Effects of diet and water supply on energy intake and water loss in a mygalomorph spider in a fluctuating environment of the central Andes

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A R T I C L E   I N F O
Article history:
Received 12 April 2011
Received in revised form 20 July 2011
Accepted 22 July 2011
Available online 29 July 2011

Keywords:
Diet
Metabolism
Water loss
Mygalomorph spider

A B S T R A C T
The metabolic and water evaporation strategies in spiders may be part of a set of physiological adaptations to tolerate low or unpredictable food availability, buffering spiders against environmental fluctuations such as those of the high mountains of the central Andes.

The aim of this study is to analyze experimentally the variations in metabolic rate and the rate of evaporative water loss at high temperatures in a high mountain mygalomorph spider population (Paraphysa sp.).

We found that the low metabolism of this spider was not affected by water restriction, but its metabolism was depressed after 3 weeks of food deprivation. The spider did not show seasonal metabolic changes but it presented seasonal changes in the rate of evaporative water loss at high temperatures.

Females with egg sacs reduced their metabolic rate and evaporative water at high temperatures.

These findings constitute a set of possible adaptations to a highly fluctuating Mediterranean environment, which is completely covered with snow for many months and then progresses rapidly to a very dry climate with high temperatures.

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1. Introduction

The metabolic rate of spiders is about half that reported in other ectoderms of similar mass (Anderson, 1970, 1974; Carrel and Heathcote, 1976; Angersbach, 1978; Greenstone and Bennett, 1980; Anderson and Prestwich, 1982, 1985; Paul et al., 1987; Strazny and Perry, 1984; Schmitz and Perry, 2001; Canals et al., 2008), which has been considered as an energetic adaptation to their predation strategy. This has led to many studies trying to relate predator type to metabolic rate (Carrel and Heathcote, 1976; Angersbach, 1978; Greenstone, 1978; Greenstone and Bennett, 1980; Paul et al., 1987; Strazny and Perry, 1984; Schmitz and Perry, 2001), and to the proposal that the low energy consumption of spiders is a strategy of “sit and wait” predators in a similar way to that found in ticks (Lighton and Fielden, 1995) and in the antlion Myrmeleon hyalinus (Scharf et al., 2009). It has also been proposed that metabolic performance would be enhanced by the ability to depress metabolic rates under usual resting levels during transient periods of food deprivation (Ito, 1964; Miyashita, 1969; Nakamura, 1972; Anderson, 1974; Humphreys, 1977). Canals et al. (2008) found that the mygalomorph spider Grammostola rosea showed very low oxygen consumption at rest, requiring a partial pressure difference of only 0.12–0.16 kPa oxygen between the external and internal environment to meet their oxygen demand. However, they also found that metabolic rate was reduced by food deprivation after 2 weeks, but only at higher temperatures (30 °C in this study), suggesting that metabolic depression is apparent with the high requirements imposed by the temperature–metabolism relationship. Because high metabolic rates are related to high rates of water exchange and evaporation, it is also possible that metabolic depression is a mechanism to avoid water loss through evaporation.

In mygalomorph spiders the rate of water evaporated (mg/cm² h) significantly increases at 40 °C, which means that fluctuating environments with high temperatures, can be very stressful for these spiders (Figueroa et al., 2010). Desiccation is a significant stress for terrestrial arthropods and several mechanisms to achieve desiccation resistance have been noted: (i) reducing the rate of water loss, (ii) increasing the bulk amount of water available to lose and (iii) increasing the tolerance to water loss (Gibbs et al., 1997; Bazinet et al., 2010). For example, in insects, respiratory water loss may be reduced by controlling respiratory patterns (Chown, 2002; White et al., 2007), and cuticular water loss can be controlled by variations in the permeability or epicuticular hydrocarbons (Gibbs, 2002). Also bulk water can be increased by increasing hemolymph volume (Hadley, 1994) or by accumulating
glycogen (Gibbs, 2002). The mechanisms determining water loss
tolerance are not well understood, although trehalose and heat
shock proteins have been implicated in cellular protection of
organism that survive losing large quantities of water (Watanabe,
2006; Benoit et al., 2010). Also photoperiodic diapause can in-
crease desiccation resistance. For example, in Aedes albopictus dia-
pause eggs had one-third more surface hydrocarbons and one-half
the water loss rates of non-diapause eggs (Urbanski et al., 2010).

