

THE EFFECT OF HUMIDITY ON THE OVIPOSITION AND LARVAL MORTALITY OF *Haemaphysalis leporispalustris* (PACKARD, 1869) (ACARI: IXODIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT:-CARDOSO, A. C. B.; FREITAS, L. H. T. DE; DAEMON, E.; FACCINI, J. L. H. **The effect of humidity on the oviposition and larval mortality of *Haemaphysalis leporispalustris* (Packard, 1869) (Acari: Ixodidae) under laboratory conditions.** [Efeito da umidade relativa na oviposição e mortalidade larval de *Haemaphysalis leporispalustris* (Packard, 1869) (Acari: Ixodidae) em condições laboratoriais.] *Revista Brasileira de Parasitologia Veterinária*, v. 15, n. 2, p. 58-64, 2006. Departamento de Parasitologia Animal, Instituto de Veterinária da Universidade Federal Rural do Rio de Janeiro (UFRRJ), Km 7 da BR 465, Seropédica, RJ, Brazil, 23890-000. E-mail: faccini@ufrj.br

Oviposition, reproductive and nutritional efficiency indices (REI and NEI), eclosion and larvae mortality of *Haemaphysalis leporispalustris* were studied under controlled conditions, inside desiccators, at constant temperatures of $25 \pm 1^\circ\text{C}$ and relative humidity (RH) levels adjusted at 30, 50, 70 and 95%. Fourth generation tick specimens, originated from a colony fed on rabbits (*Oryctolagus cuniculus*), were used. The experiment was conducted between Jul/1999 and Jan/2001. The pre-oviposition and oviposition periods decreased and increased, respectively, according to the increase in RH. The egg mass weight, REI and NEI, increased according to the increase in RH. The females' residual weight, under high humidity levels, was probably small due to a better energetic use for egg production (51.77 ± 11.06 mg at 95% RH). The incubation period was inversely proportional to RH increase (45.10 ± 2.47 days; 40.33 ± 1.45 days and 36.85 ± 0.37 days at 50%, 70% and 95% RH, respectively). There was no eclosion at 30% RH. Period and percentage of eclosion were directly proportional to the increase of the other RH levels. A diminished formation of embryonic points as well as the desiccation of the already formed eggs were observed. Larval longevity was negatively influenced by 50% RH, with death occurring concomitant to eclosion.

KEY WORDS: *Haemaphysalis leporispalustris*, humidity levels, oviposition, eclosion, larval mortality.

RESUMO

Oviposição, índices de eficiência reprodutiva e nutricional (IER e IEN), eclosão e mortalidade larval de *Haemaphysalis leporispalustris* foram estudados sob condições controladas, dentro de dessecadores, sob temperatura constante de $25 \pm 1^\circ\text{C}$ e níveis de umidade relativa (UR) ajustados em 30, 50, 70 e 95%. Carrapatos de quarta geração, originários de uma colônia mantida em coelhos domésticos (*Oryctolagus cuniculus*), foram utilizados. O experimento foi conduzido entre Julho/1999 e Janeiro/

2001. Os períodos de pré-postura e postura diminuíram e aumentaram, respectivamente, de acordo com o aumento da UR. O peso da massa de ovos, IER e NEI, aumentaram de acordo com o aumento da UR. O peso residual da fêmea, sob elevados níveis de umidade, foi provavelmente menor devido ao maior uso energético na produção de ovos ($51,77 \pm 11,06$ mg a 95% UR). O período de incubação dos ovos foi inversamente proporcional ao aumento da UR ($45,10 \pm 2,47$ dias; $40,33 \pm 1,45$ dias e $36,85 \pm 0,37$ dias a 50%, 70% e 95% UR, respectivamente). Não houve eclosão a 30% de UR. O período de eclosão e o percentual de eclosão foram diretamente proporcionais ao aumento dos níveis de UR. A redução da formação de pontos de embrionamento assim como a dessecação dos ovos já formados foi observado nos níveis de umidade inferiores. A longevidade larval foi influenciada negativamente pela UR de 50%, com ocorrência de morte subsequente à eclosão.

PALAVRAS-CHAVE: *Haemaphysalis leporispalustris*, níveis de umidade relativa, oviposição, eclosão, mortalidade larval.

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INTRODUCTION

Haemaphysalis leporispalustris (Packard, 1869) (Acari: Ixodidae) is a three-host tick, widely distributed throughout the American continent. Adults parasitize Lagomorphs which are the main natural hosts for all parasitic stages whereas immatures ticks infest several hosts (RHOR, 1909). *Sylvilagus brasiliensis* is the only natural host recorded in Brazil, but *Oryctolagus cuniculus*, the domestic rabbit in Brazil, is an excellent experimental host (LABRUNA et al., 2000).

