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### Authors

Schilman, P E

Lazzari, C R

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# Temperature preference in Rhodnius prolixus, effects and possible consequences

By P. E. Schilman\* and C. R. Lazzari§

Laboratorio de Fisiología de Insectos, Dpto. de Biodiversidad y Biología Experimental,  
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

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(\*) Present address for correspondence:  
Section of Ecology, Behavior and Evolution,  
Division of Biological Sciences,  
University of California at San Diego,  
9500 Gilman Drive, La Jolla,  
CA-92093-0116, USA

E-mail: [pschilma@biomail.ucsd.edu](mailto:pschilma@biomail.ucsd.edu)  
FAX: (+1 858) 534-7108

(§) Present address:  
Institut de Recherche sur la Biologie de l’Insecte  
Faculté des Sciences et Techniques  
Université François Rabelais  
Avenue Monge – Parc Grandmont  
37200 Tours, France

*Running headline:* Thermopreference in R. prolixus

**ABSTRACT**

The present work examines the thermal preference of adult R. prolixus along a temperature gradient. The mean preferred temperature differed slightly between sexes: 25.0°C for males versus 25.4°C for females. This preference was not constant, but varied daily by about 0.2°C for both sexes, and reached its highest value at the onset of the dark phase and was lowest during the light phase. A change in the preferred temperature with the level of starvation was also observed (about 1°C lower after 20 days of starvation). Changes in environmental temperature strongly affected the rate of weight loss for both sexes. When insects were maintained for 20 days in a chamber at 32 °C, they lost significantly more weight than when kept at 24 °C; both water loss and nutrient conversion processes are involved. This increase in weight loss rate with increasing temperature would cause a higher biting rate and consequently higher probability of Chagas' disease transmission. Females oviposit across a range of temperatures from 22° to 33°C with a peak at 25°-26°C. These results are compared with patterns of thermopreference in other species of triatomine, as related to differences in their distribution and tolerance to starvation.

**KEY WORDS:** Rhodnius prolixus, thermopreference, daily cycle, starvation, oviposition, nutrient conversion, biting rate, Chagas' disease transmission.

## INTRODUCTION

It is often believed that the metabolism of endothermic animals decreases with increasing temperature, whereas the metabolism of ectothermic animals increases. This increase in metabolism exhibited by ectothermic animals with rising temperature can be mostly explained by an increase in activity (Wigglesworth, 1972). Several ectothermic animals adjust their body temperature and metabolism behaviourally (Remmert, 1985; Vielmetter, 1958; Wurtsbaugh & Neverman, 1988; van Dijk, et al., 2002). The effect of environmental temperature on insects seems to vary depending on different physiological processes. Therefore, the preference for certain environmental temperatures results from the interplay between many physiological parameters and adaptations to certain ecological conditions (Remmert, 1960).

Rhodnius prolixus is an important vector of Chagas disease in northern South America and remains a classical model in insect physiology. It is distributed over Venezuela, Colombia and Central America, especially Guatemala, Honduras and El Salvador. Whereas in Venezuela and Colombia R. prolixus inhabit mainly wild environments, such as palm trees, in Central America it is adapted to domestic environments (Schofield, 1994).

In a series of studies, Okasha showed the effects of high temperature on R. prolixus. He demonstrated that sub-lethal high temperature impaired protein synthesis and caused a cessation of moulting, cell division and secretion of the brain hormone

(Okasha, 1964, 1968a). Moreover, sub-lethal high temperatures resulted in a decreased metabolic rate (Okasha, 1968b). Temperature and relative humidity also affect egg viability in this species (Clark, 1935, Schilman, 1998). Despite the large amount of literature regarding the effects of temperature on physiological processes during different developmental stages of R. prolixus, it is intriguing that little information exists concerning the spontaneous choice of temperature. Thus in this work, we focus our analyses on the thermopreference of adult R. prolixus. In Triatoma infestans, a related species, Lazzari (1991) demonstrated a strong change in thermopreference caused by the level of starvation. As R. prolixus has a higher resistance to starvation than does T. infestans, we first addressed whether R. prolixus modifies its thermopreference as a function of starvation level and determined the degree to which temperature affects nutrition conversion and water loss. Since the fecundity of R. prolixus females is strongly affected by characteristics of the oviposition substrates (Schilman et al., 1996, Lazzari et al., 1999), we then asked whether R. prolixus females show an active thermopreference for oviposition or if they just oviposit at the preferred temperature for resting. The results are compared between sexes and with other species of triatomines, and they are discussed in relation to differential starvation resistance.

## **MATERIALS AND METHODS**

### *Animals and temperature gradient*

We reared adult R. prolixus in the laboratory at 28°C and fed them weekly on heparinised bovine blood using an artificial feeder (Núñez & Lazzari, 1990). A temperature gradient was generated in a glass arena of 40 x 22 x 3 cm, which was longitudinally split in two halves of 40 x 11 x 3 cm by a central division. The floor of the arena was covered with filter paper, which provided a more suitable surface for the insects and acted as an oviposition substrate. The arena also had a glass cover to prevent the insects from escaping and to maintain a stable temperature gradient without exchange with external air currents. The glass floor was attached by means of thermo-conductive silicone grease to an aluminium sheet. This sheet had an electric heating resistance at one end and a cold-water bath with a thermostat at the other end, in order to generate an almost linear temperature gradient ranging from about 22° to 34°C. This was relatively independent of small changes in environmental temperature (Fig. 1).

