ASPECTS OF THE BIOLOGY OF *Uropsylla tasmanica* ROTHSCHILD (SIPHONAPTERA)

by

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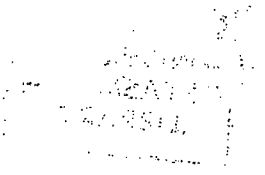

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Abstract

The flea *Uropsylla tasmanica* Rothschild is the only known flea with a parasitic larval stage (Dunnet 1970). In Tasmania the most commonly found host of *U. tasmanica* is the native cat *Dasyurus viverrinus* (Dasyuridae Marsupialia).

The life cycle of *U. tasmanica* in relation to the annual cycle of *D. viverrinus* is described. It was found that *U. tasmanica* adults and parasitic larvae only occur on the hosts from March to September (Autumn and Winter). Other species of fleas found on native cats decline in numbers during this period. Reproduction in *U. tasmanica* coincides with the reproductive period of its host.

Experimental work indicated that adult *U. tasmanica* emerge from their cocoons in response to mechanical stimulation. "Readiness" to emerge depends upon the amount of time spent in the cocoon as well as the stage of desiccation of the fleas in the cocoons. (Fleas kept at 80% RH took 104±0.2 (n=10) days to emerge whilst those kept at 20% RH took 73±.19 (n=10) days to emerge from the beginning of the experiment.) After emergence and in the absence of a host, fleas kept at high relative humidities survived for longer periods than those kept at low relative humidities (32±1.8 n=10 days at 80% RH compared with 13±3.25 n=10 days at 20% RH). On emergence from the cocoons *U. tasmanica* are not sexually mature and require a blood meal to complete sexual maturation. Newly emerged fleas prefer to feed from sub-adult rather than mature native cats during the host's non-reproductive phase. Experiments in which prolactin and hydrocortisone were administered to some of the fleas indicated that these mammalian hormones facilitated sexual maturation and reproduction in *U. tasmanica*.

The parasitic larvae of *U. tasmanica* undergo four larval stages and are morphologically different from non-parasitic flea larvae. There are some taxonomic features of *U. tasmanica* larvae which appear to converge with the myiasis-producing Dipteran larvae. Fully developed *U. tasmanica* larvae leave the host and build a cocoon in the litter of the hosts den. The larvae do not always pupate immediately but may enter a diapause the induction and duration of which appears to depend upon the daily cycle of warmth and cold to which they are subject on emergence from the host.

The phylogeny of *U. tasmanica* was investigated by comparing the chromosomes of this species with those of *Pygiopsylla hoplia* and *Lycopsylla nova*. *U. tasmanica* appears to have 2n = 14 chromosomes while the other two species have 2n = 20 chromosomes.

CONTENTS

Introduction

In discussing the life history of bird fleas, Rothschild and Clay (1956) have described how the faecal pellets of adult fleas with a high blood content provide the ideal food for their larvae. The fleas of birds are nest fleas and therefore food for flea larvae is only available during the periods when the birds are occupying their nests. In the case of migratory birds and those birds which only nest during their breeding season the fleas have a limited period in which the larvae can develop. During the periods when the nests of sand martins are occupied thousands of flea larvae may be found in one nest. These larvae are occasionally found on the bodies of the nestlings. Rothschild & Clay conclude "No doubt if they could evolve a closer relationship with the host at the larval stage their lives would be less hazardous and bird fleas as a whole might, like the feather lice, become more successful in the walk of life they have chosen".

Uropsylla tasmanica is not a bird flea but it has evolved a closer relationship with its host at the larval level. It is the only flea known where the larvae are obligatory sub-dermal parasites (Dunnet 1970, Warneke unpublished). *U. tasmanica* is not however the only flea with a parasitic larval stage. The larvae of the arctic hare flea *Holopsyllus glacialis* are ectoparasites which live and feed in the fur of the host (Freeman and Madsen 1949). The larvae of *Tunga* and *Dasypsyllus* have also been occasionally found living as ectoparasites on their host's body (Rothschild, 1975a).

The typical life cycle of a flea is as follows (Rothschild and Clay 1956, Dunnet 1970, Askew 1971). The female flea lays smooth, oval or round eggs which either fall free of the host or are glued to the

material in the host's nest. The larvae are white vermiform maggots with biting mouthparts and long body bristles which aid in locomotion. There are usually two moults during larval development. The final instar spins a cocoon from salivary gland secretions. The cocoon is egg shaped and is frequently adorned with soil particles. Pupation is of variable duration depending on climatic conditions. In some cases the adults or pupae may remain quiescent in the cocoon for long periods awaiting a change of season or the presence of a host to stimulate emergence.

The parasitic larvae of *U. tasmanica* were first recognised as fleas by Robert Warneke (Dunnet 1970) who suggested to the author that this flea might be a fascinating research topic. All that was known about the life history of *U. tasmanica* at the beginning of the project is summarised below.

It was known that *U. tasmanica* larvae are found on *Dasyurus viverrinus* and *Dasyurus maculatus*. They had also been recorded from *D. geoffroii*, *Sarcophilus harrisii*, *Thylacinus cyanocephalus*, *Felis sp.* & *Parmele nasuta* (Dunnet and Mardon 1974). The females of *U. tasmanica* were known to glue their eggs to the hairs of the host. Upon hatching the larvae descend the hairs and enter the skin of the host where they develop through the larval instars until ready to build a cocoon. When fully developed the larvae leave the host and crawl into the litter or soil in the host's den where they build a typical flea like cocoon. Pupation lasts approximately 60 days (Warneke pers comm.).

The aim of the investigation presented in this thesis was to study the life cycle of *U. tasmanica*, and in particular to examine the special adaptations which have developed in both adults and larvae relating to the parasitic larval stage. The project began with a field study of the flea in order to establish its annual cycle and to relate this,

as far as possible, to the annual cycle of the host. The host which was chosen for study was *Dasyurus viverrinus*, the native cat, because this marsupial occurs in high numbers in Tasmania and is easy to trap.

The laboratory studies which were carried out were divided into three classes. Firstly experiments were designed to investigate some aspects of the life cycle of the flea. These experiments were based on the results of the first year's trapping programme. Secondly, the parasitic larvae of *U. tasmanica* were examined with regard to their special adaptations as sub-dermal parasites. Finally, the phylogeny and evolutionary development of this unique flea was examined.

Chapter 1

Field Study of the Life Cycle of *Uropsylla tasmanica*

1.1 Introduction

Field studies of fleas in natural populations of mammals have been largely confined to the northern hemisphere. An exception is the field work which has accompanied the introduction of myxomatosis, using the rabbit flea, *Spilopsyllus cuniculi*, as a vector, into Australian populations of rabbits, (Williams 1973) and studies of other fleas found on rabbits (Shepherd and Edmonds 1973, 1978, 1979). There have been no field studies of the fleas of marsupials published so far.

In the northern hemisphere much work has been carried out on the population dynamics of the plague fleas (Rothschild 1975a Traub 1972). Other work has included study of the fleas of the field vole (Ulmanen and Myllymaki 1971) and the fleas associated with the Californian ground squirrel (Rykman 1971, a,b.). An investigation of the seasonal dynamics of the fleas infesting dogs in Egypt has been carried out by Amin (1966). There have been numerous studies of fleas which occur in small mammal populations (Elton *et al.* 1931, Evans & Freeman 1950).

The most notable feature of the results of field studies of fleas of mammals has been the seasonal variation in numbers of fleas of different species on a single host or group of hosts. Examples of this include the seasonal variation in numbers which was demonstrated in *Xenopsylla cheopis*, *Nosopsyllus fasciatus* and *Pulex irritans* by Bacot (1914), and the seasonal variation found in various species of *Ctenophthalamus* (Evans and Freeman 1950, George and Corbett 1959, Cowx 1957, Cotton 1970 and Ulmanen and Myllymaki 1971). Stickfast fleas of the genus *Echidnophaga* have been shown to peak in numbers in autumn on the rabbit (Shepherd and Edmonds 1979), while the rabbit

flea *Spilopsyllus cuniculi* peaks in spring and summer (Williams, 1973).

Another aspect of population dynamics which has occasionally been demonstrated by field studies is seasonal fluctuations in the sex ratios of some fleas. Cotton (1970) recorded higher numbers of female adults of *Ctenophthalamus nobilis*, emerging from cocoons in the nests of the field vole while similar results were obtained for adult fleas found on the bodies of voles by Evans and Freeman (1950) and Janion (1960). Seasonal fluctuations in sex ratios of fleas have been also recorded by Amin (1966) and Smit (1962),

Cole (1945) found that the sex ratio of adults of *Xenopsylla cheopis* found on the host was influenced by daily changes in temperature. On warm days with a mean temperature of 21°C to 14°C male fleas outnumbered females but this was reversed on cooler days.

There is less data in the literature concerning the distribution of fleas in the host population with regard to sex or age group of the host. Differences in flea infestation of male and female hosts have been reported by George and Corbett (1959), Janion (1961), Smit (1962) Haas (1965, 1966) and Brink-Lindroth (1968). The general trend seen in these reports indicates that male hosts are generally more heavily infested than females. The levels of flea infestations on the different age groups of mammals has been summarised by Smit (1962) and the available evidence shows that for some species of fleas and hosts there is a tendency for higher infestations on adult individuals as distinct from juveniles. Brink-Lindroth (1968) however found higher levels of flea infestation in sub-adult voles than in the adults.

The aim of the field study which is presented in this chapter was to investigate the yearly cycle of *Uropsylla tasmanica* in natural

population of native cats. This investigation included examination of the seasonal fluctuations in numbers of the species of fleas found of the native cat and the status of *Uropsylla* populations and the distribution of this flea throughout the native cat population.

The field study also included observations on the annual cycle of the native cats. It has been found in other studies on the population dynamics of fleas, that infestation rates may be influenced by physiological or behavioural factors of the host. Thus it has been suggested (Ulmanen and Myllymaki 1971, Smit 1962 and Brink-Lindroth 1968) that different levels of activity of the hosts influence flea behaviour. In the rabbit flea which is dependent upon host hormones for its own sexual maturation (Mead-Briggs and Rudge 1960) the flea can only reproduce during the breeding period of its host. Development of flea larvae into pupae is frequently dependant upon the maintenance of suitable conditions of temperature and humidity in the den or nest by habitation of the host. In *Ctenophthalamus nobilis* (Cotton 1970) the larvae pupated readily when the maternal nests were inhabited but development of adults was retarded once these nests had been abandoned by the hosts.

1.2 Materials and Methods

In the field, fleas were obtained alive from live native cats. Two study areas were used, one north and one south of Hobart. An effort was made to visit each trapping site each month for two years.

The native cats were trapped in cage traps baited with raw meat and were transferred to linen bags for examination. The fleas were removed by combing the fur with a fine tooth comb and sucking each flea into a glass tube as it appeared. As each native cat was released

the linen bag was checked for fleas before the next native cat was introduced.

A representative sample of fleas was obtained from each native cat by combing its fur according to a standardised pattern until one minute's worth of further combing failed to produce any more fleas. At no stage was any attempt made to ensure that all fleas were removed from any native cat as this would have involved killing or anaethetising each animal. This was not considered necessary and would not have been approved by the Tasmanian National Parks and Wildlife Service whose permission was required to conduct the trapping program.

The fleas obtained were analysed microscopically in the laboratory. In order to sex the *Uropsylla* it was necessary to clear the specimens in 10% KOH as the anal segments were obscured by the terminal palp like projections of the eighth abdominal tergite.

The sex and approximate age of each native cat were recorded. The native cats were aged on a weight basis into two categories, the first category being those animals which had yet to breed or were presently undergoing their first breeding season while the second category consisted of those animals which had reached sexual maturity during a previous year. Following Fletcher (1977) the two weight categories were as follows: Group 1. Males <1.5 Kg females <0.9 Kg; Group 2. Males >1.5 Kg females >0.9 Kg. Notes were made ot the native cats' condition and any special circumstances such as the presence of pouched young were recorded.

The results of the trapping data were augmented by keeping a small population of native cats in a large enclosed compound at the University. The compound measured approximately 8 m x 11 m and was

enclosed with wire mesh. In the compound the native cats were furnished with nesting boxes and it was intended that there should be more nesting boxes than native cats. The average population of native cats in the compound was eight although this varied during the two years of the study as a result of births and the release of those animals which proved difficult to handle. However there was a minimum population of three males and two females. The native cats were fed on raw meat, chicken and dried dog food. After the first year the population of native cats consisted largely of animals which had been hand reared and were therefore easy to handle. Observations of native cat behaviour were made using the animals in the compound and the fleas on these native cats provided a reservoir of fleas for laboratory study.

The trapping data was collated and analysed and is presented in this chapter in tables and figures. The raw trapping data is presented in the appendix.

1.3 Results

Description of study sites

The northern study site, Buckland on the east coast of Tasmania, consisted of an abandoned farm bordered by wet and dry sclerophyll forest. The site contained a derelict farm house and a pond which appeared to be a focus for many animals including native cats, devils (*Sarcophilus harrisii*), potoroos, bandicoots, possums, feral cats and dogs, rats and mice. The native cats appeared to come from the forest surrounding the site or from a large den which was found beneath the farm house. In this site the traps were set close together in the

small area, approximately 400 m^2 enclosing the house and pond. Frequently only one night was needed to trap enough native cats to yield a reasonable flea sample.

The southern trapping site, (Bermuda Road near Glen Huon in southern Tasmania), was a pastoral property which had been cleared and grassed. Small clumps of dry sclerophyll forest had been left along creek beds and on the hill peaks. This area was higher in altitude than the northern site and was subject to light falls of snow during winter. In the southern area the traps were laid in trapping lines and up to 100 m apart. The native cats were not as plentiful in the southern area as in the north and frequently several nights were necessary to catch enough native cats for a reasonable flea sample. The native cats in this area appeared to have their dens in the forest areas but were caught on the pastures near these small forests.

There were no climatic data available for either the two areas as neither of them were close to weather stations.

Species of fleas found on the native cats

Four main species of fleas were found on the native cats. These were *Uropsylla tasmanica* Rothschild, *Acanthopsylla rochschildi rothschildi* (Rainbow), *Stephanocircus dasyuri* Skuse and *Pygiopsylla hoplia* Jordan and Rothschild. Single specimens of *Ctenocephalides felis felis* and *Macropsylla hercules* were also found.

It was observed during the removal of the fleas from the native cats that of all the species found, *U. tasmanica* was the most difficult to remove from the fur while *S. dasyuri* was the easiest. *S. dasyuri* tended to leave the hosts during handling and were frequently collected from the linen bags and the investigator. *U. tasmanica* was rarely

observed moving about in the fur and was usually found entangled in fur mats on the spines of the comb. *U. tasmanica* were frequently removed from the tail and limbs of the host unlike the other species which were found mainly on the host's body.

The numbers of fleas removed from the native cats did not vary significantly (χ^2 test), from year to year over the period of the field study between the two trapping areas. For this reason the data concerning flea numbers on native cats has been pooled. (Fig. 1.1, 1.2, 1.3).

Fig. 1.1 shows the frequency distribution of the number of fleas collected from the native cats during the field study and using the sampling technique described in section 1.2. It can be seen from fig. 1.1 that the most common number of fleas removed from the native cats was 4. The mean number of fleas collected per host was 5.5 ± 0.36. The frequency distribution is skewed as suggested by Williams (1964) for ectoparasites.

