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Experimental studies on the reproductive biology of *Hyalomma truncatum* (Acari: Ixodidae) in Maiduguri, Nigeria

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Experimental studies were conducted between July and September, 2014 on the reproductive biology of *Hyalomma truncatum* in Maiduguri. Engorged adult female ticks were collected from the body of slaughtered cattle and morphologically identified to the species level. For further study, they were incubated in desiccators at 28°C and 85% relative humidity (RH) to determine their pre-oviposition and oviposition periods. The eggs collected from ovipositing females were counted under a stereoscopic microscope to obtain the mean daily and total egg counts as well as the average number of eggs/gram of weight of female and the percentage of body mass conversion. Batches of eggs laid were also incubated under similar laboratory conditions to determine their incubation periods. The results showed that the mean±SE values of pre-oviposition, oviposition and incubation periods for *H. truncatum* were 7.25±0.78, 10.40±1.37 and 19.90±0.97 days, respectively, while the mean±SE total egg count per head and average number of eggs per gram of weight of female *H. truncatum* were 5645.7 ± 939.14 and 8838.5±1204.1, respectively. A strong correlation was observed between pre-oviposition weight and mean egg count ($r = 0.637$, $p<0.05$), as well as between the convertible blood mass and mean egg count ($r = 0.779$, $p<0.05$). Also, female *H. truncatum* converted 53.3 to 74.3% of their total body mass (g) during oviposition. In conclusion, this study has revealed that the pre-oviposition weight of engorged female *H. truncatum* influenced their mean egg counts, oviposition pattern and efficiency of body mass conversion (%).

Key words: Biology, convertible blood mass, egg count, *Hyalomma truncatum*, Maiduguri, oviposition pattern.

INTRODUCTION

In Nigeria, 90% of the cattle population are raised under traditional pastoral husbandry system of Fulani herders in the northern region (Opara and Ezeh, 2011; Lorusso et al., 2013). Under this system, cattle are extensively grazed and become exposed to infestation with three important genera of Ixodidae; *Amblyomma*, *Hyalomma*

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and *Rhipicephalus*, including the sub-genus *Boophilus* (Opara and Ezeh, 2011; Obadiah and Shekaro, 2012; Lorusso et al., 2013). Ixodid ticks are currently recognized as the most economically important species of ecto-parasites infesting livestock in the tropics and sub-tropics (Soulsby, 1982; Lorusso et al., 2013). The direct effects of tick infestation in cattle leads to decreased productivity (Jongejan and Uilenberg, 2004; Lorusso et al., 2013). Additionally, they serve as vectors for pathogenic protozoa, virus, bacteria and rickettsia which constitute serious threats to livestock and man (Rajput et al., 2006). *Hyalomma marginatum* is the main vector of Crimean-Congo haemorrhagic fever virus, a widespread deadly disease of man (Geevarghese and Dhanda, 1987; Hassan, 2001). Several *Hyalomma* spp. are also incriminated in the transmission cycle of babesiosis, theileriosis and anaplasmosis to livestock in the tropics and subtropics (Soulsby, 1982).

The life cycle of Ixodid ticks is generally influenced by a combination of intrinsic and extrinsic factors (Shah-Fischer and Say, 1989). Temperature and humidity are recognized as the most important extrinsic factors affecting the life cycle of *Ixodidae* (Khalil and Hagras, 1988; Shoukry et al., 2000; Durrani et al., 2008), and they result from simultaneous environmental factors such as latitude, altitude, sunlight, rainfall and local wind patterns (Shah-Fischer and Say, 1989). The duration of egg incubation, female pre-oviposition and oviposition periods are all influenced by temperature, humidity and moisture conditions (Knight et al., 1978; Dipeolu, 1983; Khalil and Hagras, 1988; Shah-Fischer and Say, 1989; Shoukry et al., 2000; Durrani et al., 2008).

