J Exp Biol Advance Online Articles. First posted online on 13 November 2014 as doi:10.1242/jeb.109298
Access the most recent version at http://jeb.biologists.org/lookup/doi/10.1242/jeb.109298

Metabolism and water loss rate of the haematophagous insect, *Rhodnius prolixus*: Effect of starvation and temperature

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ABSTRACT

Haematophagous insects suffer big changes in water needs under different levels of starvation. *Rhodnius prolixus* is the most important haematophagous vector of Chagas disease in the north of South America and a model organism in insect physiology. Although, there are some studies on patterns of gas exchange and metabolic rates, there is little information regarding water loss in *R. prolixus*. We investigated if there is any modulation of water loss and metabolic rates under different requirements for saving water. We measured simultaneously CO_2 production, water emission and activity on individual insects in real time by open-flow respirometry at different temperatures (15, 25 and 35°C) and post-feeding days (0, 5, 13 and 29). We found: 1) a clear drop in the metabolic rate between 5-13 days after feeding that cannot be explained by activity and 2) a decrease in water loss rate with increasing starvation level, by a decrease in cuticular water loss during the first 5 days after feeding and a drop in the respiratory component thereafter. We calculated the surface area of the insects and estimated cuticular permeability. In addition, we analyzed the pattern of gas exchange; change of cyclic to continuous pattern was affected by temperature and activity, but it was not affected by the level of starvation. Modulation of metabolic and water loss rates with temperature and starvation could help *R. prolixus* to be more flexible in tolerating different periods of starvation, which is adaptive in a changing environment with the uncertainty of finding a suitable host.

KEY-WORDS: flow-through respirometry, respiratory water loss, cuticular permeability, CO_2 emission rate

1. INTRODUCTION

Desiccation resistance is vital for survival and colonization of terrestrial habitats. Particularly in insects there must be a fine and efficient control on water loss because of their high surface area to volume ratio. Insects lose water through various pathways: transpiration through cuticle, evaporation along open spiracles through the tracheal system, and excretion (Edney, 1977; Hadley, 1994). The contribution of each of these pathways to overall water-loss is variable but cuticular water loss (CWL) generally accounts for a high proportion of the total water loss (Gibbs and Johnson, 2004; Hadley, 1994). On the other hand, the contribution of respiratory water loss (RWL) to dehydration has been analyzed mostly on insects showing discontinuous gas exchange (DGE) (e.g., Chown and Davis, 2003; Lighton, 1992; Quinlan and Lighton, 1999). There are two techniques that enable to distinguish between cuticular and respiratory water loss in insects with continuous gas exchange: the regression method (Gibbs and Johnson, 2004) and the hyperoxic switch method (Lighton et al., 2004). Using these techniques it was observed that spiracular control under continuous gas exchange can modulate RWL as effectively as DGE (Schilman et al. 2005; Gray and Chown, 2008).

Haematophagous insects that do not drink free water show big changes in water balance under different levels of starvation (Benoit and Denlinger, 2010). Immediately after feeding they have to release large amounts of water and then, depending on the species, they can spend days, months or even years without feeding (Wigglesworth, 1972). At that time there are huge pressures to keep water. The haematophagous bug *Rhodnius prolixus* Stål 1859 (Hemiptera: Reduviidae) is an important vector of Chagas disease in northern South America and Central America and remains a classical model in insect physiology. It is distributed over Venezuela and Colombia, where it mainly inhabits wild environments such as palm trees, while in Central America (especially in Guatemala, Honduras and El Salvador) it has adapted to domestic environments (Schofield, 1994). Abiotic factors such as temperature and water availability are important for the distribution and abundance of insect species, which frequently show adaptations to their environments (Chown and Nicolson, 2004). *R. prolixus* are mostly associated to xeric regions such as dry savannah areas, making them an interesting model to assess the control of water loss.

On *R. prolixus* males metabolic rate (MR) was studied in relation to the gas exchange pattern and the effect of temperature (Contreras and Bradley, 2009; Contreras and Bradley, 2010). Although it is considered that the pattern of DGE could represent an advantage for hygric efficiency (White et al. 2007; Terblanche et al. 2010), all of the previous work focuses on patterns of gas exchange and MR and there is little information regarding water loss through the cuticle and spiracles in *R. prolixus.* The aim of the present study is to investigate any modulation on water loss rate and MR under different requirements for water and nutrients conservation. To do this, we simultaneously measured CO_2 production, water vapor emission and activity on individual insects in real time by open-flow respirometry at different feedings states and temperatures. In addition, we estimated the surface area of

the insects, discerned between cuticular and respiratory water loss by the regression method and calculated cuticular permeability.

2. RESULTS

Metabolic rates

A typical run is shown in Fig. 1. Gas exchange patterns changed from continuous to cyclic in presence and absence of activity, and varied also across treatments as reflected on the variability coefficient (VC) of CO_2 emission rate (VCO_2) (Table 1). Repeated measures ANOVA of ln-transformed VC revealed that temperature had a significant effect on the variability of metabolic rate (MR) within each recording ($F_{2,32}$=53.7; P<0.0001). The interaction between factors was not significant ($F_{6;96}$=1.67; P=0.14). Insects measured at 35°C showed smaller VC, indicating a higher degree of continuity of gas exchange, whereas the variability coefficient for insects measured at 15°C and 25°C was statistically homogeneous. Throughout the days after feeding VC remained constant as the proportion of runs where cyclic gas exchange was observed (Table 1). The probability of cyclic gas exchange presence was consistent with the VC results (Fig. A1). We also calculated Pearson´s coefficients of correlation between the VC of VCO_2 and total WLR (Fig. 2). Because insects were measured repeatedly over the days, and in order to maintain independence between samples, for this analysis we used the mean variables of each insect tested. Pearson product-moment correlation indicated a significant negative association between VC of VCO_2 trace and total water loss at 25°C (r =-0.69; P=0.013), marginally at 35°C (r =-0.58; P=0.05), while at 15°C there was no association between these variables (r =-0.46; P =0.15)(Fig. 2).

Body mass did not differ across temperatures ($F_{2,32}$=0.17; P=0.89, repeated measures ANOVA; Table 1). Overall, MR (µl h^{-1}) increased with temperature and decreased with nutritional state (Fig. 3) and the interaction of these factors was significant ($F_{6,96}$=4.81; P=2x10^{-4}). MR remained constant during the first two nutritional intervals tested, with a tendency of decreasing between 5 and 29 days after feeding. At 15°C a significant decrease was registered on the 29th day after feeding, while at 35°C was on the 13th day after feeding, and then remained constant through the 29th day. For measurements made at 25°C there were no significant differences between the rates of 0 and 5 days after feeding, as well as 0 and 13 days after feeding, however the MR on the 29th day differed significantly from all the starvation levels assessed (Fig. 3, Table 1).

As a way to account for Specific Dynamic Action (SDA) we calculated the quotient between MR measured on the hours following feeding and 29 days after feeding. The increase in MR as a consequence of feeding was 1.96-fold at 15ºC, 2.26-fold at 25ºC and 1.86-fold at 35ºC. The effect of temperature was not significant ($F_{2, 32}$=2.41; P=0.11, one way ANOVA). Overall the mean increase in VCO_2 was 2.03-fold. The four nutritional states did not differ on temperature sensitivity of the

metabolic rate, or slope (ANCOVA of log-transformed metabolic rate vs. temperature ($F_{3,132}$=2.51; P=0.06). The lines possess a common slope of 0.033 ± 0.001, which corresponds to a Q_{10} of $10^{((10)(0.033))}$ or 2.13 (Lighton, 2008). Intercepts did differ significantly ($F_{1,135}$=604.72; P<1x10^{-5}).

As well as MR, activity values (expressed as the absolute difference sum [ADS] of activity signal measured in volts; for detailed explanation of activity measurement see sections 4.2 and 4.6) showed a significant interaction between temperature and starvation ($F_{6,96}$=3.55; P=3.2x10^{-3}). This relationship was mainly due to the increased movement of insects measured on the 29th post feeding day at 35°C (Table 1 and Fig. A2). Activity was lower on insects measured at 15°C and 25°C than at 35ºC, and it was not affected by the starvation level (Tukey *a posteriori* test; Fig. A2).

