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Kakuda, Hiroyuki
Zoological Laboratory, Faculty of Agriculture, Kyushu University

Shiraishi, Satoshi
Zoological Laboratory, Faculty of Agriculture, Kyushu University

Uchida, Teruaki
Zoological Laboratory, Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/23945

出版情報：九州大学大学院農学研究院紀要. 35 (1/2), pp.17-26, 1990-12. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 35 (1・2), 17-26 (1990)

Seasonal Fluctuations of Populations and Effects of Temperatures on Development and Growth in the Tick, *Haemaphysalis flava*

Hiroyuki Kakuda, Satoshi Shiraishi* and Teru Aki Uchida

Zoological Laboratory, Faculty of Agriculture,
Kyushu University 46-06, Fukuoka 812, Japan

(Received May 15, 1990)

As a part of ecological studies on *Haemaphysalis flava,* the seasonal fluctuations of tick populations in the vicinity of Fukuoka City were investigated, and the species and the number of ticks on hares were checked in winter. Moreover, the effects of temperatures on development and growth in *H. flava* were analysed.

In the woodland, *H. flava* was found on vegetation throughout the year, but in the grassland no ticks were found in summer. *H. longicomis* disappeared from vegetation during the November-February period of hibernation. *H. flava* was dominant on the hares captured in winter. In *H. flava,* oviposition, hatching and moulting did not occur at temperatures of 15% and below, and the developmental zero was 12.3℃. The critical low temperatures for moulting of larvae and nymphs were 14.5 and 17.0℃, respectively. High temperatures exerted a harmful influence upon oviposition and hatching. The temperature required for *H. flava* to be active was lower than that for *H. longicornis,* but the lowest temperature for development and growth in *H. flava* was higher than in *H. longicornis,* and the upper limiting temperature in the former tick was lower than in the latter : thus, it was revealed that *H. flava* develops and grows within a narrower range of temperatures than does *H. longicornis.*

INTRODUCTION

Haemaphysalis flava is widely distributed in Japan and parasitises various kinds of mammals and birds. In the Kyushu district, the tick has been collected from such species of medium- and large-sized mammals as the Japanese hare, Japanese raccoon dog, dogs, the Tsushima yellow marten, Korean weasel, horses, the Japanese wild hog and cattle (Yamaguti *et al.,* 1971; Kakuda *et al.,* 1989). Notwithstanding its broad distribution, there have been only a few ecological studies on this tick in Niigata Prefecture (Saito, 1959), Chiba Pref. (Asanuma and Sakurai, 1958) and Saitama Pref. (Fujimoto *et al., 1986,* 1987 ; Fujimoto and Yamaguti, 1987a).

On the other hand, *H. flava* is known as a vector for tularemia (Asanuma *et al.,* 1956 ; Asanuma and Sakurai, 1958 ; Fujita et *al.,* 1985). Recently, epidemiology on such tick-borne diseases as Lyme disease (Kawabata *et al., 1987* ; Hashimoto *et al.,* 1989 ; Honma, 1989 ; Miyamoto *et al.,* 1990) and Japanese spotted fever (Mahara, 1987 ; Takada *et al.,* 1988 ; Mahara and Fujita, 1989) has been regarded as important in Japan. However, there have been few ecological studies, except for the cattle tick, *Haemaphysalis longicomis,* a known vector for theileriosis caused by *Theileria sergenti* (Namba, 1958 ; Yoshida, 1975, 1980 ; Chikaki, 1976 ; Shiraishi *et al.,* 1982, 1989 ; Ito et

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* To whom reprint requests should be addressed.

al., 1983, etc.). Therefore, the aim of the present study is to elucidate the life history of *H. flava* by field observations, to clarify the effects of temperatures on development and growth by laboratory experiments, and to discuss the physiological and ecological nature of this tick in comparison with that of *H. Zongicoynis.*

MATERIALS AND METHODS

Seasonal fluctuations of tick populations : The field investigation was conducted in Kasuya Forest of Kyushu University, Fukuoka Prefecture during the period from June 1987 to May 1988. One study area (grassland) was a pasture dominated by the bahiagrass, *Paspalum notatum* (altitude ca. 60 m) and grazing did not take place throughout this period. Another area (woodland at the same altitude as the pasture) was a mixed forest consisting of the Japanese red pine, *Pinus densiflora* and broad-leaved trees. The forest was shaded even in the daytime and its floor was densely covered with weeds. In both study areas, ticks were collected by the drag method with a flannel (1 x 1 m) in each habitat (ca. 200 m^2) once a month at 10 : 00-12 **: 00.** All the attached ticks on the flannel were counted.

