

NOTES ON THE LIFE-HISTORY OF THE RABBIT FLEA *CAENOPSYLLA LAPTEVI IBERA* BEAUCOURNU & MARQUEZ, 1987 (SIPHONAPTERA: CERATOPHYLLIDAE) IN EASTERN SPAIN

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Summary :

The rabbit flea *Caenopsylla laptevi ibera* occurs in arid environments of central and eastern Spain. Although the fleas breed during the coolest, most humid part of the year, the larvae survive and grow in sand at only 50-60 % relative humidity. At 22°C and 80 % relative humidity eggs hatch in six days and the cocoon stage is reached 10-11 days after hatching. Female fleas emerge from pupation at about 17 days after cocoon spinning; males emerge a little later at a mean of 20 days. Adult fleas are mainly found on the host *Oryctolagus cuniculus*. Measurements of burrow microclimate confirmed that in south-eastern Spain burrow humidity was adequate for the development of *C. l. ibera* larvae over most of the year. However, breeding may be restricted for at least part of the year, as the larvae of *C. l. ibera* apparently cannot complete development at 25°C or above. In the laboratory, fleas can enter a prolonged quiescent period while in the cocoon. This is possibly a facultative, pre-pupal diapause and the likely mechanism that accounts for the disappearance of adult fleas from the field by spring and their reappearance each autumn.

KEY WORDS : *Caenopsylla laptevi ibera*, flea, rabbit, Spain, life-history.

Résumé : NOTES SUR LE CYCLE DE VIE DE LA PUCE DE LAPIN *CAENOPSYLLA LAPTEVI IBERA* BEAUCOURNU & MARQUEZ, 1987 (SIPHONAPTERA: CERATOPHYLLIDAE) EN ESPAGNE ORIENTALE

La puce de lapin, *Caenopsylla laptevi ibera*, est présente dans la région aride de l'Espagne centrale et orientale. Bien que les puces se reproduisent pendant les mois les plus humides et les plus frais, les larves ne survivent et ne se développent, dans le sable, que pour des humidités relatives de 50 % à 60 %. A 22°C et à 80 % HR, les œufs éclosent au bout de six jours et le stade nymphal est atteint 10-11 jours après l'éclosion. La femelle émerge 17 jours après la formation du cocon, tandis que le mâle émerge un peu plus tard (20 jours en moyenne). Les adultes se trouvent, pour la plupart, sur l'hôte *Oryctolagus cuniculus*. Les mesures des microclimats effectuées à l'intérieur des terriers confirment qu'en Espagne du sud-est, les terriers sont assez humides pour le développement de *C. l. ibera*, quasiment toute l'année. Toutefois la reproduction peut être limitée, la larve ne pouvant pas finir son développement au-delà de 25°C. En laboratoire, la puce peut rester en quiescence, à l'intérieur du cocon, pendant une longue période. Il peut s'agir d'une diapause facultative au stade pré-pupe, ce qui expliquerait la disparition printanière des puces adultes et leur réapparition à chaque automne, en conditions naturelles.

MOTS CLÉS : *Caenopsylla laptevi ibera*, puce, lapin, Espagne, cycle de vie.

INTRODUCTION

Fleas of the genus *Caenopsylla* are found around the Mediterranean basin extending eastwards as far as Afghanistan. Some are parasites of rodents (Ctenodactylids) but *Caenopsylla laptevi* Mikulin & Zagniborodova, 1958 is a parasite of foxes in Egypt and Turkmenistan (Beaucornu, 1980). However, the closest relatives of this flea are parasites of the European rabbit, *Oryctolagus cuniculus* (L.), and these have been subdivided into subspecies *Caenopsylla laptevi relict*a Beaucornu, Gil-Collado & Gilot, 1975 and *Caenopsylla laptevi ibera* Beaucornu & Marquez, 1987. These subspecies may reflect the past separation of rabbits and their accompanying parasites into two

populations, possibly during periods of glaciation (Beaucornu, 1980). *C. l. relict*a is found in southeastern France (Bouches-du-Rhône and Vaucluse) whereas *C. l. ibera* occurs in eastern Spain. This latter subspecies is known from near Ciudad Real (Rodriguez *et al.*, 1981), Granada (Jiménez & Marquez, 1986), from Arpal near Zaragoza (Osácar, 1996) and from El Alquian in the Province of Almería (this study) as shown in Figure 1.