Fig. 1.2 shows the mean number of fleas collected from the native cats per month. It was found that the infestation of native cats by fleas of all species was bimodal with peaks in April and November. Analysis of fig. 1.2 reveals that the first highest peak in flea numbers which occurred in April came about as a result of a sharp increase in the numbers of *Uropsylla* during this month coupled with the high numbers of fleas of other species (Fig. 1.3). The decline in flea numbers which is observed in May and June came about as a result of the decline in the numbers of the other species of fleas while the numbers of *U. tasmanica* remained fairly high. The apparent drop in numbers of *Uropsylla* in May (Fig. 1.3) was caused by low

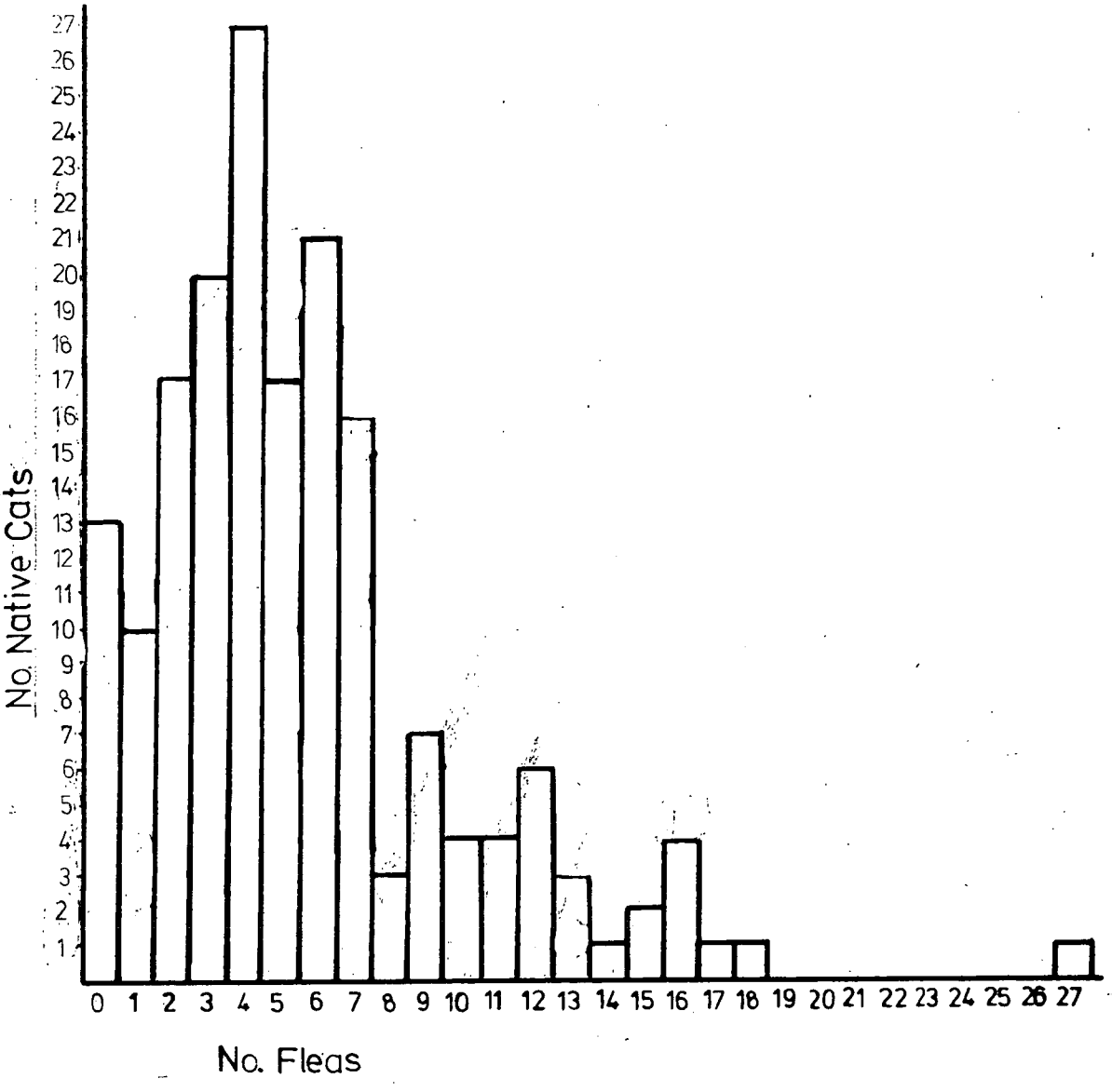


Fig. 1.1. Frequency distribution of the numbers of fleas collected from native cats.

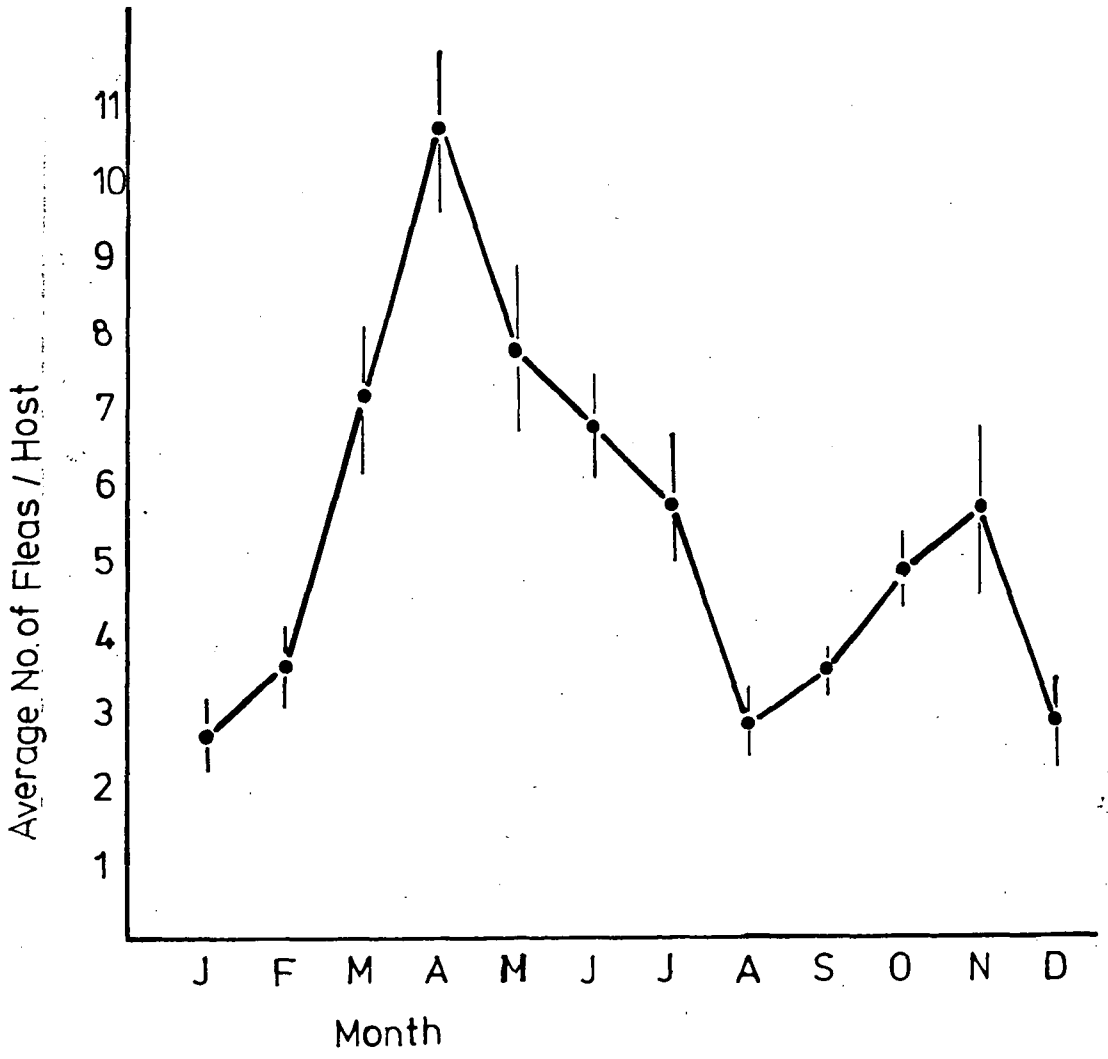


Fig. 1.2. Mean number of fleas of all species collected from native cats/month. Standard errors of the means are indicated on the graph.

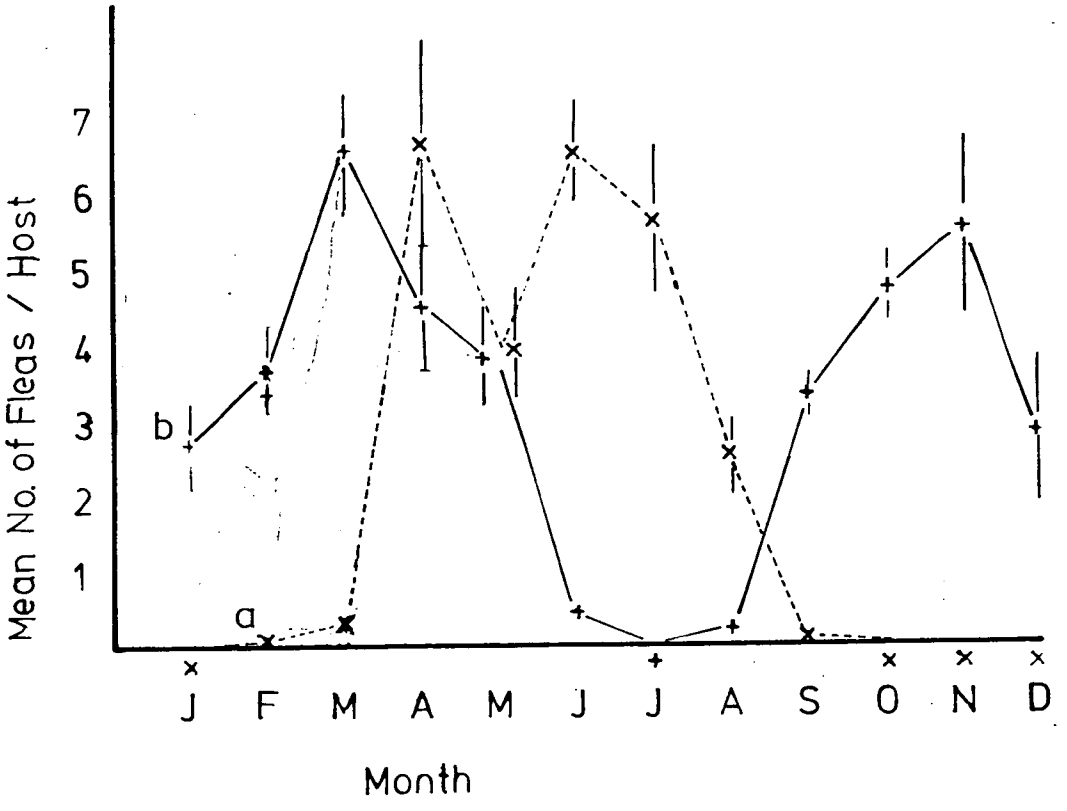


Fig. 1.3. (a) Mean no. *U. tasmanica* collected /month (------)

(b) Mean no. fleas of other species collected /month (———).

Vertical lines represent the standard errors of the means.

numbers of this flea in the southern trapping area (Fig. 1.4). This effect was not apparent in the northern trapping area. (Fig. 1.5).

The second peak in total flea numbers on the native cats in November was caused by the increase in the numbers of the other species of fleas during October and November following the decline in the numbers of *U. tasmanica* in August and September.

The percentage species composition of the fleas found on the native cats was found to differ between the two study areas. In the northern trapping area the main species of fleas found were *U. tasmanica*, *P. hoplia* and *A. r. rothschildi*. *S. dasyuri* was present in low numbers. In the southern trapping area however *S. dasyuri* was the dominant flea during the summer months, while *P. hoplia* and *A. r. rothschildi* were present in small numbers. For this reason the monthly species composition of the fleas found on the native cats in the two study areas is presented in two spearate graphs, Fig. 1.4 and 1.5.

Fig. 1.4 shows the monthly species composition of the fleas found on the native cats from the southern trapping area. It can be seen from this figure that *S. dasyurus* was present in high numbers during January, February and March (84% to 93% of all fleas caught) but by April it constituted only 40% of the fleas caught. In June and July *S. dasyuri* was not recorded from the native cats. The numbers of *S. dasyuri* began to increase in August and by October this species constituted 19% of all fleas caught. By December *S. dasyuri* had reached the 90% level. *P. hoplia* and *A. r. rothschildi* were present in small numbers during the months of January to May but were not recorded during June or July. *P. hoplia* exhibited a small peak in May where it reached a level of 12% of the fleas caught while *A. r. rothschildi*

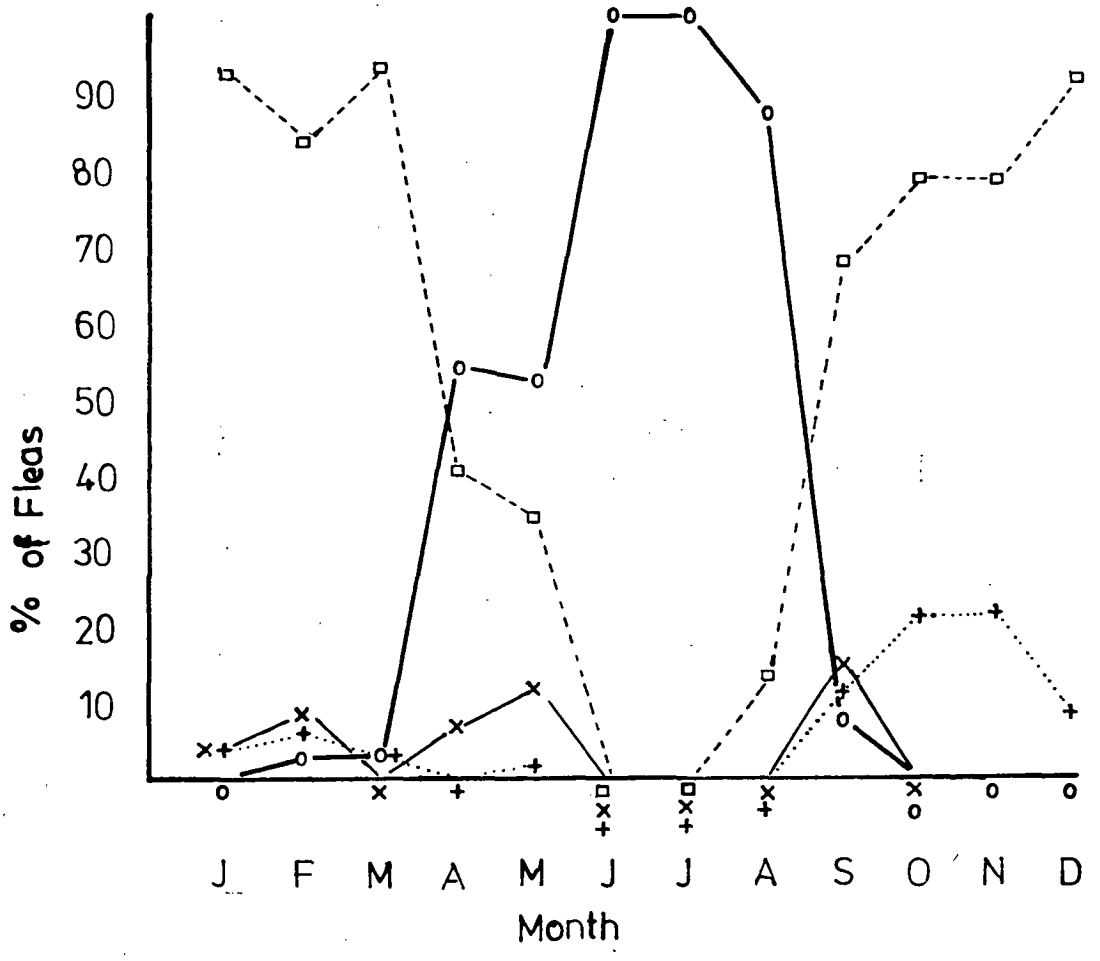


Fig. 1.4 Species composition of monthly flea samples, southern area.

(a) = *U. tasmanica* o—o

(b) = *P. hoplia* × — ×

(c) = *A. r. rothschildi* +·····+

(d) = *S. dasyuri* □----□

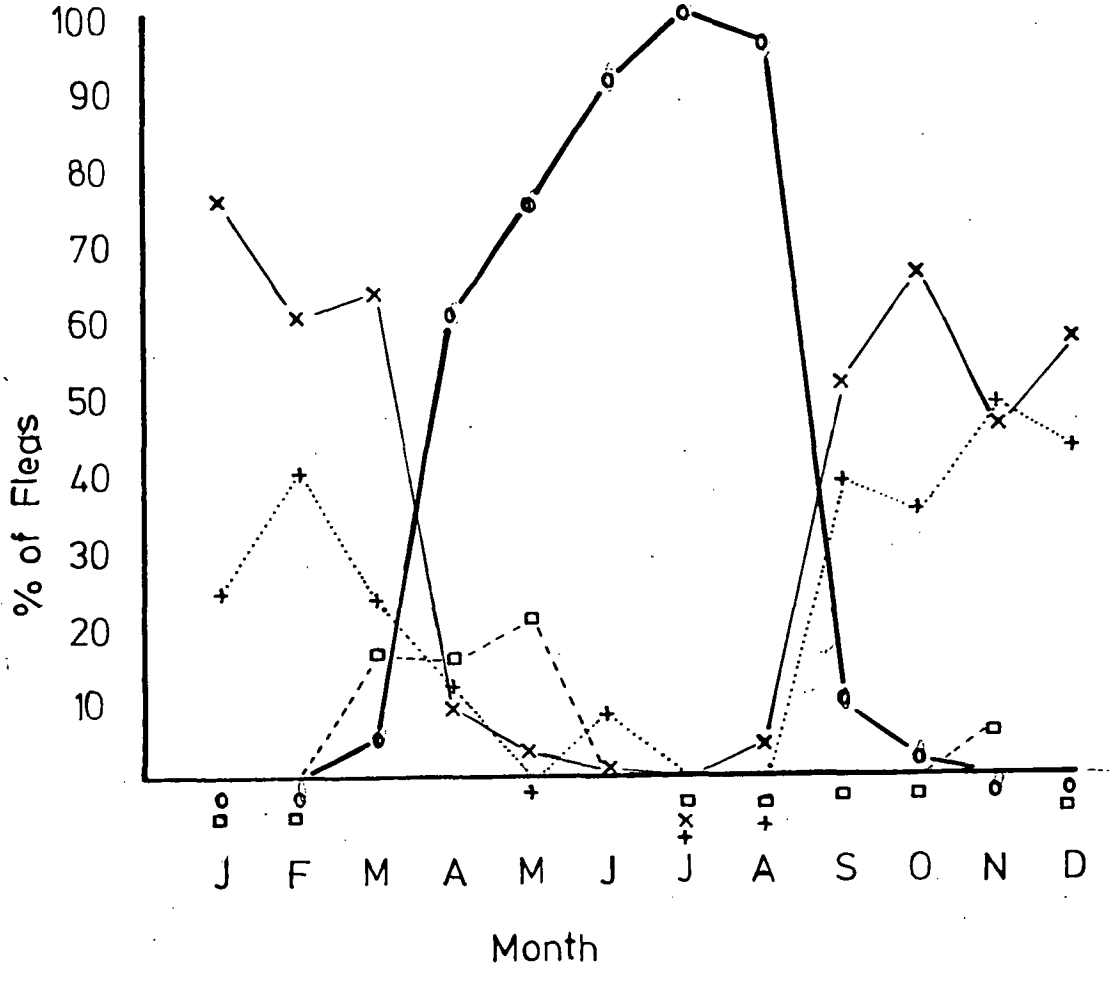


Fig. 1.5. Species composition of monthly flea samples, northern area

(a) = *U. tasmanica* o—o

(b) = *P. hoplia* x — x

(c) = *A. r. rothschildi* +·····+

(d) = *S. dasyuri* □-----□

peaked in October and November reaching a level of 21% of all fleas caught.

