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RESEARCH ARTICLE

Haematophagy is costly: respiratory patterns and metabolism during feeding in *Rhodnius prolixus*

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ABSTRACT

Feeding on the blood of vertebrates is a risky task for haematophagous insects and it can be reasonably assumed that it should also be costly in terms of energetic expenditure. Blood circulates inside vessels and it must be pumped through narrow tubular stylets to be ingested. We analysed the respiratory pattern and the energetic cost of taking a blood meal in *Rhodnius prolixus* using flow-through and stop-flow respirometry to measure carbon dioxide emission, oxygen consumption and water loss before and during feeding. We observed an increase of up to 17-fold in the metabolic rate during feeding and a change in the respiratory pattern, which switched from a discontinuous cyclic pattern during resting to a continuous pattern when the insects started to feed, remaining in this condition unchanged for several hours. The energetic cost of taking a meal was significantly higher when bugs fed on a living host, compared with feeding on an artificial feeder. No differences were observed between feeding on blood or on saline solution *in vitro*, revealing that the substrate for feeding (vessels versus membrane) and not the nature of the fluid was responsible for such a difference in the energetic cost. Water loss significantly increased during feeding, but did not vary with feeding method or type of food. The mean respiratory quotient in resting bugs was 0.83, decreasing during feeding to 0.52. These data constitute the first metabolic measures of an insect during blood feeding and provide the first insights into the energetic expenditure associated with haematophagy.

KEY WORDS: Respiration, Blood-feeding, Metabolic rate, Disease vectors, Chagas

INTRODUCTION

Vertebrate blood is the main or even the sole food for many arthropod species. Blood is rich in nutrients (proteins, lipids, carbohydrates, water, etc.) and, except for the potential presence of parasites, it is otherwise sterile.

Feeding on blood is, however, not an easy task, nor is it obtained without risk. This food is not freely available in nature; instead, it circulates inside vessels hidden below the skin surface of active animals, usually larger than the insect, that are able to defend themselves from bites.

Haematophagy has appeared independently several times in the evolutionary history of arthropods (Lehane, 2005), at least 20 times according to some authors (Mans et al., 2008). Under the influence of strong selective pressures, specific morphological, physiological and behavioural adaptations arose, which allowed these animals to successfully adopt this particular way of life.

Among haematophagous insects, two different feeding methods can be observed: telmophagy and solenophagy (Lavoipierre, 1965; Lehane, 2005). Telmophagous insects produce a wound with their cutting mouthpieces and quickly lick the blood flowing from the surface of the injury. In contrast, solenophagous insects possess tiny mouthparts that pierce the skin and enter the lumen of large capillaries, venules and arterioles. The latter method causes less damage and no pain in the host, allowing the insect to remain undetected. However, solenophagy requires sucking blood through a very narrow conduct (~10 µm diameter), increasing the time needed to obtain a full meal, particularly in relatively large insects, such as triatomine bugs. To solve the trade-off between injuring the host skin as little as possible and feeding as quickly as possible, solenophagous insects have developed powerful ingestion pumps derived from the cibarial and/or the pharyngeal pumps (Lehane, 2005). Thanks to these pumps, which are able to generate high differences in hydrostatic pressure (negative to suck blood and positive to push it into the digestive tract), feeding times remain on the order of a few minutes for most solenophagous insects.

The contraction activity of feeding pumps, as any other muscular activity, requires energy and can be assumed to be energetically costly (Guarneri et al., 2000). At present, however, no data are available concerning the energetic cost associated with obtaining a blood meal in any haematophagous insect.

One way of analysing the energetic cost of feeding is to compare the metabolism at resting with that during feeding activity. Different blood-sucking arthropod species have been analysed in terms of metabolic activity, such as fleas, bedbugs, ticks and mosquitoes (Lighton et al., 1993; Gray and Bradley, 2003, 2006; DeVries et al., 2013). However, the species that has been best characterized in terms of respiration dynamics and metabolism is the triatomine *Rhodnius prolixus* (Bradley et al., 2003; Contreras and Bradley, 2009, 2010; Rolandi et al., 2014; Heinrich and Bradley, 2014). Hence, this bug constitutes a good model system with which to evaluate the energetics of feeding in blood-sucking insects. In addition, this species is a classical model in insect physiology and a major vector of the causative agent of a major health problem in Central and South America, Chagas disease.

Very little knowledge exists on evolutionary ecology of triatomines (Menu et al., 2010). To fill this gap, we need quantitative estimates of the costs and benefits associated with different activities (e.g. feeding, locomotion, egg laying, etc.) and life history traits (tolerance to starvation, reproductive strategy, dispersal, etc.). As a first step in this direction, we analyse in this paper the cost associated with feeding in *R. prolixus*.