Several authors working in different geographical regions of the world have published data on the reproductive biology of various species of *Hyalomma* (Dipeolu, 1983; Ammah-Attoh, 1984; Khalil and Hagras, 1988; Shoukry et al., 2000; Durrani and Shakoori, 2009), but to date, there is no report on reproductive biology of *Hyalomma* species infesting livestock in the semi-arid zone of North-eastern Nigeria, hence the need for the experimental study.

MATERIALS AND METHODS

Study design

Experimental studies were conducted from July to September 2014 to determine some developmental parameters and ovipositional behaviour of *Hyalomma truncatum* at 28°C and 85% relative humidity. The period of this study corresponds to peak rainfall and humidity in Maiduguri, and is also known to favour bionomics of Ixodid ticks under natural conditions in Nigeria (Dipeolu, 1983).

Tick collection and identification

Engorged female ticks were collected by forceps from cattle during slaughter at the Maiduguri central abattoir and taken to the Veterinary Parasitology Postgraduate Research Laboratory, University of Maiduguri, where morphological identification was carried out to the species level with the aid of a stereoscopic microscope (MOTIC SM2-140 SERIES). *Hyalomma* species were recognized by their large sizes (5 to 6 mm), brown scutum and conscutum, striated integuments, and the presence of festoons in both sexes. *H. truncatum* was distinguished by the presence of smooth, shiny and dark conscutum bearing a large depressed area in the caudal part of males (Walker et al., 2003).

Determination of developmental parameters and egg counts

The first experiment was conducted to determine pre-oviposition period, oviposition period, daily mean egg counts and total mean egg counts of *H. truncatum*. Individual ticks were weighed using a Metra® Precision Electronic Balance (Model JA103P) and placed into separate test tubes (Pyrex®:15 x 125 mm) loosely capped with cotton wool. The test tubes were set up in vertical positions on a perforated plastic rack in a desiccator maintained at 28°C and 85% RH for the commencement of egg laying (Durrani et al., 2008). The eggs laid by female ticks were collected at 10:00 h daily and counted under a stereoscopic microscope (MOTIC SM2-140 SERIES). Counting was aided by drawing vertical lines on a Petri dish to serve as a counting chamber. The daily batches of eggs collected were incubated under similar conditions like the first experiment to determine their incubation periods (Durrani and Shakoori, 2009).

Determination of oviposition patterns

To determine the oviposition patterns of engorged female *H. truncatum*, ovipositing females under experimental conditions were divided into three groups based on their pre-oviposition weights. Thus, females weighing 0.1-0.4, 0.5-0.8 and 0.9-1.1 g were categorized as groups I, II and III, respectively. Daily mean egg counts of each group was calculated throughout the oviposition period and plotted on a line graph to show the oviposition patterns.

Determination of egg laying efficiency

The body mass conversion (%) through oviposition was calculated using the formula of Dipeolu et al. (1991) viz:

Body mass conversion (%) = pre-oviposition weight - post-oviposition weight x 100

The number of eggs per gram live weight of ovipositing females was also calculated using the formula of Linthicum et al. (1991). No. of eggs/g = Total number of eggs laid by female ticks in cluster ÷ total weight of female ticks in the same cluster.

Statistical analyses

Data generated from the various experiments were summarized as mean±SEM. The relationships between pre-oviposition weight and weight loss through oviposition (convertible blood mass) on one hand and the number of eggs laid on the other hand, were estimated using one-way linear regression analysis model (Y=A+BC) (Freedman, 2005), and $p<0.05$ was considered significant.

RESULTS

The mean ± SEM values of some parameters on the

Table 1. Mean ± SEM values of some developmental biology parameters of *H. truncatum* under experimental conditions.

Parameter	Mean ± SEM (range)
Pre-oviposition weight (g)	0.61±0.04 (0.3-1.1)
Post-oviposition weight (g)	0.25±0.04 (0.1-0.60)
Pre-oviposition period (days)	7.25±0.78 (00-15.00)
Oviposition period (days)	10.40±1.37 (00-19.00)
Number of eggs/gram female weight	8838.5±1204.1 (00-17635)
Total egg count	5645.7±939.14 (00-14108)
Ova incubation period (days)	19.90±0.97 (17.00-26.00)

g= gram, SEM = standard error of the mean.