### *Experimental procedure*

We marked insects by colour on the thorax with acrylic paint for identification and weighed them individually to the nearest 0.1 mg using an analytical balance (Mettler AJ100). They were placed in a communal jar for feeding and 24 h later they were weighed again. Because starvation level is a parameter that affects thermopreference in other ectothermic animals (Lazzari, 1991, Pires et al., 2002, Porter

& Tschinkel, 1993, Sogar & Olla, 1996, Van Dijk *et al.*, 2002), insects that did not feed were discarded.

Among the fed insects, 15 males and 15 females were chosen at random, and each group was placed in one half of the temperature gradient arena. We tested thermopreference in groups of insects because triatomines aggregate under natural conditions. The tendency to aggregate in this species should not be excluded from the assays, since it assures us that the strong individual thigmotaxis, also characteristic in this species, is accounted for. Since previous studies tested thermopreference in other triatomines with groups of insects (Lazzari, 1991, Canals *et al.*, 1997, Pires *et al.*, 2002, Guarneri *et al.*, 2003) results can be easily compared between studies. The positions of the insects were recorded by means of an infrared-sensitive video camera, which had its own illumination system composed of 6 IR light emitting diodes (LED's) (900 nm) and 4 extra pairs of IR-LED's placed at each corner of the arena in order to provide homogeneous illumination. This light is invisible for the bugs (Reisenman *et al.*, 1998). The camera (and the illumination system) remained switched on throughout the experiment, and the video output was recorded every hour over 20 days. In addition, a 12-12 white light-dark (LD) cycle was imposed (light from 08:00 to 20:00, 15 lux, by means of a 9 Watt fluorescent lamp). The lamp was placed 1.5 meters above the temperature gradient. This set-up meant that there was no interference with the temperature gradient as a result of the LD cycle.

The x, y coordinates of the position of each insect were read frame-by-frame from the video sequences by means of a digitising pad linked to a computer. These data allowed the mean position of each insect to be calculated in relation to the temperature gradient.

For the weight loss experiments, the insects were weighed and fed as described before, but were weighed again 4 h later. It is known that during the first 3 hours after feeding, *R. prolixus* eliminate most of the excess water from the blood meal (Maddrell, 1964). Thus, in our results we excluded differences in weight loss due to different rates of removal of redundant water immediately after feeding. Among fed insects two groups of 22 insects each (11 females and 11 males) were chosen at random and placed in two cylindrical acrylic containers with a piece of corrugated cardboard inside. The cylindrical containers were 9.5 cm long x 8.5 cm in diameter; each container had a circular end with a fine plastic mesh to allow air circulation. These containers were placed in a dark incubator at either 24° or 32 °C ( $\pm 2^{\circ}\text{C}$ ). These chambers were 39 x 38 x 22 cm boxes made of thermo-isolated material with a thermostat-controlled electric heating resistance as floor. An 8 mm thick plastic net was placed on the floor to separate the holding containers from the heating floor. The 24 °C chamber was placed inside a refrigerator to ensure that the outside temperature remained below 24°C. After 20 days in these chambers, we re-weighed each insect and calculated the weight loss.



*Statistical analysis*

Thermopreference positions obtained with the temperature gradient corresponded to 14400 values recorded for 30 insects (15 females and 15 males) over 20 days. The oviposition thermopreference data comprised of 134 eggs laid in two independent assays by a total of 30 females. As the data were repeated records from insects that could not be individually recognised, no statistical analysis could be performed.

For the weight loss experiments, a total of 44 insects were used. Only the surviving insects at the end of the assay, *i.e.*, 22 insects at 24°C and 13 (6 females and 7 males) at 32°C were considered for the analysis. The weight of the same insects before feeding, four hours after feeding and 20 days after feeding was analysed using a two-way “repeated measures” ANOVA, with sex and temperature as independent factors. A comparison (one-way “repeated measures” ANOVA) was performed to analyse the weight-loss for each sex independently. The weight-loss was expressed as a percentage of the blood ingested and was analysed by two-way ANOVA (sex and temperature as independent factors). Multiple comparisons between groups were performed by a *posteriori* Tukey test for unequal *N* (Zar, 1984).

**RESULTS**

### *Thermopreference*

During the 20 days of the temperature gradient experiment males and females differed slightly in their thermopreference. Males preferred a mean temperature of 25.0°C, whereas females exhibited an average preference for 25.4°C. However, this apparent difference was not a result of a static behaviour, because the thermopreference of each sex changed with starvation level (Fig. 2, 4) and time of day (note the peaks of the successive days on the graph in Fig. 2). The insects moved to the highest daily temperature at the beginning of the dark phase and to the minimum temperature at the onset of the light (Fig. 3). Individuals also moved towards lower temperature values with increasing starvation (Fig. 4).