U. tasmanica was found in small numbers in February and March (2% of all fleas caught) but increased to 54% in April. By June and July *U. tasmanica* constituted 100% of the fleas caught but dropped sharply in numbers from August to September and was not recorded in October.

From the data collected in the northern trapping area it was found (Fig. 1.5) that both *P. hoplia* and *A. r. rothschildi* were present in high numbers during the summer months with *P. hoplia* being recorded more frequently than *A. r. rothschildi*. The highest percentage of *P. hoplia* (75%) was recorded in January while the highest percentage of *A. r. rothschildi* (49%) was recorded in November. The highest level reached by *S. dasyuri* was 21% of all fleas caught in May. *U. tasmanica* was not recorded in the northern trapping area in February but first appeared in March. In July *U. tasmanica* constituted 100% of the fleas caught. The percentage of *U. tasmanica* caught on the native cats dropped sharply after August and in September this species constituted only 9% of all fleas caught. In the northern study area a small number of *U. tasmanica* were recorded in October (2% of all fleas caught).

Although no specimens of *S. dasyuri*, *P. hoplia* or *A. r. rothschildi* were collected from the native cats during June and July in the southern trapping area or in July in the northern trapping area the dens of the captive native cats were found to contain adult *P. hoplia* and *A. r. rothschildi* throughout the year.

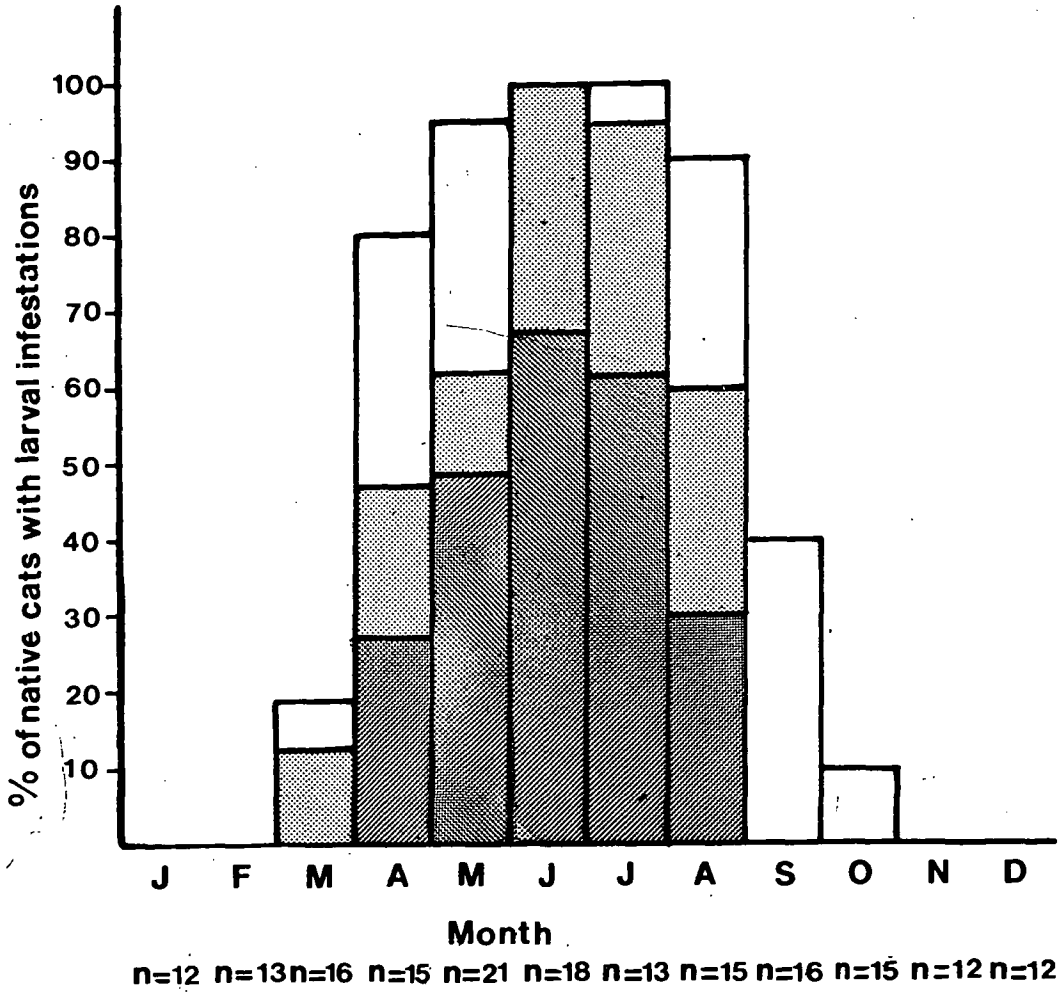


Fig. 1.6 Extent and Intensity of larval infestations in native cats

light

medium

heavy

n = no. of native cats examined

Reproductive activity of Uropsylla

Fig. 1.6 shows the extent and intensity of larval infestations of *Uropsylla* in native cats of both study areas. The infestations were ranked on a scale of three: light, medium and heavy (Ch. 4 pp.95)

It can be seen that in March the numbers of larvae were fairly low with light or medium infestations affecting only 20% of the native cats. By May however, 96% of all animals were affected, 52% heavily. In June and July all animals caught had larval infestations and in both of these months a high percentage of infestations were heavy (67% in June and 61% in July). In August the percentage of heavy infestations dropped although 90% of the animals were infested. In September only 40% of the animals were infested and these were all light infestations. By October only 10% of the native cats had infestations. Notes made with the trapping data indicate that not only were the October infestations light, sometimes consisting of only one or two maggots but in some cases these maggots were dead. No larvae were found from November to February inclusive.

Distribution of larval infestations throughout native cat populations

Table 1.2 shows a breakdown of the larval infestation figures to show their extent and intensity in male and female native cats. This table shows that the females suffered slightly less from larval infestation than the males. However it can be seen that the sample size for the males was greater than that of the females. This was because the female native cats were more difficult to catch during the months from May to August. In May a total of seven female native cats were caught compared with 14 males. In June only 5 females were caught

while 13 males were caught. In July 7 females and 11 males were caught and in August 6 females and 9 males were caught. When these results were tested using contingency tables and a value of χ^2 was obtained no significant difference was found at the 5% level.

Comparison of infestation levels in age group 1 and age group 2 native cats is shown in Table 1.3. There was an apparent trend towards higher infestation rates and intensities in the older animals but once again the results were not found to be significant. It was found during the trapping schedule that the most heavily infested animals caught were two large and presumably old males. I have observed only one instance of larval infestation of pouched young native cats.

Table 1.1 Larval Infestation in Male and Female Native Cats

Grade of Infestation	Males		Females	
	No.	%	No.	%
None	17	21.51	20	35.71
Light	15	18.98	13	23.21
Medium	14	17.72	10	17.85
Heavy	33	41.72	13	23.24
Total	79		56	

Table 1.2 Larval infestation in young and old native cats

Grade	<2 year old		>2 year old	
	No.	%	No.	%
None	14	42.42	29	30.2
Light	8	24.24	15	15.625
Medium	5	15.015	19	19.79
Heavy	6	18.18	33	34.375
Total	33		96	

Table 1.3 Monthly Sex Ratios of *U. tasmanica*

Month	No. fleas	No. Males	% Males	No. Females	% Females
March	4	2	50.0	2	50.0
April	103	50	48.5	53	51.5
May	83	39	46.9	44	53.1
June	117	53	45.3	64	54.7
July	74	37	50.0	37	50.0
August	40	14	35.0	26	65.0
Sept.	3	0	0.0	3	100.0

Table 1.3 shows the ratios of sexes of *Uropsylla* for the months of March to September when these fleas are found in the field. It can be seen that the ratio of male to female fleas was approximately even from March to July but that the percentage of males decreased during August.

Annual Cycle of the Native Cat

The annual reproductive cycle of the native cat is shown in Fig. 1.7. It can be seen that testes development began as early as January (Godsell pers. comm.), in preparation for the coming breeding season. Pouch development in the females is apparent in March and mating takes place in May. The oestrus period of the animals in the compound was short with the females all entering oestrus at the same time.

The young were born at the end of June and remained in the pouch until mid August. This was followed by a weaning period and the juvenile native cats were first caught in the field during September to October. During September the testes size in the males was reduced

(Fletcher 1977).

The nesting behaviour is based on observations of animals in the enclosure over a 2 year period, and no data is available about this aspect of native cat behaviour in the field. It was found that during the summer months the animals in the compound shared a common den but in April the females began to move out into the smaller nesting boxes. Generally the largest male had control of the main den at this time. Just prior to and during the mating period this animal was consistently found alone in the common den while the other two males occupied one of the smaller nesting boxes. During the months of October to November the native cats resumed the summer habit of sharing the common den.

1.4 Discussion

The trapping data indicated that *U. tasmanica* and the other species of fleas found on the native cats fluctuate in numbers throughout the year. *U. tasmanica* utilised the winter months for its reproductive phase and neither adults nor larvae were found on the native cats during the warmer months. The other species of fleas were found on the hosts during the warmer months but in particularly high numbers during November and March. During March and April there was some overlap between *U. tasmanica* and the other species. Overall there was a decrease in the number of fleas found on the native cats during January and February which are the hottest and driest months of the year.

The winter occurance of *U. tasmanica* sets it apart from the other fleas which were found on the native cats and also from many other species of mammal fleas which peak in numbers during the summer months. These species include the rat fleas *Xenopsylla cheopis* and *Nosopsylla fasciatus*

(Bacot 1914, Traub 1972a) *Ctenophthalmus nobilis*, (Evans and Freeman 1950, George and Corbet 1959, Cowx 1967, Cotton 1970) and many of the fleas which infest the field vole (Ulmanen and Myllymaki 1971) where the different species peak in numbers during different months from Spring through to Autumn with the exception of *Malareus p. pedias* and *Rhadinopsylla integella* which peaked in the cooler months.

It is thought that seasonality in fleas occurs because of the changes in conditions of temperature, humidity and the avilability of food in the nests of the hosts throughout the year which influence the emergence of adult fleas from the cocoons (Ioff 1941, Cotton 1970). Thus many fleas breed during the warm months when conditions are generally most favourable for larval development.

Unlike other species of fleas, however, *U. tasmanica* larvae are not free living in the host's nest but develop as parasites in the host. (Dunnet 1970). Therefore they are not subject to the external conditions of temperature and humidity of the nest and it is possible that this enables the fleas to breed during the cold winter months. This does not however explain why *U. tasmanica* does not breed during the warm months.

As well as the seasonality of *U. tasmanica*, the trapping data indicated that the breeding season of this flea coincided with the pre-breeding and breeding season of its host. This may be accidental or it might suggest a link between reproduction in *Uropsylla* and its host. It is possible that *Uropsylla*, like the rabbit flea (Rothschild and Ford 1965) derives some hormonal stimulus from the host which promotes reproduction at this time. It is also possible that the behaviour of the native cats during the breeding season provides the fleas with conditions which are suitable for reproduction.

No significant differences were found between the levels of larval infestation on native cats of either sex or age group. Therefore

the fleas probably do not prefer hosts of any sex or age category.

The decline in the numbers of male fleas found towards the end of winter (Table 1.3) suggests that the female fleas outlive the males. Instances of unequal sex ratios in fleas have been recorded by Ulmanen and Myllymaki (1971) Cole (1945), Smit (1962), and many other authors who have generally reported a surplus of females during the peak of the fleas' seasons. In the case of *U. tasmanica* however the sex ratios were approximately even for most of the winter period and the males only appeared to decrease in numbers towards the end of the reproductive season.

The trapping data showed that in the two populations of native cats studied, one North and one South of Hobart, composition of the flea fauna varied. In the northern study area *P. hoplia* was the dominant flea during summer with high numbers of *A.r. rothschildi* and small numbers of *S. dasyuri*. In the southern population of native cats *S. dasyuri* was the dominant species during the spring and summer months. The incidence of *P. hoplia* and *A.r. rothschildi* was low compared with this species. (Figs. 1.4, 1.5).

The phenomenon of different compositions of flea fauna on a single species of host in different localities has been reported by Holland (1958) in two closely related species of *Atyphloceras*, a genus of fleas found on the nearctic vole. Ash (1952) and Rothschild (1952) have shown that the three most common bird fleas in Britain, *Ceratophyllus gallinae*, *C. garei* and *Dasypsyllus fallinae*, are zoned according to the preferred nesting sites of their hosts. Murray and Vestgens (1967) reported that *Parapsyllus magellanicus* only infests the nests of rock hopper penguins which are in sheltered positions. Gabbut (1961) following his investigations of the distributions of small mammals and their associated fleas concluded that as well as showing host preferences fleas also exhibited habitat

preferences. It may be then that the northern study area favoured the development of *P. hoplia* and *A.r. rothschildi* while the southern study area favoured *S. dasyurus*. *U. tasmanica* was able to thrive in both study areas but, as has already been stated, at a different time of year, to the other genera.

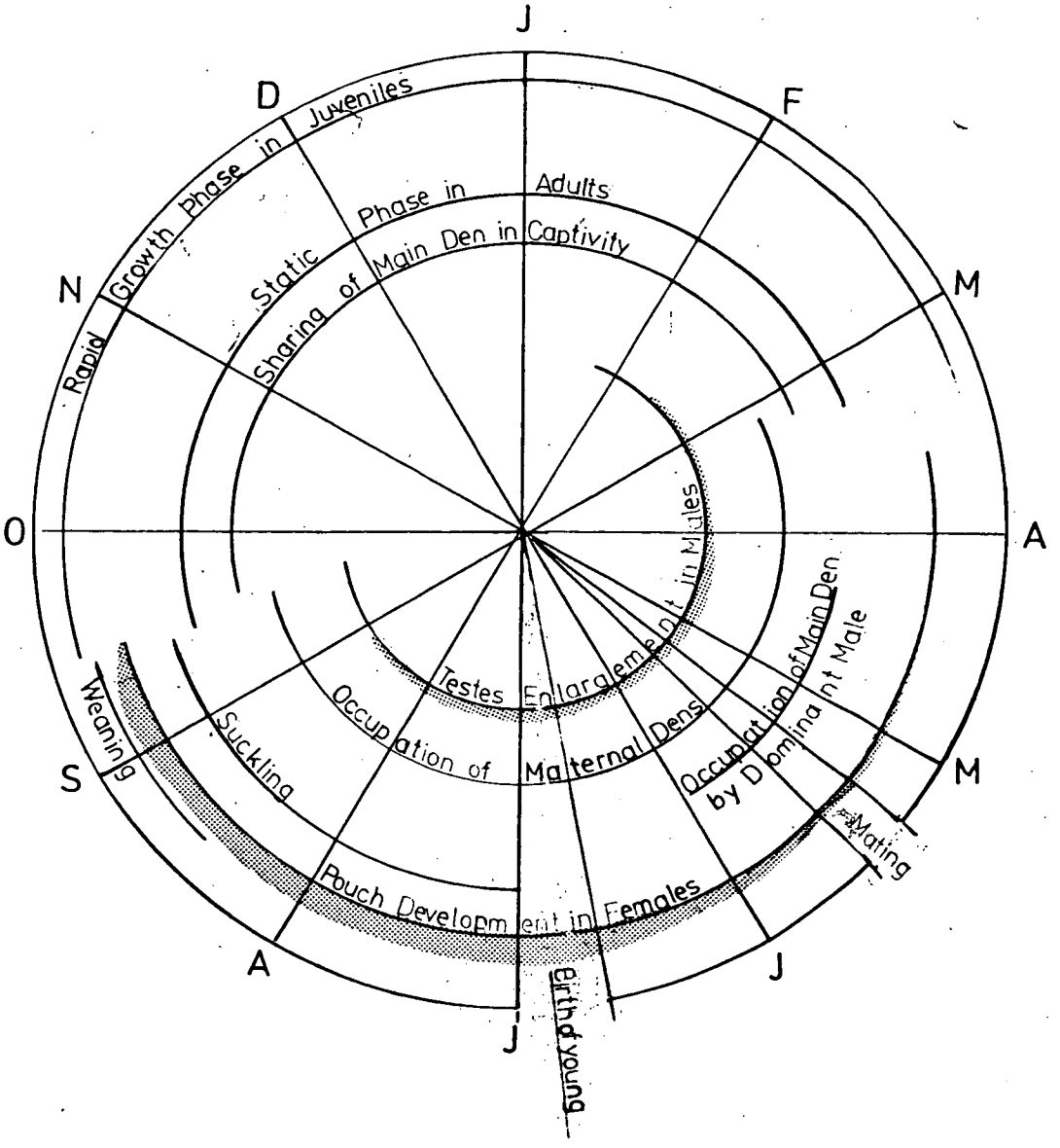


Fig. 1.7 Diagramatic representation of the annual cycle of *D. viverrinus* based on observations on captive animals.