*C. l. relict*a and *C. l. ibera* are considered to be specific parasites of the European rabbit (Beaucornu, 1980). *C. l. ibera* can transmit myxomatosis in the laboratory (Cooke, unpubl.) and is probably involved in the field transmission of myxoma virus in central and eastern Spain, where it is relatively common (Osácar, 1996). In 1987 and 1988 *C. l. ibera* was evaluated for possible introduction into inland Australia to facilitate the biological control of the introduced European rabbit by spreading myxoma virus. However, another flea, *Xenopsylla cunicularis* Smit, 1957, also a specific parasite of the rabbit, was eventually chosen for that

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purpose (Cooke, 1990). *X. cunicularis* was subsequently imported into Australia and laboratory bred fleas were released to establish populations in arid areas where rabbits remained a problem because of competition with domestic livestock and destruction of natural ecosystems. Since then, *C. l. ibera* has been further studied (Osácar, 1996) for its potential to transmit both myxomatosis and Rabbit Haemorrhagic Disease (RHD). Myxomatosis spread through the wild rabbit population of France and Spain in 1952-1954 and the initial outbreaks in RHD of wild rabbits were seen in July 1988 (Rogers *et al.* 1994).

In Europe, where wild rabbits are part of the natural fauna, the biology of fleas has been considered as a step towards reducing the impact of myxomatosis. Launay & Chapuis (1984) sought efficient insecticides for dusting rabbit burrows to remove European rabbit fleas *Spilopsyllus cuniculi* (Dale, 1878). Furthermore, rabbit populations increased when insecticide treatment of burrows reduced the incidence of myxomatosis among wild rabbits on a British experimental site (Trout *et al.*, 1992). More recently, the idea of using laboratory-bred fleas to carry attenuated myxoma viruses, or even recombinant myxoma viruses containing some genetic material from Rabbit Haemorrhagic Disease virus (RHDV), has been suggested as a means of simultaneously immunising rabbits against both myxomatosis and RHD (Anon., 1998).

In Australia, attention has again turned to *C. l. ibera* because it is a vector with some unique characteristics that may enhance the efficacy of RHD outbreaks. It has recently been confirmed in South Australia that the occurrence of RHD in spring causes lower mortality than it does in autumn or winter. This is because it is mostly young rabbits that are affected in spring and some, infected while less than nine weeks old,

may recover (Morrisse *et al.*, 1991). On the other hand autumn or early winter outbreaks largely affect young adults and mortality is very high. Consequently, vectors are being re-evaluated to see whether their seasonal activity patterns could influence the impact of disease on wild rabbit populations.

Irrespective of the underlying interests in fleas as vectors of myxoma virus or RHDV, it is useful to begin with a broad understanding of flea biology. This paper summarises observations on the basic life history of *C. l. ibera* obtained from field observations and the breeding of fleas in the laboratory.

METHODS

FIELD STUDY SITE

The study site, 10 km east of El Alquian, Almeria Province, Spain (36°57' N, 2°24' W), was visited on four occasions during 1987 and 1988 to collect fleas and record data on the micro-climate of rabbit burrows. Fleas were also collected there in April 1991. The climate at El Alquian is arid; rainfall averages about 180 mm annually, and most rain falls during winter. The study site is a deeply dissected, rocky plateau; steep slopes and dry riverbeds provide a wide range of habitats from the point of view of insolation and drainage. The soils are loamy and are rich in calcium sulphate (gypsum) and other calcium and sodium salts. The flora of the site is a mixture of salt steppe and esparto steppe vegetation such as *Atriplex* spp. and *Salsola* spp. The stony hills have false esparto, *Lygeum sparto*, as the dominant vegetation. Some terraced hill-sides are irrigated from springs or by redirection of runoff water after rains to grow cereals, prickly pear (*Opuntia* sp.) or citrus and olive trees.