Some pathogens that might infect humans such as *Francisella tularensis*, "Spotted Fever Group" Rickettsia and spirochetes resembling *Borrelia burgdorferi* have been isolated from this tick (KOLLARS; OLIVER, 2003). In USA (SONENSHINE; CLIFFORD, 1973) and Costa Rica (FUENTES et al., 1985) *H. leporispalustris* have been associated with *Rickettsia rickettsii* epidemiological chain, with participation of others *Sylvilagus* species. In Brazil, however, studies like these are lacking up to date.

The geographical distribution of ixodids is directly related to abiotic factors such as temperature and relative humidity of the air. While the effect of the abiotic have been studied extensively in several species of ticks, few studies on the effect of temperature and humidity are the solely information for Neotropical populations of *H. leporispalustris* (FREITAS et al., 2000; CARDOSO et al., 2002; FREITAS et al., 2004).

The aim of this work was to study, under experimental conditions, the effect of different humidity levels in female reproductive parameters, and larvae eclosion and mortality. With this in mind, finding the most suitable humidity level for this species, we can presume the most suitable environment for life cycle development.

MATERIAL AND METHODS

This experiment was conducted at the Laboratório de Ixodologia, located at the Estação para Pesquisas Parasitológicas W.O. Neitz, Departamento de Parasitologia Animal, Instituto de Veterinária, of the Universidade Federal Rural do Rio de Janeiro (UFRRJ) (22°45'S, 43°41'W, Alt: 33 m), from July/1999 to January/2001. Ticks used in this experiment came from a single engorged female collected from a field, caught non identified, wild rabbit near the campus of the UFRRJ. Fourth generation females from a colony fed on *O. cuniculus*, maintained in an acclimatized chamber, under controlled conditions (27 ± 1 °C, relative humidity (RH) higher than 80% and scotophase), were used to infest 10 crossbred rabbits *O. cuniculus* (California x New Zealand breed), adults males and females having had no previous contact with ticks or acaricides. Fifty males and fifty females, fasted for approximately 20 days, were placed on the ears of each rabbit according to the protocol in Neitz et al. (1971) in order to obtain the engorged females to constitute the experimental groups.

Relative humidity study: four 250 mm diameter desiccators with 30%, 50%, 70% and 95% relative humidity kept constant

by means of saturated solutions of potassium hydroxide (PETERSON, 1964) were prepared. This solution also maintained the temperature inside the desiccators at 25±1°C that were maintained at room temperature. Humidity inside the desiccators was checked daily through a thermo hygrometer.

Experimental procedure: After collection, engorged females, recovered on modal day of detachment, were taken to the laboratory and cleaned with a soft paintbrush. These were then individually weighed on an analytical scale, identified and distributed according to weight, in order to obtain experimental groups with similar average weights.

Four groups of 15 engorged females, all detached on the detachment modal day, were formed. Each group was placed on a 150 x 20 mm Petri dish, dorsally attached with adhesive tape and transferred to the different relative humidity desiccators (30%, 50%, 70% and 90%).

All eggs of each female were collected, weighed and transferred to 3 ml capacity transparent glass flasks, at 24-hour intervals after the beginning of the oviposition. The open mouth of the flasks was covered with a fine meshed cloth, held with an elastic band. Each female's entire oviposture was placed in the same flask, which was then labeled and placed under the same humidity conditions as the female.

For each experimental group the following biological parameters were observed daily: preoviposition period (from the detachment to the beginning of the oviposition), oviposition period (from the first to the last egg oviposited), egg mass weight for each female, nutritional and reproductive indices (NEI and REI respectively), according to Bennett (1974a), egg incubation period (from the beginning of oviposition to the eclosion of the first larvae), larval eclosion period (from the eclosion of the first to the last larva), larval eclosion percentage (percentage of eclosed larvae in relation to the egg mass, by means of visual estimate), larval mortality period (from the first to the last larva death), oviposition rate (percentage of eggs laid per day), accumulated eclosion rate and accumulated mortality rate.

Statistics: With the exception of oviposition, eclosion and mortality rates, ANOVA followed by the Tukey-Kramer test ($p < 0.05$) were used to compare the means of all studied parameters. When necessary, values expressed as percentage were transformed into arc sine, before applying the statistical tests.

