

Bionomics of ticks collected from Sinai Peninsula, Egypt

1. Biology of *Hyalomma schulzei* (Olenev) (Acari: Ixodidae) under laboratory conditions

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ABSTRACT

Hyalomma schulzei (Olenev) was reared on rabbits and held under laboratory conditions at 29°C, 75% RH, and 12:12 (L:D) photoperiod with high productivity. The completion of the life cycle required an average of 123.1 days. The weight of females increased 35-fold and males became one-third wider, as a result of feeding. Females converted 56.1% of engorged weight into eggs and produced 6888 eggs per gram of engorged body weight. The smallest egg weighed 44.4 µg and occurred on day 15 of oviposition. The largest egg weighed 233.3 µg and occurred on days 24 and 25 of oviposition. An inverse relationship between egg weight and rate of egg production was observed. A high correlation between the weight of females and the number and weight of eggs laid, and between the mean daily female weight loss during the oviposition period and the mean daily egg number and weight was observed. The effects of 21, 25, 29, and 34°C on developmental period were investigated at 75% Relative humidity. Egg incubation, nymphal premoult, and female preoviposition and oviposition periods were all prolonged with decreasing temperature, with egg incubation and the preoviposition periods being the most greatly affected. Larval nymphal and female feeding periods of this two-host tick were not affected by temperature.

KEYWORDS: *Hyalomma*, biology, Sinai, Egypt

INTRODUCTION

Ticks transmit a greater variety of infectious agents than do other arthropods (Hoogstraal 1985). Inquiries into the nature of tick biology may therefore contribute to the effective control of economically important tick species by rational biological manipulations and thus to less dependence on pesticides. The large camel tick *Hyalomma schulzei* (Olenev) has a restricted distribution extending from the Baluchistan province of Pakistan through Afghanistan, Iran, Iraq, Syria, Lebanon, Jordan, north Saudi Arabia and to its western limit, the Sinai Peninsula of Egypt (Hoogstraal & Tattchel 1985). Adult stages of the tick show a marked host preference for camels, whilst the immature infest burrowing rodents, hares, and hedgehogs (Hoogstraal *et al.* 1981; Diab *et al.* 1987).

Environmental temperature has a profound effect on oogenesis and oviposition of the tick (Londt 1977). It is the principal extrinsic factor that regulates the metabolic rate and influences both the efficiency of blood-meal utilization and the length of preoviposition and oviposition periods, as well as subsequent developmental rates and the viability of embryonating eggs (Diehl *et al.* 1982; Ouhelli & Bandy 1984; Koch & Tuck 1986).

In Egypt, *Hyalomma schulzei* has only been recorded from the Sinai Peninsula (Hoogstraal 1956; Shoukry *et al.* 1993). Very little is known about this species and as a consequence its histology, medical and veterinary relationships are incomplete. Indeed there has only been one previous study on the tick (Al-Asagh 1992) and this investigated its life cycle under one temperature degree.

The aim of this study is to determine the effects of different temperature ranges on the development duration of the tick. Several aspects of the tick's ovipositional biology together

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with weights and measurements of the tick were also included.

MATERIALS AND METHODS

Tick colonization: A colony of *H. schulzei* was established from an engorged female tick. These were fixed onto the shaved back of New Zealand rabbits whilst kept inside aluminum capsules (as described by Varma 1964) thus allowing them to feed. Except during feeding, all tick stages were kept inside sealed disecators containing saturated sodium chloride to obtain 75 ± 1 % relative humidity according to Winston & Bates (1960). The disecators were housed in an environmental chamber adjusted to $29 \pm 1^\circ\text{C}$ and 12:12 (L:D) photoperiod. The capsules were examined daily and engorged ticks were placed into glass vials (50 by 25 mm.) covered with cloth and secured with rubber bands.

Determination of body measurements and weight Measurements of unfed larvae were determined 10-20 days hatched and engorged nymph and females on the day of dropping from the host. Fed males detached, weighed and measured during the period of female drop-off.