SAMPLING FOR FLEAS

Fleas were mainly sought to establish laboratory colonies, consequently they were not always collected using standardised methods (see Osácar, 1996) useful in providing information on their seasonal abundance. On some occasions fleas were collected from rabbits hunted with shotguns during late afternoon. Dead rabbits were immediately placed in strong plastic bags to prevent fleas being lost and later placed in a deep tray of white plastic for close examination. Fleas were collected in an aspirator as they left the rabbit or were combed from the fur using a fine flea comb. On other occasions, soil was scraped from the entrances of rabbit burrows using the method described by Launay (1982) and spread thinly in a white tray to be examined closely for fleas. Any fleas found were collected using the aspirator and taken to the laboratory in an insulated container kept at less than 10°C by adding ice.



Fig. 1. – Locations indicated by nearest major centre, where *C. l. ibera* is known to occur in eastern Spain.

In the laboratory, fleas were immobilised by dropping them onto the surface of a beaker of water and examined under a binocular microscope (30X) for sorting according to species and sex. In addition to *C. l. iberi* three other species of rabbit fleas were collected. These were mostly *S. cuniculi* and *X. cunicularis*, but *Odontopsyllus quirosi quirosi* (Gil-Collado, 1934) was also found on rare occasions.

BURROW MICROCLIMATE

The methods used have been described previously (Cooke, 1990) but briefly, soil temperature was measured at 1 m depth using a Digitemp thermistor probe. Burrow humidity was measured using an aspirating hygrometer in which a battery-powered fan was used to draw filtered air through an insulated tube inserted into the warren. This air passed over dry and wet-bulb thermometers and relative humidity (RH) was calculated from the difference in readings. On each visit, measurements were taken from the same two burrows in each of three separate rabbit warrens. One warren was on a "terrace", another on a north-facing slope, while the third was on a south-facing slope.

LABORATORY COLONY OF FLEAS

C. l. iberi obtained from the field were put onto young domestic rabbits kept in plastic containers 0.5 m deep with a floor area of 0.4 m² containing a layer of 2-3 cm of clean, fine sand. The rabbits were given dry pelleted food *ad lib.* but only small amounts of water daily to minimise urine output (Cooke, 1982, 1990). In this way the rabbits could be kept in good health and the sand below them kept relatively dry. Any wet sand was carefully removed and replaced with dry sand each day when the rabbits were fed and watered. The dry sand from below the rabbits was collected, daily or every two-three days according to requirements, and sieved to remove rabbit faeces and uneaten food pellets. It was then spread thinly in a white tray and examined for fleas that had left the rabbits. Any fleas found were replaced on the rabbits in clean containers with fresh sand. The sand and the eggs it contained were placed in strong black plastic bags and a few grams of dried powdered rabbit blood and powdered yeast were added to supplement the normal larval diet of flea faeces. The bags were labelled, according to the identity of the rabbit and collection date, then placed in a dark cupboard at room temperature (22°C) to be inspected every two-three days to observe development of the larvae. After the larvae spun cocoons the sand in the bags was examined every two-three days but when fleas began to emerge they were collected daily to observe the emergence patterns of adults of both sexes.

In early attempts to breed *C. l. iberi*, a major problem was excessive contamination with urine and resultant high humidity. If the sand was damp, no flea larva developed, and even in slightly drier sand there was a risk of fungal infestation destroying all the food available to the larvae. *C. l. iberi* were reared through several generations in the laboratory using these methods even during the summer period when adult fleas are generally not observed in the field. Although most fleas had to be used maintaining the breeding colony, limited numbers of larval and adult fleas were available for initial laboratory investigations.

RATES OF DEVELOPMENT THROUGH LARVAL AND PUPAL STAGES

Gravid female *C. l. iberi* were kept in 15 ml glass vials overnight and the eggs they laid were kept at 22°C (laboratory temperature) and 25°C, both at 80 % relative humidity, to observe the rates of development from egg to adult. Four or five g of sand containing a small amount of dried blood and yeast was added to each tube to provide food and a suitable substrate in which the larvae could burrow and pupate. The flea larvae were examined daily, if necessary by tipping them into a petri dish, for examination under a binocular microscope (30X).