Water loss measurements

Repeated measures ANOVA of square root-transformed WLR revealed significant differences depending on the feeding state ($F_{1,32}$=40.39; P<1x10^{-4}) and temperature ($F_{2,32}$=74.24; P<1x10^{-4}). The interaction between both factors was not significant ($F_{6,96}$=1.55; P=0.23). WLR had a significant decrease between 0 and 5 days post-feeding, and continued to decrease less steeply as the starvation increased (Fig. 4). We used the regression method in order to assess the cuticular and respiratory components of WLR (Gibbs and Johnson, 2004). All regressions showed significant positives slopes (Table A1). Four regressions were excluded from analysis because their R^2 were lower than 0.1, the remaining R^2 varied between 0.21 and 0.96 (Table A1). A similar change to WLR was observed on the CWL rates profile, with a significant positive effect of temperature ($F_{2,28}$=38.84; P<1x10^{-4}) and a negative effect of the feeding state ($F_{3,84}$=23.89; P<1x10^{-4}). There was a significant decline of water loss through the cuticle between the hours following feeding (and diuresis) and the 5th post feeding day, without further changes throughout the next days (Table 2). On the other hand, for RWL, there was a significant interaction between temperature and post feeding day ($F_{6,86}$=3.38; P=0.005; Table 2); RWL reached its lowest value on days 13th and 29th for insects measured at 35°C and 25°C respectively, while it remained constant throughout the days for insects measured at 15°C.

Using equation 4 which estimates wingless surface area, together with WLR and CWL rates we calculated gross cuticular permeability (GCP) and cuticular permeability (CP) respectively. There was a negative effect of temperature [GCP: ($F_{2,32}$=5.72; P=7.5x10^{-3}); CP: ($F_{2,28}$=14.43; P< 1x10^{-4})] and nutritional level [GCP: ($F_{3,96}$=25.27; P<1x10^{-4}); CP: ($F_{3,84}$=13.83; P<1x10^{-4})] on GCP and CP. The interaction between both factors was not significant on any of the variables tested. Estimates of CP of insects measured at 35°C were significantly lower than insects at 15 and 25°C and GCP values were significantly lower at 35°C than at 15°C. The effect of nutritional level followed the same profile as CWLR, for GCP and CP (Table 2).

3. DISCUSSION

Metabolic rate

Although the present study was not specifically designed to test the SDA response, *i.e.*, the metabolic response that accompanies meal ingestion, digestion, absorption and assimilation (Secor, 2009), when we focus on the effect of digestion on the MR of *R. prolixus* we registered a postprandial metabolic scope of 2. Previous works on 5th instar nymphs of *R. prolixus* showed metabolic scopes of almost 8 and 14 (Bradley et al., 2003; Heinrich and Bradley, 2014). These higher metabolic scopes previously found could be explained by the larger amount of blood ingested by nymphs compared to adult males, together with the effects of other physiological processes such as development and molting, which occur following feeding. In addition, we chose to work only with males in order to remove the effect of oogenesis on the metabolic rate (Davey, 1993). MR did not decrease immediately, but remained high, decreasing between the 5th and the 13th day after feeding. On crickets, between the first days of food deprivation, carbohydrate reserves were consumed and then on, metabolism was powered by lipids (Sinclair et al., 2011). It must be noted that we did not take into account the possibility of a change in fuel occurring as starvation increased, since undigested blood is stored in the anterior midgut and is transported into the posterior midgut for digestion and absorption as energy is required. The metabolic demands of the insects tested are mainly movement and digestion. The higher metabolic rates between days 0 to 5 post-feeding are not explained by different activity levels, since the activity ADS levels did not vary throughout the days (except for the high values registered at 35°C on the 29th day, see figure A2). In *R. prolixus*, the time for consumption of a blood meal has been estimated to be *ca.* 26 days on insects reared at 24°C and *ca.* 13 days on insects reared at 32°C (Schilman & Lazzari, 2004). The significant drop in the VCO_2 observed between days 5 to 13 after feeding due to cessation of the SDA effect could be used as an indicator of nutritional status, showing the transition from fed to fasted insect.

In addition to the increase of metabolic rate with digestion, there was an increase with temperature. The sensitivity of the metabolic rate to temperature Q_{10} was about 2 (Table 1), similar to most of the tracheate arthropods studied so far. This value is slightly lower than 2.48, the Q_{10} found in the giant red velvet mite (Lighton and Duncan, 1995), which is used by Bradley et al. (2003) for temperature corrections of the metabolic rate in *R. prolixus*. Temperature not only affects the rate of CO_2 production, but also the pattern of release (Basson and Terblanche, 2011). We found, consistent with Contreras and Bradley (2010), that higher temperatures (significant differences at 35°C) and activity negatively affect the occurrence of cyclic patterns. On the latter research, VCO_2 was measured on 1 week fasted *R. prolixus* males at same temperatures we used. They observed DGE at 15°C, cyclic pattern at 25°C and continuous at 35°C. Instead we registered gas exchange patterns that ranged from cyclic to continuous. We did not observe DGE *sensu stricto* in any insect maybe due to the low flow rate used (Gray and Bradley, 2006). However, Terblanche and Chown (2010) showed that flow rates are only likely to be a problem at extremely low ones in very small insects (*i.e.*, at the lower operational limit of the gas analyzer). On the other hand, the relation between nutritional state and gas

exchange pattern was unclear, suggesting a doubtful relation between water needs and cyclicity. A similar lack of association between water needs and cyclic gas exchange was recently found in the Table Mountain cockroach, *Aptera fusca* (Groenewald et al. 2013).

Water loss rates and cuticular permeability

As well as it was observed for metabolic rate, WLR was lower at higher levels of starvation. Using the regression method (Gibbs and Johnson, 2004), we were able to analyze the respiratory and cuticular contribution of total water loss. The general decrease in the rate of water loss as blood reserves diminished can be explained by a decline in the cuticular water loss during the first 5 days after feeding and by a drop in the respiratory component thereafter (Fig 4, Table 2).

After carefully measuring every part of the body of *R. prolixus*, we estimated the surface area based on body mass and length of the 3rd tibia. Since all vectors of Chagas disease have similar body shape, this model will be useful for any of the triatomine species. In addition, because they are hemimetabolous insects, nymphs and adults are also similar in body shape therefore the model could be applied to all species and larval stages. Based on WLR and estimates of the surface area, we calculated cuticular permeability, which was between 1.76 and 4.11 $\mu g\ h^{-1}cm^{-2}torr^{-1}$ depending on the starvation level and temperature, in agreement with values of cuticular permeability of fed nymphs of the 5^{th} instar of *R. prolixus.* The latter was measured as the percentage of weight lost in 24 hours at 30°C (Wigglesworth 1945). Consequently we modified Wigglesworth's data to compare with our results; cuticular permeability was 1.68 $\mu g\ h^{-1}cm^{-2}torr^{-1}$. This result is very similar to our lowest value measured, although a different developmental stage was used and the surface area was estimated using Meeh's formulae (our surface area measurements were between 26 and 51% (average 37%) higher than Meeh´s formulae estimates). Moreover WLR was measured using a gravimetric technique that has been shown to yield lower values than water loss measurements made with open flow respirometry (Schilman et al., 2007). Compared to other species, cuticular permeability of *R. prolixus* was low, which is a characteristic for arthropods adapted to xeric environments (see Table 6 from Edney, 1977 and Table 3.1 from Hadley, 1994). However, it was higher than the lowest cuticular permeability measured so far in the tenebrionid beetle, *Onymacris plana* from the Namib Desert (0.75 $\mu g\ h^{-1}cm^{-2}torr^{-1}$; Nicolson et al., 1984) and the lowest measured by open flow respirometry in another tenebrionid beetle, *Eleodes obscura* with 0.9 $\mu g\ h^{-1}cm^{-2}torr^{-1}$ (Schilman et al., 2008).