Some studies have focused on the relationship between evapo-
ration and the environment living species of spiders (Vollmer and
MacMahon, 1974; Edney, 1977; Hadley et al., 1981; Hadley and
Quinlan, 1989). Thus for example Hadley et al. (1981) showed that
lycosid species from xeric environments have lower evaporation
rates than species that live in caves, and Hadley and Quinlan
(1989) suggested that the low rate of evaporation of the black
widow spider Latrodectus hesperus allowed it to invade successfully
the southwestern desert habitats in North America.

The metabolic and water evaporation strategies in spiders may
be part of a set of physiological adaptations to tolerate low or
unpredictable food availability (Mc Nab, 2002) buffering spiders
against environmental fluctuations such as those of Mediterranean
climates (Canals et al., 2008). The high mountains of the central An-
des, where there are large fluctuations in temperature and humid-
ity and where snow covers large areas for much of the year,
conditioning the availability of prey, is a stressful environment for
mygalomorph spiders that live and breed there, and should affect
their physiological machinery. Mountain spiders may manifest
metabolic depression from food deprivation as a way of saving en-
ergy (Ito, 1964; Miyashita, 1969; Nakamura, 1972; Anderson, 1974;
Humphreys, 1977; Canals et al., 2008) or manifest differences in the
rate of water evaporation associated with different environmental
conditions (Vollmer and MacMahon, 1974; Edney, 1977; Hadley
et al., 1981; Hadley and Quinlan, 1989; Figueroa et al., 2010).

The aim of this study is to investigate experimentally the vari-
atious in metabolic rate and the rate of evaporative water with
food and/or water deprivation in a high mountain mygalomorph
spider population.

2. Materials and methods

2.1. Animal model and study area

Sampling was conducted in the mountainous area of Farellones
(2200 m) in an area dominated by low shrubs, principally
Acaena andina and A. splendens (Rosaceae). This area is covered by
snow most of the year, from autumn to mid-spring, and may vary from year to year. The tem-
perature in the warm season varies from 20 to 50 °C in the soil, but
reach 50 °C in rocks exposed to the sun. The terrestrial fauna is com-
posed of small rodents, lizards, insects such as ants and phasmids
and arachnids, which during the cold season live under snow cover.

A resident population of a single species Paraphysa sp., charac-
teristic of this area, was used as a study model (Figueroa et al.,
2010; Veloso et al., in press). This is a mygalomorph spider with
body mass ranging between 6 and 10 g which inhabits the central
mountains above 2000 m. It is a crepuscular and nocturnal spider,
although males of this species occasionally can be seen at noon.
During the day it can be found in shelters under flat stones and
in the cold season it is inactive. Its reproductive period occurs
between December and January.

2.2. Sample and experimental design

During the austral spring and summer of 2009 and 2010, 64
adult females of this species were carefully captured using manual
removal of rocks to avoid cuticular damage, and the spiders were
immediately taken to the ecophysiology laboratory of the Faculty
of Sciences of the University of Chile in Santiago. The first capture
period (Dry sample) was conducted during the Dry season in mid-
December 2009 (early summer) and the second capture period
(Wet sample) immediately after the snow melted, which in 2010
happened during the first days of November (Spring).