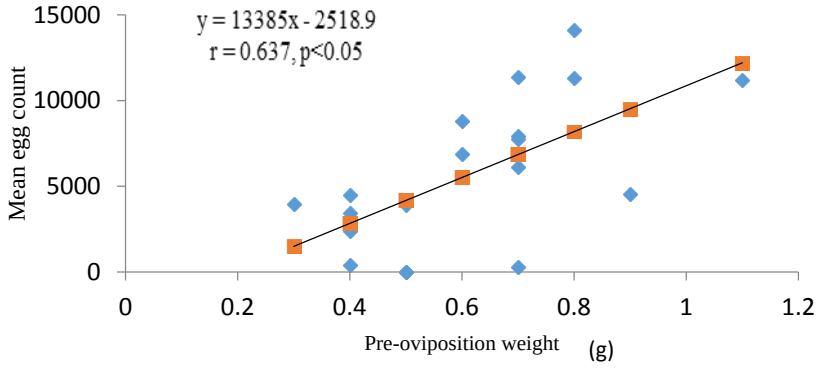

Figure 1. Correlation between pre-oviposition weight and mean egg count of *H. truncatum*.

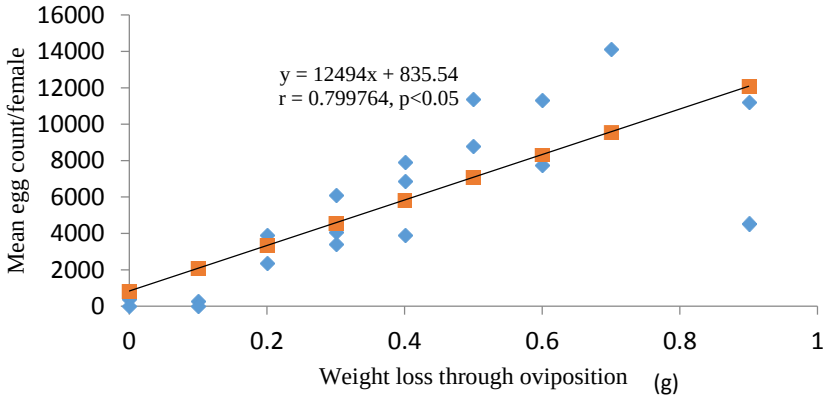

Figure 2. Correlation between convertible blood mass and mean egg count of *H. truncatum*.

biology of *H. truncatum* at 28°C and 85% RH are presented in Table 1. The mean±SEM values for pre-oviposition, oviposition and ova incubation periods of engorged female *H. truncatum* recorded in this study were 7.25±0.78, 10.40±1.37 and 19.90±0.97 days, respectively, while the mean egg/gram of engorged female weight and total egg counts were 8838.5±1204.1 and 5645.7±939.14, respectively.

A strong correlation (r = 0.637, p<0.05) was observed between pre-oviposition weight and mean total egg count of engorged female *H. truncatum* (Figure 1), as well as between the convertible blood mass and their mean total egg count (r= 0.779764, p<0.05) (Figure 2).

The mean daily egg count of *H. truncatum* is presented in Figure 3. The daily egg count increased progressively within the first 5 days to attain a plateau on day 6,