### *Effects of temperature on nutrient conversion rate*

Figure 5 illustrates the weight of the same insects before feeding, four hours after feeding and 20 days after feeding for two environmental temperatures (24° and 32°C) and both sexes: females (top) and males (bottom plot). The results revealed a strong effect of temperature (24° vs. 32°C) on weight loss after 20 days for both females (One-way “repeated measures” ANOVA;  $F_{1,32} = 23.5$ ,  $\underline{P} < 0.0001$ ) and males (One-way “repeated measures” ANOVA;  $F_{1,32} = 17.1$ ,  $\underline{P} < 0.0005$ ). These differences (see Fig. 5) were observed in groups of insects that did not differ in original weight either before (females:  $F_{1,32} = 0.009$ ,  $\underline{P} > 0.05$ ; males:  $F_{1,32} = 1.83 \cdot 10^{-6}$ ,  $\underline{P} > 0.05$ ), or after feeding (females:  $F_{1,32} = 0.159$ ,  $\underline{P} > 0.05$ ; males:  $F_{1,32} = 1.024$ ,  $\underline{P} > 0.05$ ; Fig. 5).

Figure 6 illustrates weight loss as a function of environmental temperature. The results show that the percentage of blood consumed after 20 days is significantly higher when insects were kept at 32° than at 24°C (two-way ANOVA, see legend to Fig. 6). In addition, both sexes show different responses at the two temperatures. At 24°C insects of both sexes consumed a similar percentage of blood (about 75%), whereas at 32°C females consumed significantly higher amount of blood (ca. 150%) than males (about 115%) (two-way ANOVA, see legend to Fig. 6).

#### *Thermopreference for oviposition*

Females of R. prolixus laid eggs under a wide range of temperatures (from 22° to more than 30°C), but most (>90%) were laid between 23° and 29°C, with a peak at 25°-26°C (Fig. 7).

## **DISCUSSION**

Our results show that thermopreference in R. prolixus is dynamic. The time of day as well as the degree of starvation affect the preferred temperature (Fig. 2 - 4). This species exhibits a narrow range of thermopreferences, with daily variations of just 0.2°C (Fig. 3) and there is only a small shift in relation to the time since feeding (Fig. 4). These changes in the thermopreference are markedly low when compared with a decrease of 4°C after 10-12 days of starvation and a daily variation of about 1.7°C

reported for T. infestans (Lazzari, 1991). These inter-specific differences could be explained by differences in starvation resistance (Guerenstein & Núñez, 1994). The same pattern of thermopreference, *i.e.* a daily variation, as well as a decrease in preferred temperature with increasing starvation was also observed in two other triatomines T. brasiliensis (Guarneri *et al.*, 2003) and Panstrongylus megistus (Pires *et al.*, 2002). This suggests that this thermopreference behaviour could be a generalised phenomenon in triatomines. In a previous study Canals *et al.*, (1997) found a thermopreference of  $24.8 \pm 5.8^{\circ}\text{C}$  for Triatoma spinolai. Although we found similar values of thermopreference in R. prolixus, results are hard to compare due to differences in experimental design (see detailed discussion in Pires, *et al.* 2002). When we compared thermopreference between male and female R. prolixus we found that females consistently preferred higher temperatures than males, (Figs. 2 - 4) a difference that could be explained by differences in metabolic demands. While males might remain at lower temperatures in order to save energy, females might stay at a slightly higher temperature in order to produce eggs. In other words, females might trade off metabolic energy conservation for enhanced egg production. However, both males and females, move towards lower temperatures with increasing levels of starvation (Fig. 4), suggesting a behavioural strategy that decreases metabolic rates.

Metabolic activity in R. prolixus depends on both temperature and starvation. Oxygen consumption in unfed fourth instar larvae at  $28^{\circ}\text{C}$  is half that at  $36.5^{\circ}\text{C}$ ; oxygen consumption also decreases with increasing starvation (Okasha, 1968b). A low

level of oxygen consumption is reached 10 days after feeding, about half that observed 1 day after feeding (Okasha, 1968b). As in some fishes (Wurtsbaugh & Neverman, 1988; Sogard & Olla, 1996; van Dijk et al., 2002), the fire ant (Solenopsis invicta) (Porter & Tschinkel, 1993) and T. infestans (Lazzari, 1991), R. prolixus can modulate metabolic activity and rate of nutrient conversion by means of behavioural thermoregulation. That is, just after feeding the insects might remain at relatively high temperatures in order to increase their metabolic rates and nutrient transformation. With increasing levels of starvation and decreasing nutritional reserves, it would be beneficial to move towards lower temperatures in order to decrease rates of metabolism and water loss. As shown in Figs. 5 - 6, the temperature at which the insects stay strongly affects weight loss, probably through both water loss and nutrient conversion. A possible explanation for the larger proportional weight loss in females (Fig. 6) might be that the production and deposition of eggs equates to nutrient conversion.

One of the most important parameters controlling the epidemiology of vector-borne diseases is the biting rate of the vector, which is a reciprocal of the period of time between successive bloodmeals (Dye, 1992). An increase in the biting rate may be caused by the interruption of feeding which produces a shorter period of time between bloodmeals, as is shown to occur in stable flies and tsetse flies (Schofield and Torr, 2002). Other causes include an increase in metabolic and nutrient conversion rates. Considering that R. prolixus takes a blood-meal every time it has consumed 100% of the previous meal, we can estimate (from Fig. 6) that for R. prolixus females, a blood-

meal will be taken every ca. 26 days when the environmental temperature is 24°C and ca. 13 days when it is 32°C. Considering a constant probability of infection per bite, this increase in nutrient conversion rate with increasing temperature would mean a two fold increase in the biting and Chagas disease transmission rate. Temperature effects on disease transmission have been extensively reported. For example for the dengue vector Aedes aegypti, the duration of development was inversely related to temperature (Tun-Lin et al., 2002). Moreover, both the transmission potential of orbiviruses by the biting midge, Culicoides sonorensis and filariasis by mosquito vectors are greater at higher temperatures due to a decrease in the extrinsic incubation period (Lardeux & Cheffort, 2001; Wittmann et al., 2002).