In the present study we employed two different respirometric procedures (flow-through and stop-flow) to characterize the different

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variables that allow the estimation of energetic parameters in *R. prolixus* during feeding under different conditions, *in vivo* (live host) and *in vitro* (artificial feeder), and provided different types of meals (blood or saline). The objective of our experimental study was to shed some light on the metabolism and energetic expenditure during feeding events in a haematophagous insect, as well as to provide the first quantitative data on different respirometric variables. Another aim of our work is to establish bridges connecting ecophysiology, evolutionary ecology and epidemiology of triatomines.

MATERIAL AND METHODS

Experimental animals and set-up

Fourth-instar nymphs of *Rhodnius prolixus* Stål 1859 from our laboratory colony were fed on heparinized sheep blood in an artificial feeder and used for experiments 15 to 20 days after their moult to the fifth instar. Groups of insects were maintained in 0.5 litre plastic jars at 25±1°C, 60±5% relative humidity and subjected to a 12 h:12 h light:dark cycle.

Thirty unfed fifth-instar nymphs were separately weighed to the nearest 0.1 mg and then individually placed in respirometric chambers. Chambers were made with 5 ml disposable syringes (Terumo) to measure the basal metabolic rate. Similar chambers, but having a 1 cm diameter hole sealed with latex, were used for simultaneous feeding and respirometric measurements (Fig. 1).

Feeding

Insects were allowed to feed on a living host (human finger) or on an artificial feeder. The meals offered in the feeder consisted of either heparinized sheep blood or saline solution (NaCl 0.15 mol l^{-1} containing ATP 10^{-3} mol l^{-1}). The food was deposited in a 3 ml plastic cylinder sealed with a latex membrane at the bottom and its temperature was kept at 37±1°C by means of a circulating-water heater. A small piece of filtre paper was provided to the insect inside the chamber as substrate. The finger or the feeder membrane was put in contact with the membrane of the respirometric chamber to allow the insects to feed. Insects were allowed to feed *ad libitum* and to a fully engorged state (i.e. getting a distended abdomen). Afterwards, individuals were weighed and their respirometric variables were measured.

Respiratory measurements

Metabolic measurements were carried out in a thermostatized room at a constant temperature of 25±1°C. Flow-through respirometry following the methods already validated by other authors (Bradley et al., 2003; Lighton, 2008; Contreras and Bradley, 2010) was used to measure the CO_2 production, O_2 consumption and water loss. Measures were performed during resting (standard metabolic rate) and during and after feeding using Sable Systems respirometric equipment. Expedata software controlled an eight-channel multiplexer switching a water (Drierite)- and CO_2 (Ascarite)-scrubbed airflow of 66 ml min^{-1} (Sierra mass flow controller). The airflow was passed by the multiplexer through one of the respirometric chambers, and was then conducted through Bev-A-Line tubing no longer than 30 cm (to reduce errors related to water vapour and CO_2 absorbance) to a water vapour analyser (RH-300, Sable Systems, Henderson, NV, USA), after which the water was scrubbed using magnesium perchlorate. Then, the current entered an infrared CO_2 analyser (CA-10, Sable Systems) followed by a dual fuel cell oxygen analyser (OXYLLA II, Sable Systems). An electronic interphase (UI-2, Sable Systems) gathered the data from the instruments and fed them to a computer through Expedata.

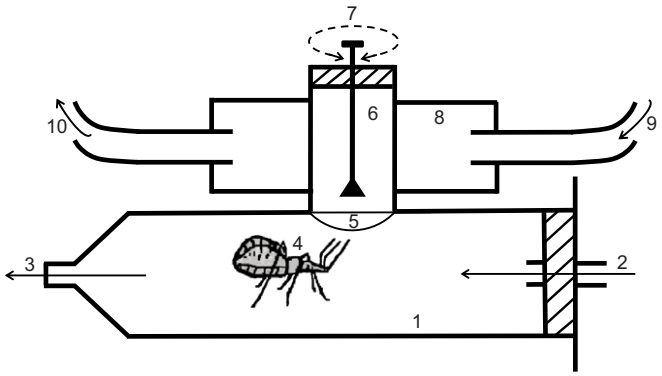

Fig.1. Respirometric chamber for measuring CO_2 release, O_2 consumption and water loss during feeding. 1, Plastic syringe; 2, incurrent air; 3, excurrent air; 4, fifth-instar *Rhodnius prolixus*; 5, latex membrane; 6, artificial feeder; 7, magnetic stirrer; 8, heating shirt; 9–10, incurrent–excurrent water flow at 37±0.5°C.

Baseline measurements using an empty chamber were made before and after each recording event to determine zero CO_2 and to correct for the instruments.