The steep and significant decrease in the cuticular water loss and cuticular permeability observed between the hours following feeding and the 5^{th} day was probably due to a change in the surface area exposed due to unfolding of inter-segmental membrane and the separation of the wings from the abdomen as a consequence of engorgement. The latter is not taken into account on the estimates of cuticular permeability, though the unfolding of the inter-segmental membrane is accounted for. However, each local region of the cuticle has different levels of sclerotization, with different cuticular permeabilities existing between them (Andersen, 2010). The inter-segmental membrane is less

sclerotizated with a higher permeability per unit of surface area than the rest of body parts (Andersen, 2010). However we cannot discard the existence of another factor down-regulating cuticular permeability as time after feeding increases. In another blood feeding arthropod, the lone star tick, higher cuticular permeability was observed in feeding ticks, and after host drop-off, a decrease in water loss together with a 3-fold boost in the surface wax deposition was measured (Yoder et al., 1997). This might favor water loss through the cuticle as an inexpensive way to concentrate blood. Our insect model has a different life cycle and feeding behavior, nonetheless the hypothesis that modulation of cuticular water loss rates and cuticular permeability after feeding might occur by deposition of extra surface wax or change of the hydrocarbon chain composition on the days following feeding remains to be tested. A change in cuticular composition is likely since in a closely related triatomine, *Triatoma infestans*, a 3-fold increase of epicuticular lipids was measured between young and old adult males (Juárez et al., 1984).

An effect on cuticular permeability with the feeding state was also observed in another haematophagous arthropod, the rabbit-tick *Haemaphysalis leporispalustris*, where engorged nymphs show a decreasing cuticular permeability with increasing starvation during the first two weeks after feeding (Davis, 1974). At high temperatures, changes on cuticular permeability have been observed as a result of melting of cuticular waxes, being over 50ºC for *R. prolixus* nymphs (Wigglesworth, 1945). We therefore expected cuticular permeability to remain constant in the range of temperatures tested, *i.e.*, between 15 and 35°C. We found however a significant lower cuticular permeability of *R. prolixus* at 35°C compared to 25 and 15°C. All assays at different temperatures were performed in dried moving air, so even though the water loss rate was significantly larger at higher temperatures, the correction for the water vapor saturation deficit results in a lower CP at 35°C. This unexpected result could not be explained as a result of dehydration because assays were short-term recordings (about 30-35 minutes). It could neither be explained by better control of the spiracles (Schimpf et al., 2009; Wigglesworth, 1972) because we discerned between respiratory and cuticular water loss and the lower CP at 35°C is maintained even after estimated corrected CP, *i.e.*, calculated only from CWL. On the other hand and because of the short duration of the recordings, a possible explanation to the lower CP at higher temperature could be the high initial rate of water loss ascribable in part to the moisture adsorbed by the highly hygroscopic surface of the cuticle that last the entire recording at lower temperatures (15 and 25°C in our case). A faster rate of water loss during the first part of the recordings than the last part of it was observed in many insects including: beetles (Schilman et al., 2008), locust (Loveridge, 1968), *Drosophila* (Lighton and Schilman, 2007; Schilman et al., 2011), cockroach (Gray and Chown, 2008) and ants (Lighton et al., 2004; Schilman et al., 2005). A similar abnormal relationship between saturation deficit and rate of water loss was found in locusts at 30°C, where the curvilinear relationship fall away from expected values at high saturation deficits (Loveridge, 1968). This resulted in a saving between 1.5 and 2.5 mg of water per locust per hour at 25 % R.H. and between 2.7 and 4.0 at 0% R.H. The anomalous relationship between saturation deficit

and rate of water loss could be explained by shrinkage of the cuticle because of a quick initial water loss from the cuticle and the concomitant decrease in intermicellar pore dimension, reducing the water diffusion rate. No matter which is the mechanism underlying our observations, it does result in substantial reduction in transpiratory water loss through the cuticle at high saturation deficits, and may be of considerable significance, for conserving water reserves at times when reduction in water loss is important.

Respiratory water loss

The contribution of respiratory water loss to the significant drop of total water loss was apparent only after the 5thday after feeding. This phenomenon is related to the significant decrease of CO_2 emission rate, simultaneously measured to WLR, from day 13th after feeding. RWL rates varied with temperature and starvation consistent with VCO_2. Only considering desiccation tolerance, a drop of 0.1 mg h^{-1} in RWL (e.g., difference between 0 and 13 days post-feeding at 35°C; Table 2) in an insect of 50 mg (Table 1) with 35% of body mass as critical water content (Schilman et al., 2007) represent about two more weeks of survival. The significant decrease of metabolic and RWL rates with increasing starvation would work as an evolutionary strategy to survive in a changing environment with the uncertainty of finding a suitable host by saving both, nutrients and water.

When we expressed the relative magnitudes of the different routes of water loss as percentage of total water loss, RWL values were between 10 and 35%, depending on temperature and feeding state. These values are relatively high compared with values from literature mainly as a consequence of a highly water proofed cuticle, as first stated by Zachariassen (1991) for a desert tenebrionid beetle and later discussed by Chown (2002). Regarding the respiratory patterns, if we relate them to the water loss through the spiracles, we observe that a lower contribution from the respiratory route occurs on insects expressing cyclic gas exchange (mainly those insects measured at 15°C and 25°C). A higher contribution of the respiratory water loss pathway occurs on insects measured at 35°C, which express continuous gas exchange. There is a positive relation between the RWL rates and the MR in *R. prolixus* males (note that this is required in order to apply the regression method). A similar positive relation was previously found in five ant species analyzed (Schilman et al., 2005) as well as in species from two families of beetles; this correlation was stronger in species from dry than mesic environments (Zachariassen et al., 1987). Moreover, Woods and Smith (2010) proposed a universal model that predicts WLR scales to gas exchange with an exponent of 1 based on results of 202 different species including 30 species of insects. The increase in RWL with increasing MR supports the hypothesis that species adapted to xeric environments have a lower standard metabolic rate compared to species adapted to mesic ones (e.g. the harvester ant *P. rugosus*; Lighton and Bartholomew, 1988). It also indirectly supports the idea of RWL reduction in species with DGE, although not necessarily as a consequence of the pattern itself, but as previously observed on *R. prolixus*, the change in respiratory pattern are given by a variations of MR (Contreras and Bradley,

2010). Higher temperatures increase metabolic rates and spiracles remain open during longer periods resulting in a continuous pattern. On a mechanistic hypothesis, the DGE pattern could be explained by a reduction in brain activity for energy saving and delegating the opening control of spiracles to thoracic and abdominal ganglia (Matthews and White, 2011).

We think that RWL in insects has been underestimated for being a small component of total water loss, but it is very important for a small insect trying to survive in arid environments. Thus, more comparative studies focusing on the importance of RWL (e.g., Chown and Davis, 2003) should be encouraged in order to appreciate the real importance and processes of selection to reduce the spiracular component of water loss rate in insects. Specially on small ectotherms, such as insects, whose metabolic and water loss rates are more susceptible to increasing temperature and declining rainfall, as predicted in many regions because of global warming (Chown, 2011; Chown et al., 2011).

4. MATERIALS AND METHODS

4.1. Animals

Twenty to thirty days post ecdysis adult males of *R. prolixus* were used throughout the study. The insects were reared in the laboratory at 28°C and 12:12 light-dark (L/D) cycle (light on 08:00am) and they were fed weekly on live hens. Respirometric measurements were performed at fixed intervals during a total period of 29 days and between measurements, the insects were kept at rearing temperature and L/D conditions.