Life cycle duration periods: The pre-feeding period of larvae and adults was determined by daily exposure of newly hatched larvae or molted ticks to a short period of human breath as described by Garris (1984) and Logan *et al.* (1989). To determine the pre-feeding periods, unfed larvae (1000) and adults male: females (10:10), were placed to feed on rabbits as described above. Ticks were monitored daily. Observations also enabled us to determine feeding and premoulting intervals. Observations were also made to determine preoviposition and oviposition periods. The minimum duration of the incubator period was determined by observing 46 egg masses and calculating the number of days from the start of oviposition until the first egg hatch. The duration of the life cycle was determined by calculating the mean period of minimum egg incubation, larval pre-feeding, larval-nymphal attachment, nymphal pre-moulting, female pre-feeding, female feeding and female preoviposition period.

Reproduction efficiency: The total number of eggs laid by females ($n=25$) per day was calculated as follows: the average weight of one egg from a sample of 50-80 eggs taken daily from each mass was determined and then the weight of each mass was divided into the average egg weight (Davey *et al.* 1980). The reproduction efficiency index (REI, number of eggs per gram of females) and the conversion efficiency index (CEI, gram eggs per gram females) were determined by the method of Drummond & Whetston (1970). The REI provided an evaluation of the number eggs a female was able to lay per gram of body, while the CEI measured the ability of the females to convert body weight into egg weight. The nutrient index, the real conversion index was determined as described by Bennett (1974) by the formula:

$$\text{Nutrient index} = \frac{\text{Weight of eggs}}{\text{Initial weight of engorged tick} - \text{Residual weight of tick}} \times 100$$

The residual weight of a tick is the weight of the female after oviposition had ceased.

The effect of temperatures on the developmental stages: Four groups of eggs pooled from 10 females were held in incubators at 21 ± 1 , 25 ± 1 , 29 ± 1 , $34 \pm 1^\circ\text{C}$ 75 % RH and 12:12 (L:D) photoperiod. All developmental stages were maintained before, during and after feeding on rabbits at the same temperatures at which the respective egg masses were held.

Statistical analyses: Statistical analyses were conducted using the MINITAB computer program (Version 10, 1994). The correlation coefficient (r) was determined to clarify the relationships between several ovipositional parameters. The relationship between female weight on one hand and number of eggs or egg weight of eggs laid on the other hand, was estimated using a one-way linear regression analysis model ($Y=A+BC$) for both disturbed and

undisturbed female groups. The students' test was used to compare the data of these groups.

RESULTS

The weights and measurements of different stages of *H. schulzei* (unfed larvae, fed nymphs and adults) reared at 29°C temperature, 75 % RH and 12:12 (L:D) photoperiod are presented in Table 1.

Table 1: Weights and measurements of *H. schulzei* reared at 29°C, 75 % RH, and 12: 12 (L: D) photoperiod

Tick stage	n	Measurements, mm (mean±se)			
		Weight, mg (Range)	Hypostome (Range)	Length (Range)	Width (Range)
Unfed larva	100	0.045 ± 0.001 (0.0458 – 0.0462)	0.14 ± 0.01 (0.12 - 0.15)	0.69 ± 0.0 (0.64 – 0.71)	0.55 ± 0.05 (0.54 – 0.59)
Engorged nymph	100	54.97 ± 1.32 (21.30 – 88.10)	0.34 ± 0.00 (0.29 – 0.40)	7.05 ± 0.12 (5.0 – 8.6)	5.6 ± 0.05 (5.0 – 6.2)
Unfed female	50	38.22 ± 0.75 (18.1 – 67.8)	0.95 ± 0.01 (0.8 – 1.2)	6.05 ± 0.001 (5.8 – 6.3)	4.7 ± 0.03 (4.2 – 4.9)
Unfed male	50	43.62 ± 0.02 (22.0 - 85.4)	0.93 ± 0.01 (0.8 – 1.1)	6.15 ± 0.05 (5.3 – 6.5)	4.66 ± 0.04 (3.7 – 5.0)
Engorged male	50	44.07 ± 0.02 (20.5 - 59.8)		7.18 ± 0.40 (5.8 – 9.0)	6.14 ± 0.34 (4.9 – 7.1)

