyl acetate. The change in total evaporative water loss at each temperature between the intact *versus* occluded conditions estimated the contribution to the loss of water produced by the book lungs, since when two spiracles were occluded the exposed book lung surface was half of the intact condition. In addition, we calculated the ratio between evaporative water loss and mass-specific CO<sub>2</sub> production (i.e. (mg H<sub>2</sub>O/g h)/(ml CO<sub>2</sub>/g h)) and compared the rates of water loss with and without spiracle occlusion.

### 2.3. Evaporative water loss and CO<sub>2</sub> production at hypercapnic and normal atmosphere and at two temperatures

Ten new individuals were captured (Mb = 7.9 ± 2.03 g) and were measured in normal air flow and then exposed to a hypercapnic air atmosphere (CO<sub>2</sub> concentration = 6.7%), to force the opening of the spiracles (Davies and Edney, 1952) at temperatures of 25 and 35 °C, measuring total evaporative water loss with a hygrometer connected to metabolic chambers (Sable System) with the above mentioned design.

### 2.4. Analyses

The involvement of book lungs and the role of the opening of the spiracles in evaporative water loss were estimated by two ways:

- (i) From spiracle occlusion. Since these spiders were measured with and without occlusion of half of their spiracles, if the change in evaporative water loss among these conditions is due to this factor alone, we can deduce the following: evaporative

water loss is due to two components, the surface of the skin (A) and through book lungs (P). When the spiracles are intact the loss is due to the additive effect of these two components:  $TEWLi = A + P$ . When half of the spiracles are occluded the water loss may be expressed as  $TEWL = A + P/2$ . Thus, combining this two relationships, the P component may be estimated as  $P = 2(TEWLi - TEWL)$ . For example, a spider with  $P + A = 7.4 = TEWLi$  and  $P/2 + A = 4.98 = TEWL$  implies  $P = 4.84$ ; the percentage of participation of book lungs would be  $4.84/7.4 = 65\%$  of  $TEWLi$ .

- (ii) From exposition to hypercapnic environments. The change in water loss between normal and hypercapnic environments estimated the effect of the spiracle opening.

The  $CO_2$  production, evaporative water loss and metabolism-specific evaporative water loss (ratio between water loss and  $CO_2$  production) and  $Q_{10}$  were studied with analysis of variance for repeated measures, considering as factors: (i) temperature and (ii) spiracle condition (occluded or free, repeated measures).

We estimated the values of  $Q_{10}$  ( $CO_2$  production change with a temperature difference of  $10^\circ C$ ) (Schmidt-Nielsen, 1998). For animals with no spiracles occluded, we estimated the share of metabolic water production in evaporative water loss from the ratio between these variables, analyzing the effect of temperature by ANOVA. The rate of total evaporative water loss and mass-specific water loss in the “normal” and “hypercapnic environment” conditions at different temperatures were compared with repeated measures ANOVA.

### 3. Results

As environment temperature increases an increase in  $CO_2$  production was observed, which was particularly noticeable at  $40^\circ C$  ( $F_{3,112} = 79.42$ ,  $p < 0.001$ ). There were no differences in  $CO_2$  production between individuals with and without spiracular occlusion ( $F_{1,112} = 0.12$ ,  $p = 0.73$ ) nor interaction between temperature and occlusion ( $F_{3,112} = 0.38$ ,  $p = 0.77$ ) (Fig. 1). Without spiracle occlusion the  $Q_{10}$  values were:  $Q_{10} 25-35^\circ C = 2.8 \pm 0.79$ ,  $Q_{10} 30-40^\circ C = 3.8 \pm 1.52$  ( $t_{14} = 2.65$ ,  $p < 0.05$ ) whereas with spiracles occluded those were:  $Q_{10} 25-35^\circ C = 2.4 \pm 0.48$  and  $Q_{10} 30-40^\circ C = 3.4 \pm 0.89$  ( $t_{14} = 4.15$ ,  $p < 0.01$ ). In both cases we can see an exaggerated increase in  $CO_2$  production at high temperatures ( $30-40^\circ C$ ).

Water loss increased significantly at a temperature of  $40^\circ C$  ( $F_{3,112} = 168.07$ ,  $p \ll 0.001$ ). While at 25, 30 and  $35^\circ C$  water

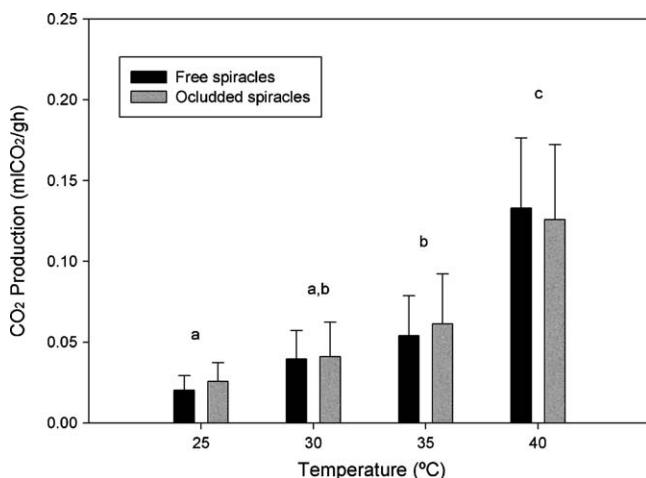


Fig. 1. Carbon dioxide production at four temperatures, with and without spiracular occlusion. Different letters indicate differences between treatments in multiple comparisons with Tukey test.