Female R. prolixus laid eggs under a range of temperatures (Fig. 7). Within this range a rapid development of embryos can occur (Clark, 1935). Only a few eggs were laid close to 34°C which is the upper limit of temperature for hatching in this species (Clark, 1935). The unimodal thermopreference to oviposit in R. prolixus contrasts with results on other triatomines. T. infestans exhibits a bimodal pattern of thermopreference to oviposit, with a main peak at 29°C and a secondary one at 26°C (Lazzari, 1991). T. brasiliensis mainly prefers to oviposit across a wider range of temperatures (Guarneri et al., 2003). It should be mentioned that in contrast to other triatomines, R. prolixus fix their eggs to the substrate. As a consequence, eggs remain where females deposited them and do not get displaced due to the bug's activity during the assay. In contrast to T. infestans, in R. prolixus the thermopreference for oviposition did not differ from the

preferred temperature at resting. For this reason we cannot discern whether R. prolixus females actively choose certain oviposition substrates according to temperature or whether they oviposit where they find themselves. However, we cannot completely exclude the former possibility because of the relevance of the properties of substrates on fecundity in this species (Schilman et al., 1996, Lazzari et al., 1999).

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**FIGURE CAPTIONS**

**Figure 1** Floor and air temperature of the arena with the temperature gradient (mean  $\pm$  1 SE;  $\underline{N} = 4$ ).

**Figure 2** Thermal preference of male and female R. prolixus as a function of days since feeding. Each value represents the mean preference of 15 insects measured every hour.

**Figure 3** Daily variation of thermal preference of male and female R. prolixus. The deviation from the mean daily value for 15 insects was calculated for each hour and averaged over the 20 days, i.e. each point of the plot is the average of 7200 values.

**Figure 4** Thermal preference of R. prolixus males and females as a function of starvation time. Each plot represents the mean of 15 males and 15 females recorded every hour, i.e. an average of 360 values for each point of the plot.

**Figure 5** Weights (mean + 1 SE) of female and male R. prolixus as a function of temperature and time since feeding. Two-way “repeated measures” ANOVA, with sex and temperature as independent factors, revealed significant differences depending on feeding state ( $F_{2, 64} = 323.1$ ,  $\underline{P} < 0.0001$ ), sex ( $F_{1, 32} = 9.2$ ,  $\underline{P} < 0.005$ ) and temperature ( $F_{1, 32} = 5.8$ ,  $\underline{P} < 0.05$ ) but not the interaction between sex and temperature ( $F_{1, 32} =$

0.069,  $\underline{P} > 0.05$ ). A comparison (one-way “repeated measures” ANOVA) was performed to analyse the weight-loss for each sex independently as well as an a posteriori analysis (Tukey for unequal N); ns = no significant difference.

**Figure 6** Mean (+ 1 SE) weight loss after 20 days, expressed as the percentage of blood ingested, as a function of environmental temperature. Significant differences were found by two-way ANOVA (independent factors: temperature and sex) between sexes ( $F_{1,31} = 5.039$ ,  $\underline{P} < 0.05$ ), temperatures ( $F_{1,31} = 59.1$ ,  $\underline{P} < 0.0001$ ) and for the interaction of these two factors ( $F_{1,31} = 7.76$ ,  $\underline{P} < 0.01$ ). Posteriori analysis (Tukey for unequal N): a – b and b – c,  $\underline{P} < 0.05$ ; a – c,  $\underline{P} < 0.0005$ ; bars with the same letter do not differ significantly.

**Figure 7** Frequency distribution of eggs ( $\underline{N} = 134$ ) laid in two independent assays by 30 females as a function of temperature.

