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Research article

Effect of Temperature on the Oviposition Capacity of Engorged Adult Females and Hatchability of Eggs of Dog Ticks: *Rhipicephalus sanguineus* and *Heamaphysalis leachi leachi* (Acari: Ixodidae)

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ABSTRACT: Effects of temperature on the oviposition capacity of engorged adult females of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* and on the hatching pattern of their eggs were investigated under laboratory conditions. The temperatures of maintenance were 15^0C, 20^0C, 25^0C, 30^0C and 37^0C at 85% relative humidity (R.H). The pre-oviposition periods of engorged adult females of *R.sanguineus* and *H. leachi leachi* increased as the incubation temperature became low from 30^0C to 15^0C. There was a significant difference ($p < 0.05$) in the pre-oviposition periods of *R. sanguineus* and *H. leachi leachi* at all the maintenance temperatures. The number of eggs oviposited by adult females of *R.sanguineus* and *H. leachi leachi* decreased as the incubation temperature dropped from 30^0C to 15^0C. The mean numbers of eggs produced respectively by 0.06g and 0.12g *R. sanguineus* female ticks at 37^0C were 278.00 + 3.46 and 955.33 + 4.90 while no egg was laid by the same weights of female *H. leachi leachi* at the same temperature. The eggs of both species did not hatch at 150C. At 370C the eclosion period of *R. sanguineus* was 17 days while the eggs of *H. leachi leachi* did not hatch. The mortality rates of eggs of *H. leachi leachi*, (56.2, 16.8 and 16.6%) were higher than those of *R. sanguineus*, (17.6, 10.3 and 10.2%) at 20^0C, 25^0C and 30^0C respectively. It is concluded that *R.sanguineus* has greater resistance to the deleterious effect of extreme temperatures than *H. leachi leachi*

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Keywords: Dog tick, temperature, *Rhipicephalus sanguineus*, *Heamaphysalis leachi leachi*, oviposition, hatchability

INTRODUCTION

The ticks *Rhipicephalus sanguineus* Latrielle, 1806 and *Haemaphysalis leachi leachi.* Audouin, 1826 are the main vectors of *Babesia canis* and *B.gibsoni* agents of canine babesiosis, a highly fulminating disease of dogs worldwide (Oduye and Dipeolu, 1976, Bobade *et al*.; 1989; Craig, 1990).

Development and survival of the ticks depends very much on successful oviposition and hatching of as many eggs as possible. This is because apart from adverse climatic factors, which cause mortality in a large proportion of the developmental stages of ticks, they also have to contend with the problem of finding suitable hosts for blood meal which is essential for their development, survival and propagation.

Numerous workers have reported on a number of factors that influence the various stages of the life cycle of ticks (Soulsby, 1982; Georgi *et al;* 1990; Koch and Tuck, 1986, Koch 1982, Jacobs *et al*., 2004) and noted that the most important factor was temperature. This is because temperature regulates rate of metabolism, influences digestion, conversion and absorption of blood meal, pre-oviposition and oviposition periods, hatching pattern of eggs and survivability of larvae and larval attachment to their hosts (Koch and Tuck, 1986; Davey 1988; Van Der Lingen *et al.*, 1999). There are varied reports on the effect of temperature on ixodid ticks. For example Dipeolu (1984a) in his study of bionomics of cattle ticks *Boophilus decoloratus* and *B. geigyi* observed that the females of these two ticks did not oviposit at 15 and 37^oC and related this to their

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distribution in Nigeria. This author also noted that *B.decoloratus* produced more eggs than *B. geigyi* at all temperatures.

Yano *et al*., (1987) in their studies on the effects of controlled temperatures on development and growth of *Haemaphysalis longicornis* in Japan reported that as the temperature became low, the periods of pre-oviposition and oviposition, egg hatching and moulting were prolonged. These authors also noted that at 12^0C oviposition, egg hatching and moulting of the larva and nymph did not occur. Yano *et al*., (1987) further noted that the number of eggs per mg body weight decreased markedly at 15^0C and the hatch-ratio was lowered with dropped temperatures. The biology of *Ixodes rubicundus* under laboratory conditions have been reported by Van Der Lingen *et al*., (1999) to have extended pre-oviposition and oviposition periods and increase production of eggs at 25^0C and 93% R.H when compared with the number of eggs produced at 10^0C and 93% R.H. *R.sanguineus* has been reported to require temperatures greater than 18^oC to complete their life cycle (Soulsby, 1982). It has also been reported by Inokuma *et al*. (1996) that the post parasitic period of larvae and nymphs and pre-oviposition and length of oviposition of engorged females of *R. sanguineus* increased as temperatures decreased from 37^oC to 23^oC; and that ticks did not develop at temperatures below 14^oC. As these ticks are abundant in tropical countries (Ojo, 1990) temperature is thought to be the most important factor for their survival.