Effect of temperature on *H. schulzei* life cycle duration (Table 2): The pre-attachment period of *H. schulzei* was 1.5, 1.2 and 1.3 days for larvae, males and females, respectively. The egg incubation and nymphal premoult periods were the parameters affected most by the change of temperatures and were prolonged by a decrease in temperature. Egg incubation and nymphal premoult increased 3.9, 2.9, 1.6 and 4.7, 3.8, 1.3 folds at 21°C, 25°C, and 29°C, respectively, than at 34°C. The percentage of moulting ranged from 88.9 - 90.6 % at the temperature degree tested. The preoviposition period was longer at lower temperatures, but at 29°C and 43°C it was almost the same. The oviposition period was also longer at 21°C and 25°C than at 29°C and 34°C. The attachment periods of larvae-nymph and adult females on rabbits held at the four temperatures did not differ, significantly. The larva-nymph attachment and female feeding periods occupied 7.2, 12.5, 20.9, 22.9 and 5.1, 6.1, 12.0 and 17.4% at 21°C, 25°C, 29°C and 34°C, respectively, from the whole tick life span. The life cycle of *H. schulzei* required a minimum of 306.14, 198.76, 120.31, and 94.79 days for completion at 20°C, 25°C, 29°C, and 34°C temperature regimes respectively. Thus, one to three generations per year could be reared under laboratory condition according the temperature degree used.

Table 2: The effects of temperature on the life cycle of *H. schulzei* reared on rabbits and held at 75 % RH, and 12:12 (L: D) photoperiod. (* Numbers of egg masses observed).

Tick stage	Period	n	Mean period (days± se) (Range)			
			21°C	25°C	28°C	34°C
Egg	Incubation	25*	72.29 ± 1.3 (67- 75)	53.12 ± 1.55 (51 – 60)	31.0 ± 0.20 (29 – 36)	18.48 ± 0.41 (14 – 22)
Larva-nymph	Attachment	150	22.15 ± 0.65 (21 – 28)	24.47 ± 0.53 (20 – 31)	24.42 ± 0.53 (21 – 28)	22.00 ± 0.60 (21 – 26)
Nymph	Premoult	150	85.08 ± 1.8 (78 – 90)	68.48 ± 1.4 (65 – 70)	22.79 ± 0.24 (16 – 30)	17.98 ± 0.51 (15 – 20)
Female	Feeding	50	15.60 ± 0.57 (14 – 17)	13.10 ± 0.24 (12 – 15)	14.52 ± 0.44 (12 – 22)	16.65 ± 0.70 (15 – 21)
	Preoviposition	50	72.33 ± 1.32 (68 – 78)	18.32 ± 0.39 (16 – 30)	10.72 ± 0.46 (6 – 16)	8.08 ± 0.25 (8 – 10)
	Oviposition	50	37.95 ± 2.20 (32 – 43)	26.29 ± 1.50 (20 – 32)	20.96 ± 0.42 (17 – 24)	18.86 ± 0.8 (18 – 21)

Reproduction efficiency: The average daily egg output through the oviposition period of *H. schulzei* reared at 29°C is illustrated in Figure 1. The mean daily egg output was 460.9 ± 70.5 . More than 96.5 % of the total number of eggs were produced during the first 17 days of oviposition. The number declined steadily after that day. Maximum egg production occurred on day 5 of oviposition (1188 eggs /female) (Fig.1). The smallest mean egg weight ($44.37\mu\text{g}/\text{egg}$) occurred on day 15 of oviposition, while the greatest mean egg weight ($233.33\mu\text{g}/\text{egg}$) occurred on days 24 and 25 of oviposition. A decrease in the mean weight of eggs oviposited during the period of high egg output and an increase in the mean weight of eggs oviposited during periods of lower egg output were observed. The inverse relationship between the daily mean number of eggs laid and mean egg weight through the oviposition period ($r = 0.55$, $P < 0.01$) is illustrated in Fig. 2. There was a significant correlation ($r = 0.45$, $P < 0.05$) between the total egg output and the maximum number of eggs laid in one day (Fig. 3). The relationship between the weight of females and the number and weight of eggs laid was highly correlated ($r = 0.868$, $P < 0.01$) ($r = 0.854$, $P < 0.01$) respectively (Figs. 4 & 5).

