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Haemaphysalis punctata (Canestrini and Fanzago, 1877) of Madeira Island II: Two contiguous environments at 560 m of height in the urban-rural area of Funchal county.

Torres de Almeida, V.C.

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Torres de Almeida, V.C.
Direcção Regional de Pecuária, Secretaria Regional do Ambiente e dos Recursos Naturais, Região Autónoma da Madeira, Portugal

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Abstract: Between September 1992 and August 1993 a sheepfold and a contiguous small pine forest, located at 560 m high in the Madeira Island southern slope, were studied on a monthly basis by the flag method. Except in summer, sheep were grazing in sheepfold. Pine forest where bared of sheep population. Cats, rats, mice, birds and dogs are the other relevant animals. *Haemaphysalis punctata* larvae were collected in July and August, nymphs between September and June, and adults from October to June. The summer larvae questing activity found was similar to the generally described in literature. The adults questing activity was alike to the generally accepted, with the exception of the non existence of peak in spring or autumn but a winter acme. With the available data it's acceptable, but not probable, even a life cycle shorter than 3 months, minimum pointed out in the bibliography. No significative differences were found in the monthly relative questing contributions between the sheepfold and the pine forest. This probably represents the absence of relevant ecological differences between the two environments in what concerns to the *H. punctata* needs.
Key Words: *Haemaphysalis punctata*, Ticks, Ecology, Life cycle, Madeira Island.

Resumen: El trabajo presenta el estudio realizado mensualmente, entre septiembre de 1992 y Agosto de 1993, en el pasto de un aprisco avino, así como en un pequeño y contiguo pinar, localizados a 550 m sobre el nivel del mar, en la vertiente sur de la Isla de Madeira. El Studio se realizó siguiendo el método de la bandera. Excepto en verano, las ovejas se localizaron siempre pastando en el aprisco antes mencionado. El pinar estaba desprovisto de población ovina, siendo gatos, ratas, ratones, pájaros y perros son las especias habituales en dicho hábitat. Cronobiológicamente, en Julio y Agosto se detectaron larvas de *Haemaphysalis punctata*, ninfas entre Septiembre e Junio, mientras que desde Octubre a Junio se pudieron detectar adultos. Las larvas no alimentadas mostraron una actividad similar a la descrita previamente en la literatura. Del mismo modo, la actividad de los adultos fue semejante a la generalmente aceptada, con la diferencia de no detectarse una máxima actividad en primavera u otoño, sino más bien en invierno. Con los datos disponibles puede considerarse aceptable, aunque poco probable, el desarrollo de un ciclo incluso más corto de 3 meses, mínimo denunciado en la literatura. Por otra parte, no se observan diferencias significativas en la actividad relativa mensual de individuos no alimentados, detectada en el aprisco y en el pinar. Esto probablemente demuestra una ausencia de diferencias ecológicas relevantes entre los dos ecosistemas en relación a las necesidades *H. punctata*.

1. Introduction

Although *Haemaphysaiis punctata* (Canestrini and Fanzago, 1877) may be generally admitted as a mesophyl and in some manner hydrophobic tick, hardly colonising moist (several authors cited by Arthur, 1963 and Feider, 1965; Aeschlimann et al., 1968); and rainy environments (Coty el al., 1986; Morel, 1965 referred by Gilot et al., 1989; Estrada-Peña et al., 1990), its geographical distribution suggests an enlarged set of adaptive capacities. Additionally it is also known the ability of this species to inhabit from cold (several authors cited by Arthur, 1963 and by Feider, 1965; Aeschlimann et al., 1968) to warm environments, if not excessive (Gilot, 1985). Being the life cycle probably in the dependence of the geographic area (Sevenet, 1937, cited by Feider, 1965; Feider, 1965; Garben ei al., 1981; Gilot, 1985; Estrada-Peña et al., 1990; Dias, 1994).

Nevertheless the ecology of *H. punctata* may be admitted as still insufficiently studied. Taking into account the British records, Arthur (1963) considers questionable its lack of capacity to colonise moist and rainy habitats. Also Gilot et al. (1989) show its ability to occur between 1,100 mm and 1,400 mm of rainfall, even the majority of the literature points out to much less extents rainfalls, lower than 700 mm (Coty et al., 1986; Morel, 1965, cited by Gilot et al., 1989, Estrada-Pefla et al., 1990).