The aim of this controlled laboratory investigation was to study the effect of selected temperatures on the oviposition capacity of engorged adults females and hatchability of eggs of ticks of dogs: *R. sanguineus* and *H.leachi leachi*. An understanding of effect of temperature on the life cycle of these ticks is important so as to unravel their current and potential biogeographic distribution, seasonality and mortality factors as suggested by Van Der Lingen *et al*., (1999). Information on the effect of temperature and timing on biological process are also important to the success of control measures against these ticks of dogs..

MATERIALS AND METHODS

Effect of Temperature on Oviposition capacity of Adult Female Ticks

Collection and maintenance of ticks

Ticks in various stages of engorgement were collected individually by careful detachment with pairs of forceps from household dogs brought to Veterinary Clinics in Ibadan, Nigeria. Care was taken to ensure that the mouthparts were not left behind while removing the ticks with thumb forceps (Bowman, 1999). The detached ticks were collected into universal bottles plugged tightly with cotton wool and immediately conveyed in kilner jars to parasitology laboratory section of the Department of Veterinary Microbiology and Parasitology University of Ibadan, Nigeria. They were identified using morphological characters described by Soulsby (1982) and Wall and Shearer (1997). Individual tick weights were also determined and recorded using a sensitive sartorious balance (Type 2472).

Five ticks of the same weight (0.06g and 0.12g) each of *R. sanguineus* and *H. leachi leachi* were used for this study They were put in incubator (Gallenhamp cooled $C0_2$ incubator) regulated to temperature of 15^oC, with saturated solution providing a relative humidity (R.H) of 85%.

The pre-oviposition and duration of oviposition (in days) and the number of eggs laid by each tick were recorded. The pre-oviposition period was taken as the day the ticks were detached and subjected to temperature treatment to the time they started to lay eggs. The oviposition period was the number of days it took each tick to lay all its eggs and died.

Egg collection and counting

After the pre-oviposition period, the eggs laid by each tick were collected at 08.00 hours every morning. The collection of eggs was carried out by carefully taken out the ovipositing tick and putting it into another clean and dry Bijou bottle and plugged tightly with cotton wool (Dipeolu and Ogunji 1980a). This procedure was carried out throughout the oviposition period. This was possible because of the wax, which made the eggs to stick together. Counting was done under dissecting microscope using a tally counter. Counting was facilitated by addition of xylene which dissolved the wax which had hitherto made the eggs stick together (Dipeolu and Ogunji, 1980a). All the eggs laid by five ticks of the same weight each of *R. sanguineus* and *H. leachi leachi* were pooled together. This procedure was repeated for incubation temperature of 20^0C, 25^0C, 30^0C and 37^0C.

Effect of Temperature on Hatchability of Eggs

Source of eggs

The eggs used for this study were eggs oviposited by engorged adult females of *R.sangunineus* and *H. leachi leachi* collected at various occasions from household dogs brought to Veterinary Clinics in Ibadan.

The ticks were collected individually by forcible detachment with pairs of forceps and were immediately conveyed to the laboratory in dual-purpose kilner jars. They were identified into species and individually put into bijou bottles plugged with cotton wool to oviposit.

To study the effect of temperature on the hatchability of the eggs, one Bijou bottle containing 0.05gm of freshly laid eggs of *R. sanguineus* and another one containing a similar weight of the eggs of *H. leachi leachi* were maintained in the incubator (Gallenhamp cooled CO_2 Incubator) at 25°C and 85% relative humidity (RH), and regularly observed until hatching began. From the day hatching started until it ended the hatched larvae were separated at 8.00 hour every morning from the unhatched eggs. This was possible because before hatching the eggs usually clumped together. Once hatching started the larvae move away from the clump. The separation of larvae from unhatched eggs was therefore achieved by scooping the lump(s) of unhatched eggs into another bijou bottle and covered with its lid while 10% formalin was added to the first bijou bottle which then contained only hatched larvae (Dipeolu, 1982).