Respiratory quotients (RQ) were determined using the stop-flow method described by Lighton (2008). An empty chamber and an experimental chamber were washed for 5 min with CO_2 and water-scrubbed air (flow of 66 ml min^{-1}), which passed then through the same instruments, as previously described. In resting bugs, accumulated CO_2 emission and O_2 consumption were recorded for 20 min for both the empty and the experimental chamber. During feeding, the amount of gas accumulation in the experimental chambers depended on the feeding duration of each insect.

Metabolic calculations, data analysis and statistics

Data on CO_2 emission, O_2 consumption and H_2O loss rate measurements were analysed using Expedata. The initial 5 min of recording of the experimental chambers and the first 3 min of the empty chambers were excluded from the analysis to eliminate effects of the accumulated CO_2 derived from insect respiration and/or valve action. The respiratory pattern (V_{CO_2} min^{-1}), real-time O_2 consumption (V_{O_2} min^{-1}) and water loss (V_{H_2O} min^{-1}) were determined during resting, during feeding and after feeding up to defecation. We calculated the mean rate of V_{CO_2} emission, O_2 consumption and water loss using the methods and equations established by Lighton (2008). We calculated the mass-independent and mass-specific energy expended to ingest 1 mg of food as specific dynamic effect (SDE) after Sarfati and collaborators (2005). To determine an energy equivalent of metabolic rate measured as the rate of CO_2 produced, we also followed the methods used by Lighton (2008). Depending on the normality and homoscedasticity of the data, data were analysed using a paired Student's *t*-test to compare resting and feeding variables, and one-way ANOVA or Kruskal–Wallis to compare meals. Calculations of metabolic rate were based in each case on the corresponding RQ, i.e. either in resting unfed insects or during feeding.

RESULTS

Food ingestion

To assess the cost of feeding, first we had to characterize the dynamics associated with feeding on a natural host and on an artificial feeder, as well as on different diets.

Fig. 2 depicts the feeding parameters associated with each situation, as the mass increases, the feeding duration, the feeding rate and the normalized feeding rate. The insects increased their mass significantly

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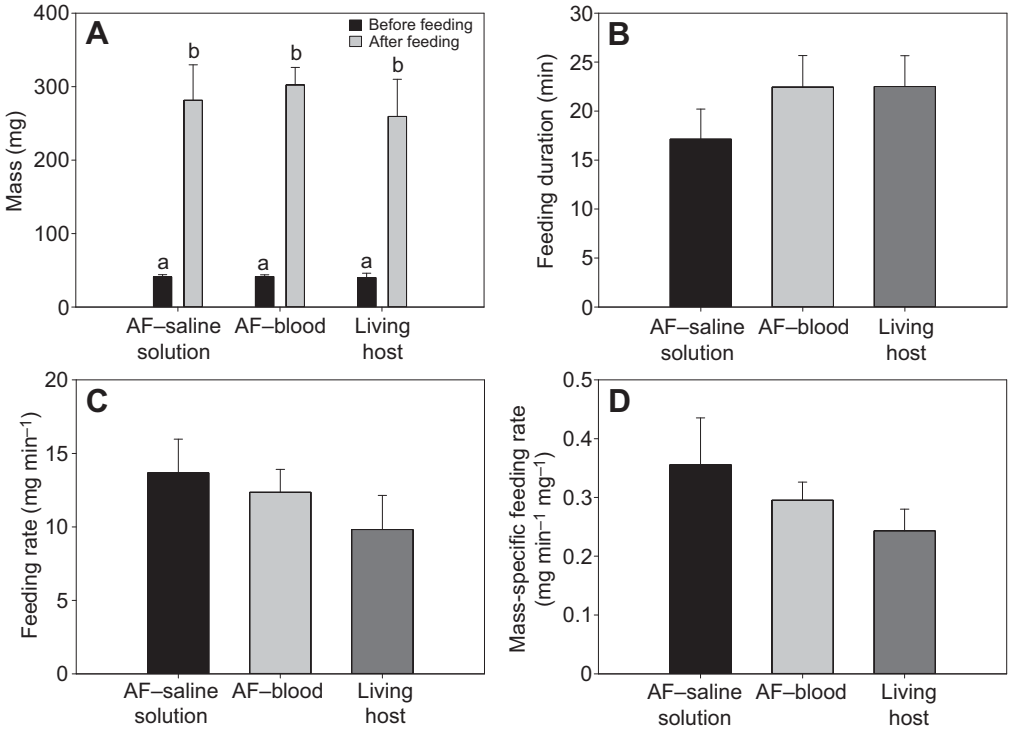

Fig. 2. Feeding parameters of *Rhodnius prolixus* fed on blood or saline solution, *in vivo* or *in vitro*. (A) Body mass during resting and after feeding, (B) feeding duration, (C) mass-independent feeding rate and (D) mass-dependent feeding rate of insects fed from an artificial feeder (AF) or a living host. Data are means±s.e.m. (n=6 for artificial feeder treatments and n=4 for living host). Different letters in A indicate a significant (P<0.05) difference between before and after feeding.

after feeding, regardless of feeding method, i.e. living host or artificial feeder (AF), or the type of meal offered (AF–saline solution: t_5=5.1925, P<0.005; AF–blood: t_5=11.379, P<0.001; living host: t_3=4.571, P<0.05; Fig. 2A). Even though saline solution was ingested in a shorter time and host feeding was slightly slower than feeding on an artificial device, no statistically significant differences were found among treatments for these variables (Fig. 2B–D).