RESULTS AND DISCUSSION

The reproductive parameters of engorged females and pre-feeding periods of larvae of *H. leporispalustris* females fed on rabbits and maintained at four humidities are presented in Tables 1 and 2, respectively.

As seen in Table 1, two females died before starting oviposition at 30% and 95%, probably due motives others than the effect of humidity. Of the parameters evaluated, two (pre-oviposition period and residual weight of females) decreased as humidity increased and four (oviposition period, egg mass, REI and NEI) increased as humidity increased. The

Table 1. Mean reproductive values of engorged females of *Haemaphysalis leporispalustris* kept at $25 \pm 1^\circ\text{C}$ and four different relative humidities.

Parameters	Relative Humidity (%)			
	30	50	70	95
Female weight (mg)	15 ¹ 237.47 ^a ± 22.5 205.9 – 274.6 ²	15 237.41 ^a ± 22.56 204.9 – 274.6	15 237.23 ^a ± 22.33 204.9 – 274	15 228.63 ^a ± 33.82 145.0 – 273.8
Pre-oviposition (days)	13 6.85 ^a ± 0.99 6 – 9	15 6.73 ^{ab} ± 0.59 6 – 8	15 6.40 ^{ab} ± 0.63 6 – 8	13 6.08 ^b ± 0.49 5 – 7
Oviposition (days)	13 9.77 ^a ± 3.11 3 – 14	15 12.47 ^a ± 3.68 5 – 20	15 17.87 ^b ± 4.32 11 – 26	13 23.15 ^c ± 5.03 12 – 30
Egg mass weight (mg)	13 46.18 ^a ± 29.38 1.2 – 114.7	15 68.43 ^{ab} ± 31.39 22.4 – 140.4	15 93.57 ^b ± 27.81 32.1 – 129	13 132.06 ^c ± 31.4 48.3 – 163.8
Female Residual weight (mg)	13 118.65 ^a ± 24.75 73.0 – 158.8	15 98.69 ^{ab} ± 28.62 60.20 – 149.70	15 80.80 ^b ± 29.14 48.20 – 140.60	13 51.77 ^c ± 11.06 39.30 – 79.20
REI (%)	13 18.68 ^a ± 11.11 0.53 – 41.77	15 28.75 ^{ab} ± 12.59 1.31 – 52.72	15 39.71 ^b ± 12.33 15.06 – 55.15	13 56.25 ^c ± 8.61 32.62 – 64.87
NEI (%)	13 34.02 ^a ± 16.45 1.81 – 56.89	15 47.34 ^{b,c} ± 13.32 23.67 – 71.23	15 58.54 ^c ± 10.78 29.97 – 72.22	13 72.46 ^d ± 7.56 51.86 – 81.71

Mean ± sd. Values in the same line followed by different letters are significantly different ($p < 0.05$);

¹Number of females; ² Range.

Table 2. Larvae data of *Haemaphysalis leporispalustris* kept at $25 \pm 1^\circ\text{C}$ and three different relative humidities.

Parameters	Relative Humidity (%)		
	50	70	95
Incubation (days)	10 ¹ 45.1 ^a ± 2.47 42 – 49 ²	15 40.3 ^b ± 1.45 39 – 44	13 36.8 ^c ± 0.37 36 – 37
Eclosion (days)	10 4.5 ^c ± 2.55 1 – 9	15 13.2 ^{b,c} ± 3.30 8 – 18	13 13.2 ^a ± 3.24 10 – 21
Eclosion Rate (%)	10 6.2 ^b ± 15.47 0 – 50	15 90.0 ^a ± 19.98 20 – 98	13 87.2 ^a ± 23.53 10 – 100
Longevity (days)	0	15 152.9 ^a ± 20 106 – 186	13 98.8 ^b ± 15.28 79 – 126

Mean ± sd. Values in the same line followed by different letters are significantly different ($p < 0.05$); ¹Number of egg mass; ² Range.

most statistical significant differences were found between the lower and higher humidity levels.

There was not eclosion at 30% RH. At 50 %, eclosion occurred only in 10 out of 15 (67%) females at very low rates and all the larvae died immediately after eclosion (Table 2).

Lancaster and McMillan (1955) also observed a decrease in the pre-oviposition period of *Amblyomma americanum* as the humidity increased. Nevertheless, the authors call attention to the fact that at lower than 47% and 69% RH there were no oviposition and larval eclosion, respectively. These are certainly interspecific differences.