The number of the larvae was ascertained under a dissecting microscope. This was repeated every day until no larva was seen to hatch. These unhatched eggs which existed in small clumps were observed for another two weeks before they were declared unhatched and dead.

They were then taken out of bijou bottle and counted through the addition of xylene (Dipeolu and Ogunji, 1980a) under the dissecting microscope.

The eclosion period was taken to be the period from the time of incubation to the time the first larva (e) emerged from the egg(s). The duration of hatching was considered to be the time it took all the eggs to hatch into larvae from the time the first larva (e) emerged to the time, after all the eggs have hatched into larva (e). Mortality was the number of eggs that failed to hatch. The eggs that failed to hatch within these periods were considered dead. The eclosion period, duration of hatching and number of hatched and unhatched eggs were recorded. This procedure was repeated for incubation temperature of 15°C, 20°C, 30°C and 37°C, all at 85% R.H.

All the data obtained were subjected to Statistical analyses – Chi-square test, Student's t test using the computer package SPSS version 1.1 2002.

RESULTS

Effect of Temperature on engorged adult females

Figs. 1-.3 show the effects of various temperature conditions on the pre-oviposition period, duration of oviposition and number of eggs laid respectively by *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi*. The pre-oviposition periods of engorged adult females of *R.sanguineus* and *H. leachi leachi* increased as the incubation temperature became low from 30°C to 15°C (Fig.1). There was a significant difference ($p<0.05$) in the pre-oviposition periods of *R. sanguineus*, and *H. leachi leachi* at all the maintenance temperatures.

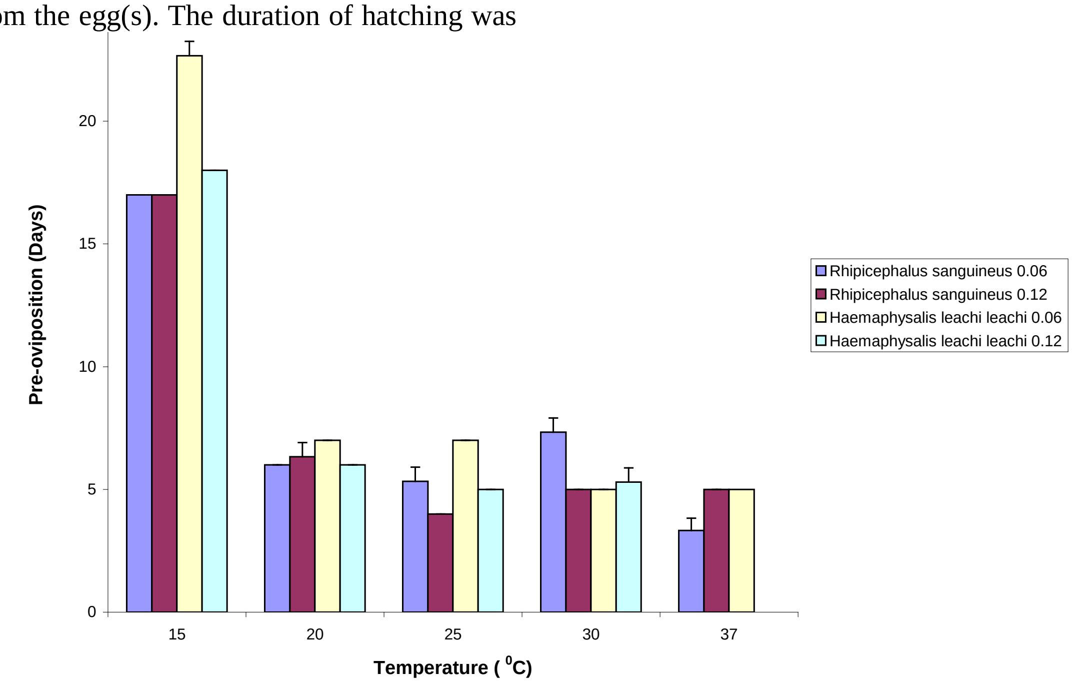

Figure 1:
Mean Pre-oviposition Period of Adult Females of the same weights of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* at different Temperature