Respiratory patterns and RQ

Prior to feeding, resting bugs exhibited a discontinuous gas-exchange cycle (DGC) of CO_2 emission (Fig. 3A, left panel), as previously described by Contreras and Bradley (2009). Once an insect began to feed, either on a live host or the artificial feeder, the pattern switched to continuous and CO_2 production notably increased (Fig. 3A, right panel). A similar increase and change in the dynamics were observed for O_2 consumption (Fig. 3B). In both cases, the respiratory pattern remained continuous for several hours after feeding, before switching back to DGC.

Water loss, in turn, did not exhibit any cyclic component (Fig. 3C), and was always continuous during resting and feeding, but showed a clear increase during the ingestion of a meal. As a consequence of diuresis, and given that drops of urine rapidly

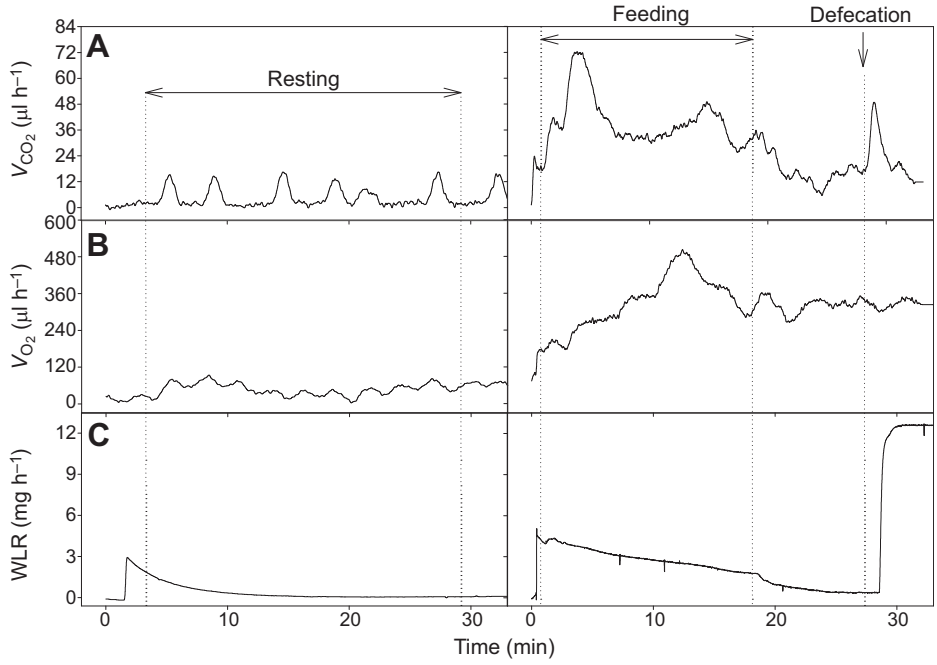

Fig. 3. Example of gas exchange and water loss for a single *R. prolixus* during resting (left) and during feeding (right). (A) Rate of CO_2 release (V_{CO_2}); (B) rate of O_2 consumption (V_{O_2}); and (C) water loss rate (WLR). During resting, a discontinuous gas-exchange cyclic pattern was observed, which changed to a continuous pattern during feeding. A high amount of water loss was observed due to defecation.

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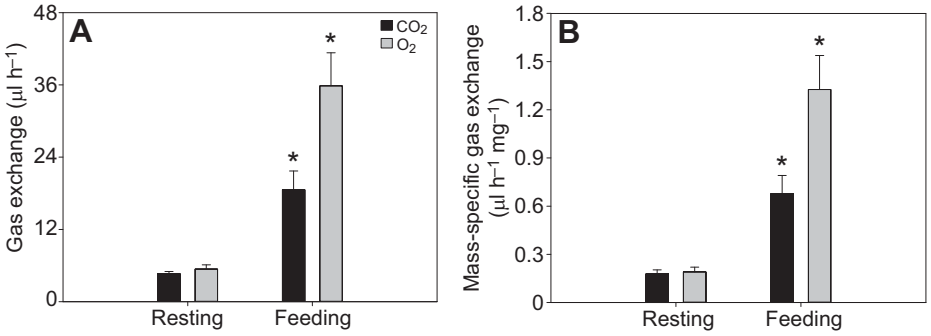

Fig. 4. Carbon dioxide emission and oxygen consumption of *R. prolixus* before and during feeding, as revealed by stop-flow respirometry. (A) Gas exchange before (resting) and during feeding; (B) mass-specific gas exchange before and during feeding. Note that the amount of O_2 consumed, as related to CO_2 emission, is different in resting from that during feeding. Data are means±s.e.m. (n=7). Asterisks indicate a significant (P<0.05) difference between CO_2 emission and O_2 consumption at resting and during feeding.

evaporate, a further increase in water loss was observed (Fig. 3C, right panel).