Studies conducted by Ouhelli and Pandey (1984) and

Ouhelli (1994), with different species of the genus *Hyalomma*, and Guimarães da Silva et al. (1997) with *Anocentor nitens*, corroborate the results observed for *H. leporispalustris* in relation to the decrease in pre-oviposition period according to an increase in relative humidity. On the other hand, Hitchcock (1955), cited by Bennett (1974b), while working with *Boophilus microplus*, reported that oviposition was not severely affected by relative humidity levels between 40 and 100%. Bennett (1974b), while working with the same tick species, reported that low saturation deficits ($\leq 29\%$), seriously compromise oviposition and egg viability, showing a higher resistance to the intermediate and minimum humidity levels for this species, since oviposition was compromised only at 29% RH. Santos (1999), while studying *B. microplus* at the same humidity levels used in the present study, corroborates our results, mentioning that alterations related to the decrease in relative humidity were milder, confirming the higher resistance of this species to saturation deficits. Ouhelli et al. (1982) stated that there was no egg mass weight alteration for *B. annulatus* exposed to different RH levels at constant temperatures. This fact leads to a speculation of a higher adaptability of this species to low saturation deficits.

The result obtained by the present study differed from those obtained by Ekpenyoung and Akinboade (1991) for *A. variegatum*, in which the same values of pre-oviposition and oviposition periods were obtained at different relative humidity levels. These results however, must be treated cautiously. Diaz et al. (1991), while studying the influence of temperatures between 24 and 34°C and several levels of relative humidity (100, 80, 75.5 and 70%) on *A. nitens*, reported that relative

humidity has little influence over pre-oviposition and oviposition periods and eclosion, attributing the observed variations to temperature. This leads us to question the narrow range of relative humidity levels used in this study, since Despins (1992), working with the same tick species, different temperatures (20, 25, 30 and 35°C), and a wider range of RH levels (40, 61, 75 and 91%), observed that, at constant temperatures, different RH levels influenced oviposition periods, reproductive efficiency indices, eclosion percentages and incubation periods, corroborating the results obtained in this study and confirming the effects of relative humidity on the ticks' development.

The studies of Rhor (1909) and Hooker et al. (1912), with *H. leporispalustris*, are difficult to compare since no relative humidity limits were fixed for the experiments. As such, it is only possible to state that, in those experiments, several alterations occurred due to the relative humidity variation. On the other hand, Labruna and Leite (1997), under laboratory conditions (27°C and RH ≥ 80%), verified shorter pre-oviposition and oviposition periods (4.3 and 21.6 days, respectively) for *H. leporispalustris*, when compared to those at 95% RH. This leads us to suggest that this difference might be explained by intrinsic differences between the populations.

In relation to the nutritional and reproductive efficiency indices (Table 1), we verified that they increase according to the increase of the RH level. This demonstrates the females' better egg production yield under higher levels of relative humidity, confirmed by their low residual weight (Table 1), ranging between 39.30 and 79.20 mg for the group exposed to 95% RH, when contrasted to the minimum and maximum values (73.00 and 158.80 mg respectively) verified in the group maintained at 30% RH. The average of the REI values found in the present study at 95% RH (56.25%) are similar to those obtained by Labruna and Leite (1997) for *H. leporispalustris* (54.9%) at a relative humidity level equal or above 85%. This leads us to conclude that RH levels above 85% favor the nutritional conversion of this ixodid species.

The analysis of these results, as well as those parameters related to oviposition, indicate that low relative humidities influence the capability to transform nutrients in eggs. Thus, in general, one can consider that both the conversion rate of the females in eggs and the consumption of metabolism energy for egg production are affected by the decrease in humidity.

The studies of Diaz et al. (1991), Despins (1992) and Guimarães da Silva et al. (1997), with *Anocentor nitens*, and Santos (1999), with *B. microplus*, showed similarities to the results obtained in the present study, demonstrating the considerable influence of relative humidity levels on the biological performance of the engorged females. Nevertheless, Guglielmo (1992), observing the biology of *A. triguttatum triguttatum* at different temperatures and under different saturation deficits, attributes to temperature the alterations observed for the reproductive efficiency index.