4.2. Respirometry

We used flow-through respirometry to measure real time water vapor emission and CO_2 production in unrestrained adult males of the haematophagous bugs, *R. prolixus*. For all measurements we used the high-resolution TR-2 Sable System International (SSI; Las Vegas, Nevada, USA) flow-through respirometry system (Lighton et al., 2004; Schilman et al., 2005). Briefly, air free of CO_2 and H_2O was drawn at a flow rate of *ca.* 55 ml min^{-1} by a SS4 sub-sampler (SSI), which unites a pump, needle valve and a linearized mass flow meter, through low-permeability, Bev-A-Line tubing (to minimize errors associated with CO_2 and water vapor absorbance) and a RC-M precision miniature respirometer chamber (volume *ca.* 13 ml; SSI). Time response was less than 15 seconds. The water vapor and CO_2 produced by the haematophagous bugs were measured by a SSI RH-300 water vapor analyzer (set to measure water vapor density in a range of 0 to 10 µg ml^{-1} and 0.0001 µg ml^{-1} of resolution) and a Li-Cor (LI-6251) CO_2 infrared analyzer (Lincoln, NE, USA; resolution 0.1 ppm CO_2), respectively. Specimen temperatures were controlled to 15, 25 or 35°C by a SSI's Pelt-5 temperature controller and SSI's PTC-1 Peltier Effect cabinet. In order to equilibrate the temperature of the respirometer chamber with that inside cabinet, the air flow passed through a copper coiled tube (*ca.* 6.5 meters long) placed inside the cabinet. In addition, the activity of the insects were simultaneously monitored and recorded

by an AD-2 activity detector (SSI) and the temperature measured by a thermocouple attached to a SSI TC-2000 thermocouple meter (accuracy 0.2 and resolution 0.01°C). The analog outputs from the analyzers measuring CO_2, water vapor, inset´s activity, temperature of the chamber and air flow rate were connected to a A/D converter (SSI UI-2, 16 bit basic accuracy = 0.05%) and stored in a computer by ExpeData data acquisition software (SSI).

Previous to the measurements, both CO_2 and water vapor analyzers were calibrated. The CO_2 analyzer was zeroed with nitrogen and spanned at 97 ± 5 ppm with a certified span gas (Grupo Linde Gas S.A., Buenos Aires, Argentina). The water vapor analyzer was zeroed with nitrogen and spanned by bubbling air through pure water at an accurately known temperature (measured by a thermocouple attached to a TC-2000) *ca.* 5°C lower than room temperature. The RH-300 was set to its dew point mode and adjusted to read the correct water temperature, *i.e.*, temperatures reading from the TC-2000 and RH-300 matched.

4.3 Measuring respiratory water loss: Water loss regression method

We analyzed the data with the regression technique developed by Gibbs and Johnson (2004). This method is useful because it allows the estimation of RWL in insects performing continuous gas exchange. Briefly, we plotted WLR against CO_2 release for each individual insect using all values over 10 min of the last part of respiratory recording. Extrapolation to the intercept provides an estimate of corrected cuticular water loss, *i.e.*, without the spiracular component. The slope of each regression line estimates the hygric cost of gas exchange for that recording, *i.e.*, the incremental increase in water loss associated with CO_2 release. RWL is calculated with the equation:

$$RWL = RS\ [CO_2] \quad (1)$$

Where RWL is estimated by the regression method (Gibbs and Johnson, 2004), RS is the slope of the regression expressed in mg H_2O h^{-1}/µl CO_2 h^{-1}, and CO_2 is the VCO_2 in µl CO_2 h^{-1}. For a detailed explanation of the method, see Gibbs and Johnson (2004).

4.4 Experimental Procedure

We identified the insects by painting a color code on the legs with acrylic paint and weighed them individually to the nearest 0.1 mg using an analytical balance (Mettler AJ100, OH, USA). Insects were placed in a communal jar for feeding and 4 h later they were weighed again. It is known that during the first 3 h after feeding, *R. prolixus* eliminate most of the excess water from the blood meal (Maddrell, 1964). Therefore, in our results we excluded differences in weight loss due to different rates of removal of redundant water immediately after feeding. We discarded insects that did not feed because we wanted to analyze the effect of starvation on the metabolic rate and water loss rate. Each insect was randomly assigned to a temperature treatment (15, 25 and 35°C) and respirometric and mass measurements were performed at different times after feeding (0, 5, 13 and 29 days).

Simultaneously twelve fed insects of the same batch were used for surface area estimation. Surface area was calculated for each post-feeding interval tested at respirometric assays (see 4.5 for description of the method).

Each assay began with a 3 to 5 minutes of baseline recording, which was paused before placing the insect inside the chamber. After 10 minutes allowing the system to stabilize, recording was resumed and it lasted approximately 25 minutes, then the recording was paused again, the insect was removed from the chamber and final baseline was recorded.

4.5 Cuticular permeability and surface area estimation

To obtain cuticular permeability we measured water loss rate and estimated the surface area and its variation as a function of feeding state: 0, 5, 13 and 29 days after feeding. Twelve specimens were photographed (Nikon S6300) on dorsal, lateral and ventral view, weighed to the nearest 0.1 mg and the abdomen maximum thickness measured with a digital caliper. Data set includes only those individuals for which all variables could be measured in all four times.

Figure 5 shows a scheme of the geometric shapes we used to calculate the surface area of insects. The head was approximated to the surface area of a cylinder minus surface area of the ellipses and adding the corresponding ellipsoid surface areas of the eyes. The surface area of antennae and rostrum were calculated as the sum of two cylinders. Each leg was constructed as the sum of three cylinders while the thorax was taken as the sum of a trapezium (anterior region) and rectangle (posterior region).The abdomen surface area was built as the area of an ellipse (dorsal) and ellipsoid (lateral). Finally, the left wing was digitally photographed and its surface area calculated with morphometric software, TPSdig (version 1.39). We also used this software to obtain other magnitudes, such as length and width of thorax and abdomen on dorsal and ventral views respectively. After 29th day after feeding specimens were killed and the rest of the measurements were performed using a Leica MZ8 stereomicroscope (Wetzlar, Hesse, Germany) with a graduate ocular. The total (TSA) and wingless (WSA) surface area was calculated as the sum of the individual surface areas described above (for median and confidence intervals of calculated surfaces see table A2).

$$TSA = A_{head} + A_{rostrum} + 2[A_{antenna}] + 2[A_{Leg\,I}] + 2[A_{Leg\,II}] + 2[A_{Leg\,III}] + 4[A_{Tegmen}] + 4[A_{Wing}] + A_{dorsal\,thorax} + 2[A_{lateral\,thorax}] + A_{ventral\,thorax} + A_{dorsal\,abdomen} + A_{Ventral\,abdomen} \quad (2)$$

$$WSA = A_{head} + A_{rostrum} + 2[A_{antenna}] + 2[A_{Leg\,I}] + 2[A_{Leg\,II}] + 2[A_{Leg\,III}] + A_{dorsal\,thorax} + 2[A_{lateral\,thorax}] + A_{ventral\,thorax} + A_{dorsal\,abdomen} + A_{Ventral\,abdomen} \quad (3)$$

Surface area decreased with starvation mainly by changes in abdomen surface area, with a fixed factor related with insect size (Fig. A3).

We applied mixed-effects regression model for longitudinal data (Fig. A4). Two models were performed to estimate insect surface area: model I for wingless body surface area (eq. 4) and model II for total body surface area (eq. 5) (including both side surface-area wings).

The intercept and slope population parameters represent the overall (population) trend, while the individual parameters express how subjects deviate from the population trend (Hedeker and Gibbons, 2006).We used the population parameters to predict surface-area of insects, because is the average across the individuals. To control for inter-individual variability in the model we included the length of the tibia 3 (Ti 3) for each individual. Insect squared mass was found to be a good predictor of time after feeding (correlation index: -0.84).

Selection of the model

Model selection was made using Akaike's information criterion and the Bayesian Information Criterion. With these criteria, the model with lower value has a better fit (Singer and Willett, 2003). Nested model comparisons were performed by maximum likelihood (Singer and Willett, 2003). In both cases, the incorporation of squared mass, the Ti 3 variables and the random effect markedly reduced fitting indicators. The final model was fitted by restricted maximum likelihood.