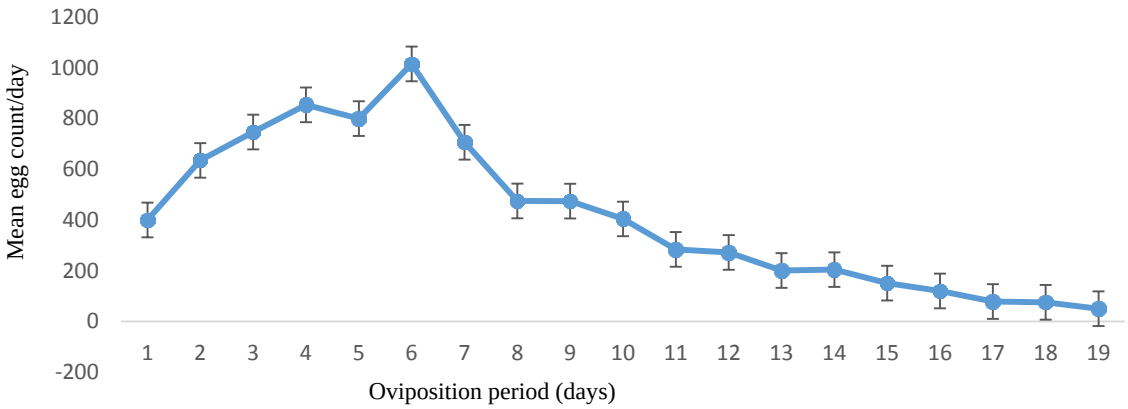

Figure 3. Total mean daily egg counts of *H. truncatum* under laboratory conditions.

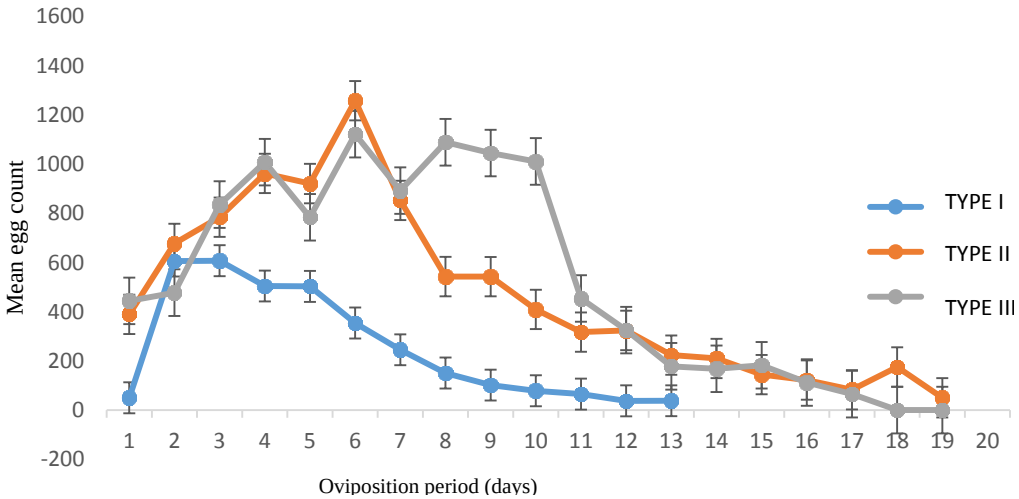

Figure 4. Oviposition patterns of three weight groups of *H. truncatum* under laboratory conditions.

followed by a regressive phase from day 7 forwards, with the lowest counts recorded on day 19. The three patterns of oviposition observed in female *H. truncatum* of different weights are presented in Figure 4. Type I was observed in group I ticks and is characterized by an initial high onset, and the early attainment of peak egg laying on day 3, with a short oviposition period of 13 days. By contrast, type II oviposition pattern observed in group II ticks was characterized by an initial low onset of egg laying, attainment of peak oviposition on day 6, and had a longer oviposition period of 19 days. Type III pattern was identified in group III ticks, and had a similar pattern of onset as group II, attaining peak egg laying on day 6, but having a lower mean egg count on the day of peak oviposition.

The efficiency of body mass conversion (%) through oviposition by three weight groups of female *H. truncatum* is presented in Figure 5. Ticks weighing 0.1-0.4, 0.5-0.8 and 0.9-1.2 g converted an average 53.3, 57.2 and 74.3% of their total body mass during oviposition, respectively.