Madeira Island has good conditions for the study of *H. punctata* ecology. It has several vertical climatic specificies, from a sub-tropical climate near by the sea level, to a cold, dry and very rainy climate at altitudes superior to 1,730 m, passing through a set of mild, moist and more or less deeply rainy conditions, with a sub-tropical insulation and light and dark day period ratio. In addition, the specie becomes visible from 100 m to 1,500 m approximately and in opposite to what's pointed out in the literature, it is very frequent, occurring in some places with large populations (Almeida, 1997). Established that's its ability to support well even the cold, and more moist and rainy habitats in Madeira island (Almeida, 1995, 1996; 1997) and adults and nymphs being continuously active below 738 m of height, but not above 1,150 m (Almeida, 1997), it was convenient to perform a more intensive study to make use of the islands good circumstances of study.

The *H. punctata* activity and life cycle in two contiguous environments at 560 m of height climatic conditions, 15°C of annual average ambient air temperature, 81% of moisture and 1,442 mm of annual rainfalls, regards to the aim of the present work.

2. Material and Methods

Between September 1992 and August 1993 ticks were collected on a monthly rate from two contiguous environments, a sheepfold, set up through an enclosure area covered by a carpet of herbaceous vegetation, and a small pine forest, also of soil covered by a carpet of herbaceous vegetation. These areas are located at 560 m of height in the urban-rural zone of the Funchal county near by residences.

In the last three days of each month the environment was sampled always three times a day, at the sunrise, with the sun vertically in the sky, and at sunset. A white flannel flag with 1m of surface was employed. The soil vegetation was dragged by the flag in all directions for five footsteps of similar ampleness. After that the ticks present on the flag were collected and placed in 70% alcohol without glycerine. The whole process was repeated fifteen times.

3. Results

H. punctata were found adults from October to July, in pine forest area from October to April, and nymphs from September to June, in pine forest area also from October to April. Those ticks were collected mainly in January and February. Larvae, qualitatively sampled, were found in July and August in sheepfold environment but in pine forest area only in August. Although more adults (c^2=393.3511; d.f.=1; p<0.000000, significant at level 0.01) and nymphs (c^2=0.56358; d.f.=l; p>0.452828, no significant at level 0.05) were collected in sheepfold than in pine forest environment no differences were found in monthly relative contributions (Adults, c^2=8.4621; d.f.=8; p<0.001. Nymphs, c^2=9.3138; d.f.=8; p<0.001). Nevertheless significative differences at level 0.01 were found in adults and nymphs ratio between pine forest and sheepfold (c^2=154.89; d.f.=l; p=0.0000).

The evolution of the total of adults and nymphs removed every month from the vegetation can be seen in Fig. 1. Allocating the natural numbers 1 to 5 to the months September to January and February to August the amount of ticks collected per month was adjusted to the equation y = A* e ^ (B*x). In what concerns to adults in the first period the data is well described (r = 0.99878). Variance explained: 99.755%. Standard error of the estimate of A = 0.312828; t (3) = 4.157469; p level= 0.025304. Standard error of the estimate of B = 0.04948; t (3) = 19.21418; p level= 0.00031) by the equation y = (1.3005714)* 2.71828^ 0.953716)*x]. To the second period the number of picked adult ticks is also well described (r = 0.97977). Variance explained: 95.995%. Standard error of the estimate of A=59.3813; t (5) = 4.9321; p level = 0.0044. Standard error of the estimate of B = 0.14647; t (5)= -4.79643; p level=0.00490) by the equality y= (292.8744)* 2.7 1828 ^ [(0,7025395)*x]. In what concerns to nymphs the data of the first period is not well described by an exponential equation. Nevertheless the collected ticks from February to August are quite well described by this relation (r = 0.99306). Variance explained: 98.617%. Standard error of the estimate of A = 25.48 58; t (5) = 6.3273; p level = 0.0015. Standard error of the estimate of B = 0.13519; t (5) = -8.00748; p level = 0.00049 by the equation y = (161.22827)* 2.71828 ^ [(-1.082498)*]

4. Discussion

The summer unfed larvae activity is similar to what is generally described for the different geographic areas.