SURVIVAL OF LARVAE AT DIFFERENT HUMIDITIES

A fine brush moistened with water was used to transfer groups of five newly hatched larvae into eight 15 ml gauze-capped vials containing sand, dried powdered blood and yeast. The vials were then placed separately in sealed plastic containers over carefully adjusted concentrations of sulphuric acid which maintained humidities between 30 % and 100 % RH in steps of 10 % (Solomon, 1954). The larvae were examined daily to record survival and rates of development.

SURVIVAL OF ADULT FLEAS AT DIFFERENT HUMIDITIES

Adult fleas, bred in the laboratory and collected one-two days after emergence from pupation were also placed in 15 ml gauze-capped vials and held at 22°C in the sealed plastic containers where sulphuric acid maintained humidities ranging from 40-100 % RH in steps of 10 %. A piece of paper in each vial provided a substrate on which the fleas could rest. The fleas were checked daily and survival times recorded.

SURVIVAL OF ADULT FLEAS ON LABORATORY RABBITS

When fleas used in breeding colonies were transferred to new rabbits, the opportunity was taken to count all fleas. Two colonies were established using fleas freshly-

emerged from pupation and no new fleas were added to the population between counts. This made it possible to record the survival rate of groups of breeding fleas from emergence onwards. For analysis, data from the two colonies were combined by expressing results in terms of percentage survival of the founding populations.

RESULTS

DISTRIBUTION AND BEHAVIOUR OF THE FLEAS ON THE RABBIT

Field data from El Alquian (Almeria) showed that, on freshly killed wild rabbits, *C. l. iberica* were largely distributed around the head and chest, and less commonly near the rabbits' hindquarters. In laboratory colonies of *C. l. iberica*, fleas were seldom found away from the rabbits, living free in the sand. They were mostly seen on the head and neck of the rabbits, and occasionally seen at the base of the ears. In addition, flea faeces could often be seen quite thickly in the fur at the back of the rabbits' necks.

Only occasionally were *C. l. iberica* found in significant numbers in rabbit burrows (see Table I). These were usually unfed and apparently newly emerged from pupation. One burrow yielded eight adult fleas. Soil taken from the same burrow yielded two further adult fleas, which emerged from cocoons soon after transfer to the laboratory. *C. l. iberica* larvae in the same sample completed development in the laboratory and spun cocoons. Collections of fleas made in April 1988 and April 1991 showed that, contrary to expectations, some *C. l. iberica* persisted in the field in Almería until spring (Table I).

April 1988

Source of fleas	<i>C. l. iberica</i>	<i>X. cunicularis</i>	<i>S. cuniculi</i>
From 20 burrows	1	6	8
From 8 rabbits	36	5	77

April 1991

Source	<i>C. l. iberica</i>	<i>X. cunicularis</i>	<i>S. cuniculi</i>
From 10 burrows	8*	9	8
From 14 rabbits	1	4	328

*All fleas were found in one burrow and were newly emerged from pupation.

Table I. – Numbers of fleas collected from burrows and rabbits at El Alquian, 1988 and 1991.

TEMPERATURE AND HUMIDITY OF RABBIT BURROWS IN ALMERIA

Temperature and relative humidity (\pm standard deviation) of the air in rabbit burrows at El Alquian are given

Date	Température (°C)	Relative Humidity (%)
16-12-1987	19.0 \pm 2.9	72.6 \pm 8.1
09-02-1988	17.4 \pm 2.4	54.0 \pm 12.1
20-04-1988	22.5 \pm 2.6	49.3 \pm 21.5
17-07-1988	29.1 \pm 2.0	55.7 \pm 8.3

Table II. – Temperature and humidity of air in rabbit burrows at El Alquian, 1987-1988. Means with standard deviations (n = 6).

in Table II. These show that temperatures within the burrows varied over a range of about 12°C from winter to summer but the relative humidity of air was much more constant, seldom approaching dryness or saturation.

EGG LAYING

Freshly caught female *C. l. iberica* in 15 ml tubes laid eggs in short lines of four, or possibly eight eggs. This suggested that they might lay four-eight eggs daily. However, from the ratio of gravid females to new adult fleas that eventually emerged from cultures, the maximum rate of egg laying is unlikely to exceed four eggs daily.