DISCUSSION

The duration of developmental periods of *H. truncatum* recorded in this study are comparable with previous reports. The pre-oviposition period of *H. truncatum* in this study was comparable with the earlier reports of Knight et al. (1978). The mean oviposition period recorded in this study is also comparable with previous reports elsewhere for various *Hyalomma* species by Ammah-Attoh (1984), Khalil and Hagras (1988) and Durrani and Shakoori (2009). However, our pre-oviposition period does not agree with Linthicum et al. (1991), Al-asgah (1992) and Chen et al. (2011) who reported 11.9±0.8, 10.6±0.56 and

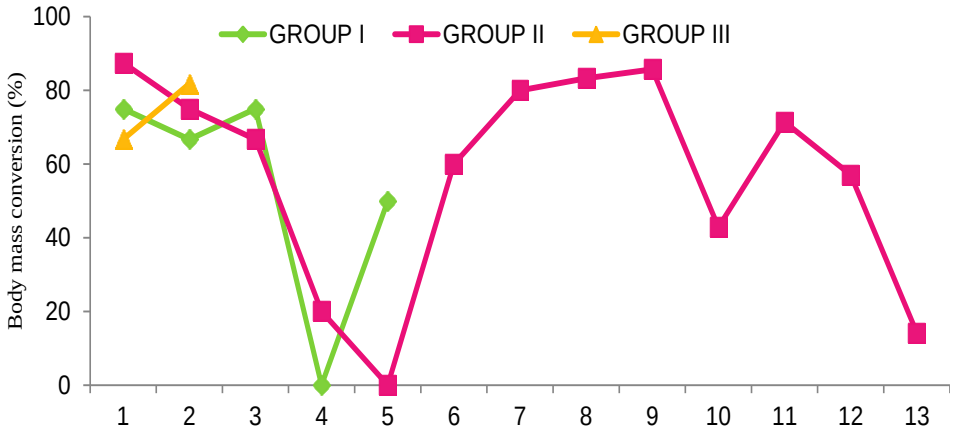

Figure 5. Percentage body mass (g) conversion by three weight groups of *H. truncatum* under laboratory conditions.

12.2±2.05 days, respectively. Similarly, the oviposition period recorded in this study differs from previous reports by Soulsby (1982), Dipeolu (1983) and Chen et al. (2011) who reported 37-59, 15 and 39.7±1.59 days, respectively. Furthermore, the results also disagreed with Shoukry et al. (2000) who reported a higher mean oviposition period (20.96±0.42) in *Hyalomma schulzei* at 28°C. The variations observed from these different studies may be associated with differences in laboratory conditions, especially temperature and humidity under which the ticks were reared. Temperature is widely recognized as the most important extrinsic factor that regulates the bionomics of Ixodid ticks (Soulsby, 1982; Khalil and Hagras, 1988; Shah-Fischer and Say, 1989; Dipeolu et al., 1991; Durrani et al., 2008). Other extrinsic factors known to affect the bionomics of Ixodid ticks include photoperiodic regime (light: day), rainfall and vegetation (Ouhelli et al., 1982; Shah-Fischer and Say, 1989; Adejinmi and Akinboade, 2012).