Chapter 2

Factors Affecting the Emergence of Adult *Uropsylla tasmanica* from the Cocoon

2.1 Introduction

Fleas which exhibit seasonal variation in numbers fall into two categories. Firstly there are those which remain as adults in the nests of the hosts during the unfavourable season where they may feed occasionally but do not breed (Sviridov *et al.* 1963). Secondly there are those which remain quiescent in their cocoons either as adults or pupae during the unfavourable period (Rothschild and Clay 1956). Inactive periods during which reproduction or development does not take place coincide with adverse conditions such as low temperature or the absence of a host (Rothschild and Clay 1956).

Once the adults or pupal fleas have entered a period of quiescence there are two main factors which either bring about emergence or which stimulate reproductive or feeding activity. The first of these is a change in climate which signals the return of favourable conditions. Some species of *Xenopsylla* were found (Sviridov *et al.* 1963) to remain inactive as adults in the depth of the host's burrows during winter, however during spring these fleas moved towards the burrow entrances where they commenced feeding. The sand martin fleas (*Ceratophyllus styx*) overwinter as adults or pupae in cocoons in the abandoned nests of the host. These fleas emerge as adults in spring in time for the return of the sand martins to the nests (Rothschild and Clay 1956). The other fact which has been found to stimulate emergence of adult fleas from the cocoons is the presence of a host. *Ctenopthalamus nobilis* emerges from the cocoons in response to either a slight increase in temperature or to mechanical disturbance (Cotton 1970).

The importance of moisture content of the material surrounding the cocoons containing imaginal fleas has been stressed by Humphries (1967 a, b) in studies on the emergence of the hen flea *Ceratophyllus*

gallinae. Humphries found that under dry conditions these fleas were incapable of performing the sequence of behaviour which led to emergence despite the fact that they were as yet not displaying extreme symptoms of desiccation. He also found that desiccated fleas frequently failed to successfully complete the imaginal moult. Thus in dry conditions many fleas died during the imaginal moult while others were incapable of emerging from the cocoons. Humphries noted that the fleas which were unable to complete the behavioural sequence of emergence were able to survive beyond this time but died in the cocoons within several days.

It was found (Chapter 1), that *Uropsylla tasmanica* only utilises the cooler months of March to September for its reproductive phase. From September to late February or March the fleas are not found in the field on native cats. It has been found however, (Chapter 6) that *Uropsylla* are present as adults in their cocoons in October which implies that these fleas remain dormant during spring and summer, possibly in their cocoons.

The aim of the experiments presented in this chapter was to investigate the factors which control the summer quiescence of *U. tasmanica* adults and the factors which stimulate the fleas to emerge from cocoons.

As well as this the survival of adult fleas following emergence was investigated.

Humidity Experiment

In this experiment 5 tubes each containing ten adult fleas in their cocoons were placed in desiccators with relative humidities of 20%, 40%, 60%, 80% and 99% respectively. The cocoons were checked

visually every second day and one cocoon of each batch was opened every two weeks. If the flea in any cocoon was dead another cocoon was opened and so on until a living flea was found. Living fleas found in the cocoons were replaced in the desiccators in artificial cocoons consisting of glass tubing of 1.5 mm internal diameter 1 cm long with its end plugged with cotton wool. In this experiment the living fleas were handled with soft foil forceps and great care was taken not to stimulate the fleas by vibration. The desiccators were kept at 15°C for the duration of the experiment.

The use of artificial cocoons is discussed in greater detail in Chapter 5.

In an experiment designed to examine the survival of fleas in their cocoons twenty adult fleas still in the cocoons were placed in a desiccator with a relative humidity of 80% in November. The desiccator was then placed in a quiet corner of the 15°C constant temperature room and was undisturbed until mid April when the trapping data indicated that adult *Uropsylla* had already entered the native cat population. At this time the cocoons were opened.

2.2 Results

Desiccation Experiments

The group of fleas kept at 99% relative humidity all died without emerging from the cocoons. The cause of death is not known although the cocoons were heavily coated with a fungal growth and it is possible that this also killed the fleas inside. Of the other groups of fleas used in the experiment some 25% died without emerging. Of these fleas 6 had died without pupating while the other two had pupated but had died as pupae.

The results of the desiccation experiments are shown in fig. 2.1. Graph (a) shows the mean time of emergence of the fleas in each experimental group. It can be seen from the graph that the fleas emerged earlier under conditions of low relative humidity. The fleas kept at 20% R.H. emerged approximately 73 days after the commencement of the experiment while those kept at 80% R.H. emerged after approximately 104 days. The shape of the curve which flattened out at 60% R.H. suggested that at higher humidities the passing of time influenced the readiness of the fleas to emerge. Thus the difference between the mean time of emergence between the fleas kept at 80% and 60% was approximately 7 days while the difference between the mean time of emergence of the fleas kept at 60% and 40% R.H. was approximately 14 days. The difference in mean time of emergence between the fleas kept at 40% and 20% R.H. was approximately 14 days.

Graph (b) (Fig. 2.1) shows the mean time of survival of the fleas of each group in the absence of a host. The differences between the points on graphs (a) and (b) show the survival period of fleas in each group following emergence. It was observed that fleas survived longer, approximately 32 days, at 80% R.H. than at lower relative humidities. At 20% R.H. the fleas survived only 13 days after emergence, and as the relative humidity at which the fleas were kept increased so did the period of survival.

The significance of these results was tested by means of variance analysis. The differences between mean times of emergence of the experimental groups and the mean period of survival following emergence were found, using F distribution tables, to be highly significant (at 1% level).

It was noted that for each group of fleas the amount of mechanical

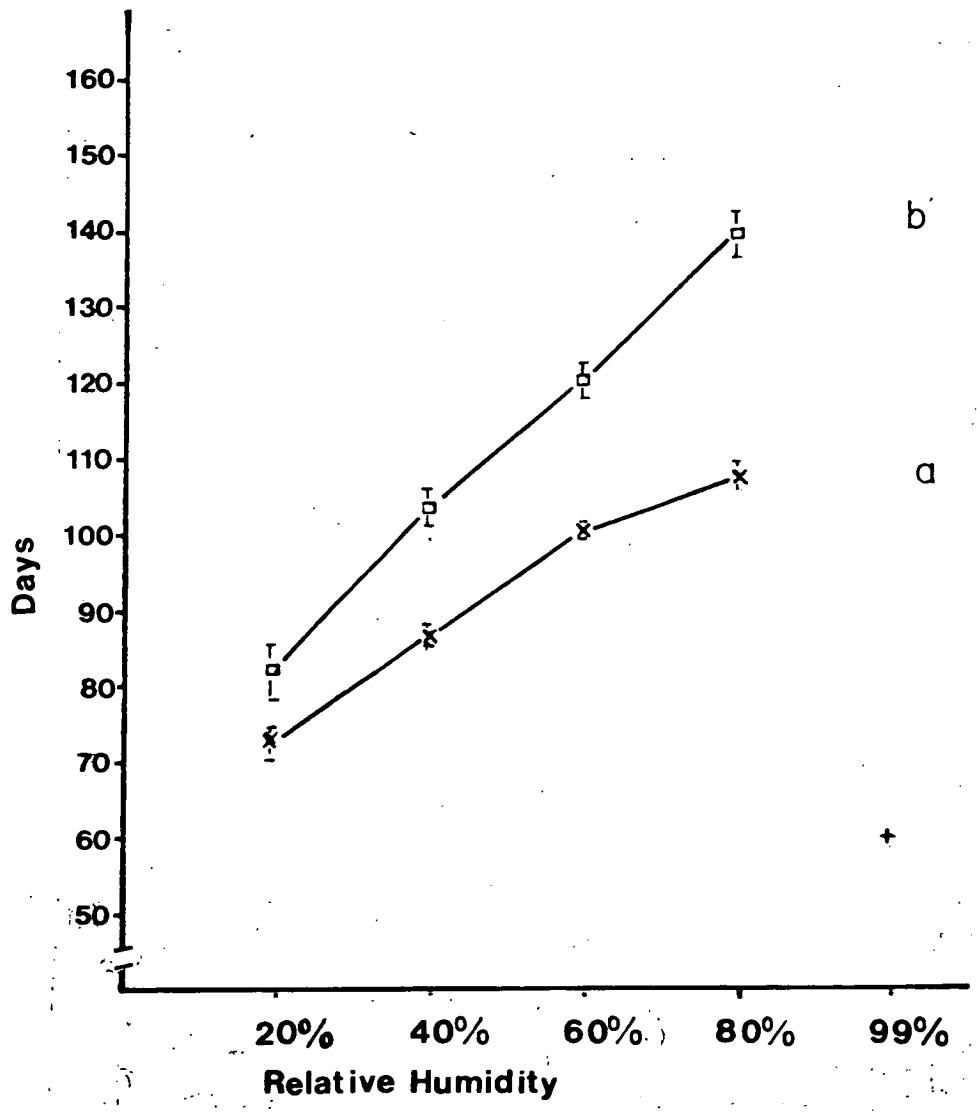


Fig. 2.1 Mean Time of Emergence (a) and Survival (b) of *Uropsylla* in different conditions of Relative Humidity at 15°C. Vertical lines and bars represent the standard errors of the means.

disturbance required to activate the quiescent fleas decreased as time passed until finally the act of removing the tube containing the cocoons from the desiccators was sufficient to arouse their occupants.

Survival of fleas in cocoons

In the experiment where the fleas were left in cocoons at 80% R.H. and not disturbed at all, it was found upon opening the cocoons in mid April that all of the adult fleas were dead. The fleas exhibited symptoms of desiccation or starvation as in all cases the abdominal plates were telescoped and the bodies were shrunken.

2.3 Discussion

The results suggest that aestivation was broken by vibration but that two factors affected the fleas' sensitivity to this stimulus. Firstly, it was found that at low relative humidities the fleas emerged from their cocoons earlier than those fleas kept at high relative humidities. Secondly it was found that regardless of the relative humidity at which the fleas were kept, their sensitivity to vibration increased with the passing of time.

The adaptive value of these results needs to be discussed in relation to the Tasmanian climate as well as the annual cycle of the host so far as it is known. In Tasmania the climate is variable but generally the driest months are January, February and March (see Table 2.1). While it was not possible to measure the relative humidity of native cat dens in the field it is possible that they would be subject to desiccation during the dry months, particularly if they were not occupied at this time. The observations on the captive native cats suggest that over summer the maternal nests are not in use (Chapter 1). It has been found that the litter in the dens in the native cat

compound was normally dry during summer but wet during winter. It is postulated that the dens of native cats in the field which are constructed at or below ground level would gradually dry out during the summer months when rainfall is at its lowest and the temperature is high.

Table 2.2 Average Monthly Rainfall of Trapping Areas.

	Average Monthly Rainfall	
	Buckland area	Huonville Area
January	43 mm	44 mm
February	55 mm	49 mm
March	46 mm	48 mm
April	69 mm	72 mm
May	79 mm	75 mm
June	60 mm	71 mm
July	67 mm	82 mm
August	67 mm	77 mm
September	51 mm	69 mm
October	67 mm	70 mm
November	78 mm	76 mm
December	66 mm	67 mm

Data supplied by Hobart Weather Bureau.

The importance of the moisture content of the material surrounding flea cocoons has been stressed by several authors (Petri and Todd, 1923, Uvarov, 1931). In *Xenopsylla* it has been shown (Mellanby, 1933, Edney 1947, Sharif 1948a) that the various developmental

stages in the cocoon are adversely affected by desiccation while Humphries (1963) has suggested that lack of water can affect the mortality and fecundity of bird fleas by preventing the successful operation of behavioural mechanisms concerned with development and reproduction.

Humphries (1967a) in a study of the behaviour of hen fleas in the cocoon has concluded that desiccation adversely affects the fleas in the cocoon at two stages. The first of these is the imaginal moult where inadequate water content prevents this taking place successfully, while desiccation can also lead to the breakdown of the behavioural sequence of emergence which causes death even though the fleas still retain physiological capacity to live for several days beyond this time.

Humphries also found (1967b) that in the absence of hosts imagines of *Ceratophyllus gallinae* were able to remain in the cocoon for several months and still emerge as healthy adults. He found that these fleas were able to counteract water loss by active uptake of water from the atmosphere so long as the relative humidity was above 82%. Below this level of relative humidity the fleas became desiccated and died. He also found, in conditions which allowed the fleas to take up water from the atmosphere, that the mechanism for this eventually failed thus causing the fleas to die in the cocoons.

The results of the desiccation experiments carried out on the *Uropsylla* adults in the cocoons suggest that their sensitivity to mechanical disturbance increases with desiccation. The results also suggest that the flea's sensitivity to mechanical stimulation increases with time. Thus it appears that emergence of adult *U. tasmanica* is under dual control.

It has been postulated that the humidity of the soil and litter of native cats' dens is controlled by climate and occupation by the hosts. In dens with high relative humidity the results predict that adult fleas would emerge after a certain period as a result of the raising of their sensitivity to mechanical stimulation. The results also suggest that fleas which emerge into conditions of high relative humidity (>60%) could survive for many days in absence of a host, if for instance, they emerged in response to a casual visit of an animal into the den.

In conditions of low relative humidity however, which might occur during a very dry summer, the results suggest that the fleas' sensitivity to mechanical stimulation would be raised by desiccation. In this case fleas in danger of death through loss of moisture would be stimulated to emerge early by the presence of a host. However, since fleas which have emerged into conditions of low relative humidity survive for only a few days, only those fleas which succeeded in finding a host within a few days would survive.

It is possible that the raising of the fleas sensitivity to vibration as a result of desiccation is a mechanism which ensures the survival of some fleas, those in occupied dens, in very dry summers.

The results of the experiment where the fleas were not given any mechanical stimulation suggests that in the absence of such disturbance the fleas do not emerge. It is possible therefore that in the absence of a host (i.e. in an uninhabited den) the fleas survive for longer periods in the cocoons than they might if they were to emerge and not find a host. The fact that all fleas were dead by mid April suggests that either that 80% R.H. was too low for the fleas to actively uptake

atmospheric moisture (Humphries 1967b) or the period was too long and the mechanism by whichatmospheric moisture is taken up failed (Humphries 1967b).

In the relative humidity experiments it was found that within each experimental group emergence of the adult fleas took place at approximately the same time (73 ±0.19days at 20% R.H., 84 ± 0.18 days at 40% R.H., 98 ± 0.11 days at 60% R.H., and 104 ± 0.20 days at 80% R.H.). This implies that the adult fleas in the cocoons in any particular native cat den would reach a state of "readiness to emerge" at the same time. It is possible then that the dual control of the duration of quiescence also ensures synchronisation of emergence of adult fleas.

Conclusion

Emergence of *U. tasmanica* adults from the cocoons appeared to be in response to the presence of a host. In the absence of any mechanical stimulation the fleas failed to emerge and died in the cocoons.

Readiness to emerge from the cocoons appeared to be under the dual influence of time and degree of desiccation. At high relative humidities the fleas emerged in response to mechanical stimulation later than those fleas which were kept in conditions of low relative humidity.

It was postulated that the dual control of emergence of adult *U. tasmanica* is a mechanism which ensures the survival of some fleas during summers.

It is possible also that synchronisation of emergence of adult fleas is achieved by means of the dual control mechanism.

Chapter 3

Sexual Maturation in *Uropsylla tasmanica*

3.1 Introduction

The trapping data (Ch. 1) has shown that the reproductive cycles of *U. tasmanica* and its host *D. viverrinus* appear to be synchronised. Both *U. tasmanica* and *D. viverrinus* are winter breeders and the larvae of *Uropsylla* are found on the native cats over a period which begins during the pro-oestrous phase of the native cats and which abruptly ends when the young native cats are weaned and first appear in the field. Reproductive synchrony of fleas and hosts has been observed in many species (Rothschild and Clay 1956, Askew 1971).

Synchronisation of breeding cycles of fleas and their hosts generally ensures that the flea larvae emerge from the eggs into conditions which are favourable for development (Rothschild and Clay 1956, Rothschild and Ford 1972). The fleas of many birds breed only during the periods wherein the nests are occupied by parent birds of nestlings (Rothschild and Clay 1956). Bird fleas are generally found in the nests rather than on the hosts and it is thought that the conditions of high humidity and temperature and the availability of food which are necessary for the development of flea larvae only occur during the breeding phase of the host.

The hen flea *Ceratophyllus gallinae* will breed only during spring despite the year round breeding of its domesticated host (Rothschild and Clay 1956). Thus despite the extended period of favourable nest conditions this flea still retains the "wild" habit of a spring breeding phase.

The reproductive period of the bird fleas is generally recognised (Rothschild and Clay, 1956, Askew 1971) as being under the control of season factors. Thus the sand martin fleas *Ceratophyllus hirudinus* emerge from their cocoons in response to the rise in temperature which

occurs in Spring. Their emergence occurs just prior to the return of the sand martins to the nests to breed (Rothschild and Clay 1956).