In the laboratory, animals were kept separate in transparent
boxes of 34 × 34 × 7 cm at 20 °C ± 4 °C and photoperiod of
12 h:12 h L:D. Twenty-eight individuals were used from each sam-
ples: Dry (m0 = 5.38 ± 2.23, average ± standard deviation) and Wet
(m0 = 5.79 ± 1.64). Each sample was randomly divided into four
groups of seven individuals each and assigned to one of the follow-
ing treatments: (Group 1) water and food ad libitum (W+F+); water
was administered by maintaining a moist cotton ball in the bottom
of the box and the spiders were offered 2–3 larvae of Tenebrio mol-
itor daily as a food source (total mass: 0.2402 ± 0.031 g); (Group
2) without water, food ad libitum (W−F+); (Group 3) water ad li-
bitum, without food (W+F−); (Group 4) without water or food
(W−F−). The spiders were kept in these conditions for 3 weeks
and then were subjected to the experimental trials. If an individual
moulted it was replaced by another individual that was subjected
to identical experimental conditions. Females that spun an egg sac
were maintained in the experimental conditions, but during mea-
surements the egg sac was removed.

2.3. Evaporative water loss and CO2 production at different
temperatures

After the period of conditioning, total evaporative water loss
(TEWL) and CO2 production (VC02) were measured at temperatures
of 30°, 35° and 40 °C because the preferred temperature of this spe-
cies is near 30, and 40 °C is near the critical maximum temperature
(Figueroa et al., 2010; Veloso et al., in press). The animals were
weighed using an electronic balance SHIMADZU AUX 220
(±0.01 g) and then placed in metabolic chambers of 100 cc spe-
cially 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. Only one
trial (at one temperature) was performed each day. The animals
were separated at 6:00 h and measurements began at 14:00 h,
ensuring a fast of at least 6 h. We used a computerized open flow
system for determination of CO2 production (Sable Systems).
The equipment was calibrated at the factory for CO2 and in the lab
for O2 with a known mixture of oxygen (20%) and nitrogen (80%)
which was certified by chromatography (BOC, Chile). The meta-
obbyal chamber received dried air at a flow of 50 ml/min. The air
passed through columns of Drierite and Baralime to remove water
and CO2 respectively. We recorded the total evaporative water loss
with a hygrometer attached to the metabolic chambers (Sable Sys-
tems). The outputs of the water and CO2 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 during each metabolic trial, and
measurements were taken when the records were stabilized,
approximately 30 min after the beginning of the trial.

2.4. Analyses

To rule out effects of body size on the analyzed variables, the
body mass of the different groups in the sample periods was ana-
lyzed with a two way ANOVA. To analyze the experimental effects
on VC02 a repeated-measures factorial ANOVA was performed,
using as factors the sample period (Wet and Dry), diet (W+F+,
W+F−, W−F+ and W−F−) and temperature (repeated measure:
30, 35 and 40 °C). Females with egg sacs were excluded. Tukey multiple comparisons and one tailed planned comparisons were performed. Previous to these analyses, normality and homoscedasticity assumptions were tested. Later, a second analysis was performed using the reproductive variable (ES). This was an ANOVA including the egg sac factor (ES+) and ES−), but without considering the diet factor because of mixed numbers and because only a few females in each group had egg sacs.

3. Results

Initial body mass was similar in all experimental groups \( F_{3,48} = 0.165, p = 0.91 \) and in both capture periods \( F_{1,48} = 0.557, p = 0.46 \).

3.1. Carbon dioxide production

We did not find a global effect of the capture period on \( \text{CO}_2 \) \( F_{1,38} = 0.315, p = 0.58 \), but there were clear \( \text{CO}_2 \) differences attributable to diet \( F_{1,38} = 3.95, p = 0.015 \) and to temperature \( F_{2,76} = 269.3, p < 0.001 \) without interaction among these variables \( F_{6,76} = 0.89, p = 0.51 \) (Fig. 1). Multiple comparisons showed that carbon dioxide production was different at the three different temperatures (Table 1). However, the diet effect was uneven in the Dry period. In the planned comparisons we obtained: (i) water effects: with or without food (W+F+ vs. W+F−: \( t = 2.21, p = 0.015 \) and W−F+ vs. W−F−: \( t = 1.75, p = 0.045 \)).