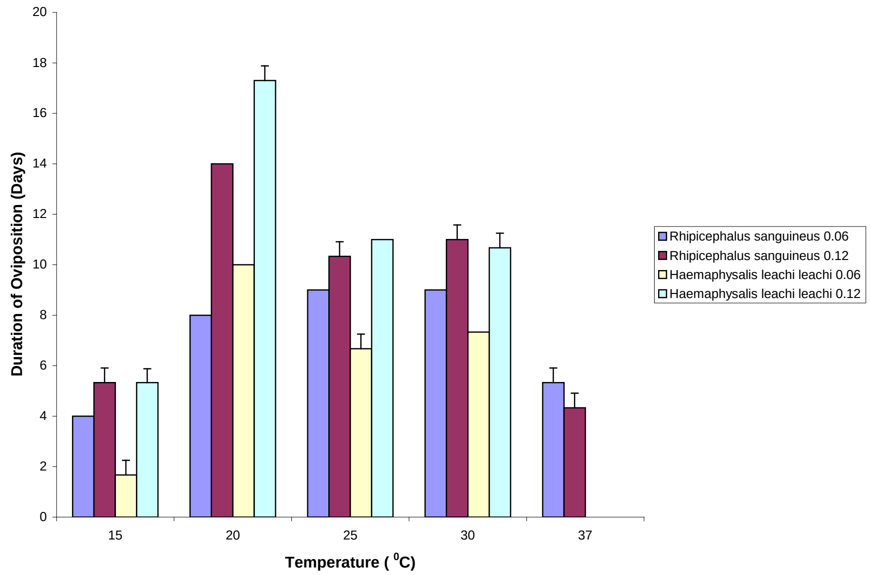

Figure 2:
Mean Duration of Oviposition of Adults Females of the same weights of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* at different Temperature

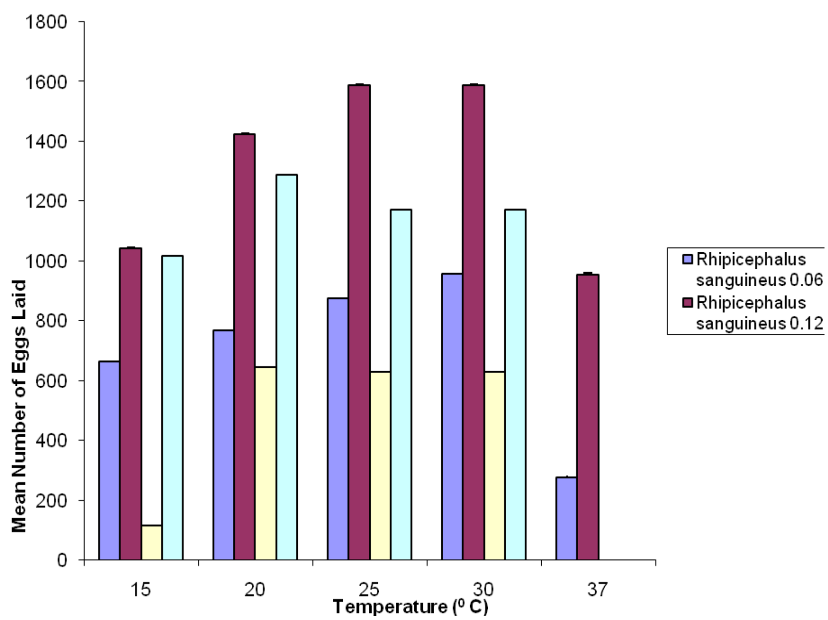

Figure 3:
Mean Number of Eggs Laid by Adult Females of the same weights of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* at different Temperature

The duration of oviposition at 15^0C was the shortest (4.00 ± 0.00 and 4.00 ± 0.55 for *R. sanguineus* and 1.67 ± 0.58 and 5.33 ± 0.58 for *H. leachi leachi*) compared with other temperatures (Fig. 2). The number of eggs laid by adult females of *R. sanguineus* and *H. leachi leachi* decreased as the incubation temperature dropped from 30^0C to 15^0C (Fig.3).