Estimated parameters were significantly different from zero (P<0.001), nonetheless the parameter associated with the Ti 3 only for model I is marginally significant (P=0.068). We used *"nlme"* package (Pinheiro et al., 2013) for R *Core Team* (R Development Core Team, 2013). As a result of this analysis we obtained the formulae used to estimate the body surface:

$$Model\ I: WSA = 146.36 + 3{,}01x10^{-3}\ [Mass^2] + 10.50\ [Ti\ 3] \quad (4)$$

$$Model\ II: TSA = 237.88\ + 2{,}94x10^{-3}\ [Mass^2] + 32.08\ [Ti\ 3] \quad (5)$$

Where body mass (Mass) is expressed in mg; length of Ti 3 is expressed in mm, and WSA and TSA are expressed in mm^2.

4.6 Analyses and statistics

Respirometry data were stored in a laptop computer and analyzed by ExpeData data acquisition and analysis software (SSI). The following corrections and conversions were made from the recordings: (1) CO_2 and H_2O baselines were subtracted assuming a linear drift, (2) CO_2 in ppm was converted to µl h^{-1} (for formulae see Lighton, 2008), (3) H_2O vapor density in µg ml^{-1} was converted to WLR in mg h^{-1} (by multiplying by flow rate in ml h^{-1}). (4) The CO_2 and water vapor signals were lag corrected because they were slightly out of phase due to the experimental arrangement, *i.e.*, analyzers were arranged in series thus the air coming out of the respirometry chamber arrives first to the H_2O and then to the CO_2 analyzer. (5) The activity signal (in volts) was copied again, into another empty channel,

and its absolute difference sum (ADS) was calculated. The ADS is the cumulative sum of the absolute difference between all of adjacent data points. The ADS was originally used as a means of translating bi-directional position measurements into an accumulated displacement vector (Lighton et al. 1993a), but has proved to be of broader utility as a measure of the short-term dynamic variability of data (e.g., Lighton and Turner, 2004).

After corrections and conversions were made, the following values were measured and analyzed from the recording: (1) mean values of CO_2 and H_2O from last twenty minutes of the recording, (2) range (difference between maximum and minimum values of the activity ADS from same last twenty minutes of recording). We saved these values in a spreadsheet for further data manipulations. The spreadsheet also included the water vapor saturation deficit from chamber temperature (formulae in Lighton and Feener, 1989), the insect surface area and, hence, gross CP (*i.e.*, combined respiratory and cuticular water loss), the CP and the respiratory component of WLR calculated by the regression method (Gibbs and Johnson, 2004).

Means are accompanied by standard error and sample sizes. The effect of temperature and feeding state on the measured variables was tested using repeated measures ANOVA. When required the variables were transformed to meet the model´s assumptions. Furthermore when deviations from sphericity existed the degrees of freedom were adjusted with the Lower bound epsilon or we used a generalized least squares approach. Regressions are by least squares, with axis transformations where noted, and are tested for statistical significance by analysis of variance. Regressions are compared by analysis of covariance (ANCOVA).

There are some approaches to establish an objective criterion to classify the continuous or discontinuous pattern of gas exchange (e.g., Marais et al. 2005; Shelton and Appel 2000; Lighton et al. 1993b). Here we categorized VCO_2 patterns using the method described by Marais et al. (2005). Briefly, the percentage of points above the middle line of each trace is computed. Traces with less than 30% of the points above the middle line are considered cyclic. To analyze the effect of temperature and movement on respiratory pattern, we constructed a logistic generalized mixed model defining temperature as a factor and activity as a continuous explicatory variable (null model: AIC 195.97, with the chosen model AIC: 110.54).

At the same time we calculated the variation coefficient (VC) to quantify the “degree of discontinuity” of CO_2 liberation on each recording (Lighton et al., 1993b; Shelton and Appel, 2000).

$$VC = \frac{s.d}{m} \quad (6)$$

Where *s.d*. corresponds to the standard deviation and *m* the mean of each recording. A smaller VC portrays a continuous and more homogeneous pattern, where spiracles remain open and gas exchange is relatively constant. We tested the occurrence of cyclic gas exchange using temperature and

nutritional state together with ADS as a proxy of activity fitting a generalized linear mixed model (GLMM) with binomial distribution.

All data was analyzed using Infostat Statistical software (Di Rienzo et al., 2011) and R version 3.0.1 (R Development Core Team, 2013).

5. ACKNOWLEDGMENTS

The authors thank Maia Orsi and Hector Schoo for critical reading and corrections of an early version of the manuscript and Laboratory of Insect Physiology (DBBE) for sharing the rearing of insects and discussions. We thank many anonymous referees and John Terblanche whose constructive criticisms improved the paper. We also thank the editor Julian Dow for his patience and for choosing excellent reviewers. PES dedicates this paper to Prof. Josué A. Núñez.

COMPETING INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: CR, PES. Respirometry experimental assay: CR. Morphometric assay and statistical analysis: MI, CR. Contributed reagents/materials/analysis tools: PES. Jointly wrote the paper: All authors participated in the critical revision of the manuscript and gave final approval of the article.

FUNDING

This work was supported in part by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) /Argentina (grant numbers PICT2008-0268 and PICT2008-0035). C.R. was also supported by a Ph.D. fellowship from Consejo Nacional de Investigación Científica y Tecnológica (CONICET)/Argentina and P.E.S. by a researcher position at CONICET and Universidad de Buenos Aires (UBA)/Argentina.

6. BIBLIOGRAPHY

Andersen, S. O. (2010). Insect cuticular sclerotization: a review. *Insect Biochem. Mol. Biol.* **40**, 166-178.

Basson, C. H. and Terblanche, J. S. (2011). Respiratory pattern transitions in three species of *Glossina* (Diptera, Glossinidae). *J. Insect Physiol.* **57**, 433-443.

Benoit, J. B. and Denlinger, D. L. (2010). Meeting the challenges of on-host and off-host water balance in blood-feeding arthropods. *J. Insect Physiol.* **56**, 1366-1376.

Bradley, T. J., Brethorst, L., Robinson, S. and Hetz, S. (2003). Changes in the rate of CO_2 release following feeding in the insect *Rhodnius prolixus*. *Physiol. Biochem. Zool.* **76**, 302-309.

Chown, S. L. (2002). Respiratory water loss in insects. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **133**, 791-804.

Chown, S. L. (2011). Discontinuous gas exchange: new perspectives on evolutionary origins and ecological implications. *Funct. Ecol.* **25**, 1163-1168.

Chown, S. L. and Davis, A. L. V (2003). Discontinuous gas exchange and the significance of respiratory water loss in scarabaeine beetles. *J. Exp. Biol.* **206**, 3547-3556.

Chown, S. L. and Nicolson, S. W. (2004). *Insect Physiological Ecology: Mechanisms and Patterns.* Oxford: Oxford University Press.

Chown, S. L., Sørensen, J. G. and Terblanche, J. S. (2011). Water loss in insects: an environmental change perspective. *J. Insect Physiol.* **57**, 1070-1084.

Contreras, H. L. and Bradley, T. J. (2009). Metabolic rate controls respiratory pattern in insects. *J. Exp. Biol.* **212**, 424-428.

Contreras, H. L. and Bradley, T. J. (2010). Transitions in insect respiratory patterns are controlled by changes in metabolic rate. *J. Insect Physiol.* **56**, 522-528.

Davey, K. G. (1993). Oxygen consumption and egg production in the blood feeding insect *Rhodnius prolixus*. *Invertebr. Reprod. Dev.* **23**, 45-49.

Davis, M. T. B. (1974). Changes in critical temperature during nymphal and adult development in the rabbit tick, *Haemaphysalis leporispalustris* (Acari: Ixodides: Ixodidae). *J. Exp. Biol.* **60,** 85-94.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M. and Robledo, C. W. (2011). InfoStat versión 2012. *Grup. InfoStat, FCA, Univ. Nac. Córdoba, Argentina. URL http//www. infostat. com. ar*.