The measures of accumulated CO_2 emission and O_2 consumption in a closed chamber using stop-flow respirometry revealed an RQ of 0.83±0.07 (mean±s.e.m., n=7) in resting bugs. During feeding, the RQ measured under the same conditions (i.e. stop-flow) was 0.52±0.02 (mean±s.e.m., n=10). Both CO_2 production and O_2 consumption were significantly higher during feeding (V_{CO_2}: t_8=5.3143, P<0.001; V_{O_2}: t_6=4.8453, P<0.005; mass-specific V_{CO_2}: t_8=5.3010, P<0.001; mass-specific V_{O_2}: t_6=5.3093, P<0.005; Fig. 4). These values were used for further energetic calculations.

Metabolism during feeding

A summary of the measures of metabolic variables measured in this study is presented in Table 1.

The computation of energetic variables revealed that actual, as well as normalized, metabolic rates significantly increased during feeding as compared with resting conditions (metabolic rate: AF–saline: t_5=8.6804, P<0.0005; AF–blood: t_5=10.1156, P<0.0005; living host: t_3=5.9131, P<0.05; Fig. 5A; mass-specific metabolic rate: AF–saline: t_5=11.1077, P<0.0001; AF–blood: t_5=7.276, P<0.001; living host: t_3=9.5273, P<0.005; Fig. 5B). This increase reached 9.7-fold for saline solution and 13.2-fold for blood *in vitro*. Bugs that fed *in vivo* displayed an increase in energetic expenditure of approximately 16.8-fold, which was significantly higher than the energetic cost of *in vitro* feeding (metabolic rate: ANOVA, $F_{2,13}$=5.833, P<0.05; Fig. 5A; mass-specific metabolic rate: Kruskal–Wallis, H_2=7.4853, P<0.05; Fig. 5B). This was consistent for the SDE (mass-independent SDE: ANOVA, $F_{2,13}$=5.516, P<0.05; Fig. 5C; mass-specific SDE: Kruskal–Wallis, H_2=7.8713, P<0.005; Fig. 5D).

Water loss

The water loss rate (WLR) was significantly higher during feeding than during resting, even after excluding diuresis and defecation events (WLR: AF–saline: t_5=8.9662, P<0.0005; AF–blood: t_5=8.3138, P<0.0005; living host: t_3=3.9971, P<0.05; Fig. 6A; mass-specific WLR: AF–saline: t_5=6.4073, P<0.005; AF–blood: t_5=6.5471, P<0.005; living host: t_3=9.8802, P<0.005; Fig. 6B). All three types of feeding rendered increases in water loss (4.6-, 8.2- and 5.2-fold for saline solution, blood in artificial feeder and living host, respectively), but no statistical differences were found among them.