The mean number of eggs produced, respectively by 0.06gm and 0.12gm *R. sanguineus* ticks at 37^0C, were 278.00 ± 3.46 and 955.33 ± 4.90 while no eggs were laid by the same tick weights of *H. leachi leachi* at the same temperature. At 15^0C significant differences ($p < 0.05$) were observed in the duration of oviposition of both tick species (Figs. 1 and 2).

There was no significant difference (P>0.05) in the number of eggs laid by *R. sanguineus* and *H. leachi leachi* at 20°C, 25°C and 30°C maintenance temperatures, while a significant difference (P<0.05) was observed in the number of eggs laid by both tick species at 15°C.

Effect of temperature on eggs

Figs. 4- 6 show the effect of various temperature conditions on the hatching pattern and mortality rates of eggs of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi*. For both species, there was no hatching of eggs maintained at 15°C.

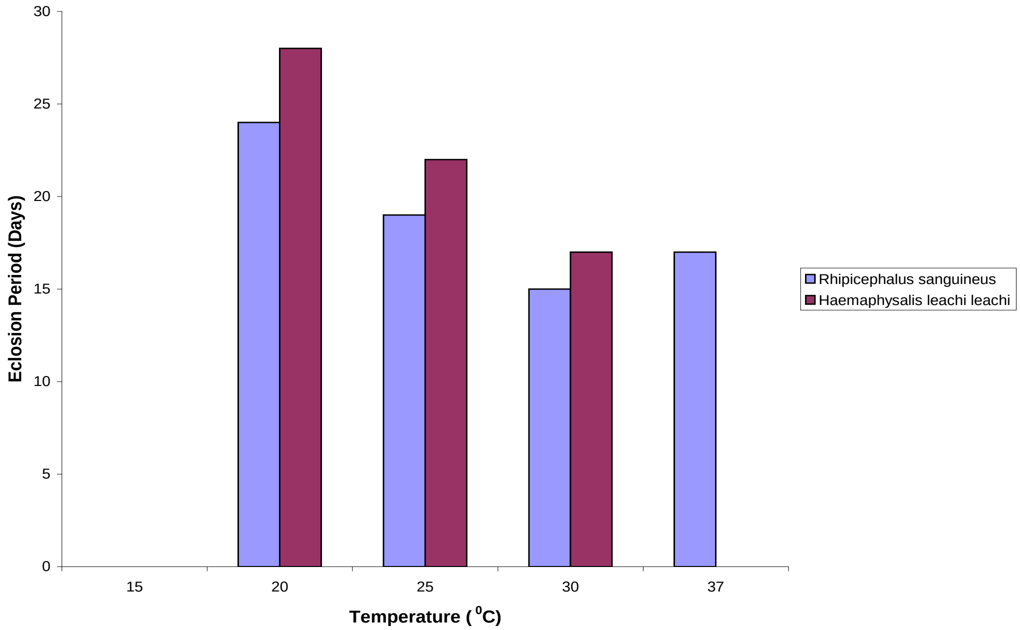

Figure 4:
The Eclosion Period of Eggs of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* at Different Temperature