The fleas of mammals are not so strictly dependant on the presence of the host in dens during the breeding period. Firstly many adult mammal fleas are commonly found on the hosts rather than in the nests and secondly many mammals inhabit nests, dens or burrows all year round thus providing continuous food and shelter for their fleas. It has been found, however, (Bacot 1914, Darskaya 1963, 1970, Darskaya *et al.* 1962, Beck 1971, Peus 1968, 1972, Hurka 1963a, 1963b, 1969, Zhovtyi 1968, Traub 1972a, Williams 1973, Shepherd and Edmunds 1973, 1978, 1979, Cotton 1970, Ulmanen and Myllymaki 1971, Evans and Freeman 1950, Cowx 1967, George and Corbet 1959) that different species of mammal fleas exhibit seasonality and in some cases reproduction in the fleas is accelerated during the breeding season of the host as a result of the extra availability of food and the conditions of temperature and humidity which are found in maternity nests. An example of this is the flea (*Ctenophthalamus nobilis*) of the field vole (*Microtis agrestis*) (Cotton 1970) which reproduces throughout the year but in greater numbers in autumn and winter. The rate of development of this flea is affected by temperature and larvae found in the breeding nests and permanent nests are able to develop quickly whereas larvae which are left in deserted breeding nests develop slowly.

There are however two species of mammal fleas in which reproduction has been found to be dependant upon the reproductive phase of the host. These fleas are the rabbit flea (*Spilopsyllus cuniculi*) Mead — Briggs (1964 a,b) and the North American hare flea (*Cediopsylla simplex*) Rothschild and Ford (1972, 1973). Reproduction in the rabbit flea has

been extensively studied (Mead-Briggs 1964 a, b, Mead-Briggs and Vaughan 1969, Rothschild and Ford 1964a,b,1966 a, b 1969, 1970, 1973, Rothschild Ford and Hughes 1970). It has been found that gamete development, copulation and sperm transfer in *S. cuniculi* do not take place when the flea is feeding on non-reproductive female rabbits or on mature buck rabbits. It was found that in order for the female fleas to become gravid it is necessary that they feed on a pregnant doe rabbit and then transfer to feed on the new born kittens following parturition. In the male fleas it was found that sperm transfer could not take place until the fleas had partaken of a blood meal although copulation might be attempted. It was also found that the maximum development of the acessory sex gland and epididymis and maximum copulation and sperm transfer took place only after the male fleas had fed on the pregnant does and then transferred to the new born kittens (Rothschild, Ford and Hughes 1970).

Experiments using mammalian hormones by Rothschild and Ford (1964b, 1966b) have shown that many mammalian hormones particularly corticosteroids, estrogen, and somatotropin stimulate egg development, copulation or sperm transfer in the rabbit flea. Other hormones particularly progesterone, lutinising hormone and testosterone promote regression of eggs in the female fleas. Overall these studies have shown that the reproductive cycle of the rabbit flea is controlled by hormone levels of the host, and reproduction in the flea is bound in this way to the reproductive phase of its host the rabbit.

Cediopsylla simplex has also been studied (Rothschild and Ford 1972, 1973) and has also been found to be hormonally bound to the reproductive cycle of its host. Considerations of hormonal links between

other fleas and their hosts include an investigation of the oriental rat flea (*Nosopsyllus fasciatus*)(Rothschild *et al.* 1970) which was found to be capable of achieving maturation in the absence of those hormones which are necessary for reproduction in the rabbit flea. Ulmanen and Myllymaki (1971) in an investigation of the fleas in populations of the field vole *Microtus agrestis* found little evidence of a hormonal link between any of the flea species studied and their host although there was no physiological investigation. Cotton (1970) in his investigations of reproduction in *Ctenophthalmus nobilis* was able to discount the possibility of a hormonal bond as the flea was able to breed irrespective of the age or sex of the host or the time of year.

It can be seen that the life cycle of *Uropsylla* as far as it is known resembles that of the bird fleas or the hormonally bound mammal fleas. *Uropsylla* has a limited breeding season which coincides with that of the host but unlike the bird fleas, the host of *Uropsylla* is at least partially available to the flea all year round and in this respect *Uropsylla* resembles the rabbit and hare fleas (See Ch. 1). Another important resemblance between *Uropsylla* and the hormonally bound species is host specificity. *Spilopsyllus cuniculi* is generally thought of as being host specific (Rothschild and Ford 1963) although gravid rabbit fleas have been found living on hares (Rothschild and Ford 1965) while the records of the British museum (Hopkins and Rothschild 1953, p.58) show that with a few exceptions *Cediopsylla simplex* has generally been collected from hares. *Uropsylla tasmanica* is possibly somewhat more host specific than the British museum records indicate (see Ch. 7).

In view of the literature summarised above concerning interactions between fleas and their hosts with regard to reproduction as well as the apparent synchrony in reproduction of *U. tasmanica* and the native cat, an investigation into sexual maturation in *U. tasmanica* following emergence from the cocoon was undertaken. Firstly it was necessary to determine whether or not the fleas were capable of reproduction on emergence or whether a blood meal was necessary for the maturation of gametes. Secondly the fleas were subjected to a series of feeding trials to determine any feeding preferences for hosts of any sex or age groups. Following this an experiment was carried out to determine whether the application of mammalian hormones to the fleas affected their sexual development. The experiments were carried out in December and early January when the fleas are normally quiescent in their cocoons and are not found on native cat populations in the field. During January the native cats are non-reproductive as mating takes place in May, although it is to be expected that gonadal development and hormonal changes would take place prior to the actual mating period. In fact it has been found the the testes of male native cats begin to increase in size late in January (Ch. 1, p.17). It was hoped that by carrying out the experiments early in January and by using castrated male native cats during the hormone experiments that the effects of the build up of host hormones prior to the breeding season would be minimised.

3.2 Feeding and Sexual Maturation in new emerged fleas

This investigation was carried out simultaneously with the feeding trials. The aim was to determine whether *U. tasmanica* were sexually mature upon emergence from the cocoons or whether a blood meal was necessary for copulation and sperm transfer to take place. The aim of the feeding trials was to establish whether the fleas, on emergence from the cocoons, displayed any preference for hosts of any sex or age category.

Materials and Methods

Fleas were taken from their cocoons during December and put into cylinders, 4 cm diameter x 12 cm long and made of clear perspex . These tubes had a fine gauze covering at one end while the other end was blocked by a screw in bung of perspex with a 1 cm hole drilled through it. The hole in the bung was normally blocked by a cork which held a strip of moist filter paper in place.

In order to feed the fleas the cork and filter paper were removed and a shaved portion of a native cat's tail was inserted. The native cats' tails were not completely nude but retained a fur covering approximately 3 mm long so that the fleas could cling to the tail but would not be lost from view (see Plate 3.1).

In the feeding preference trials the fleas were offered feeds from male and female sub-adult native cats as well as adult native cats of both sexes. For each tube the total time used by the fleas for feeding was calculated. Mirrors were placed so that the entire portion of the tail of the native cat in the feeding tube was always visible (Plate 3.1 b).

For this experiment four tubes each containing five fleas were used. More than five fleas were difficult to watch at any one time.

Plate 3.1

(a) Sub-adult native cat with tail shaved ready for a feeding trial.

(b) Feeding apparatus.

(c) Flea feeding on shaved native cat tail.

a

b

c

Each feeding session lasted one hour. The trials were continued until each native cat had amassed a total of ten available flea feeding hours. Each day four native cats were selected and the four feeding tubes were attached to their tails in turn for one hour. If the fleas failed to feed they were used again on a subsequent native cat that day but the tubes containing fleas which did feed were set aside until the next day (see Table 3.1).

Table 3.1 Feeding trial schedule

	DAY 1		DAY 2		DAY 3	
Session	1	2	3	4	5	6
Adult 1	tube 1		tube 4			
Adult 2	tube 2		tube 3			
Adult 1	tube 3		tube 2			
Adult 2	tube 4		tube 1			
Sub-adult 1		tube 1		tube 4		
Sub-adult 2		tube 2		tube 3		
Sub-adult 1		tube 3		tube 2		
Sub-adult 2		tube 4		tube 1		
Adult 2 + urine from sub-adult					tube 3	tube 4

During the feeding trials notes were made of the fleas behaviour. This was faciliated by the fact that the fleas assumed three characteristic positions whilst on the tail. The first position, the "attached position" consisted of the flea clinging to the tail with its body at a slight angle to the tail. This appeared to be a resting position. Position 2 was the "probing position" where the flea clung to the tail with the head in contact with the skin and the body at an oblique angle to the tail. This posture was never maintained for more than a few seconds

and always preceeded either walking or feeding. Position 3 was the "feeding position" where the flea's body, supported by the hind legs was kept at an angle of about 45° to the tail. That this was in fact the feeding position was ascertained by watching the fleas with the aid of a dissecting microscope whilst they fed on blood through a membrane.

After each feeding session the fleas were observed for 30 minutes and, following Humphries (1967b) description of sexual behaviour in the hen flea *Ceratophyllus gallinae* two categories of sexual behaviour were recorded for *Uropsylla*. Behaviour of type 1 consisted of pre-copulatory behaviour where the male flea, with its antenna raised would nudge the female's abdomen and attempt to push its head and thorax underneath her body. Behaviour of type 2 was recorded when copulation took place.

In the feeding trials two adult male and female native cats were used as well as two sub-adult male and female native cats. The sub-adult native cats were approximately seven months old and would have reached sexual maturity in the following breeding season in May. In a further experiment an adult female native cat whose tail had been rubbed with urine from the sub-adult native cats was offered to the fleas as host.

At the end of the experiment which lasted three days the fleas were killed and their stage of sexual development was evaluated microscopically from sections and compared with the level of sexual development of newly emerged unfed fleasand unfed fleas which had been removed from the cocoons three days previously.

All fleas were prepared for sectioning and stained by the method described by Rothschild (1975) and outlined below:

Firstly fleas were fixed in Dubosq Brasil, initially at 30° for 12 hours and then at room temperature. They were then transferred to Supercedrol (Gurr) for 24 hours with three changes of solution. Following this the fleas were vacuum embedded in paraplast for 1 hour and finally mounted in paraplast blocks. Sections were cut at 10 μ and transferred to clean glass slides coated with Meyer's albumin. The sections were stained with Mallory's stain as described by Rothschild (1956).

Procedure for assessing and comparing sexual development

The degree of sexual maturation in male fleas was assessed by examination of the testes and the stage of development of the sperm bundles. In the rabbit flea (Rothschild *et al.* 1970) spermatogenesis is complete before emergence of the adult flea from the cocoon. When the adult male flea emerges the sperm are arranged in bundles of approximately 250 sperm per bundle, in the testes. The sperm in each bundle mature synchronously and generally in the testes of any flea there is a high proportion of sperm bundles at the same stage of maturation. The stage of sperm bundle development in the newly emerged rabbit fleas varies but in all cases the opening between each testis and epididymis is blocked by a testicular plug which prevents the migration of sperm out of the testes. Rothschild *et al.* have described seven stages in the development of the sperm bundles where the last stage coincides with the unblocking of the epididymis. In the last stage the sperm bundles begin to break down and the sperm are forced into the epididymis In the last stage the sperm bundles begin to break down and the sperm are forced into the epididymis by the muscular contractions of the wall of the testes. When sperm are present in the epididymis the flea is thought to be mature.

Associated with the development of the sperm bundles in the male rabbit fleas several other histological changes take place in the accessory glands, lumen of the epididymis and the vas deferens.

In both sexes of rabbit fleas, sexual maturation is accompanied by changes in the salivary glands and the mid-gut. In rabbit fleas of both sexes but particularly in the female the defecation rate increases markedly prior to egg laying thus ensuring a plentiful supply of food for the developing larvae. At this time copious amounts of saliva are produced by fleas and the blood is only partly digested in the mid-gut thus causing the rectal ampulla to swell with an accumulation of partly digested blood which is then passed out with increasing frequency before the eggs are laid.

In newly emerged rabbit fleas the fat body contains a varying number of "albuminoid bodies" which using the staining technique already described stain bright blue or orange. These inclusions decrease as the flea matures. In unfed fleas the fat body, once the inclusions have disappeared, seem to consist of a series of apparently empty cells with a granular nucleus, which stains red with Mallory's stain. Once feeding has commenced the cells increase in size and granular inclusions are found in the cytoplasm. During maturation the fat body cells again increase in size and both the nucleus and cytoplasm contain dense aggregations of protein inclusions which stain red or brownish purple except for the non-staining fat droplets. After egg laying and the exhaustion of sperm from the testes the fleas' fat bodies become depleted and ragged in appearance.

Rothschild *et al.* (1970) have pointed out that the sequence of events in the sexual maturation of the rabbit flea are not always followed by other species and therefore the descriptions of maturation

for the rabbit flea have been used only as a guide in assessing the development of the *U. tasmanica* following emergence from the cocoon. But generally, the male *U. tasmanica* were thought to be sexually mature when sperm were observed in the coils of the epididymis. Evaluation of the immature fleas was based on the stage of sperm bundle development the state of the salivary glands and the appearance of the fat body as well as the size and appearance of the accessory glands.

In female fleas the degree of sexual development can be gauged from the development of the eggs. Mature eggs contain yolk and have a chorion. In the case of the *U. tasmanica* females the deposition of yolk and the formation of the chorion was not frequently observed and did not occur in any of the fleas used in the feeding trials. For this reason the females were compared on the basis of egg size and number. The procedure for comparison of egg development between females of each group was as follows: From the stained sections of each group one female of each group was chosen, this was the female which had the greatest number and the largest eggs; the most complete section of each female was then chosen, many sections were fragmented and only complete median sections were chosen; the eggs found in each section were then counted and measured with the aid of a camera lucida. For small eggs measurements were taken in the direction of the egg column, fleas have panoistic ovaries so that the eggs appear to be serial. For large eggs the measurements were taken along the long axis. Following this the groups were compared statistically for egg number, egg size and size range.

As an extra check on sexual maturation in the female fleas notes were made on the state of the salivary glands and the fat body.

Results

Feeding Preference Trials

The results of the feeding trials are summarised as histograms in Figure 3.1. It can be seen from Figure 3.1 that prefeeding behaviour took place more frequently on the sub-adult native cats and the adult native cats carrying the juvenile urine, (10-16 incidents) than on the adult native cats (0-1 incidence). When these results are subjected to a χ^2 test the differences in prefeeding incidence were found to be significant at the 1% level.

Comparison of the time spent feeding by the fleas on the different native cats (Fig 3.1) shows that fleas fed on the sub-adult native cats. On these hosts some 10% to 23% of available flea feeding hours were used for feeding. On the adult native cats the only record of actual feeding involved a 0.8% use of the available flea feeding hours on one host. The fleas did not feed at all on the other three adult native cats. On the adult native cat which carried the sub-adult urine it was found that while the prefeeding behaviour of the fleas was similar to that on the sub-adult native cats through the actual time spent feeding was only 1.5% of the available flea feeding hours. When χ^2 tests were applied to these results the difference between the amount of flea feeding on the sub-adult native cats and the adult native cats was found to be significant at the 5% level while the difference between the adult native cats and the adult native cat bearing sub-adult urine were not significantly different at the 5% level.

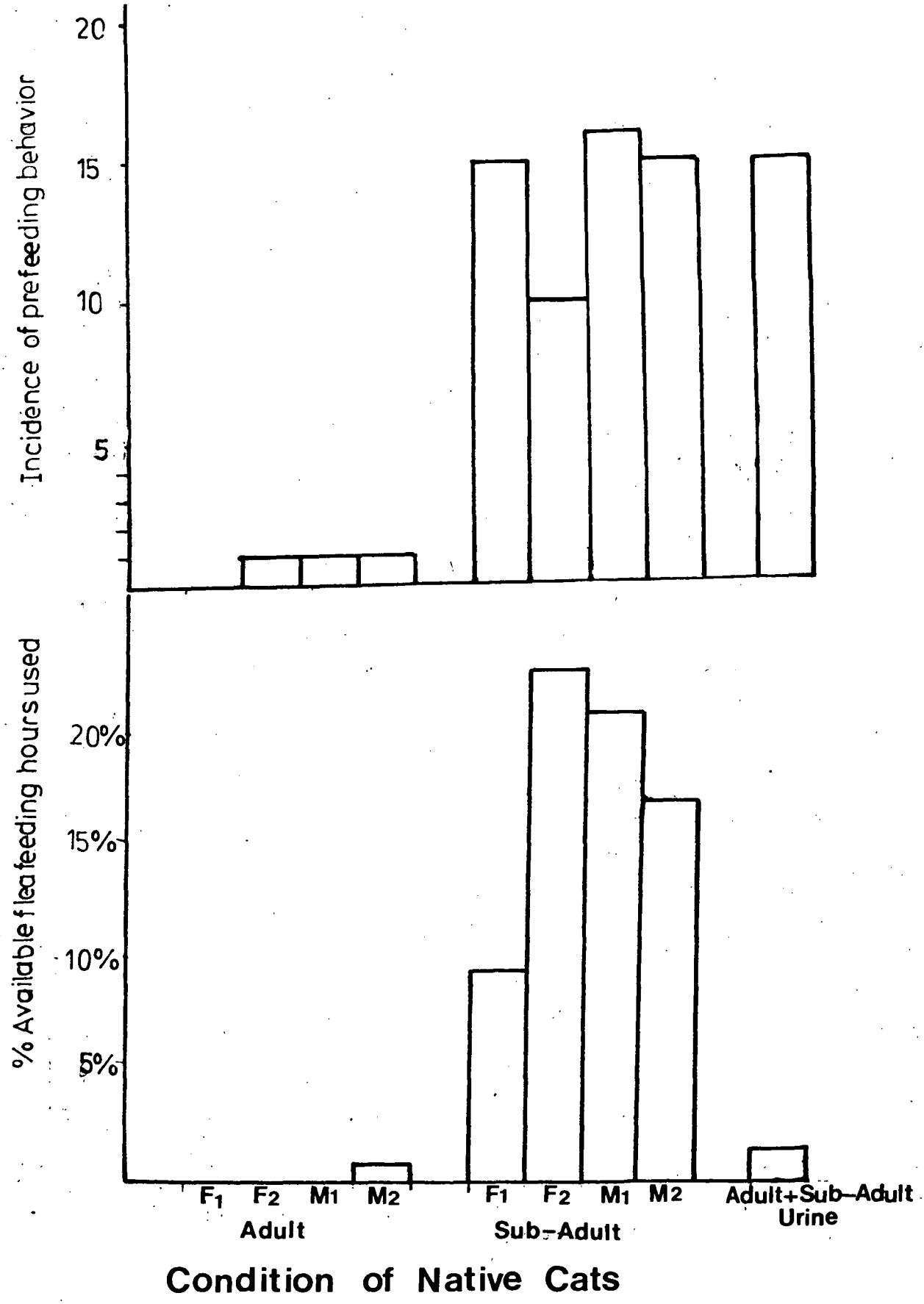


Fig. 3.1 Feeding Trials

(a) Incidence of pre-feeding behaviour on all native cats.