Ticks from hares in winter : Ticks on five Japanese hares, *Lepus brachyurus brachyurus* captured in Kasuya District, Fukuoka Pref. from December 1988 to January 1989 were removed upon visual inspection.

Effects of temperatures on development and growth in *H. flava*: Unfed nymphs and adults collected by the drag methods, and laboratory-hatched larvae were fed on the rabbits to complete engorgement and detachment. The detached ticks, including all the stages, were placed in Petri dishes (diam. 6 cm) whose bottoms were covered with wet filter papers (ca. 100 % RH), and reared in dark incubators maintained at five constant temperatures (10, 15, 20, 25 and 30°C). The premoulting periods and moulting ratios of detached larvae and nymphs, and the preoviposition and oviposition periods and the number of eggs deposited per mg of body weight of detached females were examined. The incubation period and hatching ratio for eggs gathered from egg batches deposited by females placed at 25°C were analysed at seven different temperatures (10, 15, 20, 25, 27, 29, and 30°C).

RESULTS

Seasonal fluctuations of populations in two *Haemaphysalis* species in grassland and woodland

Only two species, *H. flava* and *H. longicornis* were collected in both study areas ; thus, seasonal fluctuations of their populations were separately described according to the species (Figs. 1 and 2).

1) *H. flava*

Fig. 1A and 1B indicate the seasonal fluctuations of *H. flava* populations in the grassland and woodland, respectively. No larva was collected in the grassland. In the woodland, however, a great number of larvae were found from August to October with a peak in August. The active period of nymphs in both study areas extended from autumn of 1987 to the next spring with few nymphs in summer. The nymphs in the grassland and woodland had similar bimodal fluctuations with two peaks in November and March, although those in the latter were more abundant than in the former. The adults showed a similar fluctuation pattern to the nymphal pattern in the grassland, whereas a very small number of adults were collected in the woodland without a bimodal fluctuation.

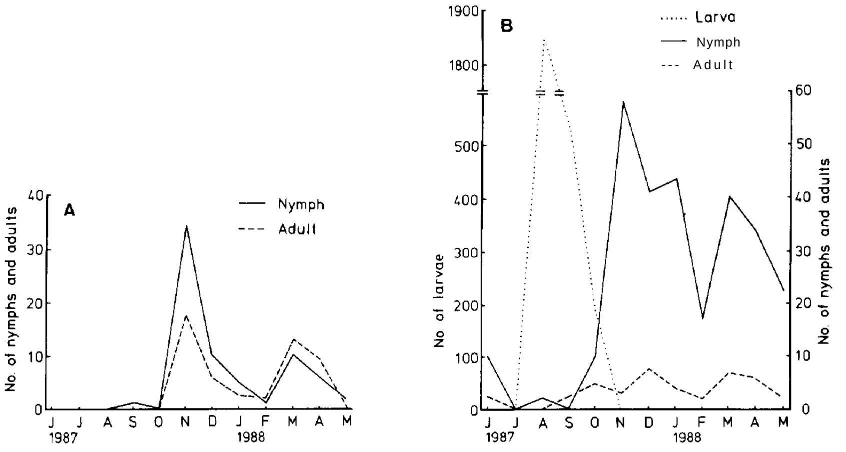

Fig. 1. Seasonal fluctuations of *Haemaphysalis flava* populations in the grassland (A) and woodland (R) in Kasuya Forest of Kyushu University, Fukuoka Prefecture, from June 1987 to May 1988. Note the absence of larvae in the grassland during the summer.

2) ***H.*** *longicornis*

The seasonal fluctuations of *H. longicornis* in the grassland and woodland are shown in Fig. 2A and 2B, respectively. Although the larvae were more abundant in the grassland than in the woodland, in both study areas their fluctuations had a unimodal pattern with a peak in September. In both areas, a large number of nymphs were collected in spring with a maximum in April ; a considerably high incidence was recognized also in June, but in the woodland there was no peak in September seen in the grassland. In both areas, the total number of adults was much smaller than that of the immatures, with a few adults in summer. Every developmental stage of *H. longicornis* disappeared from vegetation during the winter from November to February.

Ticks from hares in winter

A total of 2,043 ticks, consisting of *H. flava, H. kitaokai, Ixodes ovatus* and *I. nipponensis,* were collected from five Japanese hares captured during the hunting season (Table 1). *H. flava* was most abundant in number (1,952), occupying 95.5 % of all collected ticks : according to the developmental stages, the adults (1,088 ♂♀, en *bloc)* were most abundant (55.7 %), the nymphs were next abundant (39.1 %) and the larvae were least (5.2 %). Other ticks were much smaller in number than *H. flava,*

i. e. *H. kitaokai,* 1 ; *I. ovatus,* 9 and *I. nipponensis,* 81, all of which consisted of adults.