TEMPERATURE AND THE RATE OF DEVELOPMENT OF LARVAE

At 22°C and 80 % relative humidity the eggs of *C. l. iberica* hatched after six days. The larval stages lasted for a further 10 or 11 days and spinning of cocoons was normally completed by the 17th day after hatching.

At 25°C and 80 % relative humidity the eggs hatched in five days but the survival of larvae was poor. All died within 20 days after hatching and none were able to spin cocoons although a few reached the prepupal stage. The temperature of 25°C appears to be near the limits for normal development of the larvae of *C. l. iberica* although this should be reconfirmed using a bigger range of temperatures and larger numbers of fleas.

EFFECTS OF HUMIDITY ON THE SURVIVAL AND DEVELOPMENT OF LARVAE

Newly hatched larvae of *C. l. iberica* were able to survive and develop between 60 and 100 % RH. However, at 50 % RH they fed poorly and all died within seven days of being put into the experimental treatment. At 30 and 40 % RH the larvae died within 48 hours of being transferred into the humidity chambers. The lowest level of humidity that larvae can apparently tolerate lies between 50-60 % RH. Examples of survival at different humidities are given in Figure 2. Larvae took longer to complete development and spin cocoons at 70-80% RH than at higher humidities (Fig. 3).

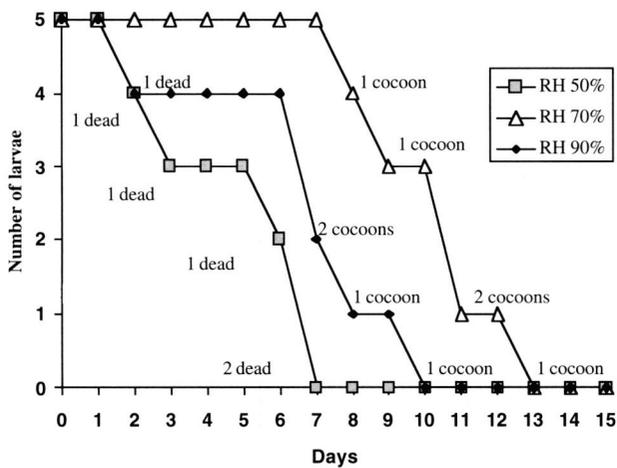


Fig. 2. – Examples of survival and development of *C. l. iberla* larvae at three different humidities, all at 22°C.

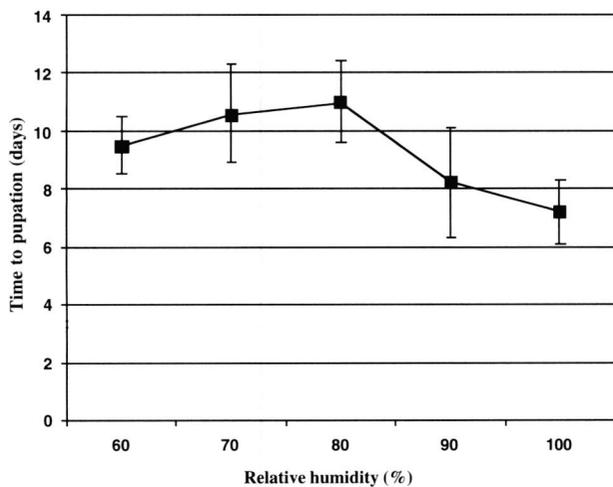


Fig. 3. – Mean time (days \pm s.d.) taken to completion of cocoon spinning at different humidities.

EFFECTS OF FOOD SHORTAGE ON LARVAL DEVELOPMENT

In some cultures of *C. l. iberla* insufficient food was provided for the larvae. As the food became scarce the larvae began crawling about on the surface of the sand, and, although they persisted in this state for up to 25 days, they did not pupate. However, 30 of these starving larvae all completed their larval stages when transferred into fresh sand to which more powdered blood and yeast had been added. It was an additional 12-17 days after this transfer that they spun cocoons, a little longer than the 12-13 days taken by normal, well-fed larvae in culture bags. Those larvae that remained in the original culture eventually died.