Edney, E. B. (1977). *Water balance in land arthropods.* Springer-Verlag.

Gibbs, A. G. and Johnson, R. A. (2004). The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. *J. Exp. Biol.* **207**, 3477-82.

Gray, E. M. and Bradley, T. J. (2006). Evidence from mosquitoes suggests that cyclic gas exchange and discontinuous gas exchange are two manifestations of a single respiratory pattern. *J. Exp. Biol.* **209**, 1603-1611.

Gray, E. M. and Chown, S. L. (2008). Bias, precision and accuracy in the estimation of cuticular and respiratory water loss: A case of study from a highly variable cockroach, *Perisphaeria* sp. *J. Insect Physiol.* **54**, 169-179.

Groenewald, B., Bazelet, C.S., Potter, C.P., and Terblanche, J.S. (2013) Gas exchange patterns and water loss rates in the Table Mountain cockroach, *Aptera fusca* (Blattodea: Blaberidae) *J. Exp. Biol.* **216**, 3844-3853

Hadley, N. F. (1994). *Water relations of terrestrial arthropods*. Academic Press.

Hedeker, D. and Gibbons, R. D. (2006). *Longitudinal Data Analysis*. John Wiley & Sons.

Heinrich, E. and Bradley, T.J. (2014). Temperature-dependent variation in gas exchange patterns and spiracular control in Rhodnius prolixus. *J. Exp. Biol.* **217**, 2752-2760.

Juárez, M. P., Brenner, R. R. and Gros, E. G. (1984). The epicuticular lipids of *Triatoma infestans*—I. Glycerides. *Comp. Biochem. Physiol. Part B Comp. Biochem.* **78**, 427-431.

Lighton, J. R. B. (1992). Direct measurement of mass loss during discontinuous ventilation in two species of ants. *J. Exp. Biol.* **293**, 289-293.

Lighton, J. R. B. (2008). *Measuring Metabolic Rates: A Manual for Scientists*. Oxford: Oxford University Press.

Lighton, J. R. B. and Bartholomew, G. (1988). Standard energy metabolism of a desert harvester ant, *Pogonomyrmex rugosus*: Effects of temperature, body mass, group size, and humidity. *Proc. Natl. Acad. Sci. USA* **85**, 4765-4769.

Lighton, J. R. B. and Duncan, F. D. (1995). Standard and exercise metabolism and the dynamics of gas exchange in the giant red velvet mite, *Dinothrombium magnificum*. *J. Insect Physiol.* **41**, 877-884.

Lighton, J. R. B. and Feener, D. H. J. (1989). Water-loss rate and cuticular permeability in foragers of the desert ant *Pogonomyrmex rugosus. Physiol. Zool.* **62**, 1232-1256.

Lighton, J. R. B. and Schilman, P. E. (2007). Oxygen reperfusion damage in an insect. *PLoS One* **2**, e1267.

Lighton, J. R. B. and Turner, R. J. (2004). Thermolimit respirometry: an objective assessment of critical thermal maxima in two sympatric desert harvester ants, *Pogonomyrmex rugosus* and *P. californicus*. *J. Exp. Biol.* **207**, 1903-1913.

Lighton, J. R. B., Weier, J. A. and Feener, D. H. J. (1993a). The energetics of locomotion and load carriage in the desert harvester ant *Pogonomyrmex rugosus*. *J. Exp. Biol.* **61**, 49-61.

Lighton, J. R. B., Fielden, L. J. and Rechav, Y. (1993b). Discontinuous ventilation in a non-insect, the tick *Amblyomma marmoreum* (Acari, Ixodidae): characterization and metabolic modulation. *J. Exp. Biol.* **245**, 229-245.

Lighton, J. R. B., Schilman, P. E. and Holway, D. A (2004). The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. *J. Exp. Biol.* **207**, 4463-4471.

Loveridge, J. (1968). The Control of Water Loss in Locusta *Migratoria Migratorioides* R. & F I. Cuticular Water Loss. *J. Exp. Biol.* **49**, 1-13.

Maddrell, S. (1964). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stal. *J. Exp. Biol.* **41**, 163-176.

Marais, E., Klok, C. J., Terblanche, J. S. and Chown, S. L. (2005). Insect gas exchange patterns: a phylogenetic perspective. *J. Exp. Biol.* **208**, 4495-507.

Matthews, P. G. D. and White C. R. (2011). Discontinuous gas exchange in insects: is it all in their heads? *Am. Nat.* **177**, 130-134.

Nicolson, B. Y. S. W., Louw, G. N. and Edney, E. B. (1984). Use of a ventilated capsule and tritiated water to measure evaporative water losses in a tenebrionid beetle. *J. Exp. Biol.* **108**, 477-481.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Core Team (2013). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-113.

Quinlan, M. C. and Lighton, J. R. B. (1999). Respiratory physiology and water relations of three species of *Pogonomyrmex harvester* ants (Hymenoptera: Formicidae). *Physiol. Entomol.* **24,** 293-302.

Schilman, P. E. and Lazzari, C. R. (2004). Temperature preference in *Rhodnius prolixus*, effects and possible consequences. *Acta Trop.* **90**, 115-122.

Schilman, P. E., Lighton, J. R. B. and Holway, D. A. (2005). Respiratory and cuticular water loss in insects with continuous gas exchange: comparison across five ant species. *J. Insect Physiol.* **51**, 1295-1305.

Schilman, P. E., Lighton, J. R. B. and Holway, D. A. (2007). Water balance in the Argentine ant (*Linepithema humile*) compared with five common native ant species from southern California. *Physiol. Entomol.* **32**, 1-7.

Schilman, P. E., Kaiser, A. and Lighton, J. R. B. (2008). Breathe softly, beetle: Continuous gas exchange, water loss and the role of the subelytral space in the tenebrionid beetle, *Eleodes obscura*. *J. Insect Physiol.* **54**, 192-203.

Schilman, P. E., Waters, J. S., Harrison, J. F. and Lighton, J. R. B. (2011). Effects of temperature on responses to anoxia and oxygen reperfusion in *Drosophila melanogaster*. *J. Exp. Biol.* **214**, 1271-1275.

Schimpf, N. G., Matthews, P. G. D., Wilson, R. S. and White, C. R. (2009). Cockroaches breathe discontinuously to reduce respiratory water loss. *J. Exp. Biol.* **212,** 2773-2780.

Schofield, C. J. (1994). Triatominae (Biología y Control). *Eurocommunica Publ. Reino Unido* **51**, 313-319.

Secor, S. (2009). Specific dynamic action: a review of the postprandial metabolic response. *J Comp Physiol B.* **179,** 1-56.

Shelton, T. G. and Appel, A. G. (2000). Cyclic carbon dioxide release in the dampwood termite, *Zootermopsis nevadensis* (Hagen). *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **126**, 539-545.

Sinclair, B. J., Bretman, A., Tregenza, T., Tomkins, J. L. and Hosken, D. J. (2011). Metabolic rate does not decrease with starvation in *Gryllus bimaculatus* when changing fuel use is taken into account. *Physiol. Entomol.* **36**, 84-89.

Singer, J. D. and Willett, J. B. (2003). *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. Oxford University Press.

Terblanche, J. S. and Chown, S. L. (2010). Effects of flow rate and temperature on cyclic gas exchange in tsetse flies (Diptera, Glossinidae). *J. Insect Physiol.* **56**, 513-521.

Terblanche, J. S., Clusella-Trullas, S. and Chown, S. L. (2010). Phenotypic plasticity of gas exchange pattern and water loss in *Scarabaeus spretus* (Coleoptera: Scarabaeidae): deconstructing the basis for metabolic rate variation. *J. Exp. Biol.* **213,** 2940-2949.

White, C. R., Blackburn, T. M., Terblanche, J. S., Marais, E., Gibernau, M. and Chown, S. L. (2007). Evolutionary responses of discontinuous gas exchange in insects. *Proc. Natl. Acad. Sci. USA* **104**, 8357-8361.