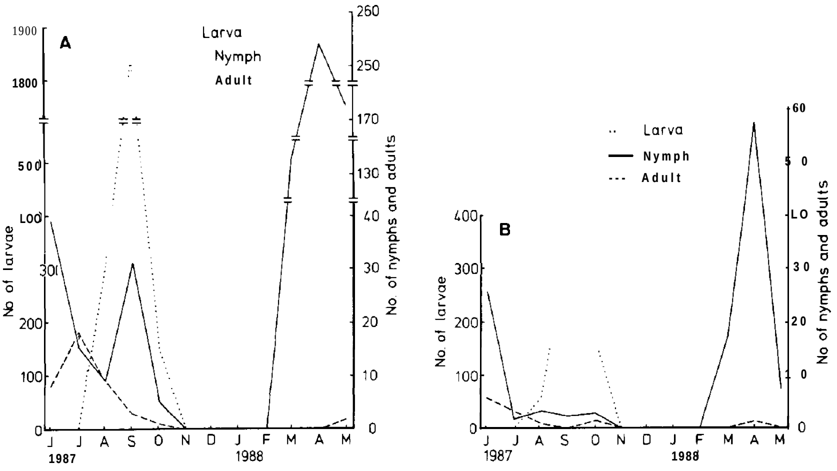

Fig. 2. Seasonal fluctuations of *Haemaphysalis longicornis* populations in the grassland (A) and woodland (B) in Kasuya Forest of Kyushu University, Fukuoka Prefecture, from June 1987 to May 1988. Note the absence of the tick during the winter in both the grassland and the woodland, which contrasts conspicuously with the fluctuation pattern of *H. flava* in Fig. 1.

Table 1. Ticks on five Japanese hares, *Lepus brachyurus brachyurus* captured in Kasuya District, Fukuoka Prefecture from December 1988 to January 1989.

Tick species	Developmental stage				Total
	L	N	♂	♀	
Haemaphysalis flava	101	763	897	191	1,952
H. kitaokai	0	0	1	0	1
Ixodes ovatus	0	0	2	7	9
I. nipponensis	0	0	37	44	81

L, larva ; N, nymph.; ♂, adult male ; ♀, adult female.

Effects of temperatures on development and growth in *H. flava*

1) Oviposition

As shown in Table 2, all females did not oviposit even 3 months after detachment at 10°C and 15°C. The preoviposition period was shortened from a mean of 26.0 days to 8.5 days as the temperatures rose from 20°C to 25°C, but it became longer (11.0 days) at 30°C than at 25°C. Thus, the critical low temperature for oviposition could not be calculated. The oviposition period was longest at 20°C (27.0 days on an average) and was 9.5 days at both 25°C and 30°C. The number of eggs deposited per mg of body weight was most abundant at 20°C (9.2) and was 6.7 at 25°C and 30°C.

Table 2. Oviposition ability at constant temperatures and 100%RH in detached females* of *Haemaphysalis flava.*

	Temperature (°C)				
	10	15	20	25	30
Preoviposition period (days)			26.0±11.63	8.5±0.58	11.0±4.83
Oviposition period (days)			27.0 i4.55	9.5±5.32	9.5t1.29
No. of eggs deposited/mg body weight			9.2±0.70	6.7k2.11	6.7k1.53

A symbol of - shows no oviposition even after 3 months.
Values are M±S. D.
*The number of females used is 4 specimens for each temperature.

2) Egg hatching

As given in Table 3, the incubation period was shortened as the temperatures became higher. However, no egg hatched even 3 months after deposition at the temperatures of 10°C and 15℃, and all eggs died at 30°C. The hatching ratio decreased with increased temperatures, being highest at 20°C (96.6%). The developmental zero was 12.3℃, which was theoretically calculated from a regression line (Y = -0 . 029+0.0023X, r=O. 97) of the inverse of the incubation periods Y on the temperatures X (20, 25, 27 and 29°C).

Table 3. Incubation period and hatching ratio at constant temperatures and 100% RH in *Haemaphysalis flava.*

	Temperature (°C)						
	10	15	20	25	27	29	30
No. of eggs examined	200	200	640	805	532	300	735
Incubation period (days)*			53.6zk6.96	37.8i1.96	27.7i2.71	25.8t4.72	
Hatching ratio(%) †	0 (0)	0 (0)	96.6 (618)	89.4 (720)	76.7 (408)	50.3 (151)	0 (0)

A symbol of - shows no hatching.
*Values are M±S. D.
†The numbers of eggs hatched are in parentheses.