Female spiders with egg sac \( n = 11; 5 \) in the Dry sample and 6 in the Wet sample) showed lower \( \text{CO}_2 \) \( F_{1,52} = 5.12, p = 0.027 \). In the same analysis the effect of temperature was confirmed (now for all individuals, with and without egg sac) \( F_{2,104} = 194.24, p < 0.001 \) as well as the lack of sample period effect on \( \text{CO}_2 \) \( F_{1,52} = 0.43, p = 0.52 \) and the lack of variable interactions \( p > 0.05 \) (Table 2 and Fig. 2).

3.2. Evaporative water loss

There was an increase in evaporative water loss with increase of temperature \( F_{2,76} = 178.8, p < 0.001 \). There was also a difference in water loss between capture periods \( F_{1,38} = 5.7, p = 0.022 \) which was only significant at 40 °C, without differences attributable to the diet factor \( F_{1,38} = 2.23, p = 0.10 \). A positive interaction between capture period and temperature was found \( F_{2,76} = 6.20, p = 0.003 \); water loss was greater during the Wet capture period, but again this was only evident at 40 °C (Table 3 and Fig. 3). Analyzing exclusively water loss data at 40 °C, the capture period effect on water loss was confirmed \( F_{1,39} = 3.90, p = 0.05 \), but diet effects \( F_{3,39} = 1.1; p = 0.35 \) and interaction effects on water loss were not found \( F_{3,39} = 2.4, p = 0.08 \). The total water loss during the time of the trials (2.5 h) at 40 °C was 68.97 mg (1.28% of the initial body weight \( m_{bi} \)) in the Dry sample and 103.10 mg (1.78% \( m_{bi} \)) which means that these spiders could loss 12.3% \( m_{bi} \) and 17.2% \( m_{bi} \) respectively, probably reaching their maximal limit of desiccation tolerance (Vollmer and MacMahon, 1974).

Comparing the evaporative water loss between females with and without egg sacs, the greater water loss at 40 °C \( F_{2,104} = 83.26, p < 0.001 \) and the increase of water loss in the Wet capture period was confirmed \( F_{1,52} = 6.90, p = 0.011 \), with a marginal decrease of evaporative water loss in females with egg sacs \( F_{1,52} = 3.28, p = 0.07 \) which is evident in the temperature–egg sac interaction \( F_{2,104} = 4.11, p = 0.02 \) (Fig. 4). Multiple comparisons revealed that these differences only occurred at 40 °C (Table 4). When we analyzed only data at 40 °C we found the same

Fig. 1. Carbon dioxide production (\text{mLCO}_2/\text{gh}) at three temperatures in Paraphysa sp. captured in two periods (Dry and Wet) and with four different diets [see text].
results for the capture period effect ($F_{1,51} = 7.32, p = 0.009$) and the egg sac effect ($F_{1,51} = 3.65, p = 0.06$).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>30°C Dry</th>
<th>30°C Wet</th>
<th>35°C Dry</th>
<th>35°C Wet</th>
<th>40°C Dry</th>
<th>40°C Wet</th>
</tr>
</thead>
<tbody>
<tr>
<td>W+F+ ab</td>
<td>35.4 ± 12.4</td>
<td>19.9 ± 4.3</td>
<td>45.7 ± 14.0</td>
<td>29.8 ± 5.5</td>
<td>67.6 ± 13.7</td>
<td>60.8 ± 6.4</td>
</tr>
<tr>
<td>W-F- a</td>
<td>34.5 ± 14.5</td>
<td>28.4 ± 12.7</td>
<td>48.7 ± 18.2</td>
<td>42.1 ± 17.6</td>
<td>70.4 ± 15.8</td>
<td>59.7 ± 29.7</td>
</tr>
<tr>
<td>W+F- c</td>
<td>13.5 ± 6.5</td>
<td>18.0 ± 12.4</td>
<td>23.8 ± 6.2</td>
<td>30.1 ± 11.6</td>
<td>41.8 ± 7.6</td>
<td>66.0 ± 10.8</td>
</tr>
<tr>
<td>W-F+ b</td>
<td>21.8 ± 7.9</td>
<td>20.9 ± 10.1</td>
<td>31.8 ± 8.9</td>
<td>33.8 ± 11.4</td>
<td>58.4 ± 9.9</td>
<td>65.9 ± 10.9</td>
</tr>
<tr>
<td>W+F+ c</td>
<td>20.1 ± 5.2</td>
<td>17.8 ± 4.3</td>
<td>29.1 ± 6.0</td>
<td>22.3 ± 7.2</td>
<td>40.4 ± 9.1</td>
<td>52.0 ± 11.2</td>
</tr>
</tbody>
</table>