(b) % of time spent feeding by fleas on all native cats.

Sexual Behaviour following Feeding Sessions

The incidence of sexual behaviour observed following the feeding sessions is summarised in Table 3.2 3.3. It can be seen from this table that the greatest incidence of sexual behaviour took place following the feeding sessions on the juvenile native cats. Overall, following the feeding sessions on the sub-adult native cats a total of ten copulations were observed while no copulations took place following feeding sessions on the adult native cats. Following feeding sessions on adult female 2 which had been smeared with urine from the sub-adult native cats, one copulation was observed as well as pre-copulatory behaviour. Two instances of pre-copulatory behaviour were observed following the sessions on the adult native cats. However after the feeding sessions on the sub-adult native cats precopulatory behaviour was observed in all cases. On two occasions precopulatory behaviour was during the feeding sessions on the sub-adult native cats but was never observed during the sessions on the adult hosts. Sexual behaviour was not observed to occur in the tubes before the feeding sessions.

Table 3.2 Sexual Behaviour following Feeding Sessions

Host	Tube No.	Day	Sexual behaviour observed
Sub-Adult 1	4	2	2 x copulation
Sub-Adult 1	1	1	type 1 + 1 copulation
Sub-Adult 2	2	1	1 x copulation
Sub-Adult 2	3	2	type 1 + copulation
Sub-Adult 1	3	1	type 1 + 2 x copulation
Sub-Adult 1	1	2	type 1 + 2 x copulation
Sub-Adult 2	4	1	1 x copulation
Sub-Adult 2	2	2	type 1 + 1 x copulation
Adult 1	3	1	NN
Adult 1	4	2	type 1
Adult 2	3	1	NN
Adult	2	2	NN

Table 3.2 cont.

Host	Tube No.	Day	Sexual behaviour observed
Adult 1	2	1	NN
Adult 1	3	2	NN
Adult 2	1	1	Type 1
Adult 2	4	2	NN
Adult 2	3	3	Type 1
Sub-Adult urine	4	3	Type 1 + 1 copulation

No. of copulation recorded following exposure to sub-adult native cats = 10

Copulation following exposure to adults = 0

Copulation following exposure to adult + sub-adult urine = 1

Table 3.3 Total Sexual Behaviour on all Native Cats.

Native Cats	ffh.	Copulations	Attempted Copulations
Sub-Adult	40	10	7
Adult	40	0	2
Adult + Sub-Adult Urine	10	1	2

ffh = available flea feeding hours.

Newly Emerged Unfed Fleas

Examination of the sections of 5 newly emerged male fleas revealed that these fleas were in all cases sexually immature. The testes in all cases contained sperm bundles in which the spermatids were enclosed in cytoplasmic caps at both ends. The sperm bundles exhibited synchronised crimping of the sperm tails. In two individuals some twisting of the sperm bundles was evident (Plate 3.2a) although the majority of the bundles were aligned with the long axis of the testes. In sections of one individual a testicular plug was observed but was not seen in other specimens. In all cases the lumena of the epididymis were undeveloped. The sperm bundles were judged to be at a state of development which would correspond to stage 3-4 in sperm bundle development in the rabbit flea as described by Rothschild *et al.* (1970).

The accessory glands in the newly emerged male fleas were small, approximately 1/8th the length of the testes and none of the accessory glands examined had a lumen.

The salivary glands in newly emerged fleas of both sexes were composed of large sub-spherical cells without a visible lumen. They appeared to be composed of fewer cells than the salivary glands of the rabbit flea and resembled those of the rat flea (Rothschild *et al.* 1970) (Plate 3.2 a).

In all newly emerged *U. tasmanica* the fat body contained the "albuminoid bodies" described by Rothschild *et al.* (1970) for the newly emerged rabbit flea and thought to be remnants of the pharate adult stage where they are present dense aggregations in the fat body cells (Plate 3.2 a).

On emergence the five female *U. tasmanica* examined were found to contain many small oocytes (Table 3.4). The spermatheca was filled with a pale blue staining, homogeneous secretion. The oviducts were visible in the sections but a lumen was only infrequently observed.

3 Day Old Unfed Fleas

Four male fleas were examined and these generally resembled the newly emerged male fleas. The state of the testes was the same as in the newly emerged group with the sperm bundles at stage 3 or 4 of development. The lumen of the epididymis was not yet developed and in two specimens a testicular plug was observed.

The five female fleas examined were indistinguishable from the newly emerged fleas with respect to the oocytes, oviducts and spermatheca.

In both sexes some changes has taken place in the fat body which appeared to be less densely packed with the blue and red staining granules which were observed in the newly emerged fleas. The fat body cells appeared to contain more and larger vacuoles than those of the newly emerged group.

Of the nine fleas examined the lumen of the salivary gland was apparent in three specimens and contained an homogeneous pale blue staining material.

3 Day Old Fed Fleas

Eleven male fleas were examined. The testicular plug was not observed in any of the specimens and in all cases the lumen of the epididymis was developed. Sperm were observed in the epididymis of eight of the fleas. In the other specimens the sperm bundles

were large, twisted and loosely bound at one end, corresponding to stage 6 in sperm bundle development of the rabbit flea. The accessory glands were very obvious with well developed lumen and the largest of these was ¼ as long as the testes. The state of development of the testes and epididymis of a flea from this group was judged to be sexually mature is shown in Plate 3.2b., Plate 3.3.

In the female fleas of this group of which nine were examined the oocytes appeared to be slightly larger than those of the unfed fleas (Table 3.4) (Plate 3,2c). None of the eggs contained yolk. In one female the spermatheca contained some (Plate 3.5b of Plate 3.5) sperm while the spermatheca of the other females contained the blue staining secretion found in the previous groups.

Comparison of the number, sex and size range of the oocytes in the female fleas of the fed, unfed and newly emerged groups (Table 3.4) shows that the number of oocytes in each group is similar although there is a higher mean oocyte size in the fed females than in the other groups.

In both sexes the blue and red inclusions of the fat body cells described for the newly emerged fleas had disappeared and the cells contained vacuoles and blue staining granular matter. The cells of the salivary gland appeared to be flattened around a wide lumen which contained slightly granular blue staining material.

Discussion

The results suggest that newly emerged *U. tasmanica* preferred to feed from sub-adult native cats and that they found the adult native cats unattractive.

The incidence of sexual behaviour following the feeding sessions showed that copulation was most likely to occur following exposure of the fleas to the sub-adult native cats. Since the amount of feeding which took place on the adult native cats was very small, 0.8 hrs, compared with 7.1 hrs, on the sub-adult native cats, it is possible that sexual behaviour is initiated by a blood meal. It is also possible that the sub-adult native cats transmitted some factor to the fleas which stimulated copulation. It was found however that the fleas which were exposed to the adult native cat which had been treated with sub-adult urine exhibited sexual behaviour following the feeding sessions despite their failure to feed. It appears therefore that sexual behaviour in this case was stimulated by a factor which was carried at least partly in the urine of the sub-adult native cats.

It was found during the three days of the feeding trials that some sexual maturation took place, mainly in the male fleas. The male fleas which were sectioned on emergence from the cocoons were all found to be immature as were those fleas which were sectioned three days after emergence but which were not fed. This suggests that male *U. tasmanica* require a blood meal before complete sexual maturation and sperm transfers can take place. Since the female fleas of all three groups were immature at the completion of the experiment, it is also possible that they too require a period of feeding in order to mature.

It has been stressed by Rothschild and Ford (1970) that copulation can take place in sexually immature fleas of both sexes. In some species the females will pair and be impregnated before there has been oocyte development. Similarly, willingness to pair in males can be stimulated in the absence of complete sexual maturation and therefore take place without sperm transfer or with limited sperm transfer.

The preference of the fleas for the sub-adult native cats as shown in the laboratory, was unexpected. This was because the field data (Chapter 1, pp16) had revealed no significant difference in the levels of *Uropsylla* infestation on hosts of different age categories.

Native cats generally are monoestrous and the mating period is fairly short with the females all coming into oestrous at the same time in May (Chapter 1 pp17). Exceptions do occur however when female native cats either fail to produce a litter or the litter is lost soon after birth. In these cases they may enter a second oestrous and mate (Fletcher 1977). Young native cats become sexually mature and participate in the reproductive season following their birth. Sexual development in preparation for the breeding season was observed as an increase in testes size in male native cats in February where pouch development in female native cats was observed in March (Chapter 1 pp17). Therefore, by the time the *U. tasmanica* enter the host population in late February to March (Chapter 1, Chapter 2), the native cats were already undergoing gonadal development and all native cats of each sex were at a similar stage of development.

In an attempt to explain the results of the feeding preference trials it is necessary to consider the possible differences between the sub-adult and the adult native cats at the time of the experiment.

The experiment was carried out in December at which time the adult native cats were in a static, non-reproductive state. The sub-adult native cats were, on the other hand growing very rapidly. Furthermore, the possibility of gonadal growth in the sub-adult native cats analagous to pre-pubic development in placentals (Scheer 1965) at this time cannot be overlooked. It is possible then that the sub-adult native cats provided the fleas with certain factors associated with somatic and/or gonadal growth which were not present in the static adult native cats during the non-reproductive phase but which are present in all native cats at the time when the fleas enter the host population in March.

Rothschild *et al.* (1973) have described a kairomone effect of the urine of newborn rabbits on the reproductive cycle of the rabbit flea. A kairomone is inter-specific (Brown et al 1970) and in this species the presence of urine from newborn kittens was attractive to the fleas and stimulated sexual activity as well as promoting the production of eggs. The newborn kitten urine stimulated increased sexual activity in mature fleas but only increased the incidence of successful impregnation if the fleas were feeding on the newborn kittens at the time. The kairomone effect was not observed on immature fleas. The results of the feeding trials in which the fleas appeared to find the sub-adult urine attractive and sexually stimulating were similar to the rabbit flea results in that the stimulating factor was found in the urine of the sub-adult native cats and not in the adult hosts. There was however evidence that the factor was restricted to the urine of the sub-adult native cats. The results of the *Uropsylla* experiments differ from those of the rabbit flea in the age group of the hosts emitting the kairomone. In the rabbit flea the kairomone was only found in the newborn nestlings not in sub-adult rabbits. The

second major difference occurred in the effect of the sub-adult urine on the immature fleas. In *Uropsylla* the presence of the urine was associated with two things, willingness to feed and sexual activity. While feeding on the sub-adult native cats some of the male fleas were able to mature so that limited sperm transfer took place during copulation in at least one case. In the rabbit flea the kairomone only stimulated sexual activity if the fleas were sexually mature at the time of exposure to the kairomone.

It was impossible to test the effects on development in immature fleas of exposure to very young native cats since at the time when these native cats were available, the *Uropsylla* adults were already fully mature on the adult native cats.

3.3 The effects of mammalian hormones on sexual development in *U. tasmanica*

These experiments were carried out to investigate whether certain mammalian hormones influence sexual maturation in newly emerged *U. tasmanica*. The experiments were carried out for several reasons; firstly, the trapping data (Chapter 1) had indicated that the adult *Uropsylla* commenced their reproductive phase at a time when both male and female native cats were undergoing secondary sexual development, testes growth in males and pouch development in females, in preparation for the mating period in May; secondly, the trapping data had shown that the reproductive phase of the fleas ceased at the time when the newborn native cats became independent; thirdly, the results of the previous experiments (section 3.2) had suggested that while adult *Uropsylla* males were able to feed and achieve sexual maturity on the sub-adult native cats, they found the non-reproductive adults unattractive and refused to feed on them.

Since the trapping data and the experiments on feeding preferences (section 3.2) did not indicate any sexual bias in host selection it appeared unlikely that male or female sex hormones influenced reproduction in *Uropsylla*. It was decided therefore to concentrate on the effect of hydrocortisone which had been shown (Rothschild and Ford 1964b) to influence sexual development in the rabbit flea. It was intended also to investigate the effect of growth hormone on development in *Uropsylla* because the results of the feeding preference experiments had suggested that the sub-adult native cats provided the fleas with a factor which was absent in the mature hosts. Somatotropin was not obtainable for this experiment and of the hormones which are known to affect tissue growth in mammals only prolactin was available. Prolactin was therefore incorporated into the experiment.

Materials and Methods

Four feeding tubes of fleas were set up as before (Section 3.2) each containing 7-10 newly emerged fleas. The fleas in each tube were fed for one hour each day on one of two previously, (two weeks prior to the experiment), castrated male native cats. The native cats were castrated while anaesthetised with ether by breaking the spermatic cords leading to the testes and removal of the testes. Following each feeding session the fleas in the tubes were subjected to the different hormonal treatments shown in table 3.5.

The hormone solutions were made up from dried preparations of hydrocortisone (B.P. Batch 5077) and prolactin (Series NIHP - s-12 ovine). The prolactin was donated by Dr A. Gidley Baird of the Department of Veterinary Science at the University of Sydney. The concentrations of

the saturated aqueous solutions of the hormones which were used in the experiments were Prolactin approximately 2μg/l and Hydrocortisone 10μg/l. The hormones were delivered to the fleas through the hole in the perspex bung in the feeding tube from ASPAX pocket atomisers, a different atomiser being used for each preparation.

When not attached to the tails of the native cats for feeding sessions the fleas were kept at approximately 99% Relative Humidity and at room temperature. The experiment lasted 16 days and at the end of this time all surviving fleas were killed and sectioned. The effects of the various treatments on the development of the gonads was observed from sections prepared and evaluated by the methods which have already been set out in section 3.2. The female fleas were also compared with sections of a fully mature wild female.

This experiment was carried out in January 1979 and used all of the adult fleas that were available at the time. These adults were quiescent in their cocoons and as with the previous series of experiments it was necessary to remove them from the cocoons and rouse them from the quiescent state in order to carry out the experiments. The adult fleas were removed from the cocoons on the day before the experiments commenced.

Table 3.5 Hormone Experiment - Daily Procedure

Tube 1	1 hour feeding and spray of distilled H_2O
Tube 2	1 hour feeding and spray of hydrocortisone
Tube 3	1 hour feeding and spray of hydrocortisone and spray of Prolactin
Tube 4	1 hour feeding and Prolactin spray.

Results

In general it was observed that the feeding sessions had been too short and too infrequent. Of the 32 fleas alive at the beginning of the experiment only 24 survived the sixteen days, 11 females and 13 males. From the sections it was observed that in all fleas which survived the experiment, the dorsal plates were overlapping to a great extent and frequently the rectal ampullae was small and empty. The mesenteron was empty in six of the fleas examined which suggests that not all fleas fed during each feeding session as the fleas were killed immediately following the final feeding session.

Tube 1 - Fleas sprayed with distilled water following feeding sessions.

The males of this group of which 3 survived had varied sperm bundle development. In one flea there were no sperm in the epididymis although the sperm bundles appeared to be almost completely developed corresponding to stage 6 of development as described by Rothschild *et al.* (1970). The other two fleas had fully developed sperm bundles and sperm were observed in the epididymis. The accessory glands of these fleas were not well developed and from the sections the largest accessory gland was approximately ¼ as long as the testes. The salivary glands of this group consisted of flattened secretory cells surrounding a lumen packed with homogenous slightly granular blue staining material.

When the female fleas of this group (of which 3 survived), were examined it was found that in all cases the spermathecae were devoid of sperm and contained the pale blue staining secretion which was seen in newly emerged and unmated, fed female fleas. The oocytes lacked yolk deposition but were generally larger than those of the newly

emerged female it was found that the egg number was greater and the mean size of the eggs was greater. The variation in egg size of this female was also greater than in the newly emerged female where the variation was only 28.3% compared with 41.97%. When egg number, size and size variation were compared with the wild female it was seen that egg number was comparable, 18 compared with 21 while the mean egg size of the group 1 female was much smaller than that of the wild female, .00142, compared with 0.426 mm. The variation in egg size of the group 1 female was also small compared with that of the wild female, 41.97% compared with 88.4%.

Tube 2 - Fleas sprayed with Hydrocortisone solution following feeding.

In all three surviving males of this group, sperm bundle development was complete and sperm were observed in the epididymis. The accessory glands appeared to be similar in size and development to those of the previous group, while the salivary glands were indistinguishable from the previous group.

The four surviving females of this group lacked sperm in the spermathecae. The oocytes however appeared to be generally larger than those of the previous group and minimal yolk deposition, diagnosed as red staining inclusions in the eggs, was observed in some eggs.