3) Moulting

The premoulting periods and moulting ratios of the detached larvae and nymphs are shown in Table 4. The higher the temperatures, the shorter the premoulting periods of both stages, and the premoulting period took longer time in the nymphs than in the larvae at each temperature. At 10°C and 15℃, however, moulting did not occur in both stages even 3 months after detachment. High moulting ratios were recorded in both larvae and nymphs at 20, 25 and 30°C. The critical low temperature for larval moulting was 14.5℃, which was calculated from a regression line ($Y = -0.083 + 0.0057X$, $r = 0.77$) of the inverse of the premoulting periods Y on the temperatures X (20, 25 and 30°C). As for nymphal moulting, it was 17.0℃ ($Y = -0.083+0.0049X$, $r = 0.87$).

Table 4. Premoulting period and moulting ratio at constant temperatures and 100% RH in detached larvae and nymphs of *Haemaphysalis flava.*

		Temperature(°C)				
		10	15	20	25	30
Detached larvae	N	20	20	**35**	**112**	**38**
	Premoulting period (days) *			**24.1 t3.40**	**19.2k3.19**	10.4±1.87
	Moulting ratio(%)	0 (0)	0 (0)	**85.7** (30)	**94.6** (106)	**78.9** (30)
Detached nymphs	N	5	5	6	5	5
	Premoulting period (days) *			71.2±3.11	28.0±9.82	17.0± 4.32
	Moulting ratio(%)+	0 (0)	0 (0)	83.3 (5)	100 (5)	80.0 (4)

A symbol of shows no moulting even after 3 months.
*Values are M±S. D.
'The numbers of ticks moulted are in parentheses.

DISCUSSION

The life history and effects of temperatures on development and growth in *H. flava* are discussed in comparison with those of *H. longicornis.*

Life history of *H. flava*

The genus *Haemaphysalis* exhibits the three-host life cycle ; the larva, nymph and adult each feeds once on its respective host during the life cycle, and subsequently the detached larva and nymph moult to the nymph and adult, respectively, and the engorged female, which mated on the host, oviposits on the ground after detachment.

In the woodland, the abundance of *H. flava* larvae found during the summer with a great peak in August might be due to the newly hatched larvae from deposited eggs in spring. Such a larval unimodal activity has been reported also in Niigata Pref. (Saito, 1959), Chiba Pref. (Asanuma and Sakurai, 1958) and Saitama Pref. (Fujimoto et al., 1987). Most of these larvae appear to attach to hosts and to become the nymphs within the year. Although the larvae were not collected except for the period from August to October, they were collected from the Japanese hares captured near the study areas in winter. This implies that part of the larvae overwintered on hosts, as briefly described by Yoshida (1980) and Fujimoto *et al.* (1987). In the grassland, on the other hand, the absence of the larvae in summer seems to be attributed to an inhibiting influence of environmental factors (high temperature and low humidity) to which the eggs were exposed. In this connection, the egg development in *H. flava* is retarded by high temperatures (Fujimoto and Yamaguti, 1987b) and low humidities (Fujimoto, 1988), and all eggs did not hatch at 30°C (present study). Thus, the larval absence in the grassland during the summer might result from very high temperatures (over 30°C) of the ground surface exposed to the sunlight and consequent low humidities.

The nymphal and adult *H. flava* are collected from vegetation throughout the year with declines in number in both summer and winter (Fujimoto et al., 1987). In both areas of the present study, a small number of nymphs and adults in both summer (July-September) and winter (February) might be due to the inhibitions for the tick activity, especially climbing up vegetation by high and low temperatures in summer and winter, respectively. In this connection, the larva, nymph and/or adult were collected on vegetation throughout the year with a small number of nymphs and adults in summer in the woodland, whereas no tick was collected in summer in the grassland ; therefore, it was revealed that grassland is a basically unsuitable environment for *H. flava.*

The seasonal fluctuation of *H. flava* populations on wild animals can not be discussed in detail because of a few host mammals captured only in winter (the hunting season). Since numerous ticks parasitised the Japanese hare during this season, the hare seems to be a most important host in winter for *H. flava.* Taking the above into consideration, the suggested life history of *H. flava* is schematically illustrated (Fig. 3).

As for *H. longicornis,* in spite of the presence of numerous ticks in both study areas, the absence of ticks during the period from November to February is closely related to hibernation under the ground, as pointed out also by some authors (Yoshida, 1975, 1980 ; Ito *et al.*, 1983 ; Shiraishi *et al.*, 1989). In both areas, the nymphs emerging from hibernation exhibited a conspicuous peak, but the larva and adult had no peak in spring : this might result from larval death during the hibernation period as shown also by Yoshida (1975, 1980) and from a small number of adults.