The influence of relative humidity over combined parameters, such as weight and oviposition period, can best

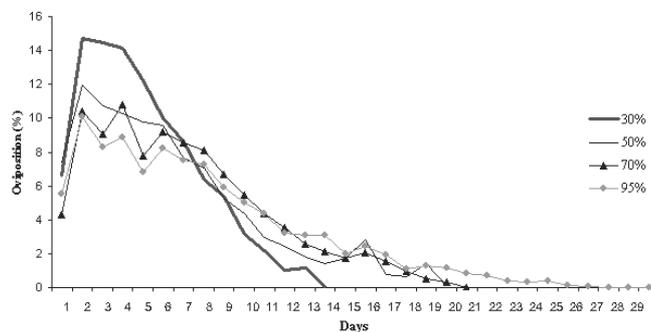


Figure 1. Daily oviposition rates of *Haemaphysalis leporispalustris* females kept at 25 + 1°C and four different relative humidities.

be analyzed by the oviposition rate (Figure 1). At 30% RH, the oviposition curve was more accentuated, with the most evident peaks on the 2nd, 3rd and 4th days, with a accentuated reduction of oviposition that ended in less time when compared to the oviposition rate observed for the remaining RH levels.

The oviposition peaks were obtained on the second laying day for 30, 50 and 95% RH, with the production of 14.72%, 11.94% and 10.09% of the total egg mass respectively, while at 70% the oviposition peak occurred on the fourth day, with the production of 10.79% of the total egg mass. The necessary periods for the laying of 50% of the total egg masses were of four, five, six and seven days, under 30, 50, 70 and 95% RH, respectively. Under 95% RH, despite the majority of the eggs being laid on the first days of oviposition, the oviposition rate was extended, when compared to the other RH levels, characterized by small egg mass quantities on the last days of oviposition, as reported for *Rhipicephalus simpsoni* in a study carried out by Baidu (1987). This data, together with the weight and oviposition period, confirms the influence of relative humidity in the development of *H. leporispalustris*, showing that the lower the relative humidity, the lower the development of females in relation to oviposition, fact that was also verified by Guimarães da Silva et al. (2000) for *Amblyomma cajennense* and Santos (1999) for *B. microplus*, under the same conditions of this experiment.

Sonenshine and Tigner (1969), while studying *Dermacentor variabilis* and *A. americanum* at six different relative humidity levels (45, 56, 65, 76, 86 and 95%), obtained results similar to those of the present experiment. They state that the majority of the eggs are deposited at the beginning of the oviposition process, disagreeing, however, when they state that oviposition is not affected by relative humidity when it is higher than 65%, as long as the temperature is constant.

Table 2 shows the values for egg incubation and eclosion periods and larvae longevity.

The 30% relative humidity level completely prevented larvae eclosion, in the same manner as observed for *A. nitens* (GUIMARÃES DA SILVA et al., 1997), *A. cajennense* (GUIMARÃES DA SILVA et al., 2000) and *B. microplus* (SANTOS, 1999), at the same RH level.

The incubation period diminished according to the increase in relative humidity, as observed by Ouhelli (1994), while

studying five tick species, and by Guimarães da Silva et al. (2000), for *A. cajennense* using the same RH levels as this study. There are statistical differences among the three conditions under which larvae eclosion occurred. On the other hand, the eclosion period tended to increase under higher RH levels, showing a higher eclosion percentage at 70 and 95% RH. The larvae eclosion percentage did not present statistical differences between 70 and 95% RH – Figure 2. On the other hand, the larvae eclosion percentage, at 50% RH was reduced and differed statistically from that of the other RH levels used in this study. It should be noted that of the 15 ovipositions, maintained at 50% RH, only 10 began the eclosion process. It should also be noted that observations of the embryony development, culminating in larvae eclosion, showed an absence or decrease in the formation of embryonic points in the ovipositions maintained at 30 and 50% RH, respectively, and the desiccation of embryonated eggs maintained at 50% RH. Guimarães da Silva (2000) observed the same pattern for *A. cajennense* under equal temperature and RH conditions of the present study.

Lancaster and Mcmillan (1955), working with *A. americanum*, and Tukahirwa (1976), with *Rhipicephalus appendiculatus*, observed a lack of eclosion in ovipositions maintained at relative humidity levels lower than 69% and 67%, respectively. Tukahirwa (1976) also observed a lack of embryonic points in ovipositions maintained at 10% RH and very few points for eggs maintained at relative humidity levels between 45 and 67%. For *Haemaphysalis spinulosa* and *Rhipicephalus simus*, Hussein and Mustafa (1987) verified that at relative humidity levels lower than 75%, the eclosion percentage decreased significantly. Guimarães da Silva et al. (1997), Guimarães da Silva et al. (2000) and Santos (1999) verified, for *A. nitens*, *A. cajennense* and *B. microplus*, respectively, a lack of eclosion for ovipositions maintained at 30 and 50% RH. These authors obtained results that were similar to those obtained by Guglielmo (1992), who observed that *A. t. triguttatum* eggs were vulnerable to saturation deficits.