When the egg size and size range in this female was compared with the female of the control group (Table 3.6) it was seen that the mean eggs size was greater, .0196 mm compared with .0142 mm while the egg number was smaller, 10 compared with 18. The variation in egg size of this female was slightly greater when this female was compared with the wild female the egg number was smaller, 10 compared with 21 and so was the mean egg size, .0196 mm compared with 0.425 mm while

variation in egg size in this female, 49.43%, was considerably smaller than in the wild female, 88.42%.

Tube 3 - Fleas sprayed with Hydrocortisone and Prolactin

This group of fleas of which 6 survived, 2 females and 4 males showed the greatest degree of sexual development.

The males all had fully developed sperm bundles with sperm in the epididymis and greatly enlarged accessory glands. In the section with the largest portion of accessory gland visible, the gland was approximately 2/3 as long as the testes. (Plate 3.3). Once again the salivary glands resembled those of the other groups.

The most developed female of this group had fully yolked and chorionated eggs though chorionation were not observed in the other two females. Two of the females had sperm in the spermatheca. In two of the females the eggs showed signs of regression having brown and yellow staining inclusions (Plate 3.4b). The mean egg size for the best developed female was 0.0164 with a coefficient of variation of 101%. This female had the greatest egg size variance of any of the groups and in this flea the variance was greater than in the wild female. (Plate 3.5).

The fat body of the male and female fleas of this group contained small areas of granular inclusions which stained orange under the modifified Mallory's stain thus indicating slight protein storage.

Tube 4 - Fleas sprayed with Prolactin

Male fleas of which 3 survived the experiment were all fully mature. The accessory glands appeared to be well developed and the largest section of accessory gland observed in the sections of these fleas measured approximately 2/3 the length of the testes. This is

comparable with the accessory gland development in the previous group. The salivary glands resembled those of the previous groups but the fat body in two of the specimens contained orange staining granular inclusions unlike those of the previous groups.

Of the 2 female fleas which survived the experiment, both had sperm in the spermathecae (Plate 3.6b). Although some of the eggs observed contained yolk many of the eggs appeared to be degenerating and contained brown and yellow staining matter. In this group, the mean egg size was .01466 mm compared with .0164 mm in the female treated with hydrocortisone and prolactin. The variation in egg size was smaller than in the previous group and smaller than in the wild female although larger than in the females of the control group of those which were treated with hydrocortisone (Table 3.6).

The salivary glands resembled those of the feeding fleas of the other groups and once again the fat body contained granular orange or red staining inclusions as well as the blue staining material found in the non-prolactin treated groups.

Discussion

It was found, that under the conditions imposed on the fleas during the course of the experiment, the least sexual development took place in the control group of fleas which were treated with distilled water while the greatest amount of sexual development took place in the group of fleas treated with Hydrocortisone and Prolactin.

Both sperm bundle and egg development appeared to be encouraged by the application of the hormones. Hydrocortisone appeared to stimulate the growth of eggs while prolactin appeared to stimulate egg growth, deposition of yolk and the formation of the chorion. The greatest amount of maturation in male fleas took place under the influence

Table 3.4 Egg development in female Fleas.

	Group	N	$\bar{x}$ mm	SD.	υ
Feeding Trials	Newly Emerged	10	.00126	.000301	28.3%
	3 Day Unfed	12	.000128	.00032	25.0%
	3 Day Fed	16	.001375	.00468	28.2%
Hormone Experiments	16 Day No hormone	18	.0142	.0058	41.97%
	16 Day Hormone	10	.0196	.0096	49.43%
	16 Day Hormone + P	22	.0164	.0167	101%
	16 Day P	24	.01466	.0098	66.8%
	Wild	21	.0426	.0386	88.42%

Pearson's coefficient of variation, $\upsilon = \frac{S}{\bar{x}} \times \frac{100}{1}$

of prolactin and prolactin plus hydrocortisone with successful transfer of sperm to the females occurring after both treatments. Under the influence of hydrocortisone alone sperm transfer did not take place.

Nothing is known about the effects or levels of prolactin in native cats while little is known about corticosteroid levels or cycles in this animal. Peripheral plasma cortisol concentrations have been measured in moderately stressed native cats (McDonald 1977) and found to be 3.2-5.6 µg/100 ml in females and 7 µg/100 ml in males. Most of the endrocrinology which has been investigated in marsupials has concentrated on the Diptotodonta. The native cats belong to the Polyprotodonta which are thought (Ride 1962) to have diverged from the main stem of the Australian radiation of marsupials very early, so that the relationship between the two groups is not very close. Endocrinological studies which have been carried out on the Dasyuridae have been largely confined to work on reproductive hormones (Woolley 1966). It was therefore difficult to estimate the doses of hormones which should be administered to the fleas, however the concentrations of hormones used in the experiments were kept within similar limits to those used in the rabbit flea experiments (Rothschild and Ford 1966a,b)

Since nothing is known about the role played by prolactin in the annual cycle of native cats of either sex it is difficult to speculate about the relevance of the apparent effect this hormone had on flea development. Recently some light has been thrown upon the role of prolactin in regulating oestrous in the tammar wallaby (Evans 1978). It was found that prolactin was partly responsible for delaying the onset of oestrus in this highly specialised marsupial. It is

thought (Evans pers. comm.) that the level of prolactin would gradually build up in the tammar wallaby prior to oestrus and would then drop sharply so that oestrus could occur. Following oestrus the prolactin levels would rise again to play a role in lactation and high levels would then be maintained in response to suckling. Thus several high peaks of prolactin could be expected in the tammar wallaby during the year.

Importantly, it can be seen that prolactin in the tammar wallaby acts as an antigonadotrophic hormone while delaying the onset of oestrus but following oestrus it acts as a gonadotrophic hormone in the development and maintenance of lactation. As a result of suckling, high levels of prolactin maintain the blastocyst diapause which is found in the tammar wallaby and some other macropods by inhibiting the corpus luteum. Prolactin in conjunction with other hormones plays a major role in the regulation of reproduction in female marsupials of the higher Diptotodonta. It is possible that prolactin plays an important part in control of oestrus in the Polyprotodonta but this has not been demonstrated.

In the series of studies on maturation of the rabbit flea it was found the hydrocortisone directly affected the rate of sexual maturation of both male and female fleas while prolactin along with somatotropin stimulated copulation (Rothschild and Ford 1966a,b). These were however only a small part of the range of hormonal effects of the host upon the fleas and the full extent of the relationships between the rabbit flea and its hosts is complex and subtle. Hormonal dependence upon the host has been demonstrated in *Cediopsylla simplex* which parasitises the North American hare (Rothschild and Ford 1972) but instance of such hormonal links between fleas and their host would appear to be exceptions rather than common adaptations. It has

been found (Rothschild & Ford 1973) that *Xenopsylla cheopis* and *Nosopsyllus fasciatus* are not dependant upon hosts' hormones.

While the results of the experiments suggest that *U. tasmanica* may be dependant upon its host's hormones, the extent of this dependance and the role played in sexual development of the fleas by hormones other than hydrocortisone and prolactin remain unknown. Even the effects of these two hormones are not clear cut and it can only be said that application of hydrocortisone and prolactin appeared to accelerate sexual maturation, mainly in the male fleas, but to a lesser extent in the female fleas.

In these experiments small numbers of fleas were used since the possibility of a hormonal bond between the flea and its host was only considered at the end of the period of laboratory studies. For this reason and also because no more pharate adults were available the experiments were not repeated. During the experiments some important variables were not controlled. Thus the dosage of hormones administered to the fleas was not known, there was no way of accurately measuring the quantity of hormone solution delivered to the fleas from the ASPAX aspirators. Another variable which has been stressed as important by Rothschild *et al.* (1970) is the difference in response to hormone treatements by fleas from different batches, that is fleas which may have been reared under different conditions.

In *Uropsylla* it is not known to what extent larval development on different native cats effects maturation of the adult fleas. The fleas used in the hormone experiments had been reared on three native cats which had been kept under similar conditions but it is possible that different hosts provide nourishment for the larvae which differs in quality.

One of the main differences observed between sexual maturation in *U. tasmanica* and that described for the rabbit flea and *X. cheopis* was the absence of histological change in the salivary glands of the *U. tasmanica* during sexual development. In all feeding fleas the morphology of the salivary gland cells was similar as was the secretion in the lumena. The structure of the salivary gland which was typical of all feeding fleas can be seen in Plate 3.4b. In the rabbit flea defecation increases greatly prior to egg laying and in this way the fleas provide food for the developing larvae. There are changes to the salivary glands during sexual maturation as well as changes in the morphology of the cells of the mesenteron. The cumulative effects of these changesare increased feeding combined with the frequent defecation of only partially digested blood. Since *U. tasmanica* larvae are parasitic on the host there is no necessity for the adult fleas to provide nourishment for them and it is possible that the histological changes which take place in the rabbit flea in order to provide food for the larvae do not take place in *U. tasmanica*.

When the female fleas used in the experiment were compared (Plate 3.5a,b with the wild females it was observed that the degree of sexual maturation was greatly inferior in all experimental groups. It is possible that this was caused by the conditions of the experiment since many fleas died in the 16 days and the survivors appeared to be under-nourished at the completion of the experiment. (Plate 3.4a).

Conclusion

It was found that fleas which were removed from their cocoons during December and January were sexually immature but the males were capable of limited maturation within three days when permitted to feed on sub-adult native cats. The fleas appeared to be attracted to the urine of sub-adult native cats, the presence of which also appeared to stimulate sexual behaviour. The fleas rejected the adult native cats at the time of the experiment. It was suggested therefore that the sub-adult native cats provided the fleas with a factor which was attractive, stimulated feeding, aided sexual development and which was not found in the static and non-reproductive adult native cats. It was also suggested that *U. tasmanica* is one of the species of fleas which needs a blood meal before sexual maturation can take place.

When newly emerged fleas were exposed to various mammalian hormones it was found, within the limits of the experiment that the application of hydrocortisone and prolactin singly or together promoted sexual development to different degrees. The most fully developed group of fleas were those treated with hydrocortisone and prolactin. Sperm transfer took place in the presence of prolactin as did the greatest degree of enlargement of the accessory glands in the males. Chorionation and yolk deposition took place in the females which had been treated with hydrocortisone and prolactin while egg degeneration was recorded in the presence of Prolactin but particularly in the absence of hydrocortisone. On average there were larger eggs in the females treated with hydrocortisone than in the control group. It is postulated that *U. tasmanica* may to some extent be dependent upon its hosts' hormones.

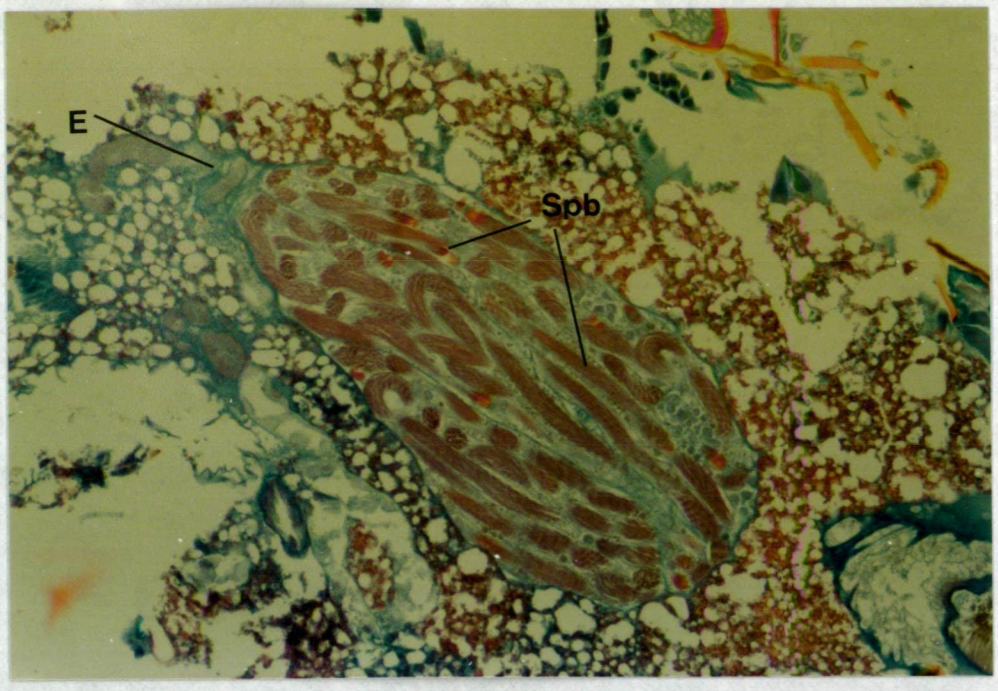
Plate 3.2

(a) Level of sperm development and fat body, in newly emerged unfed male fleas. (x 100)

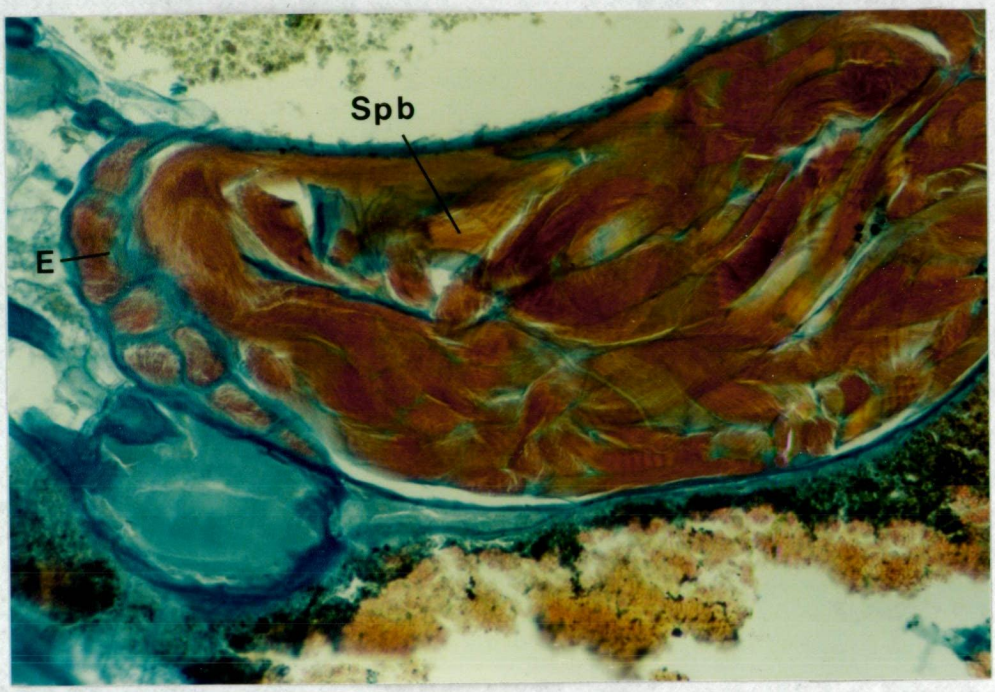
(b) Fully mature male flea showing complete sperm bundle development and prescence of sperm in epididymis. (x 200)

(c) Oocyte development in female flea which has fed for three days. (x 100)

o	=	oocyte
SpB	=	sperm bundle
E	=	epididymis



a



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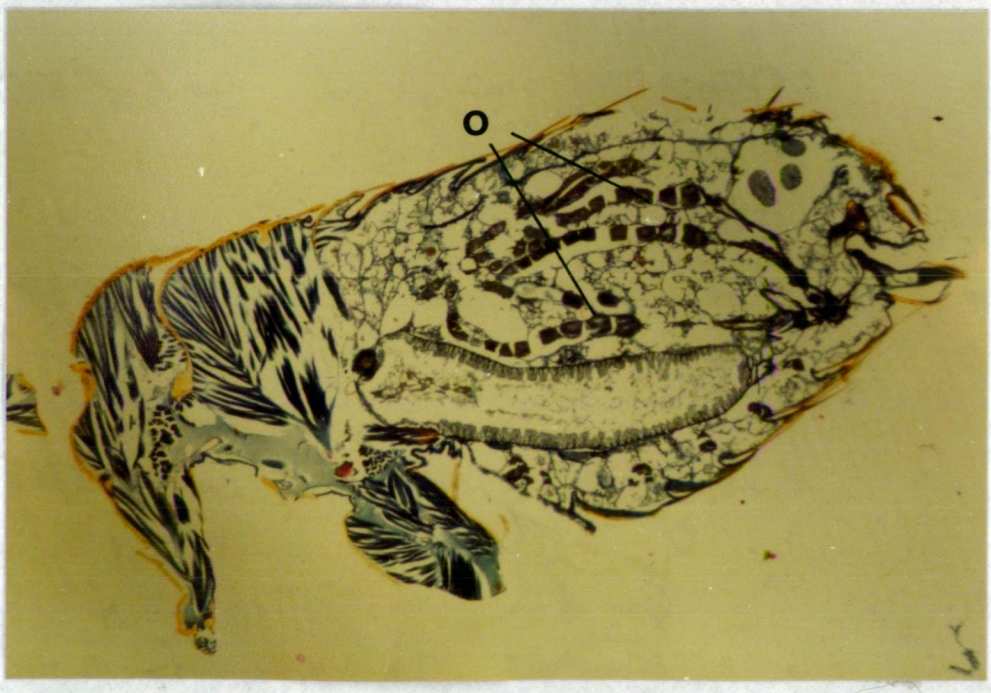


c

Plate 3.3

Complete sperm development in male flea with sperm in epididymus also showing accessory gland development.(x 350)

Agl = accessory gland.