Judging from the distinct difference in winter activity between the two tick species, it was suggested that the temperature permitting activity for *H. flava* was lower than that for *H. longicornis.*

Effects of temperatures on development and growth in *H. flava*

Oviposition by the female, egg hatching, larval and nymphal moulting did not occur at the temperatures of 15°C and below. The developmental zero was 12.3°C and the critical low temperatures for larval and nymphal moulting were 14.5 and

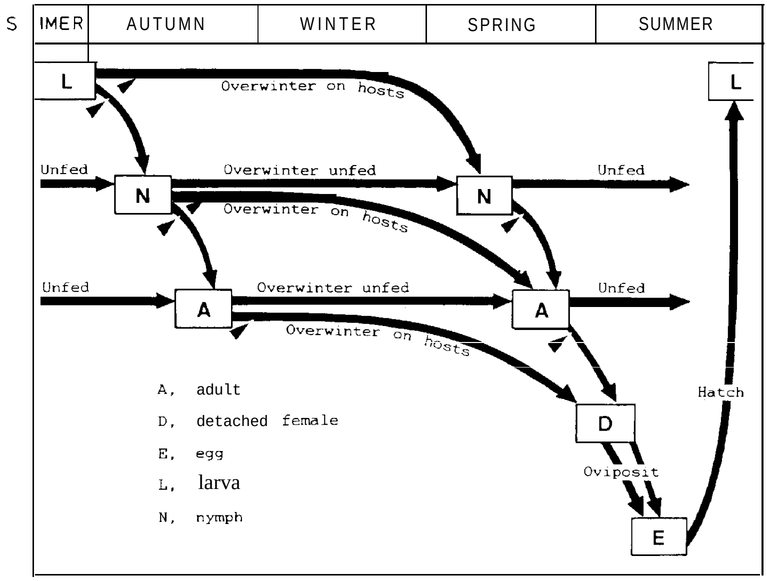

Fig. 3. Schematic diagram showing a suggested life history of *Haemaphysalis flava in* Kyushu. Arrow heads indicate ticks' attachment to hosts.

17.0℃, respectively. Regarding this, these values have been said to be 9.1, 8.9 and 13.3℃ (Fujimoto and Yamaguti, 1987b), all of wich are considerably lower than our values. Such disparities with regard to the results between the above two studies might be brought about by the geographic variation. On the other hand, the developmental zero for *H. longicornis* (bisexual race) has been said to be 10.7℃ (Shiraishi et *al.,*1982),12.2℃ (Yano *et al., 1987)* and 10.0℃ (Fujimoto and Yamaguti, 1987b), and the critical low temperature for moulting has been described to be 10.2℃ (Yano *et al.,* 1987) and 8.6℃ (Fujimoto and Yamaguti, 1987b) in larvae, and 11.8℃ (Yano et *al.,* 1987) and ca. 10°C (Fujimoto and Yamaguti, 1987b) in nymphs, all of which are lower than those for *H. flava* in this study.

The egg productivity at 25°C and 30°C was inferior to that at 20℃, and no hatching occurred at 30°C in *H. flava.* Such a harmful influence of high temperatures to oviposition and hatching agrees with the result of Fujimoto and Yamaguti (1987b). In contrast to *H. flava, H. longicornis* oviposits normally and the eggs hatch in a very high ratio at 30°C (Yano *et al., 1987* ; Fujimoto and Yamaguti, 1987b) with a low hatchability (39.8 %) even at 35°C (Heath, 1979).

Thus, it was revealed that the lowest temperature for normal development and growth in *H. flava* is higher than in *H. longicornis,* and the highest temperature in the former is lower than in the latter ; that is, it was suggested that *H. flava* develops and grows within a narrower range of temperatures than does *H. longicornis.*

The conclusion reached can be expressed as follows ; *H. flava* can be active even in the season at relatively low temperatures, but the development and growth is considerably restricted by both high and low temperatures. In contrast with this, *H.*

longicornis can develop in the broad range of temperatures. Such a disparity between the two species appears responsible for the scarcity of *H. flava,* i. e. the dominance of *H. longicornis* in pastures.

ACKNOWLEDGEMENTS

We thank Dr. K. Okano, Kyushu University Farm for facilities in collecting ticks, Mr. Y. Inoue for hunting hares and Professor E. W. Jameson, Jr., University of California for comments on the manuscript. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

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