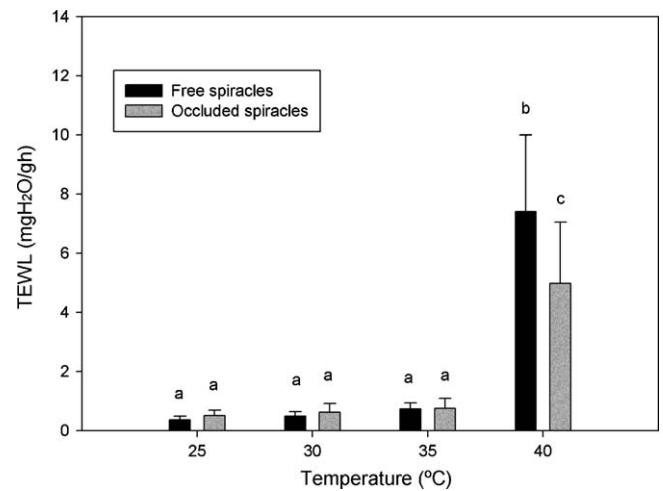


Fig. 2. Evaporative water loss at four temperatures, with and without spiracular occlusion. Different letters indicate differences between treatments in multiple comparisons with Tukey test.

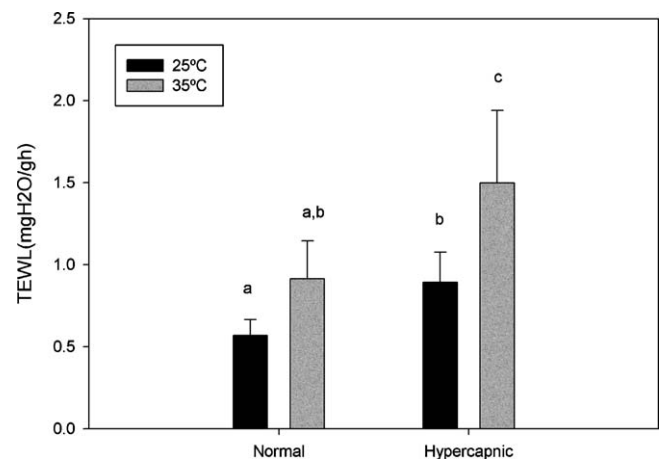


Fig. 3. Evaporative water loss at two temperatures in normal and hypercapnic atmospheres. Different letters indicate differences between treatments in multiple comparisons with Tukey test.

production was similar, at  $40^\circ C$  this increased approximately 10 times (Fig. 2). By dividing water loss by  $CO_2$  production ( $ml H_2O/ml CO_2$ ) the same results were obtained. Besides, metabolic water production (MWP) was very low in relation to the total evaporative water loss:  $0.014 \pm 0.006$ ,  $0.028 \pm 0.013$ ,  $0.034 \pm 0.018$ ,  $0.095 \pm 0.031 ml H_2O/g h$  at 25, 30, 35 and  $40^\circ C$  respectively ( $F_{3,56} = 17.228$ ,  $p < 0.01$ ). This is especially relevant at  $40^\circ C$  where MWP represents less than two percent of TEWL while at others temperatures MWP it was about 4–6% of TEWL.

Finally, we found an increase in both the total and mass-specific evaporative water loss in animals exposed to a hypercapnic environment compared to those exposed to water loss in a normal environment ( $F_{1,18} = 414.85$ ,  $p \ll 0.001$  and  $F_{1,18} = 111.69$ ,  $p \ll 0.001$  for total and mass-specific TEWL). We also found a difference in total and mass-specific TEWL between different temperatures ( $F_{1,18} = 79.58$ ,  $p \ll 0.001$  and  $F_{1,18} = 17.69$ ,  $p \ll 0.001$ , respectively) (Fig. 3).

### 4. Discussion

Among the arachnids, migalomorph spiders have comparatively low resting metabolic rates. This fact probably reflects their low energy requirements for their lifestyle, which includes months of

inactivity in holes during the winter and during periods of moulting, as well as periods of low availability of food because of its “sit and wait” predatory strategy (Shillington, 2005).