Figure 1

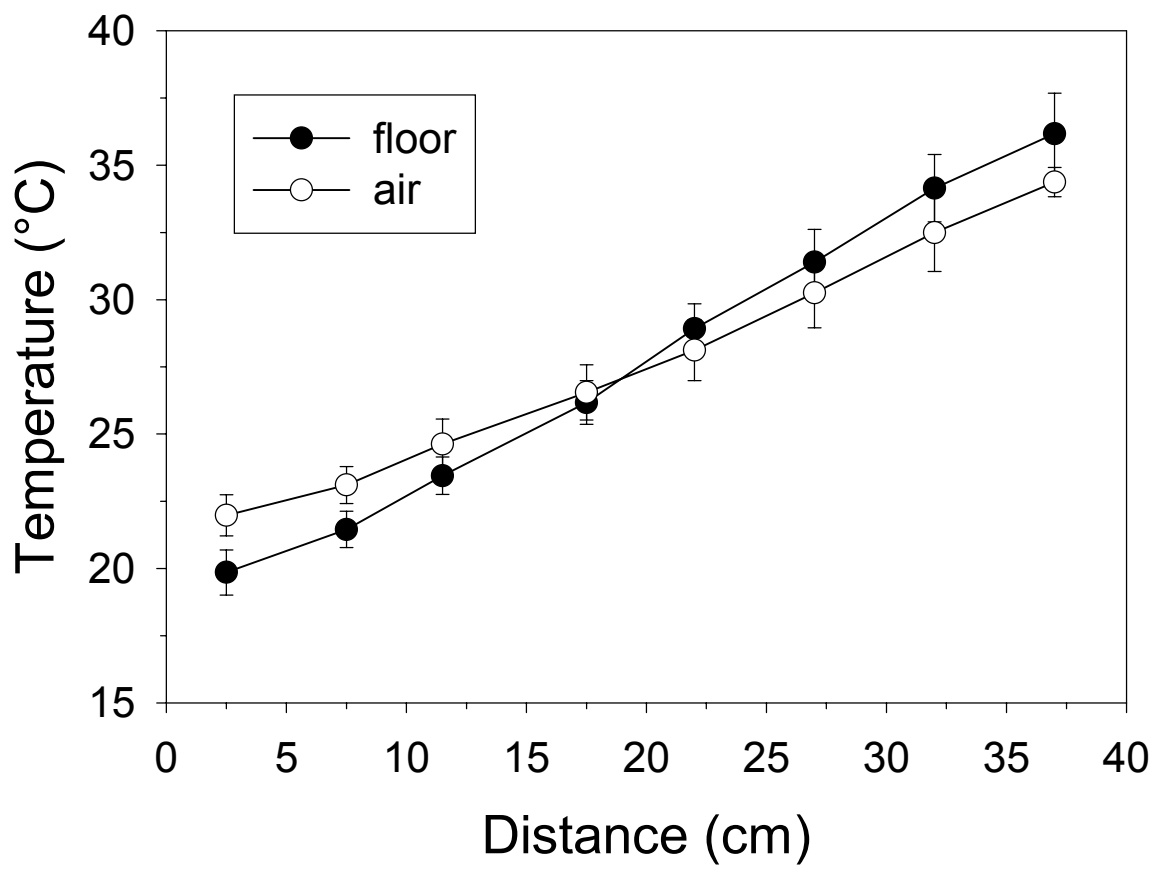


Figure 2