4. Discussion

The sampling area of the central Andes has a long season in which snow covers the whole area. After the snow melts in the spring, the weather changes to a high altitude dry climate. Under these conditions the *Paraphysa* sp. population lives, captures prey, mates and breeds. The temperature of the rocks in some areas may exceed 50°C in the dry season, while in winter the temperature in their shelters is less than 0°C (Veloso et al., in press). In our experiments we compared the response of spiders captured in the dry season (Dry) with others captured when the snow had recently melted. Our results show a spider with a low metabolism, which, calculating the expected oxygen consumption as $\log V_{O2} = 0.133 + 0.71 \log(m_b)$ with $V_{O2}$ in μl/h and $m_b$ in mg (Green-
with (ES+) and without (ES+) egg sac captured in two periods (Dry and Wet). Different

Paraphysa sp. (mgH₂O/gh) with (ES+) and without (ES+) egg sac captured in two periods (Dry and Wet).

Fig. 3. Evaporative water loss at three temperatures in Paraphysa sp. (mgH₂O/gh) captured in two periods (Dry and Wet) and with four different diets (see text).

Table 4
Evaporative water loss at three temperatures in Paraphysa sp. females (mgH₂O/gh) with (ES+) and without (ES+) egg sac captured in two periods (Dry and Wet). Different letters indicate significant differences (p < 0.05) in Tukey multiple comparisons.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Dry ES-</th>
<th>Dry ES+</th>
<th>Wet ES-</th>
<th>Wet ES+</th>
</tr>
</thead>
<tbody>
<tr>
<td>30°C</td>
<td>0.80 ± 0.19 a</td>
<td>0.77 ± 0.21 a</td>
<td>0.77 ± 0.14 a</td>
<td>0.81 ± 0.28 a</td>
</tr>
<tr>
<td>35°C</td>
<td>±0.24 b</td>
<td>1.07 ± 0.29 b</td>
<td>±0.22 b</td>
<td>1.11 ± 0.24 b</td>
</tr>
<tr>
<td>40°C</td>
<td>5.03 ± 2.33 c</td>
<td>2.69 ± 0.88 d</td>
<td>7.00 ± 3.26 e</td>
<td>5.70 ± 1.63 f</td>
</tr>
</tbody>
</table>

stone and Bennett, 1980), and considering a respiratory quotient of RQ = 0.92 (Shillington, 2002), corresponds to a 53.2% expected for spiders, typical for mygalomorph spiders (Canals et al., 2008). Its CO₂ production and evaporative water loss are temperature dependent with a sharp increase in evaporative water loss at 40 °C, which has already been reported for Paraphysa sp. (Figueróa et al., 2010; Veloso et al., in press), suggesting that this temperature is probably very close to the maximum critical temperature for the species. This is also consistent with the abrupt change in permeability attributable to the phase change of cuticle lipids (Gibbs 1998, 2002), although other factors can be implicated in the change of cuticular permeability (see Yoder et al., 2009). The capture period did not affect the metabolism, which is consistent with results obtained in other mygalomorphs such as Aphonopelma anax (Stoltey and Shillington, 2009), where no metabolic changes were observed throughout the mating season.