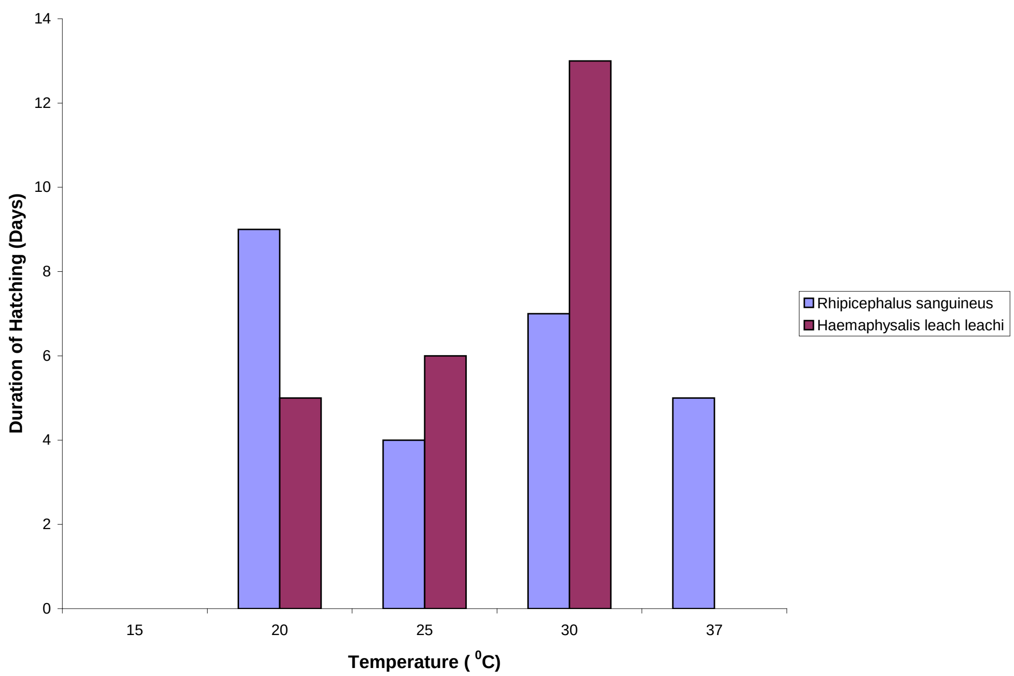

Figure 5:
The Duration of Hatching of Eggs of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* at different Temperature

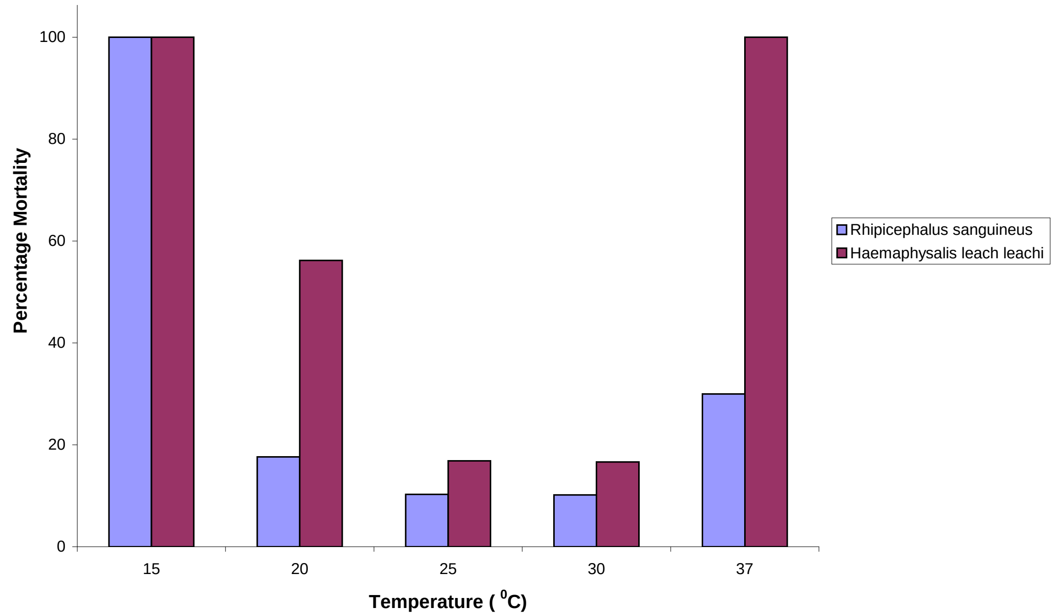

Figure 6:
The Mortality Rate of Eggs of Eggs of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* at different Temperature

The eggs of *H. leachi leachi* took longer time to eclode (28, 22, 17 days) at temperatures of 20^{0}C, 25^{0}C and 30^{0}C respectively than those of *R. sanguineus* (24, 19, 15 days) (Fig.4). At 37^{0}C, the eclosion period of *R. sanguineus* was 17 days, while the eggs of *H. leachi leachi* did not hatch (Fig. 4). The durations of hatching of the eggs of *H.leachi leachi*, (6 and 13 days) were significantly longer ($p < 0.05$) than those of *R. sanguineus*, (4 and 7 days), at 25^{0}C and 30^{0}C, respectively (Fig. 5).