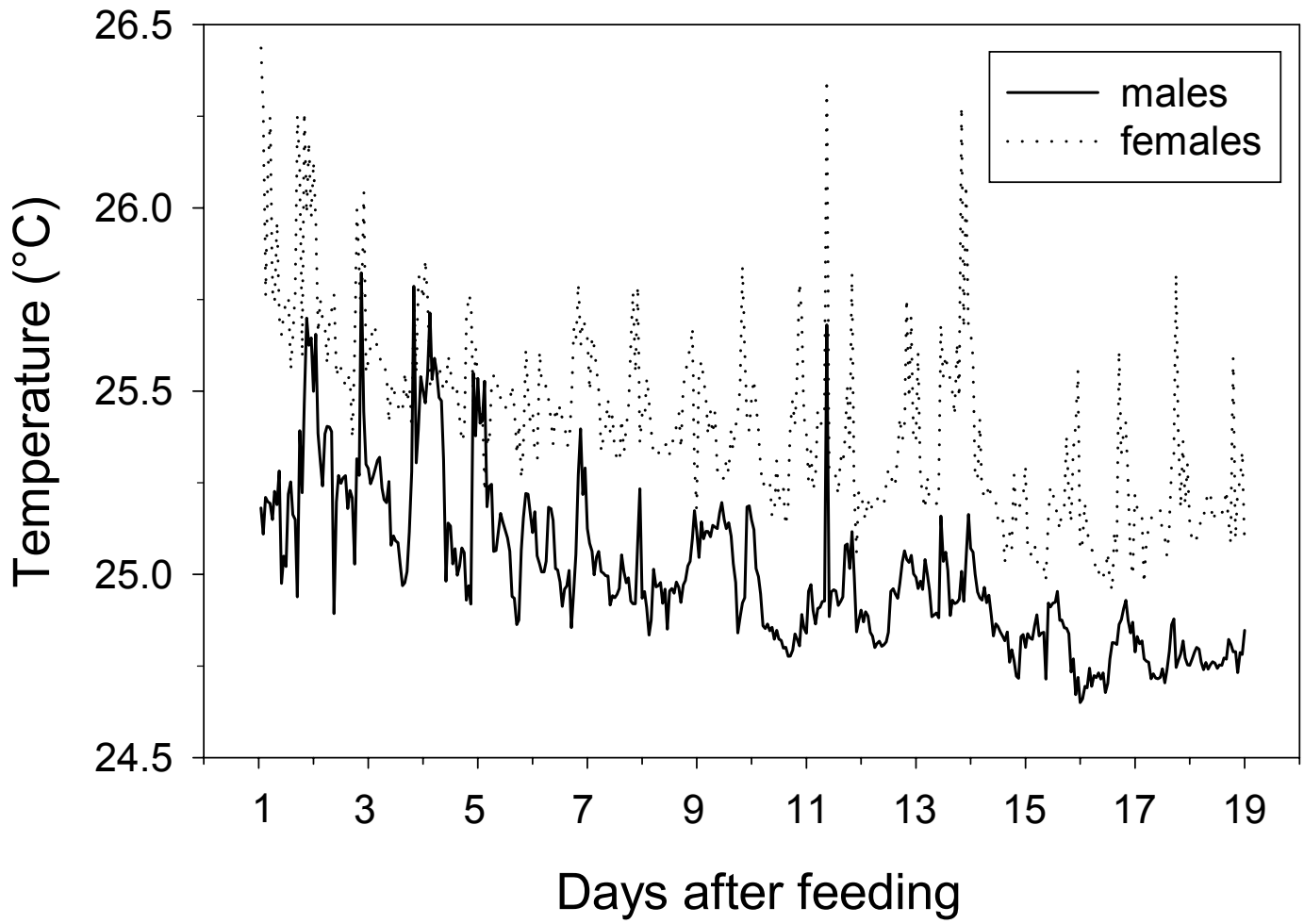


Figure 3

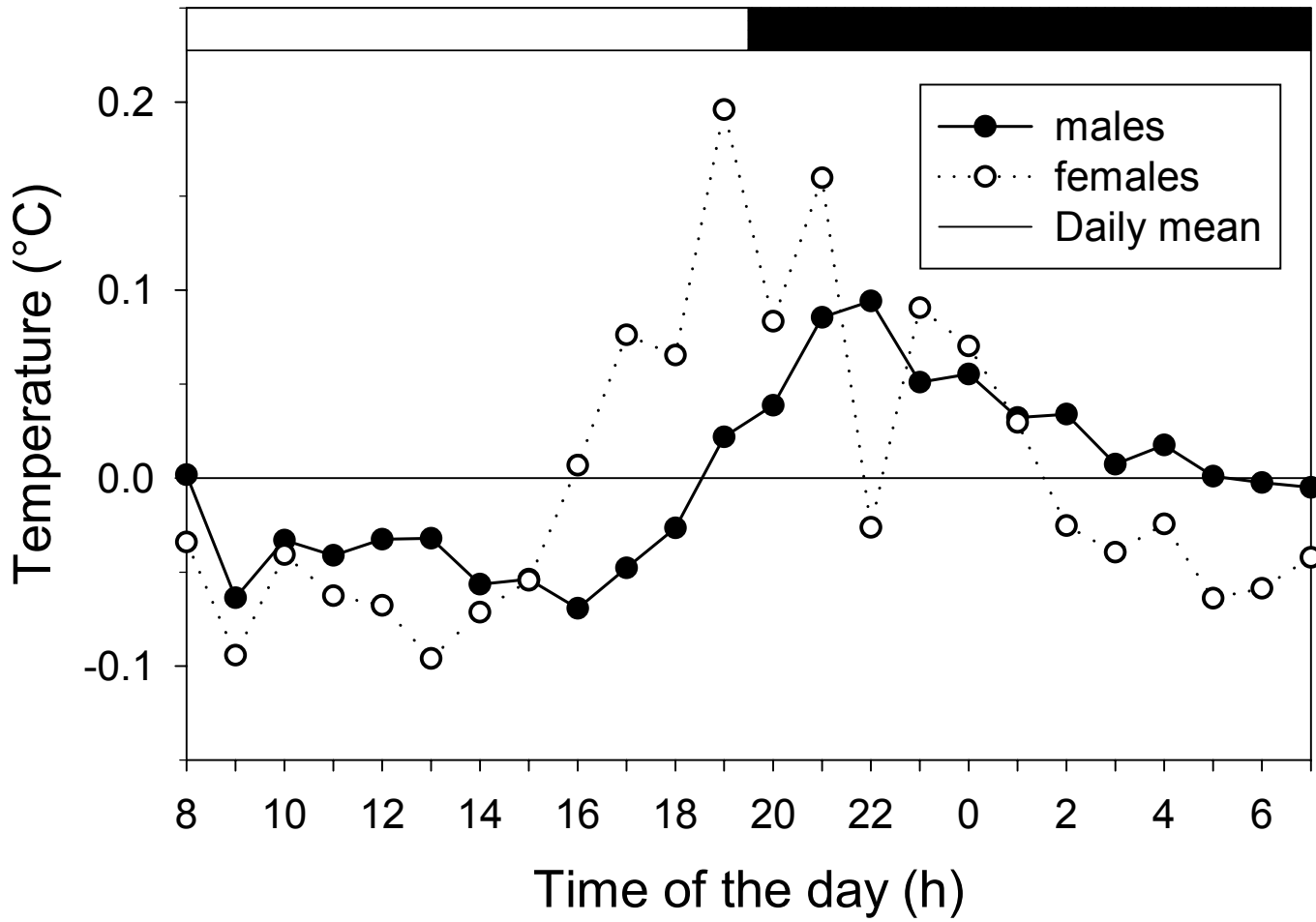