Wigglesworth, V. B. (1945). Transpiration through the cuticle of insects. *J. Exp. Biol.* **21,** 97-114.

Wigglesworth, V. B. (1972). *The Principles of Insect Physiology*. Halsted Press, New York.

Woods, H. A. and Smith, J. N. (2010). Universal model for water costs of gas exchange by animals and plants. *Proc. Natl. Acad. Sci. USA* 107, 8469-8474.

Yoder, J., Selim, M. and Needham, G. (1997). Impact of feeding, molting and relative humidity on cuticular wax deposition and water loss in the lone star tick, *Amblyomma americanum*. *J. Insect Physiol.* **43**, 547-551.

Zachariassen, K. E. (1991). Routes of transpiratory water loss in a dryhabitat tenebrionid beetle. *J. Exp. Biol.* **437**, 425-437.

Zachariassen, K. E., Andersen, J., Maloiy, G. M. and Kamau, J. M. (1987). Transpiratory water loss and metabolism of beetles from arid areas in East Africa. *Comp. Biochem. Physiol. Part A Physiol.* **86**, 403-408.

FIGURE CAPTIONS

Figure 1. Typical recording of *R. prolixus.*

The extreme left and right portions of the recording correspond to baseline values, which represent measurements made on an empty chamber. Insect (mass=117.7 mg) at 25°C on 5th day post-feeding. Traces represent CO_2 production (black trace) and H_2O release (red trace), together with the activity, measured in arbitrary units (green trace).

Figure 2. Correlation between water loss rate and variability of emission VCO_2.

Variability of CO_2 trace was calculated as the s.d. mean ratio. Each point is an average of four measures of the same insect.

Figure 3. Effects of temperature and feeding state on metabolic rate.

Measurements throughout the days for the same temperature were performed on same individuals. Different letters show significant differences of MR between post feeding days for each treatment (Tukey test; $P<0.01$).

Figure 4. Effects of temperature and feeding state on water loss rate.

Water loss rate (mg h^{-1}) for different temperature treatments at 0, 5, 13 and 29 post-feeding days. Different letters show significant differences between WLR for the three treatments ($P<0.01$).

Figure 5. Scheme of geometric figures used to estimate body surface area.

Rhodnius prolixus on dorsal (left) and lateral (right) view.

664 TABLES

665 **Table 1**. **Summary means (SE) of body mass, activity and metabolic rates** (CO_2 production). Data
666 is reported as mean and SE. N = sample size. Same letters represent statistically homogeneous values
667 between temperature treatments.

	Day 0 post feeding			Day 5 post feeding			Day 13 post feeding			Day 29 post feeding		
Temp (°C)	15	25	35	15	25	35	15	25	35	15	25	35
N	11	12	12	11	12	12	11	12	12	11	12	12
Mass (mg)	111.3 (4.2)	114.8 (4.7)	116.1 (4.1)	89.2 (3.8)	92.6 (4.3)	91.9 (3.9)	74.7 (4.0)	74.0 (3.3)	75.5 (4.2)	51.6 (3.4)	52.3 (3.1)	50.8 (2.9)
Activity (arbitrary units)	23.23 (6.07)	53.44 (20.97)	83.99 (16.64)	20.80 (5.07)	50.19 (12.47)	48.62 (10.65)	31.35 (8.88)	70.91 (15.90)	58.40 (19.73)	13.06 (1.17)	31.86 (8.32)	160.76 (28.15)
VCO_2 ($\mu l\ h^{-1}$)	9.08 (0.73)	19.74 (2.27)	45.08 (2.90)	10.98 (0.98)	24.17 (1.32)	45.61 (2.65)	7.64 (0.70)	15.76 (1.16)	26.84 (1.77)	4.93 (0.56)	8.84 (0.81)	26.27 (1.97)
VC	0.70^{A} (0.07)	0.60^{A} (0.05)	0.21^{B} (0.01)	0.67^{A} (0.04)	0.59^{A} (0.05)	0.32^{B} (0.03)	0.75^{A} (0.06)	0.63^{A} (0.07)	0.34^{B} (0.03)	0.75^{A} (0.09)	0.77^{A} (0.12)	0.28^{B} (0.02)
Q10	2.14			2.12			1.89			2.10		