The mortality rates of eggs of *H. leachi leachi* (56.2, 16.8, and 16.6%) were higher than those of *R. sanguineus* (17.6, 10.3, and 10.2%) at 20^{0}C, 25^{0}C and 30^{0}C maintenance temperatures respectively (Fig. 6). The mortality rates of both species were 100% at 15^{0}C. Also, 100% mortality rate was observed for eggs of *H. leachi leachi* at 37^{0}C (Fig.6).

DISCUSSION

The results of this study agree with the findings of previous workers (Dipeolu, 1985, Koch and Tuck 1986, Dipeolu 1991; Jacobs *et al*., 2004), who at various times reported that warm temperatures increased the number of eggs oviposited by ticks while high temperatures and excessive moisture decreased quantity of eggs laid. This means that for each tick specie there is a range of temperatures at which oviposition occurs with highest number of eggs laid and temperatures at which oviposition ceases.

The results of this investigation for *R. sanguineus* agree with the observations of (Dipeolu1984); Jacobs *et al*. (2004) who reported that oviposition was suppressed at below 15^{0}C but took place at 37^{0}C and that the optimum temperature for maximum oviposition was 30^{0}C. The results for *H. leachi leachi* were also in agreement with the observations of Dipeolu 1985 that oviposition did not occur at 37^{0}C and the highest number of eggs was laid at 27^{0}C. The increased pre-oviposition and duration of oviposition of engorged females of *R. sanguineus* and *H. leachi leachi* as temperatures decreased from 37^{0}C to 20^{0}C observed in this study is in agreement with the report of Inokunna *et al*. 1996 in their study of effect of temperature on the development of tick.

The results obtained from experiments on various temperature conditions gave an insight into what may be happening in the field. The high temperature of 37^{o}C which was detrimental to oviposition capacity of engorged female ticks and hatchability of their eggs can only be attained in the field during the hours of afternoon. For most of the morning, evening and whole night the temperature normally drops to as low as 20^{o}C – 22^{o}C and lower than 20^{o}C during the harmattan. It follows that the ticks could be exposed to fluctuating temperatures which are bound to be more variable than those used in this experiment (Enyenihi, 1972; Dipeolu, 1984).

A translation of the observations of this study to field situations indicates that each climatic condition is bound to have its own distinct effect on the biology of *R. sanguineus* and *H. leachi leachi*. Depending on the geographical zone, dry season temperatures in Nigeria fluctuates between 30^{o}C and 40^{o}C and lasts 3-9 months and raining season temperatures between 20^{o}C and 27^{o}C and lasts 6-9 months (Dipeolu and Ogunji 1977a; Dipeolu, 1991). During the dry spell only a proportion of engorged females of *R. sanguineus* and *H. leachi leachi* would be able to oviposit. In a similar manner, the cold spell of harmattan which lasts 2-4 months depending on the geographical zone, and during which temperature could be as low as 10^{o}C, would have an effect on the oviposition capabilities of engorged ticks as well as hatchability of eggs (Dipeolu and Ogunji, 1977a). From the results of this investigation, at 15^{o}C the eggs of both species did not hatch. It can therefore be said that the wet season will be the most favourable to these species of tick, because apart from moisture, temperatures between 20^{o}C and 30^{o}C are optimal. It also implied that *R.sanguineus* and *H. leachi leachi* could only establish in areas where temperatures are maintained between 20^{o}C and 30^{o}C.

The decreased egg output observed during oviposition by engorged females of *R. sanguineus* and *H. leachi leachi* at 15^{o}C and 37^{o}C could be explained by the effect of temperature on metabolic processes in tick egg production. It is possible that at 15^{o}C, the tick's metabolic processes had been retarded and that food digestion essential for egg laying may have been impaired, hence the ticks could not cope with the rate of egg laying. At 37^{o}C however, the metabolic rate in the ticks may have been too high, at least for *H. leachi leachi* that the ticks became injured permanently and could not oviposit. It is evident from this study that there are differences between these two species in their resistance to the deleterious effects of extreme temperatures. The tolerance of a wider range of temperatures by adult females and eggs of *R. sanguineus* coupled with the fact that they produce more eggs than *H. leachi leachi* as observed in this study probably explain why it is more preponderance compared with *H. leachi leachi* in Ibadan Southwestern Nigeria where this study was carried out. This however is being investigated in our laboratory. However, the two species of ticks would occur throughout the year. Hence, control measures against these ticks to be successful must be both tactical and strategic.

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