Figure 4

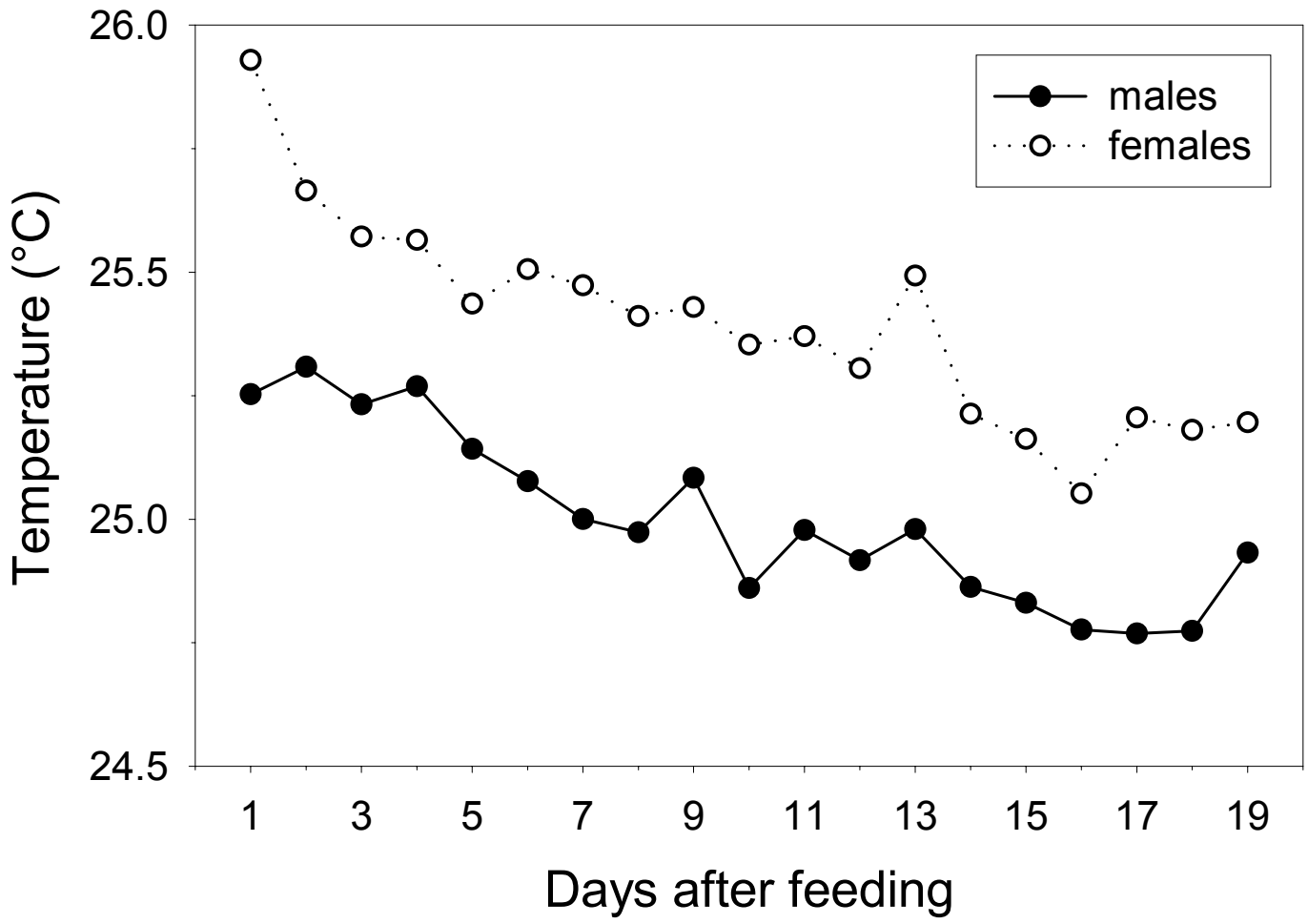


Figure 5

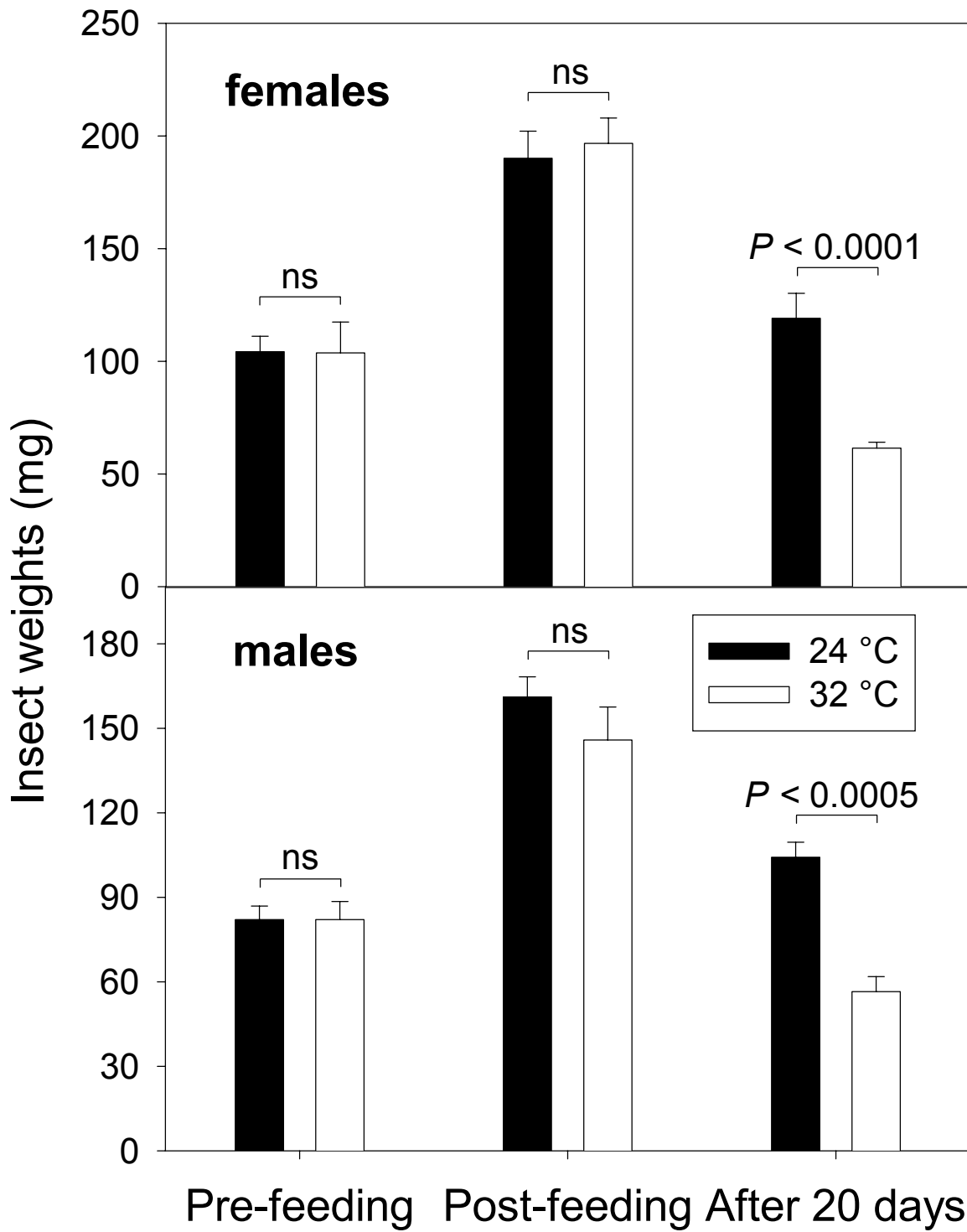