668

669 VC is the variability coefficient of CO_2 emission rate (for a detailed explanation see materials and
670 methods).

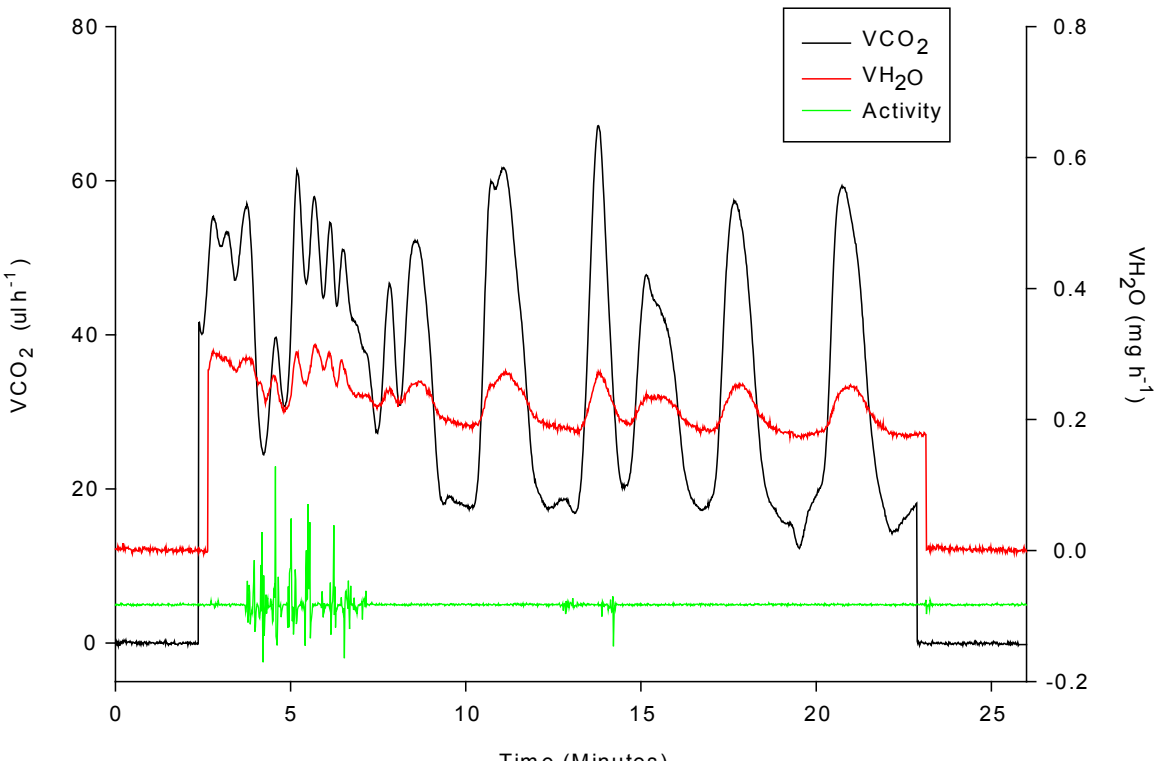
671

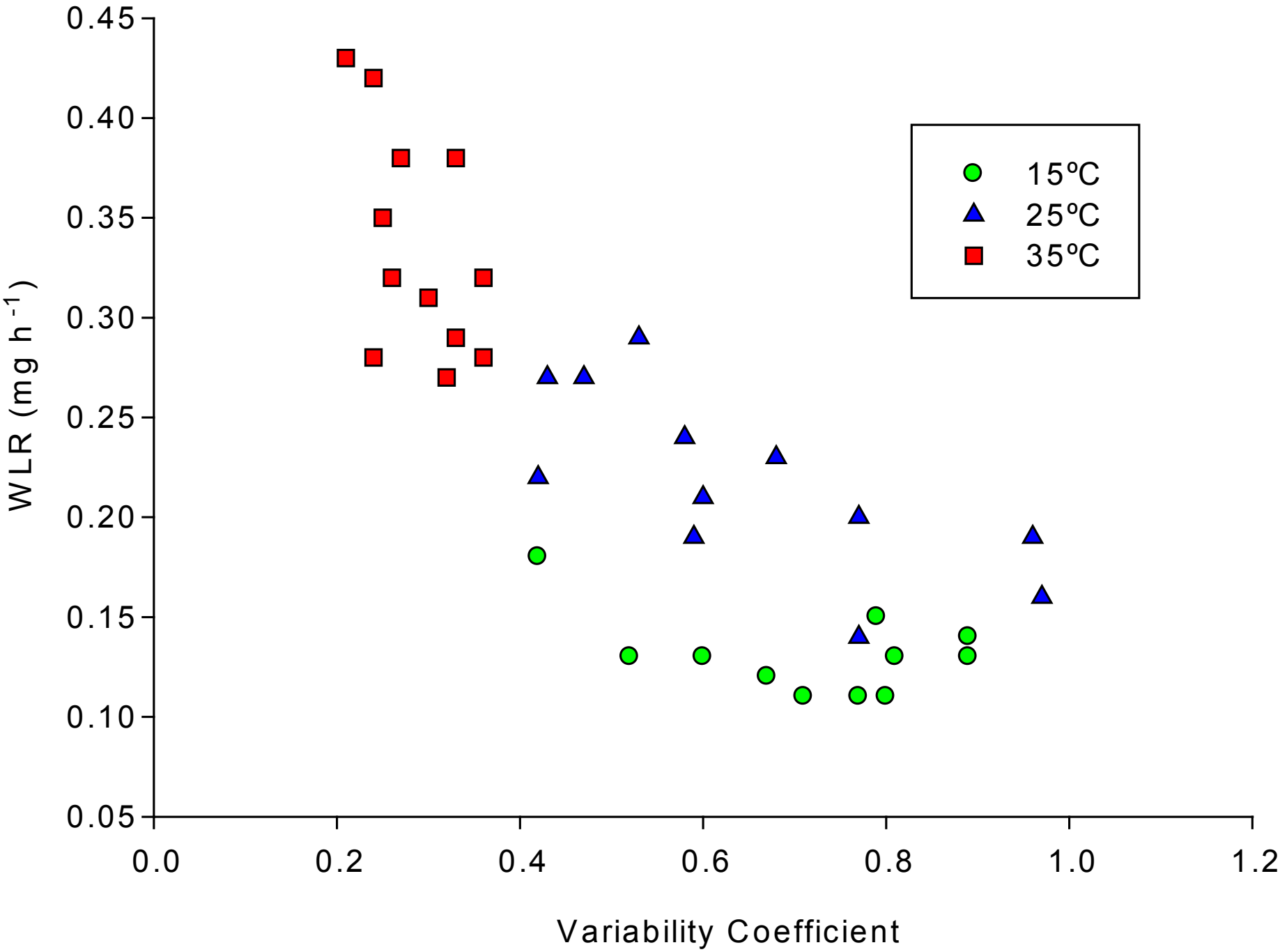
672

673 **Table 2. Summary means (SE) of water loss rates:** total (WLR), Cuticular (CWLR) and respiratory
674 (RWLR), together with estimates of gross cuticular permeability (GCP) and corrected cuticular
675 permeability (CP) for different feeding states and temperatures. Same letters indicate statistically
676 homogeneous groups, upper case letters represent comparisons between temperatures and lower case
677 letters represent comparisons between post-feeding days. WLR, CWLR and RWLR were analyzed by
678 repeated measures ANOVA followed by a *posteriori* Tukey tests. GCP and CP were analyzed by GLS
679 followed by a posteriori LSD Fisher test.

	Day 0 post feeding			Day 5 post feeding			Day 13 post feeding			Day 29 post feeding		
Temp (°C)	15	25	35	15	25	35	15	25	35	15	25	35
N	11	12	12	11	12	12	11	12	12	11	12	12
WLR (mg h^{-1})	$0.170^{A,a}$ (0.013)	$0.300^{B,a}$ (0.024)	$0.461^{C,a}$ (0.025)	$0.130^{A,b}$ (0.011)	$0.211^{B,b}$ (0.021)	$0.341^{C,b}$ (0.024)	$0.120^{A,bc}$ (0.009)	$0.180^{B,bc}$ (0.018)	$0.282^{C,bc}$ (0.021)	$0.106^{A,c}$ (0.014)	$0.177^{B,c}$ (0.019)	$0.264^{C,c}$ (0.016)
CWLR (mg h^{-1})	$0.140^{A,a}$ (0.010)	$0.224^{B,a}$ (0.025)	$0.278^{C,a}$ (0.021)	$0.101^{A,b}$ (0.010)	$0.146^{B,b}$ (0.020)	$0.221^{C,b}$ (0.012)	$0.096^{A,b}$ (0.007)	$0.124^{B,b}$ (0.009)	$0.202^{C,b}$ (0.009)	$0.082^{A,b}$ (0.016)	$0.130^{B,b}$ (0.012)	$0.169^{C,b}$ (0.011)
RWLR (mg h^{-1})	0.020^{a} (0.002)	0.056^{a} (0.010)	0.167^{a} (0.022)	0.022^{a} (0.003)	0.053^{a} (0.008)	0.115^{ab} (0.019)	0.017^{a} (0.001)	0.039^{ab} (0.006)	0.073^{ab} (0.018)	0.017^{a} (0.005)	0.020^{b} (0.005)	0.088^{b} (0.013)
Surface area (cm^2)	2.57 (0.02)	2.59 (0.03)	2.61 (0.03)	2.44 (0.02)	2.45 (0.02)	2.46 (0.03)	2.36 (0.02)	2.36 (0.01)	2.38 (0.02)	2.28 (0.01)	2.28 (0.01)	2.28 (0.01)
Surface area-Meeh' (cm^2)*	1.87 (0.05)	1.91 (0.05)	1.93 (0.04)	1.61 (0.01)	1.65 (0.01)	1.65 (0.01)	1.43 (0.05)	1.42 (0.04)	1.44 (0.05)	1.12 (0.05)	1.13 (0.05)	1.11 (0.04)
GCP (µg h^{-1} cm^{-2} $torr^{-1}$)	$5.11^{A,a}$ (0.38)	$4.89^{AB,a}$ (0.37)	$4.19^{B,a}$ (0.22)	$4.14^{A,b}$ (0.34)	$3.65^{AB,b}$ (0.36)	$3.29^{B,b}$ (0.22)	$3.93^{A,b}$ (0.31)	$3.24^{AB,b}$ (0.32)	$2.81^{B,b}$ (0.20)	$3.59^{A,b}$ (0.45)	$3.29^{AB,b}$ (0.34)	$2.75^{B,b}$ (0.17)
CP (µg h^{-1} cm^{-2} $torr^{-1}$)	$4.11^{A,a}$ (0.38)	$3.64^{A,a}$ (0.37)	$2.51^{B,a}$ (0.17)	$3.20^{A,b}$ (0.33)	$2.53^{A,b}$ (0.35)	$2.13^{B,b}$ (0.10)	$3.12^{A,b}$ (0.28)	$2.24^{A,b}$ (0.31)	$2.01^{B,b}$ (0.08)	$2.76^{A,b}$ (0.53)	$2.43^{A,b}$ (0.21)	$1.76^{B,b}$ (0.11)

680 (*) Meeh´s formula ($S=k\ W^{0.667}$), k=8.1 (species-specific constant from Wigglesworth, 1945) and
681 W=mass in g.


VCO2
VH2O
Activity
80
60
40
20
0
0.8
0.6
0.4
0.2
0.0
-0.2
0
5
10
15
20
25
VCO2 (ul h-1)
VH2O (mg h-1)
Time (Minutes)


0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
WLR (mg h-1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Variability Coefficient
15ºC
25ºC
35ºC