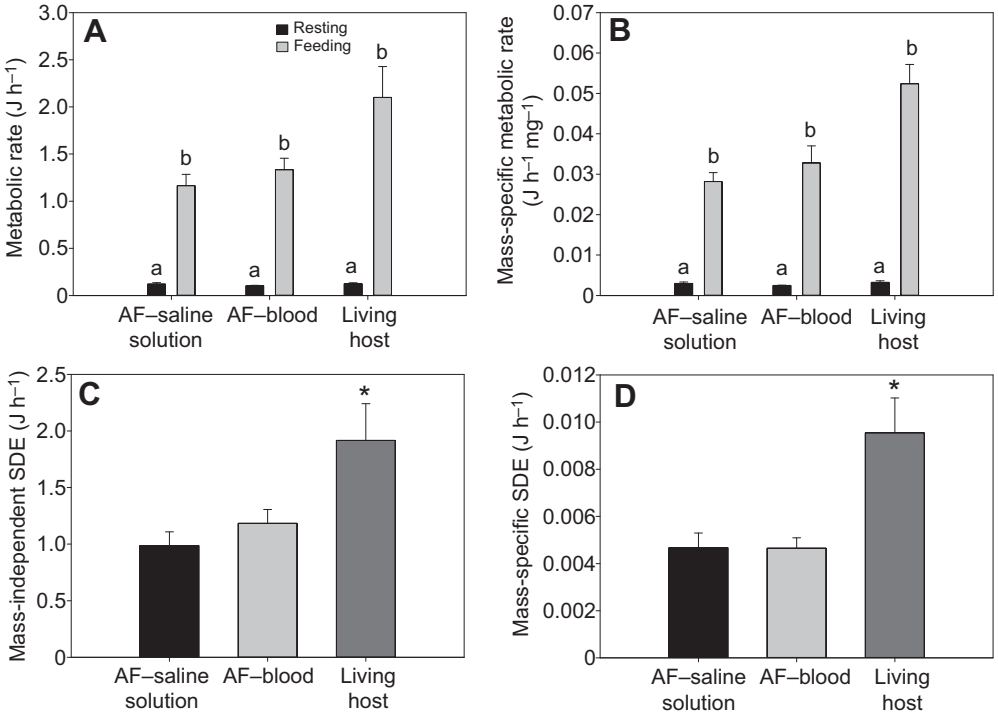

Fig. 5. Energetic parameters associated with feeding of *R. prolixus* on different foods, *in vivo* and *in vitro*. (A) Metabolic rate during resting and feeding; (B) mass-specific metabolic rate during resting and feeding; (C) mass-independent specific dynamic effect (SDE); and (D) mass-specific SDE of insects fed from an artificial feeder (AF) or a living host. Data are means±s.e.m. (n=6 for artificial feeder treatments and n=4 for living host). Different letters in A and B indicate a significant (P<0.05) difference between resting and feeding; asterisks in C and D indicate significantly higher (P<0.05) than the other groups.

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Table 1. Summary of the measured values corresponding to the different metabolic variables analysed in this study

Treatment	MR during resting (J h^{-1})	Mass-specific MR during resting (J h^{-1} mg^{-1})	MR during feeding (J h^{-1})	Mass-specific MR during feeding (J h^{-1} mg^{-1})	Mass-independent SDE (J h^{-1})	Mass-specific SDE (J h^{-1})	MR increase during feeding (fold change)	Mass-dependent MR increase during feeding (fold change)
AF-saline	0.12±0.016	0.00296±0.000375	1.164±0.121	0.0281±0.0022	0.988±0.121	0.00467±0.000623	9.7	9.5
AF-blood	0.103±0.004	0.00247±0.0000703	1.334±0.121	0.0328±0.00421	1.183±0.122	0.00465±0.000441	13	13.2
Living host	0.125±0.01	0.00323±0.000396	2.1±0.327	0.0524±0.00478	1.917±0.324	0.00954±0.001481	16.8	16.2

AF, artificial feeder; MR, metabolic rate; SDE, specific dynamic effect.
Data are means±s.e.

DISCUSSION

Artificial versus natural hosts

In agreement with previous studies on feeding dynamics in triatomines (e.g. Pereira et al., 2006), taking a meal from an artificial feeder seems easier in terms of energetic investment than taking it from a living host. Feeding *in vitro* only requires the insect to pierce a membrane and to gather a fluid (i.e. saline or blood) whose properties remain constant. Conversely, when biting a living host, insects have to find a vessel, neutralize the response of the skin and vessels to the mechanical damage, and deal with the local variations of blood flow (Soares et al., 2014).

Varying respiratory patterns according to behaviour

As previously described (Contreras and Bradley, 2009), *R. prolixus* displays different respiratory patterns according to their physiological condition. Three different patterns of gas exchange have been described in this species: continuous, DGC and cyclic. Two of them, DGC and cyclic, have been proposed in mosquitoes to be variants of the same dynamic, being the cyclic pattern of a manifestation of DGC (Gray and Bradley, 2006). In the present study, we used the same airflow (66 ml min^{-1}) and chamber size (4 ml) as that employed by Bradley et al. (2003), while Contreras and Bradley (2009) employed 200 ml min^{-1} and chambers of 2 ml. Even though we do not describe cyclic respiration in this species, as it was not evinced under the conditions of this particular study, we regularly observed it in adults and nymphs. Thus, despite the variation in measuring conditions, i.e. different airflows and chamber sizes, the three patterns clearly appear as manifestations of gas exchange in *R. prolixus*, suggesting that they are probably not just artefacts of measuring procedures.

Unfed resting bugs exhibited DGC and switched to continuous respiration during active feeding. This change was revealed in our experiments as a marked increase and dynamic change in the CO_2 emission and O_2 consumption (Fig. 3). Water loss was predominantly continuous during both phases of our experiments, i.e. before and during feeding, and a high increase in the rate of water loss was only observed after defecation, but without a change in the dynamics. Cyclic water loss, synchronized to CO_2 emission and O_2 consumption, could not be observed in resting unfed nymphs used in this experiment, although it was occasionally observed under other conditions, e.g. in resting adults. During feeding, the large increase in the exchange rate of CO_2, O_2 and water vapour may be related to the continuous opening of spiracles.