Figure 6

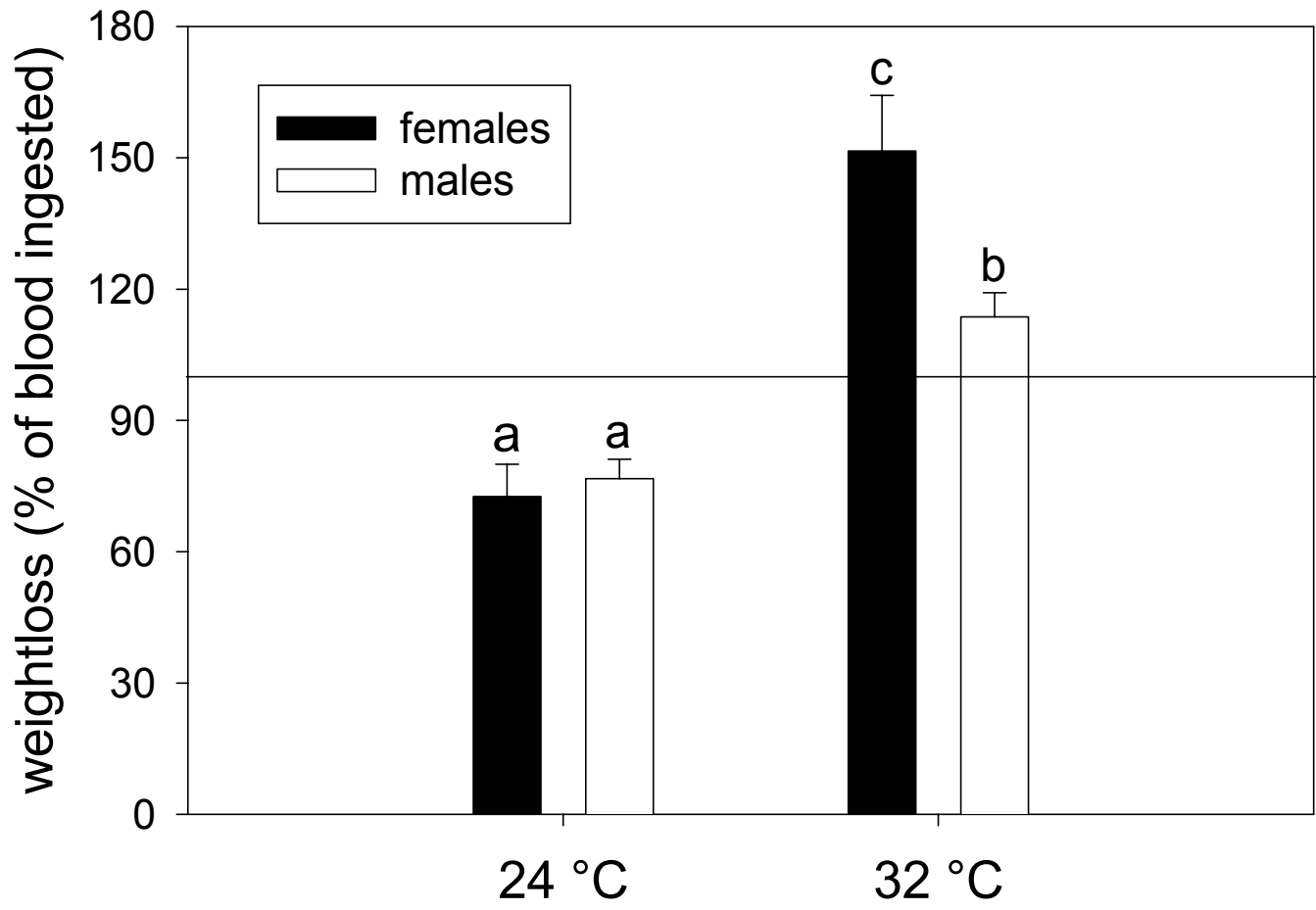


Figure 7