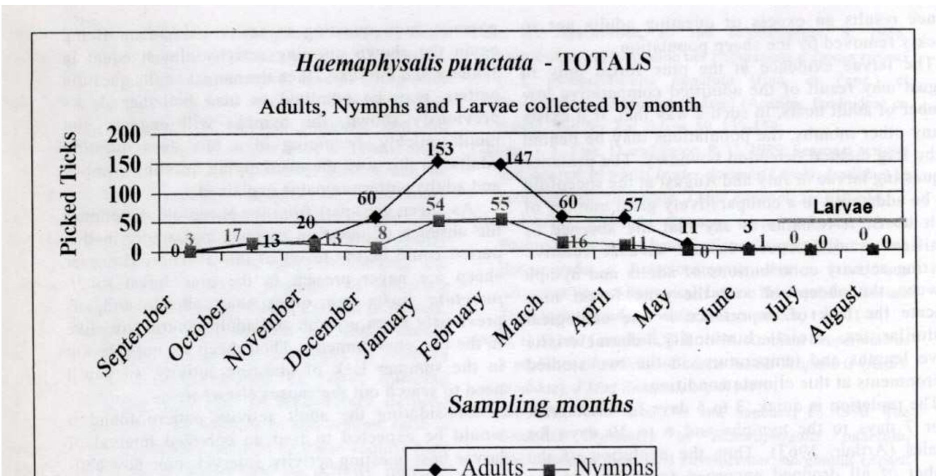

Fig. 1. Monthly evolution of the total adults, nymphs and larvae.

The unfed nymphs activity, with the acme found during winter time, is different from what is mostly referred in the bibliography, in summer (Feider, 1965) or from late spring to early fall (Stockman, 1908 cited by Arthur, 1963; Garben et al., 1981; Dias, 1994), with maximum in mid summer (Dias, 1994). Nevertheless in the north of Spain (Estrada-Peña et al., 1990) the feeding period prolongs itself for almost all the year, just interrupted in summer, whit the apex in September and October. The differences in winter activity between these two habitats, and by extension with Estrada-Peña et al. (1990) studied surrounds, can be explained by the differences in the environment air temperatures. in south slope at 560 m of height the average winter air temperature is 12.2°C, with average maximum and minimum temperatures of, respectively, 15.3°C and 9.3°C. Although the examined literature doesn't even mention any references of the existence of a motor incoordination temperature level in *Haemaphysalis* sp., in other genera it's well defined (McEnroe, 1977; Lee and Baust, 1987; Daniels et al., 1989; Duffy and Campbell, 1994; Clark, 1995). And in I. *scapularis* the motor incoordination level it's reached at 13.9°C in nymphs and at 9.2°C and 11.2°C in females and males, respectively (Clark, 1995). So with those temperature air conditions no any lack of activity by motor incoordination is expected, at least with the sun vertically in the sky.

Being the adults activity mainly not estival it is similar to what it's generally accepted (Sevenet, 1937, cited by Feider, 1965; Gilot, 1985; Estrada-Peña et al.,

1990; Dias, 1994), with the exception of the non existence of peak in spring or autumn but a winter acme.

The significative divergence in adults and nymphs questing activity ratio in the sheepfold, different from what would be expected even on the supposition .of inexistence of nymph mortality, and in the pine forest, theoretically acceptable, may be addressed to the differences in host population. In the sheepfold it's possible to find sheep, from early autumn to late spring, and silvicolous rabbits, rats and wild birds during all year long. In the pine forests there are no existence of sheep, but in addition to silvicolous rabbits, rats and wild birds, it's possible to find a little number of dogs and cats from the surrounding zone. As it's accepted, there are marked differences between adults and immature hosts. So, iii the area, sheep (Arthur, 1963), exceptionally dogs and in very rare occasions rabbits (Gilot, 1985) are expected to be the adult hosts, while birds, chiefly (Arthur, 1963; Gilot, 1985), and rabbits, but also sheep (Almeida, 1997) are awaited to be the hosts of the immature forms. At the pine forest the data can be interpreted as having any expected mortality in the evolution from nymphs to adults. it may be due to the lack of a convenient number of susceptible hosts, predation or by any expected failure in the feeding and moulting process. At the sheepfold the data interpretation isn't so easy but it may be that combined action of sheep, rabbits and birds quickly removes the questing nymphs, thence results an excess of questing adults not so quickly removed by the sheep population.

The larvae evidence at the pine forest only in August may result of the admitted comparative low number of adult hosts, in such a way that, if it exists in any other months, the populations may be behind of the flag method detection boundary. The presence of questing larvae in July and August at the sheepfold can be addressed to a comparatively great number of adult hosts. It remains to say that the absence of significative differences in the mensal relative questing activity contributions of adults and nymph between the sheepfold and the pine forest may indicate the lack of importance in the ecological dissimilarities, moist, luminosity, characteristic waive lengths and temperature, in the two studied environments at this climate conditions.