Unexpectedly low respiratory coefficient

Our calculation of the RQ from stop-flow measures of oxygen consumption and carbon dioxide emission rendered values around 0.83 for resting insects, which is close to the consensus value of 0.8 that has been assumed by several authors when only carbon dioxide was measured (Lighton et al., 1993; Sarfati et al., 2005; Lighton, 2008). This value is within the classical interval resulting from burning lipids, proteins and sugar. During feeding, however, we observed a marked increase of both oxygen consumption and carbon dioxide emission, but the ratio between the two is not that observed at resting (Fig. 4), resulting in a RQ of approximately 0.52. This result shows that the rate at which oxygen is consumed is higher than that at which carbon dioxide is produced.

Unusually low RQ values, i.e. below 0.7, have also been measured in birds, and the available evidence shows that they are not measurement artefacts (Walsberg and Wolf, 1995). Their physiological nature, however, remains unknown.

In the case of *R. prolixus*, different testable hypotheses could be proposed to explain this phenomenon. The remaining carbon dioxide could have dissolved into tissues or could have been used for other physiological processes associated with feeding. For instance, diuretic activity greatly increases during feeding, and the production of excretory products (i.e. uric acid) requires CO_2 for forming $KHCO_3$ (Wigglesworth, 1931). This retained carbon dioxide should be gradually eliminated once feeding has ended and this could explain the fact that the respirometric variables remained high and the respiratory pattern remained continuous for several hours after the ingestion of a blood meal (data not shown).

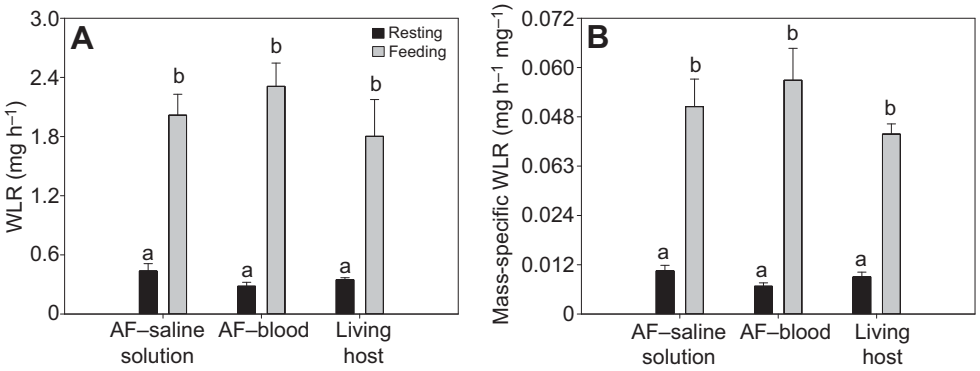

Fig. 6. Water loss associated with feeding in *R. prolixus*. (A) Water loss rate (WLR) during resting and feeding and (B) mass-specific WLR during resting and feeding of insects fed from an artificial feeder (AF) or a living host. Data are means±s.e.m. (n=6 for artificial feeder treatments and n=4 for living host). Different letters indicate a significant (P<0.05) difference between resting and feeding.

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Table 2. Comparative production of CO_2 (V_{CO_2}; µl h^{-1} mg^{-1}) as a function of nutritional state in haematophagous arthropods

Species	Unfed	During feeding	Post-feeding	References
Amblioma marmoreum	0.023–0.042	–	0.08–1.3	Lighton et al. (1993)
Parapulex chephrenis	0.17	–	0.2–1.34	Sarfati et al. (2005)
Cimex lectularius	0.127–0.218	–	–	DeVries et al. (2013)
Rhodnius prolixus	0.10–0.15*	0.78–1.45*	0.04–0.26‡	Contreras and Bradley (2010); Rolandi et al. (2014); present study

When not in the same units, data from other studies were transformed to allow proper comparisons. The only available values during feeding are those of *R. prolixus*, which were obtained in this study.
*Normalized by initial mass of unfed insects.
‡Normalized by total mass.

Alternately, the blood ingested could act as an oxygen sink, gathering it from respiratory activity to saturate haemoglobin. None of these alternatives appears as stronger than the other and further experiments are necessary.

As underlined by previous authors, errors that could result from these unexpected RQ values can be large and could present the primary limit to the accuracy of power consumption estimates based upon measurement of carbon dioxide production (Walsberg and Wolf, 1995).