Apart from taking an abnormally long time to complete their larval stages, the thirty *C. l. iberla* larvae that spun cocoons after additional feeding were aberrant in ano-

ther sense too. 17 to 21 days after cocoon spinning, six adult fleas (three females, three males) emerged after a pupation of apparently normal duration (see below), and then no further emergence occurred. On dissecting a sample of ten of the unhatched cocoons two weeks after the last emergence, it was found that they still contained healthy, unmetamorphosed prepupae. While being far from conclusive evidence, this raised the possibility that *C. l. iberla* might undergo a pre-imaginal diapause induced by environmental conditions.

EMERGENCE FROM PUPATION

In the laboratory at 22°C new adult fleas began to emerge from pupation 15 days after cocoon spinning began, however there were slight but significant differences in the peak of emergence of fleas of each sex. Female fleas most commonly emerged 17 days after spinning and the males at 20 days. The time taken from egg laying to emergence from the cocoon was 28-31 days. Although temperatures above 25°C apparently suppress the development of *C. l. iberla* larvae, development within the cocoon continues quite normally at 27°C.

SEXUAL MATURATION OF ADULT FLEAS

As the fleas emerged from pupation they were placed on rabbits and the sand from below the rabbit was sampled regularly to detect the presence of larvae. Judging from the first appearance of larvae, remembering that the eggs take six days to hatch at 22°C, the first viable eggs were laid 11 days after the emergence of the male fleas. The minimum generation time of *C. l. iberla* in the laboratory was 48 days.

SURVIVAL OF FLEAS AT DIFFERENT HUMIDITIES

The survival times (days) of unfed adult fleas kept at humidities ranging from 40-100 % RH and 22°C showed that survival time increased significantly at high humidities and that male fleas survived two-three days

Change	d.f.	Sum of squares	Mean square	Variance ratio	Probability
Humidity	1	102.08	102.08	45.07	< 0.001
Sex	1	38.30	38.30	16.91	< 0.001
Residual	23	52.09	2.27		
Total	25	192.46			

	Estimate	Standard error	t ₍₂₃₎	Probability
Constant	- 1.02	1.10	- 0.92	0.367
Humidity	0.0901	0.0160	5.61	< 0.001
Sex M	2.521	0.613	4.11	< 0.001

Table III. – Survival of male and female *C. l. iberla* at different humidities: accumulated analysis of variance and estimates of parameters for the fitted model.

longer than females. A generalised linear model was fitted using Genstat 5 (4.1) (Lawes Agricultural Trust, Rothamstead Experimental Station) to combined data from small numbers of fleas in each treatment. This model accounted for 71 % of the variance. The regression analyses and estimated parameters are given in Table III. The survival time of freshly emerged fleas was: male survival time (days) = $-1.02 (\pm 1.10) + 0.0901 \cdot \text{RH} (\pm 0.0160) + 2.521 (\pm 0.613)$
female survival time (days) = $-1.02 (\pm 1.10) + 0.0901 \cdot \text{RH} (\pm 0.0160)$

SURVIVAL RATES OF ADULT FLEAS ON LABORATORY RABBITS

In the laboratory, individual females of *C. l. iberica* were maintained for up to three months on rabbits. Male fleas disappeared quite rapidly from the rabbits. Furthermore, their smaller size made them more difficult to find than the larger females and consequently data on their survival rates were less reliable. The survival of female fleas is the most important measure from a population perspective because, even after the loss of male fleas, females continue to lay eggs fertilised by sperm held in the spermatheca. Despite relatively few data, the regression fitted to combined data from two small colonies of fleas was highly significant ($P < 0.001$). The survival rate of female fleas, shown in Figure 4, was calculated as:

$$\text{Percent surviving} = 100 \cdot e^{-0.0284 \cdot \text{days}}$$

Extrapolation of this curve suggests that about 95 % of female fleas should disappear within 100 days (three months).