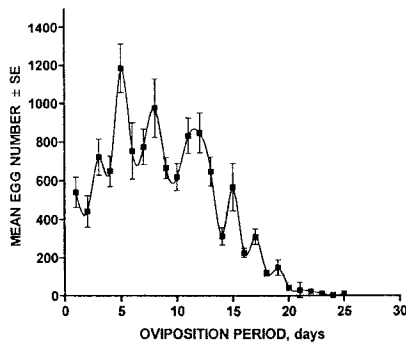


Fig. 1: Mean daily oviposition of *H. schulzei* at 29°C, 75% RH and 12:12 (L:D) photoperiod

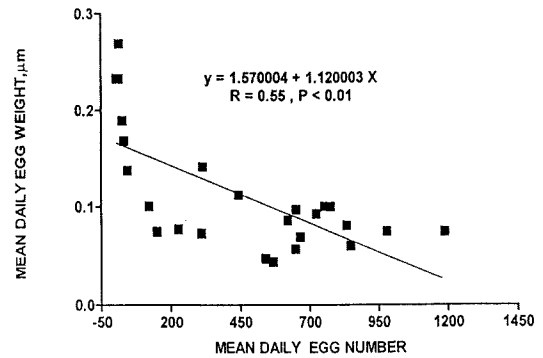


Fig. 2: The relationship between the number of eggs laid by 20 females of *H. schulzei* and egg weight.

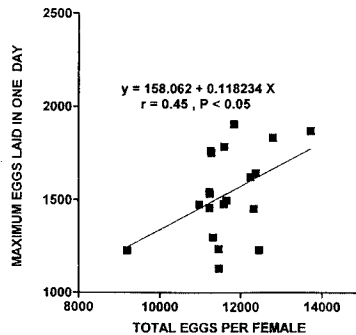


Fig. 3: The relationship between the number of eggs laid by *H. schulzei* in the day of maximum egg output and the total number of eggs laid.

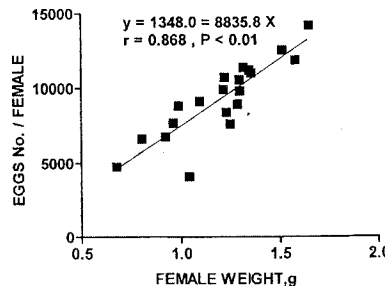


Fig. 4: The relationship between the weight of *H. schulzei* females and the number of eggs laid.

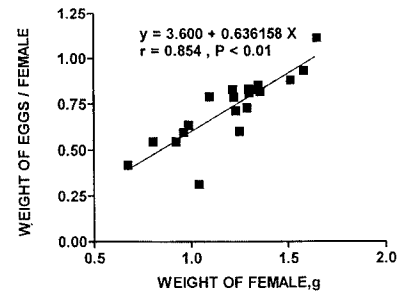


Fig. 5: The relationship between the weight of *H. schulzei* females and the weight of eggs laid.

The ovipositional biology parameters of *H. schulzei* recorded in the present study are shown in Table 3. There was a highly correlation between the mean daily female weight loss during the oviposition period and the mean daily egg number ($r = 0.75$, $P < 0.001$) and egg weight ($r = 0.84$, $P < 0.001$) produced (Figs. 6 and 7).

Table 3: The ovipositional biology parameters of *H. schulzei* reared on rabbits at 29°C, 75 % RH, and 12 : 12 (L : D) photoperiod.