The repletion is quick, 3 to 5 days for the larvae, 4 for 7 days to the nymphs and 6 to 30 days for females (Arthur, 1963). Thus the evolution of the number of all dropped engorged forms should not differ significantly from what was found to the questing activity. The egg lying is also quick, beginning from 10 to 20 days after detachment (Arthur, 1963). Moreover the minimum life cycle span found in literature, at lower temperature conditions than the present ones, is of 3 months (Stockman, 1911, cited by Dias, 1994), what implicates quick ecdyses.

The number of nymphs collected between September and January do not adjust well to an exponential equation of positive coefficient. Nevertheless this fact should not be valorised considering the remaining equation adjustments, but rather addressed to the sampling hazards. So the addition of questing nymphs should be understood as an increase of hatching, resulting the observed value of the positive balance between the hatching and the passage to active parasitism. Similarly the diminution of questing nymphs, seen between February and July and well described by an exponential equation of negative coefficient, should be regarded as the negative balance between the hatching and the passage to active parasitism. Being the shown questing activity almost equal in January and February, then the annual nymphs questing pattern may be admitted as some how bell-shaped. Likewise, the well described by exponential equations of respectively positive and negative coefficient increase and decrease of questing adults, seen respectively from September to January and between February and July, should be seen as the positive and negative balance between the hatching and the passage from questing to active parasitism. Being again the shown questing activity almost equal in January and February, then the annual adults questing pattern may be admitted as also bell-shaped. As previously shown, the nymphs will engorge and moult quickly, becoming in a few days questing adults. In this way the overlapping questing nymphs and adults pattern remains explained.

As sheep are apart from the sheepfold in summer the absence of questing nymphs and adults in this period could be the result of this absence. However sheep are never present in the pine forest so *H. punctata* hosts are other than sheep, and, as previously seem, nymph and adult patterns are alike in the two environments. Then sheep are not relevant in the summer lack of questing activity, so you'll need to search out the causes elsewhere.

Considering the adult activity pattern found it would be expected to find an enlarged inter of larvae host questing activity. Interval some how bell-shaped, likewise to what was noticed to nymphs and adults, that could not be attested. It may be due to the lack of activity by motor incoordination only bypassed in summer, to hatch delay, that's described to exceed 200 days (Arthur, 1963), or just by sampling lottery. But considering the comparatively higher temperature conditions, the lack of activity by motor incoordination or by significant hatch delay is quite unlikely. The sampling lottery remains. It's quite possible that the exhibited summer larvae questing activity it may be nothing more than his moment of maximum. By hypothesis, in the months other than July and August eventually the larvae questing population may be below flag exhibiting capacity. Nevertheless, the addition of female host reaching and repletion times, eggs laying and embryonic developing times, required for the evolution from questing female to questing larvae, do not seem sufficient to displace the hypothetical maximum larvae questing activity to mid summer. They are 6 months. At sheep it's partially explainable by the sheep absence throughout summer and then, the July and August evidenced larvae activity, would be the result of the larvae accumulation as result of the lack of the main hosts. But the pine forest problem resulting of the apparent similarity of patterns remains.

Thus, assuming the constancy of the development pattern found, *H. punctata* data collected suggests mainly a one-year life cycle laid upon summer larvae activity limitation. Nevertheless and accepting an enlarged larvae questing activity, it's possible that, when the larvae, nymphs and adults questing activity curve overlaps, all life cycle completes itself in a short time, even less than the literature refereed 3 months. Therefore it's complex to interpret the life cycle with available data. Taking into account a hypothetical individual variability of thermal response and the proportionality among the temperature and the diurnal extent of activity, the larvae shown pattern can be eventually seen as mainly dependent to temperature. The same thing can be pointed out to the nymphs. But hardly the adults ecdyses and activity will be chiefly in the dependence of temperature considering the almost systematic summer absence of questing activity shown elsewhere in Madeira island (Almeida, 1977). Being *H. punctata* nymphs and adults highly associated in the questing environments in Madeira island barely a summery lack of adults questing activity may be addressed to temperature induced aestivation. In fact the adults area and volume ratio is smaller than the nymphs are and so those acquire heat slower. Then it's possible that its activity may be chiefly in the dependence of the solar incidence angle, night and day length ratio or any other ecological components related with radiation, but all of this needs further studies.

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Corresponding author:

Dr. Victor Carlos Torres de Almeida.

Direcção Regional de Pecuária, Secretaria Regional do Ambiente e dos Recursos Naturais, Avenida do Mar e das Comunidades Madeirenses, 23 – 2.º andar, 9000-054 Funchal, Madeira, Portugal