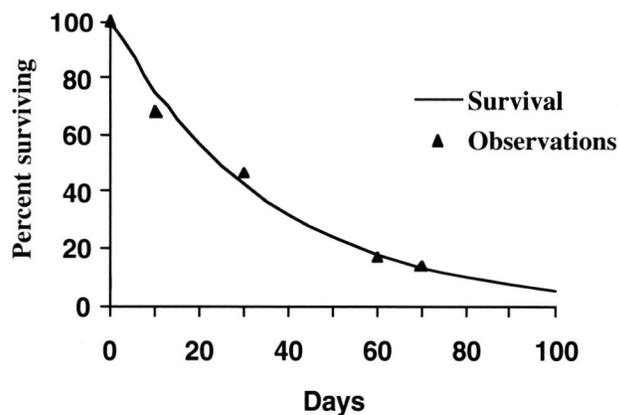


Fig. 4. – The percentage survival of female fleas in laboratory colonies over time. Fitted curve is: Percent surviving = $100 \cdot e^{-0.0284 \cdot \text{days}}$.

DISCUSSION

The rabbit flea *C. l. iberica* shows significant adaptation to arid habitats. Unfed adult fleas are more tolerant of desiccation than *S. cuniculi* but

less tolerant than *X. cunicularis* (Cooke & Skewes, 1988; Cooke, 1990). The life cycle of *C. l. iberica* includes a relatively long egg stage of six days but a short larval stage of 10-11 days, presumably reducing exposure of the larvae to low humidities. In *X. cunicularis*, a rabbit flea that withstands extremely dry habitats, the egg stage lasts seven-eight days and larval stages nine-ten days (Cooke, 1990). This contrasts markedly with the equivalent life stages in *S. cuniculi* that is adapted to life in nests kept warm and humid by newborn rabbits. The egg stage of *S. cuniculi* lasts four days; the larval stage 16 days at 22°C (Cooke & Skewes, 1988). The larvae of *C. l. iberica* are able to persist and grow at relative humidities between 50-60 %, whereas the larvae of *S. cuniculi* only survive if relative humidity exceeds 70 % (Cooke, 1990). Nevertheless, the larvae of *C. l. iberica* are not as well adapted to aridity as those of *X. cunicularis* that can complete development at between 40 and 50 % RH.

Larvae of *C. l. iberica* took a little longer to complete development and spin cocoons at 70-80 % RH than at higher humidities (Fig. 2). This indicates either that lower humidities are still stressful to larvae, or alternatively, that 70-80 % RH is optimal for larval development and that stress associated with higher or lower humidity causes premature pupation or early death.

In the laboratory, pupation usually lasted 17 days for female *C. l. iberica* and 20 days for males. A difference between sexes in the time to emergence from the pupa is usual among fleas. Male *X. cunicularis* and *S. cuniculi* also emerge later than females (Vaughan & Coombs, 1979; Cooke & Skewes, 1988; Cooke, 1990).

Observations of burrow temperature and humidity at El Alquian in 1987 confirmed that burrow humidity was adequate for the larvae of *C. l. iberica* to develop over most of the year. However, breeding may be restricted for part of the year as the larvae of *C. l. iberica* apparently cannot complete development at 25°C or above.

Adult *C. l. iberica* normally appear in the field during the autumn each year and persist into the winter but generally disappear by spring (Jiménez & Marquez, 1986; Osácar, 1996). At Arpal in the Ebro Valley of northern Spain the fleas appeared in September or October each year and most had disappeared by February although a few stragglers were seen in April, May and June (Osácar, 1996). As fleas are present in reasonable numbers on the rabbits at Arpal for about five months it seems likely that they produce more than one generation each year. This is further supported by laboratory observations that individual adult fleas rarely survive for more than three months. Two or three generations within a period of five months would certainly be possible because, at medium temperatures,

a generation is completed in about 48 days (seven weeks). The relative abundance of *C. l. iberica* on rabbits at El Alquian in April 1988 and the presence of larvae, cocoons and newly emerged adults at the same site in late April 1991 (Table II) confirmed that breeding may extend into the spring in some field populations.

Observations made during the laboratory rearing of *C. l. iberica* larvae suggested that prepupae within cocoons may enter a quiescent phase, possibly a pre-imaginal diapause, as observed in some other fleas. Teplych *et al.* (1988), working with gerbil fleas, considered that pre-imaginal diapause could be induced if the adult fleas that laid the eggs were exposed to cold weather for a month or more. However, for *C. l. iberica*, food shortage or intermittent feeding may also be implicated in initiating diapause. Benton & Surman (1989) suggested that such a pre-imaginal diapause would explain the long period between generations of the squirrel fleas, *Epitedia faceta* (Rothschild, 1915) and *Conorhinopsylla stanfordi* Stewart, 1930, that are seldom found in the adult stage in the warmer months between May and September. This implies a diapause of 100 to 150 days over summer.