Parameters	X ± SE (Range)
Engorged female weight/mg	$1,339.7 \pm 109$ (638 – 1,912)
Egg mass weight/mg	934.0 ± 0.06 (809.1 – 1,197.8)
Number eggs / female	12596 ± 1279 (9189 – 18252)
Egg weigh/mg	$0.077 \pm .008$ (0.049 – 0.099)
CEI (g. eggs / g. O)	56.12 ± 4.23 (42.68 – 70.49)
REI (no. eggs / g. O)	6888 ± 724 (5256 – 9428)
Residual female weight/mg	361.0 ± 15.6 (326.5 – 402.5)
Nutrient index	70.76 ± 4.14 (60.35 – 80.73)
Hatching %	96.21 ± 0.47 (93.80 – 98.70)

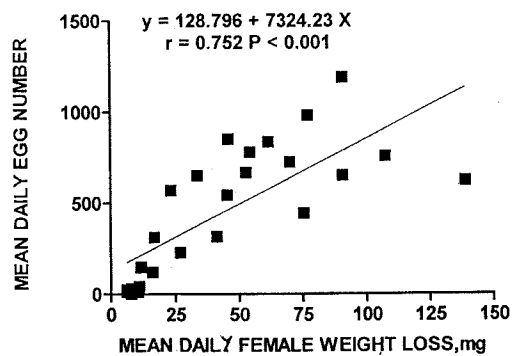


Fig. 6: The relationship between the daily female weight loss and the daily egg number laid.

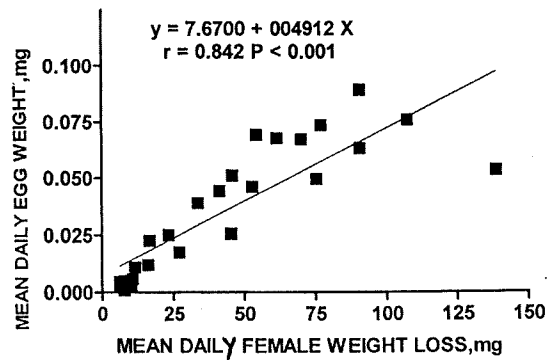


Fig. 7: The relationship between the daily female weight loss and the daily egg weight.

DISCUSSION

The present results indicate that *H. schulzei* behaves as a two-host tick, as some *Hyalomma* tick species, two to three generations could be obtained yearly under laboratory conditions according to the temperature used. The duration of the developmental periods of the different stages in the life cycle of *H. schulzei* observed in this study was higher than those reported by Hagra & Khalil (1988) for *H. dromedarri* and Khalil & Hagra (1988) for *H. impeltatum*. As in other ixodids, *H. schulzei* increased in weight, length, and width at each stage after feeding. In comparison with other *Hyalomma* the mean body length, width, and hypostome length in *H. schulzei* were longer than those of other species reported (*H. anaticum*, *H. impeltatum* and *H. truncatum*) and unfed males were wider than females, (Balashov 1972; Logan *et al.* 1989; Linthicum *et al.* 1991).

Of the periods off the host, egg incubation and adult preoviposition were the most prolonged by decrease in temperature in *H. schulzei*. This is similar to *H. dromedarri* (Hagra & Khalil 1988), *H. impeltatum*. (Khalil & Hagra 1988). The incubation period of *H. schulzei* was approximately comparable to that reported by Al-Asgah (1992) at 28°C for the same species and for *H. impeltatum* (Logan *et al.* 1989), but was longer 22.6 days than that reported for *H. rufipes* (Knight *et al.* 1978), 22.2 days for *H. dromedarii* (Hagra & Khalil 1988) and 21.2 days for *H. impeltatum* (Khalil & Hagra 1988). In other tick genera, Davey *et al.* (1980) reported 23.3 days for *Boophilus microples*, Mourad *et al.* (1982) reported 24 day for *B. annulatus*, Dipeolu (1984) reported 32 and 24 days for *B. decoloratus* and *B. geigy*, respectively, Hussein and Mustafa (1987) reported 40-42 and 20-26 days for *Rhipicephalus simus* and *Haemaphysalis spinulosa*, respectively.

The mean percentage of egg hatching observed in the present study was 96.21 %, which is comparable to that reported by Al-Asgah (1992) for the same species. Logan *et al.* (1989) reported 84.0 % for *H. impeltatum*, Linthicum *et al.* (1991) reported 48 % for *H. truncatum*. In other ixodids, Mourad *et al.* (1982) recorded 96.9 % for *B. annulatus*, Dipeolu (1984) recorded 91 % for *B. decoloratus* and 92 % for *B. geigy*, Garris (1984) recorded 61.5% for *Amblyomma vareigatum* (L.).