Although there are differences in the eclosion periods and eclosion percentage at different levels of relative humidity, the accumulated eclosion rates (Figure 2), were similar in all

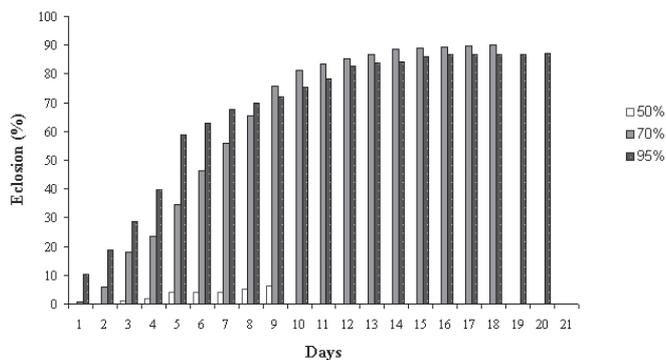


Figure 2. Cumulative eclosion rate for *Haemaphysalis leporispalustris* kept at 25 + 1° C and four different humidities.

groups maintained at different humidities, with low eclosion percentages on the first day, increasing as the days went by, with periods becoming shorter as the RH decreased.

Larvae longevity was strongly and negatively influenced by 50% RH, since deaths occurred concomitant to eclosion, preventing even the verification of mortality rates. Nevertheless, our observations might suggest that the average survival of the larvae at this RH was approximately of 1 day. Similar results were observed for *A. cajennense* at 70% RH (GUIMARÃES DA SILVA et al., 2000), thus showing interspecific characteristics between *H. leporispalustris* and *A. cajennense*, such as low larvae resistance and even of eggs, though indirectly, at low RH levels. Longevity was significantly higher for the 70% RH, when compared to the 95% RH. This fact could be attributed to the accumulation of water droplets, with the subsequent appearance of fungi, inside the flasks maintained at 95% RH. This was not observed in flasks maintained at 70% RH, preventing us from safely stating that 70% RH favors, in fact, higher longevities, under the experimental conditions adopted in this study, since, according to Knülle and Rudolph (1982) the hydro equilibrium of *H. leporispalustris* occurs at 85% RH. Below these levels, tick development would be compromised.

According to Hooker et al. (1912), larvae longevity of *H. leporispalustris* is higher than 258 days under favorable conditions, fact that leads us to believe in the negative interference of water and fungi inside the flasks at 95% RH.

The accumulated mortality rate (Figure 3) was similar at 70 and 95% RH, with a gradual increase, most accelerated for those larvae maintained at 95% RH, which after five weeks already presented a mortality rate of over 50%. On the other hand, specimens maintained at 70% RH only reached this percentage after seven weeks of exposure. Once again, the formation of water droplets and fungi contamination of the transparent flasks maintained at 95% RH prevented an accurate evaluation of the results, rendering it impossible to predict if this fact had any influence over larvae mortality and which RH is most favorable for specimen survival.

Based on the results obtained in the present study, we conclude that different degrees of relative humidity influence

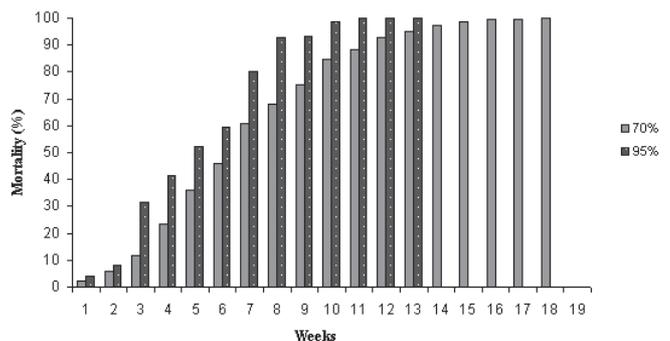


Figure 2. Cumulative mortality rate for *Haemaphysalis leporispalustris* kept at 25 + 1° C and four different humidities.

pre-oviposition and oviposition periods, productivity and nutrient conversion of engorged females, diminishing their performance according to the relative humidity decrease. In addition, 30 and 50% relative humidity values are deleterious, for they interfere with the egg embryonary development, compromising eclosion as well as larvae survival. As such, we can suggest that the minimum limit of 70% RH would be the most favorable to the development of the non-parasitic phase of engorged females and larvae survival of *H. leporispalustris*.

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