In the case of *C. l. iberica*, a pre-imaginal diapause is likely to be facultative, not obligatory, because it is possible to breed *C. l. iberica* in the laboratory even when fleas have disappeared from the field. In comparing data from sites across Spain it seems that at Granada, and in most other parts of Spain, *C. l. iberica* larvae develop during the autumn and winter then enter diapause and survive until the following autumn in cocoons (Rodriguez *et al.*, 1981; Jiménez & Marquez, 1986). At Arpal this is the predominant pattern too, although a few fleas are seen in spring (Osácar, 1996). At El Alquian, *C. l. iberica* not only persisted but also bred during the early spring. Such differences in flea population dynamics may be related to climatic differences between sites. El Alquian is drier and milder than other regions in Spain where *C. l. iberica* occurs. Burrow temperatures at El Alquian varied between 17°C and 29°C from winter to summer (this study) whereas those at Arpal varied between 10°C and 25°C (Osácar, 1996).

Practical experience in maintaining the flea colony in Seville showed that *C. l. iberica* larvae survived well only if the sand in which they lived was relatively dry. Increasing dampness limited larval food supplies because soil microorganisms destroy flea faeces (or powdered blood), the normal larval food, when humidity is high.

In the Mediterranean climate, where summers are warm and dry and winters cool and wet, conditions suitable for survival of *C. l. iberica* larvae are most likely to occur in autumn when soil moisture has been

depleted over the summer and temperature falls. However, soil moisture would be expected to build up over winter eventually making burrows too damp for larvae in all except the driest parts of Spain. Breaking of diapause in autumn (September-October) would generally give the fleas the best chance of reproducing successfully. A facultative onset of diapause would explain how fleas in warmer drier areas persist until spring and produce several generations in the same year.

As a vector of myxomatosis, *C. l. iberica* is likely to be abundant for only three to five months each year. This fact and the difficulty in maintaining an adequate laboratory colony for research purposes were among the reasons why *C. l. iberica* was not initially chosen as a vector of myxomatosis for use in arid Australia. Nevertheless, it is useful to review such decisions in the light of new evidence. Apart from autumn and winter outbreaks of RHD in Australia being more effective than spring outbreaks, it also seems that the pattern of outbreaks of myxomatosis has changed. In some parts of Australia, myxomatosis is now more common in autumn than it was before the introduction of RHD (G. Mutze, Animal and Plant Control Commission, South Australia, pers. com.). A vector like *C. l. iberica* that is most abundant in autumn, and could potentially anchor RHD into a pattern of autumn or winter outbreaks, might therefore improve the efficacy of RHD and myxomatosis as biological control agents.

Apart from being of interest in facilitating biological control in Australia, an understanding of the biology of *C. l. iberica* may also be useful in considering the conservation of rabbits in Spain. As autumn outbreaks of RHD and myxomatosis also occur in eastern Spain (C. Calvete, pers. comm.) it is likely that *C. l. iberica* becomes abundant each year at precisely the right time to be an important vector. Additionally, *C. l. iberica*, like *S. cuniculi*, is likely to be an efficient vector of viruses. Both species are essentially adapted for life in the rabbit's fur and, because unfed adult fleas do not survive for more than a few days in dry burrows, they leave rabbits that die from myxomatosis and immediately seek another rabbit. This means that fresh virus can be transferred to a new host before there is much chance of degradation. The abundance of *C. l. iberica* on freshly shot rabbits and the relatively low numbers of obtained from burrows suggests that *C. l. iberica* is normally closely associated with the host in the field. Comparative data from other species of rabbit fleas supports this idea. *S. cuniculi*, which spends most of its time on its host, was also predominantly obtained from shot rabbits. By contrast, *X. cunicularis*, which spends part of its time living in the sand of the burrow floor, was equally obtainable from either rabbits or burrows.

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