Production of CO<sub>2</sub> in *P. parvula* was well within the range of those reported for other Theraphosidae (Shillington and Peterson, 2002; Shillington, 2005). At 25 °C CO<sub>2</sub> production averaged 0.166 ml CO<sub>2</sub>/h and at 30 °C, CO<sub>2</sub> production was 0.331 ml CO<sub>2</sub>/h, similar to those results obtained by Shillington (2005) for *Aphonopelma anax*. Comparing CO<sub>2</sub> production with the expected values for arthropods from Lighton et al. (2001), *P. parvula* values correspond to 16.35 ± 7.3% of the expected value, confirming that the metabolism of this species is low and coincident with the hypothesis that an important aspect of their metabolic efficiency include very low resting metabolic rates as consequence of the anatomical characteristics of their respiratory system (lung volume and respiratory surface area) being part of a suite of physiological, anatomical and behavioural adaptations which have the effect of making energy less limiting for spiders than other animals of similar size and trophic position (Anderson, 1970; Anderson and Prestwich, 1982; Greenstone, 1978; Greenstone and Bennett, 1980). The evaporative water loss was influenced by temperature. However, while at temperatures of 25, 30 and 35 °C water loss was relatively low, it suddenly increased about 10 times at a temperature of 40 °C in the two experimental conditions (intact and occluded spiracles). The three first temperatures are usually found near or inside refuges of *P. parvula* in spring and summer months. Thus, 40 °C will be the limit temperature for this species after which evaporative water loss starts to become damaging, so it has to avoid it. Measures taken in spring and summer refuges of this species have temperatures around 30 °C.

Those with non-occluded spiracles had greater water loss than those with spiracle occlusion. The apparent contribution of metabolic water production to the water budget is practically negligible, especially at 40 °C where the ratio between these two variables (MWP/TEWL) represented only 1.3 ± 0.4% of the total. This value is less than the value obtained at lower temperatures, suggesting that the increase in evaporative water loss was not correlated to the elevation of aerobic metabolism at this temperature.

The significant increase in water loss at 40 °C could be explained by a loss of impermeability of the body surface at these temperatures, by the secretion of wetting fluid in the coxal glands and by the greater frequency of spiracle opening. According to Fincke and Paul (1989) resting spiders keep their spiracles completely closed and when performing maximum activity they open their spiracles completely, which can lead to a greater water loss. Differences in evaporative water loss at 40 °C between spiders with occluded and non-occluded spiracles suggest a contribution from the lungs of 60.7% to total water loss, although with a large variability (±40%). However, this assumes that at high temperatures there was a higher frequency of spiracle opening, a situation that was not observed directly. In the second approach we used lower temperatures (25 and 35 °C) thus avoiding the possible alteration of the permeability at high temperatures, and administered a hypercapnic (6.7% CO<sub>2</sub>), but normoxic atmosphere, which assured spiracle opening (Davies and Edney, 1952; Schmidt-Nielsen, 1998). In this case there was an increase of 58.43 ± 14.6% at 25 °C and 63.84 ± 20.58% at 35 °C. These two values are very close to the value estimated by the former method, excepting that here there was no temperature effect that could alter the permeability surface or significant changes in the secretion of water, and spiracle opening was assured. The values reported here are slightly higher than the 50% estimated by Davies and Edney (1952). From an evolutionary standpoint, one might imagine that natural selection favours the development of a regulatory mechanism that keep the spiracles open enough to allow exchange of respiratory gases but otherwise restrict

its opening as a dehydration probability increase. Thus, while gas exchange requires an increase in spiracle opening area (Fincke and Paul, 1989), conservation of water requires their closure. *P. parvula* is a species which at rest is under flat stones in a microenvironment that is completely covered by snow in winter. These two conditions can increase the environmental carbon dioxide by about 25% (Jones, 1999; Musselman et al., 2005), which coupled with the low vapour pressure associated with low temperatures, might be considered a factor of drying stress for the spiders. In spring and summer the Central Andes Range of Chile has a dry climate with high temperatures for at least 4 months, reaching over 40 °C in some areas, constituting another drying stress factor. The possibility of losing water could conditionate this species to seek temperate and oxygenate shelters under rocks and avoid to shelt in holes as other migmorph spiders do. Coupled with this, the low metabolic rate reported in this work could be part of the physiological adaptation characteristics of sit and wait predatory spiders to tolerate low or unpredictable food availability (Anderson, 1970; Anderson and Prestwich, 1982; Greenstone, 1978; Greenstone and Bennett, 1980; Canals et al., 2007), buffering them against environmental fluctuations of their habitat.

### Acknowledgements

We thank Lafayette Eaton for useful comments on a previous version of the manuscript. Andres Sazo provided invaluable assistance in the field and the laboratory. Founded by FONDECYT 1080038 grant to MCL.

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