The mean larval prefeeding period recorded in this study (1.5 days) was slightly greater than those reported by Al-Asgsh (1992). On the other hand, Logan *et al.* (1989) and Linthicum *et al.* (1991) observed less than 1 day for *H. impeltatum* and *H. truncatum*. During the parasitic period, The feeding pattern of immature stages and adults was very similar to that of other two-host *Hyalomma* species. The larva-nymph attachment period of *H. schulzei* was longer on host than females. The larvae-nymph attachment period observed in this study is similar to that reported by Hagra & Khalil (1988) for *H. dromedarri* and Khalil & Hagra (1988) for *H. impeltatum*. The female feeding period of *H. schulzei* reported here at 29°C was similar to the report by Al-Asgah (1992) for the same species, and also comparable to *H.*

impeltatum (Khalil & Hagra 1988). Moreover, it was higher than that of *H. dromedarii* and *H. impeltatum* (Hagra & Khalil 1988; Logan *et al.* 1989). On the other hand, the feeding period of *H. schulzei* did not vary at any stage with change of temperature. In contrast, the feeding period of *H. aegyptium* (L.) on poikilotherms (leather) was longer at the lower temperatures (20-30°C); wheal larvae and nymphs died without feeding at temperature below 20°C.

Egg production and duration of oviposition of *H. schulzei* fall within the range for other *Hyalomma* tick species. Knight *et al.* (1978) reported a preoviposition period of 7.1 days and a total of 6,867 eggs per female *H. refipus*. Dipeolu (1983) observed a 7-day preoviposition period for *H. impeltatum* and 15-day. Hagra & Khalil (1988) reported 4.3 preoviposition and 12.4 days oviposition periods for *H. dromedarii*. Khalil & Hagra (1988) reported 4.2 days preoviposition and 15.6 days oviposition periods for *H. impeltatum*. Logan *et al.* (1989) reported 8.9 days for *H. impeltatum*. As in other ixodids, Rachev & Kinght (1983) reported a 6.0 days preoviposition and 26 days oviposition periods for *R. oculatus*. Egg production for *H. schulzei* reached a peak on day 5 of oviposition, this was followed by a steady decline when just a few eggs were laid by only 4 of 20 females. This finding compares favorably with Al-Asgah (1992) for the same species, and with those of other *Hyalomma* species e.g. Logan *et al.* (1989) and Linthicum *et al.* (1991) for *H. impeltatum* and *H. truncatum*, respectively. The same observation was also reported for other ixodids, *B. micoplus* (Bennet 1974); *B. annulatus* (Mourad *et al.* 1982); *B. decoloratus* (Londt 1977). No bimodal oviposition like that reported by Hitchcock (1955) with *B. micoplus*, was observed in the present study.

The correlation coefficient ($r = 0.679$, $P < 0.05$) observed between the number of eggs laid on the day of maximum egg output and the number of eggs laid (Fig. 3), emphasized that the oviposition period was independent of fertility. Whereas those females depositing larger number of eggs displayed sharper peak than less fertile females. The same observation reported for *H. anatolicum* and *H. aegyptium* (Snow & Arthur 1966; Sweatman 1968).

The highly positive correlation between the weight of *H. schulzei* replete females and number of eggs laid and weight was similar to that reported for other *Hyalomma* species e.g. *H. anatolicum* (Snow & Arthur 1966), *H. aegyptium* (Sweatman 1968) and *H. dromedarii* (Bassal & Hefnawy 1972). The same findings have been observed in other ixodid tick species including, *R. sanguineus* (Sweatman 1967); *Anocentor nitens* (Drummond *et al.* 1969); *B. microplus* (Bennet 1974; Davey *et al.* 1980); *B. decoloratus* (Londt 1977) and *B. annulatus* (Ouhelli *et al.* 1982).