The lack of water in the diet did not affect the metabolism or evaporative water loss, which reveals that these spiders are well adapted to water shortages over the time period considered in this experiment, in which the average change in weight attributable to water loss in the dehydrated groups (W – F – and W – F +) was 3.55%. However, results in araneomorph spiders have been contradictory. For example, in Argyope trifasciata dehydration caused a decrease in VCO₂ at 15 °C and 25 °C, while in A. aurentia dehydration caused an increase in VCO₂ at 25 °C (Markezieh, 1993). The absence of food for 3 weeks resulted in a decrease of metabolism, which is consistent with previous findings in different species. For example, in Lycosa pseudonunulata (Ito, 1964), Pardosa atra (Miyayashi, 1969; Tanaka and Ito, 1982) Lycosa lenta, Filistata hibernalis (Anderson, 1974) and Latrodectus hasselti (Stoltz et al., 2010) a decrease in metabolic rate caused by starvation has been reported. This decline in metabolic rate has been attributed in part to a behavioral change in spiders deprived of food, from an active search for prey to a wait phase. Thus the metabolic change could be attributed to a decrease in activity. This may be particularly adaptive in sit and wait predators (Matsura, 1981; Greenstone and Bennett, 1980; Canals et al., 2008). In mygalomorphs a decrease in metabolism due to food deprivation has also been reported. For example, in G. rosea metabolic depression was seen at 30 °C in animals subjected to a short starvation period of 3 weeks (Canals et al., 2008). In contrast, the species studied here showed a decrease in metabolic rate which was similar in amount at different temperatures. Our results show metabolic differences between the groups with and without food, however, the spiders were not monitored over time. Thus we cannot determine whether feeding increased metabolism or whether starvation caused the decline. Interesting in this regard are the results of Phillip and Shillington (2010), who found that the mygalomorph spider Phormictopus cancerides fed with a cricket every 5 days increased its metabolism, while individuals fed every 30 days maintained their metabolism. The metabolic increase was attributed to specific dynamic action (SDA) or a physiological response associated with feeding (Phillip and Shillington, 2010). It has also been reported that a mygalomorph spider of central Chile Euathlus truculentus has an SDA scope of 6.5 ± 1.1, with a peak at 45 min, but the metabolic increase can last 8 h (Nespolo et al., 2011). In our experiments with Paraphysa sp. animals were separated in the morning and measured in the afternoon. This implies that if there were any effects of SDA they would be minimal. Moreover the values obtained at comparable temperatures in postabsorptive animals of the same species by Figueróa et al. (2010) are very similar to those of our animals with food (W+F and W–F+) and much higher than the group without food (W–F– and W–F–F3). This suggests that Paraphysa sp. depresses its metabolism with food deprivation of 3 weeks. Moreover, this effect is greater in the dry capture period, which may be a seasonal adjustment to a less predictable or reduced availability of prey. Spiders captured during the Wet period had a greater evaporative water loss at 40 °C than those captured during the Dry period in which temperatures are higher and humidity low. This may be due to changes in the composition of the cuticle such as the proportion of hydrocarbon compounds and lipids; this has been reported among adult females and young in Tegenaria atrica (Trabalon et al., 1996). Temporary changes in trans-cuticle water flow have been reported in other arthropods (Haldy and Quinlan, 1987; Toolson and Haldy,


Hadley, N.F., 1987; Hadley et al., 1986), but to the best of our knowledge there are no reports of seasonal changes in cuticle permeability in spiders. These findings constitute a set of possible adaptations to a highly fluctuating Mediterranean environment, which is completely covered with snow for many months and then progresses rapidly to a hot, dry climate with high temperatures. Fluctuating environments may be linked to changes in prey availability and metabolic changes associated with diet. Adaptation to rapid temperature change probably requires changes in the cuticle to avoid dehydration at temperatures above 40 °C that are common in parts of its habitat, which also forces the spider to search for adequate shelters (Veloso et al., in press). Reproduction also imposes new physiological changes and behavioral needs in this spider. Thus the presence of an egg sac is associated with a decrease in metabolism that may be an adaptation to lower food consumption in these conditions. This, associated with a possible decrease in water content, involves the search for cooler shelters (Veloso et al., in press), to avoid dehydration.

Acknowledgements

We thank Lafayette Eaton for his useful comments on the manuscript. Funded by FONDECYT 1080038 Grant to M.C.L.