The mean ova incubation period of *H. truncatum* recorded in this study is not comparable with previous reports on various species of *Hyalomma* by Knight et al. (1978) who reported a mean ova incubation period of 29.3±2.96 for *Hyalomma marginatum*. Soulsby (1982) reported ova incubation period of 34-66 days for the genus *Hyalomma*. Ammah-Attoh (1984) reported a mean ova incubation period of 33 (18-50) days for *H. marginatum rufipes* in Nigeria. Shoukry et al. (2000) reported a mean ova incubation period of 29.3±2.96 days for *Hyalomma schulzei* in Egypt, while Durrani and Shakoori (2009) also reported a mean incubation period of 15 (10-20) days. The observed differences could be attributed to variations in temperature, humidity and other important ecological conditions under which the different studies were conducted. However, the result of this study is comparable with that of Biu et al., (2012) who reported a mean incubation period of 20.60±3.04 (8-12) for *Rhipicephalus sanguineus* in Maiduguri. The total mean egg count recorded in this study falls within the expected normal range of 2,000 to 20,000 eggs in a single batch for female hard ticks (Soulsby, 1982; Walker et al., 2003). However, the total mean egg count in this study is lower than previously reported by Knight et al. (1978) for *H. marginatum*, and those of Linthicum et al. (1991) who reported a mean total egg count of 6701 for *H. truncatum*, Al-asgah (1992) who reported a total mean egg count of 10259±728 for *Hyalomma schulzei*, but was higher than that reported by Ammah-Attoh (1984) with a mean egg output of 4899 (114-1038) for *H. marginatum rufipes* in Nigeria. The discrepancy in findings may be associated with species differences in fecundity (Shah-Fischer and Say, 1989). Moreover, these studies were conducted under different experimental and ecological conditions which have been reported to regulate the fecundity and rate of development of hard ticks (Soulsby, 1982; Shah-Fischer and Say, 1989; Durrani et al., 2009), and could account for the observed differences in mean egg counts recorded in these studies.

The strong positive correlation observed between pre-oviposition weight of engorged female *Hyalomma* and their total mean egg count in this study were comparable with the findings of Dipeolu et al. (1991) who reported a direct relationship between egg production and weight of engorged *Amblyomma variegatum* females. This finding also was comparable with the reports of Shoukry et al. (2000) who reported a positive correlation between weight of replete *H. schulzei* females and the number of eggs laid. Thus, irrespective of species, the pre-oviposition weight of engorged females significantly contributes to the number of eggs produced by Ixodid ticks. In the other side, the weight of female ticks was reported to be proportional to their degree of engorgement and determines their fecundity (Soulsby, 1982; Dipeolu et al., 1991). The most important intrinsic

factor influencing propagation of a hard tick is the degree of engorgement of the females, which determines the pre-oviposition weight (Shah-Fischer and Say, 1989; Dipeolu et al., 1991). Furthermore, the strong correlation observed between weight loss during oviposition and total mean egg count of engorged female *H. truncatum* in this study was comparable with previous reports by Dipeolu et al. (1991) and Shoukry et al. (2000), and suggests that female ticks become depleted as they lay eggs, a significant part of the lost weight being converted into egg mass.

The average efficiency of body mass conversion (%) recorded in different weight groups of ovipositing females in this study falls within the range of values previously reported for some *Hyalomma* species elsewhere (Khalil and Hagras, 1988; Linthicum et al., 1991; Al-asgah, 1992), and the high body mass conversion (%) efficiency recorded in this study (53.3-74.3%) may suggest that the experimental conditions were favourable for oviposition. Moreover, the three different oviposition patterns observed in this study indicates that the oviposition behaviour of *H. truncatum* depends on their pre-oviposition weights. Also, this finding suggests that female *H. truncatum* with different pre-oviposition weights have different capacity to discharge eggs once in their life time. In the current study, there were two patterns of oviposition behavior between groups I and II-III. This agrees with Dipeolu et al. (1991) who observed two patterns of oviposition behaviour in two different weight groups of *Amblyomma variegatum*.

Conclusion

This study revealed that *H. truncatum* is a highly prolific species of Ixodidae, and represents a serious threat to livestock and control methods. Also, pre-oviposition weights of the engorged females influenced some bionomical parameters, including the mean egg counts, oviposition pattern and efficiency of body mass conversion (%).

RECOMMENDATION

For effective population control of *H. truncatum*, measures to reduce reproductive efficiency such as the use of effective repellents, anti-feedent compounds and the destruction of their habitats are recommended. For bionomical studies or bioassays involving adult *H. truncatum*, females weighing 0.5 to 0.8g are the most suitable. Studies are ongoing in our laboratory to evaluate the effects of some plants extracts on the reproductive efficiency of *H. truncatum* as potential candidates for population control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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