The mean REI reported was higher than that reported by Al-Asgah (1992) for the same species, and also higher than those reported for *H. impeltatum* (Logan *et al.* 1989) and *H. truncatum* (Linthicum *et al.* 1991). *H. schulzei* females were found to be more efficient in converting blood meals into eggs (CEI = 56.1 %), this was comparable with that 57 % of Al-Asgah (1992) for the same species. Logan *et al.* (1989) reported CEI of 55 % for *H. impeltatum* and 56 % for *H. truncatum* (Linthicum *et al.* 1991). However, a higher CEI of 79 % was reported for *H. dromedarii* (Hagra & Khalil 1988) and of 72 % for *H. impeltatum* (Khalil & Hagra 1988). The CEI of other ixodids species reported was 62 % for *B. microplus* (Bennett 1974) and 55.6 % for *B. annulatus* (Davey *et al.* 1980).

The mean nutrient index observed for *H. schulzei* was 70.76 %, which was less than that observed for *B. annulatus* (Mourad *et al.* 1982). The index indicates a high efficiency of *H. schulzei* females. The mean daily female weight loss was highly significantly correlated with both number and weight oviposited during the oviposition period. This phenomenon means that female weight loss and reproduction efficiency shows the involvement of metabolic processes in egg production. Moreover, the data on weight of female ticks in relation to the weights and numbers of eggs they produced provide indices for predictability

of the reproductive potential of these ticks, a factor which can be of immense value in estimates involving field or laboratory samples of the ticks, and of course in control work.

In conclusion, *H. schulzei* could be reared successfully in the laboratory with two to three generations per year, the tick life cycle activity appears to continue in Sinai Peninsula during the year. Further investigations are needed in the histology, physiology and medical and veterinary importance of this poorly known tick species.

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الملخص العربي

المعايير الحيوية للقراد المجموع من سيناء - مصر

١- دراسات بيولوجية علي النوع (*Hyalomma schulzei* (Oleneu) (Acari: Ixodidae) تحت الظروف المعملية

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درست الخصائص البيولوجية في دورة حياة القراد *H. schulzei* والمتغذي علي أرنب في المعمل علي درجة حرارة ٢٩ و ٧٥ % رطوبة وفترة ضوئية (١٢ ضوء / ١٢ ظلام). ووجد أن دورة حياة القراد تكتمل في مدة ١٢٣,١ يوم ، كما وجد أن أوزان الأطوار المختلفة للقراد تزداد بالتغذية حيث تزداد الأنثى بمقدار ٣٥ ضعف عن وزن الحورية الغير مغذاة . كما أن الذكور المغذاة تزداد في العرض بمقدار الثلث عن الذكور الغير مغذاة وقد لوحظ أن معدل كفاءة الأنثى في تحويل وجبة الدم تبلغ ٥٦,٥ % من وزنها تضع خلالها ٦٨٨٨ بيضة لكل جرام في المتوسط . وكان اقل وزن للبيض ٤٤,٤ ميكروجرام في اليوم الخامس عشر من التبييض ، بينما وصل اعلي وزن للبيض ٢٣٣,٣ ميكروجرام وذلك في اليوم الرابع والعشرون والخامس والعشرون من التبييض . وقد لوحظ أن هناك علاقة بين وزن البيض وعدد البيض الموضوع خلال فترة التبييض ، كما وجدت علاقة ترابط موجبة بين وزن الأنثى ووزن البيض وعده الموضوع بالمقارنة بالفقد اليومي لوزن الإناث خلال تلك الفترة. كما أجريت أيضا دراسة تأثير درجات الحرارة المختلفة (٢١ ، ٢٥ ، ٢٩ ، ٣٤ م) ونسبة رطوبة ٧٥ % علي دورة حياة القراد حيث وجد أن فترة حضانة البيض وكذلك فترة انسلاخ الحوريات وفترة التبييض وفترة وضع البيض تزداد بانخفاض درجة الحرارة ، حيث كانت فترة حضانة البيض وفترة التبييض هي الأكثر تأثرا بانخفاض درجة الحرارة . كما وجد أن فترة تغذية اليرقات والحوريات والإناث للحيوان ثنائي العائل لا تتأثر بانخفاض في درجة الحرارة .