Feeding is costly

When we compare the metabolism of bugs before and during feeding in terms of rates of variation in respiratory or energetic variables, we observe that feeding is a costly activity. The increase associated with taking a blood meal is up to three times over that of walking in the ant *Camponotus* sp. (Lipp et al., 2005) and even higher than the metabolic increase of flying over resting in terms of mass-specific rate of O_2 consumption for *Drosophila melanogaster* (Hocking, 1953; Lehmann et al., 2000; Niven and Scharlemann, 2005). It is, however, lower than the increase in oxygen consumption associated with flying in the bee *Apis mellifera* and the grasshopper *Schistocerca gregaria* (Niven and Scharlemann, 2005), and lower as well to the increase in metabolic rate during leaf-cutting in the ant *Atta sexdens rubropilosa* (Roces and Lighton, 1995).

The cost of feeding in blood is associated with the necessary muscular activity for feeding on blood. Solenophagous haematophagous insects such as mosquitoes and bugs ‘cannulate’ blood vessels with thin mouthparts that minimize the damage caused to the host’s tissues. So, the diameter of alimentary channels is just large enough to allow blood cells to circulate inside. In the case of *R. prolixus*, the apical diameter in the fifth-instar larva is approximately 8 µm and it has been estimated that a pump capable of developing between 2 and 9 atm of pressure is required for a fifth-instar larvae of *R. prolixus* to consume approximately 300 µl of blood in 15 min or less (Bennet-Clark, 1963; Smith, 1979). A conservative estimate of the necessary muscle tension to produce such pressure differences is at least 25 N cm^{-2} or 1 kg cm^{-2} (Smith, 1985). In line with this effort, our calculations show that all metabolic variables, normalized by the insect and food masses, increased several-fold during feeding, for the different feeding conditions tested.

It is worth noting that the quantitative analysis of different feeding parameters in several species of haematophagous insects has revealed that they usually feed easier on the host with which they are naturally associated than on other vertebrates (Guarneri et al., 2000; Sarfati et al., 2005). Hence, not only does haematophagous feeding have a cost, but it should also vary with alimentary eclecticism. Therefore, we hypothesize that the energetic cost of feeding will also vary from one host to another, even in insects that are essentially opportunistic in their haematophagous activity. It is worth noting that the energetic expenditure for digesting the blood (not considered here) should be added to the cost of obtaining a meal. The latter may also vary according to the host, as shown by Sarfati et al. (2005) in fleas that fed on different bat species.

The available information on *R. prolixus* and other haematophagous insects provides quantitative data on resting unfed and fed (digesting) individuals. We can then compare some energetic variables, in order to have an idea about the relative cost of haematophagy across species and under different conditions, i.e. resting, starved, feeding and digesting. Such a comparison is presented in Table 2. It can be verified that the normalized production of CO_2 is highly variable across species of blood-sucking arthropods, suggesting that their metabolic demands are not similar. This may be related to their mobility (e.g. ticks versus fleas), but our knowledge is too scarce to reasonably speculate. In particular, we need more data on metabolism during feeding. If our hypothesis proposing that the energetic cost of feeding depends on the host is correct, this cost of feeding might constitute an important selective force modelling the adaptation of blood-sucking arthropods to exploit particular vertebrate species as hosts. The comprehension of this relationship may not only shed light on the adaptation of arthropods to the haematophagous way of life, but also provide relevant information to epidemiologists about host preferences.

Our results are also of interest from the point of view of evolutionary ecology. Indeed, it is probable that the availability of preferred hosts (hosts with which bugs are naturally associated, i.e. birds in the case of *R. prolixus*) in the field is not constant in time and/or space. According to this assumption, when preferred hosts are temporally absent, bugs should choose to feed on other hosts (e.g. mammals) to which they are less adapted (Guarneri et al., 2000; Soares et al., 2014) and then pay a higher cost for feeding than they would when feeding on birds. This additional investment will probably have an impact on individual fitness in the future. To avoid this additional cost, bugs should wait for the arrival of an appropriate host, exploiting their tolerance to starvation (i.e. time escape), or disperse and look for the appropriate host elsewhere (i.e. spatial escape). The most adaptive response will depend on the relative cost of feeding on natural hosts or on an alternative host to which they are maladapted, the cost associated with dispersal and fasting, as well as the probability of finding a natural host after a given time or spatial dispersal. We propose then as relevant research avenues the comparison of the energetic costs associated with these different activities, and the investigation of the consequences, in terms of individual fitness, of such costs in order to unravel the most adequate evolutionary strategy that may be selected in different ecological contexts. The adaptive response selected could, theoretically, have strong epidemiological consequences if

it can impact the vectorial capacity of bugs, for example, affecting the defecation delay at each feeding event.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
M.L., M.H.P. and C.R.L. conceived and designed the experiments; M.L. and M.H.P. carried out the respirometry experiments; M.L. and C.R.L. performed statistical analyses; J.C. and C.R.L. contributed reagents/materials/analysis tools; and C.R.L., J.C. and F.M. provided the conceptual framework. All authors jointly wrote the paper.

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