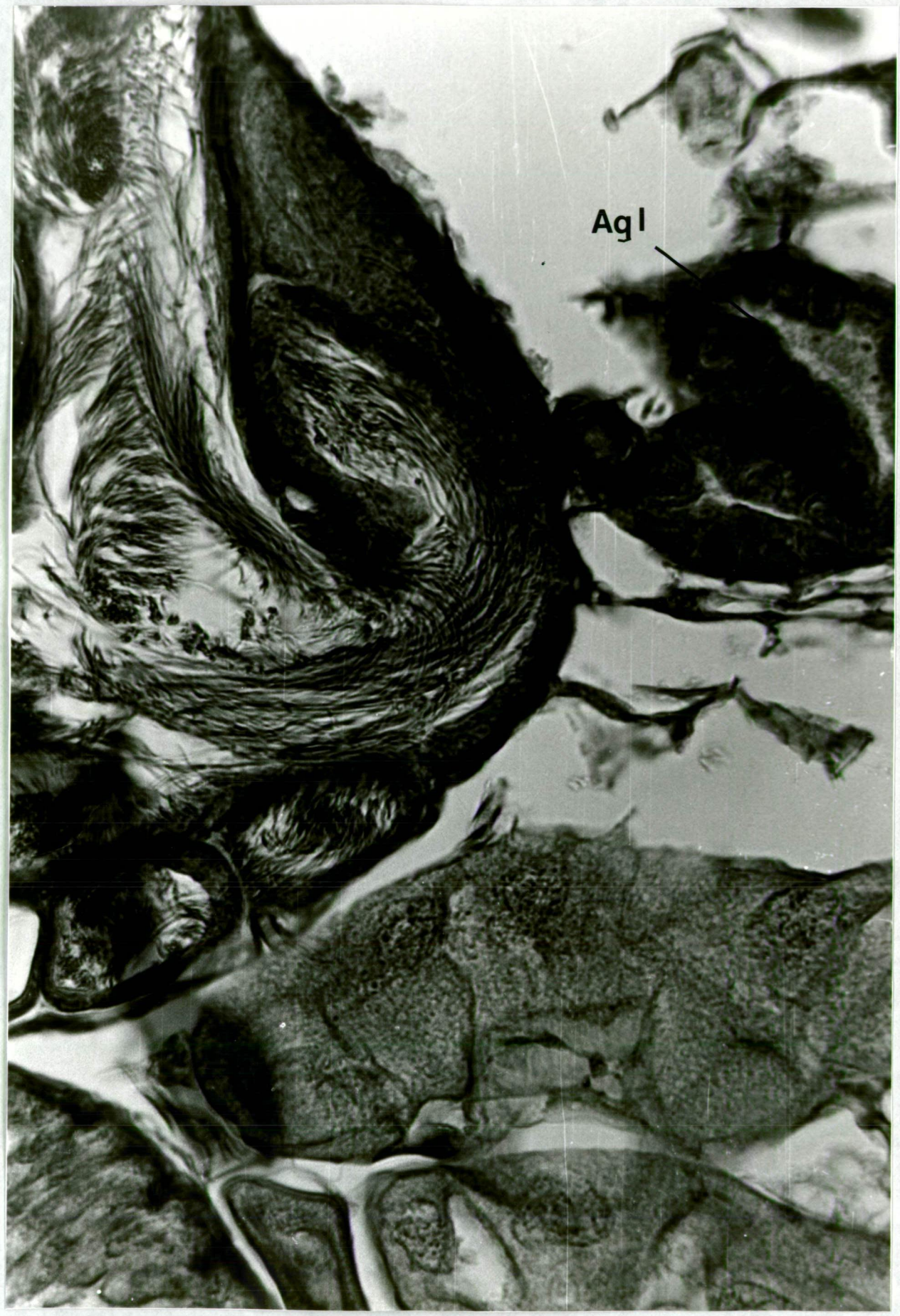

AgI

Plate 3.4

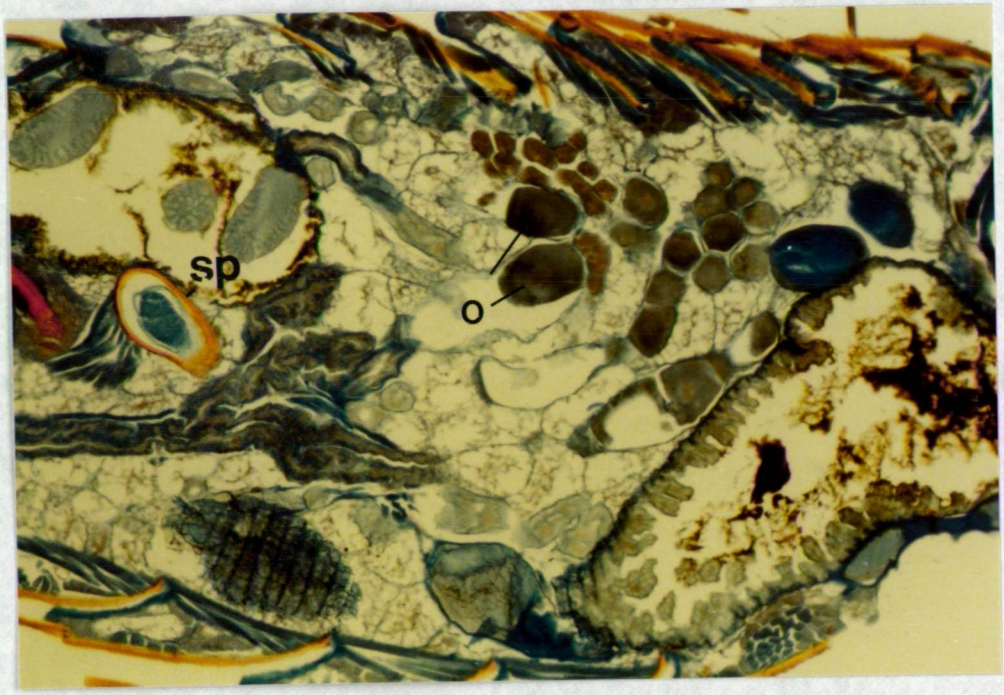
(a) Section of a female flea following the hormone experiment showing the extent of overlap of the dorsal and tergal plates as a result of insufficient feeding. (x 200)

(b) Section of a female flea which had been subjected to hydrocortisone and prolactin spray showing oocyte degeneration and the salivary gland appearance which was typical of that of the fleas used in the experiment. (x 250)

O = Oocyte

Sgl = Salivary gland

a



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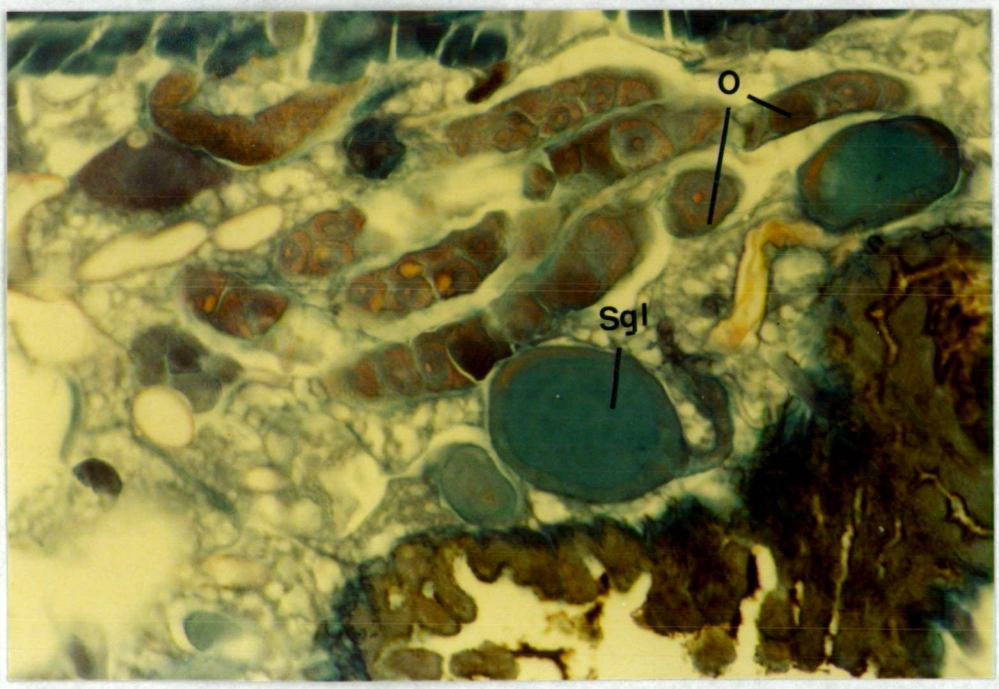


Plate 3.5

(a) Section through a wild female flea showing the presence of sperm in the spermatheca. (x 250)

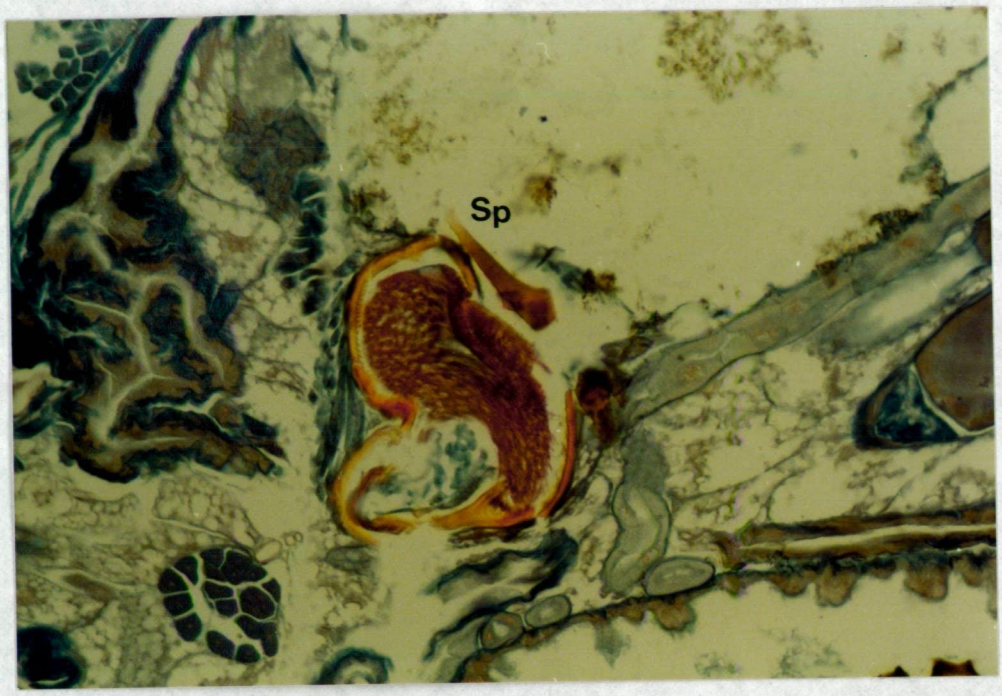
(b) Section of wild female flea showing complete development of oocytes. (x 250)

Sp = Spermatheca

O = Oocyte

Sp = Spermatheca

a



b

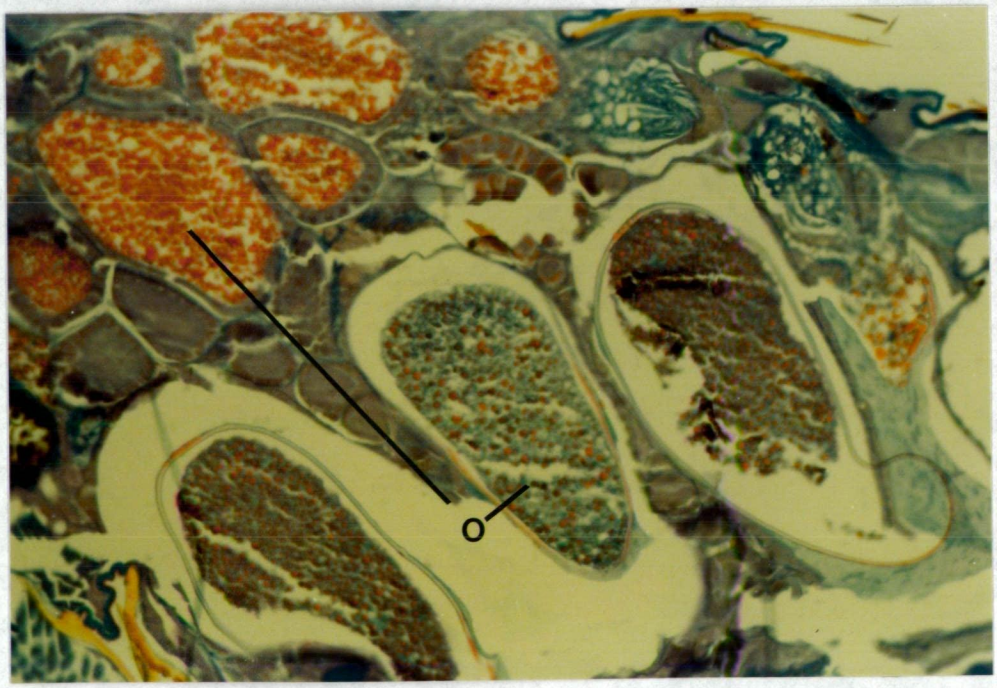


Plate 3.6

(a) Section of female flea following exposure to hydrocortisone and prolactin spray showing yolked and chorionated eggs. (x 250)

(b) Section of the same flea showing the presence of sperm in the spermatheca. (x 250)

E = Eggs

Sp = Spermatheca

a

b

Chapter 4

The Biology of the Larvae of *Uropsylla tasmanica*

4.1 Introduction

In the literature, (Dunnet 1970, Rothschild and Clay 1956, Askew 1971, Wagner 1939) flea larvae are described as being vermiform with a well developed head capsule and the body not clearly differentiated into abdominal or thoracic segments. The head capsule of fleas is described as being heavily sclerotised in comparison with the rest of the body and as having a single segmented antenna and chewing mouth parts. Flea larvae have a characteristic chaetotaxy with rows of long bristles on each body segment. They are described as having 13 body segments with a pair of anal struts on the last segment which are used for locomotion.

Flea larvae with the exception of *Uropsylla* and *Holopsyllus glacialis* are described (Wagner 1939, Rothschild and Clay 1956, Dunnet 1970, Askew 1971) as living free in the nests of the host where they feed on nest debris and the fecal pellets of the adult fleas. *Holopsyllus glacialis* larvae have been found living as ectoparasites in the fur of the arctic hare where they feed on adult fecal pellets. These larvae are described as resembling the normal type of flea larvae described above (Freeman and Madsen 1949).

The larvae of *Uropsylla tasmanica* have been described by Dunnet (1970) who has briefly mentioned some of its parasitic adaptations. The aim of this chapter is to examine the adaptations to the parasitic mode of life of the larvae of *Uropsylla*.

The adaptations evident in these larvae are mainly in their external morphology although some internal modifications have taken place. Because the larvae appear to have converged, to some extent in their evolution, with the larvae of the protelean dipteran they are compared with these as well as with other species of free

living flea larvae. The most important features of the parsitic mode of life to which the *Uropsylla* larvae would have to adapt are: Respiration; *Uropsylla* larvae are not exposed to the atmosphere to the same extent as free living larvae; Attachment to host: free living larvae need to move about freely as they forage for food, *Uropsylla* larvae are surrounded by their food but must maintain their position in the host; Food: the diet of *Uropsylla* larvae and the method of ingestion differs from that of free living larvae.

For comparisons of *Uropsylla* larvae with those of the protelean dipterans I have drawn largely upon the work of Zumpt (1965) who has published a monograph on the myiasis producing flies. For comparisons of *Uropsylla* with the free living flea larvae I have used the description of the larvae of *Nosopsyllus fasciatus* by Sharif (1937) as well as my own observations on the larvae of *Pygiopsylla hoplia* and *Acanthopsylla rothschildi* both of which are readily available in Tasmania. These latter species of fleas have free living larvae which are found in the dens of the host (*D. viverrinus*). *P. hoplia* and *A. rothschildi* appear from my observations to feed on nest debris and adult flea faecal pellets while *N. fasciatus* appears to be heavily dependent upon the adults' faeces (Molyneaux 1967). The species also comply with the general descriptions of the morphology of flea larvae which was given earlier.

Because the larvae of *Uropsylla tasmanica* are examined in some detail in several ways, this chapter is divided into five sections each with its own materials and methods, results and discussion.

4.2 The Larval Stages of *Uropsylla tasmanica*.

Materials and Methods

The determination of the number of larval stages of *Uropsylla tasmanica* was difficult because of the inaccessability of these larvae and their exuvia during the parasitic stage. Some exuvia were recovered from the skin lesions of the hosts but these were generally fragmentary.

Limited attempts were made to rear the larvae in artificial media such as nutrient agar and agar containing homogenised native cat tissues and blood. These attempts failed probably because the larvae became coated with the medium and were unable to respire.

The determination of the number of larval instars was therefore based on an examination of some 300 larvae which were obtained either as they emerged from the host or by artificially removing the earlier stages from the skin lesions of the host.

The description of the first instar was based on examination of only two larvae which emerged from eggs which had been collected from native cats. These eggs, still attached to the native cat hairs, were incubated at 25^{o}C and approximately 99% Relative Humidity. The larvae were examined microscopically by mounting them temporarily in glass cavity slides. This temporary mounting was carried out in order that they could, at a later date be subjected to Electron Microscope scanning. Unfortunately, the larvae were lost during the atmospheric drying process prior to mounting for Electron Microscope.

The later larval stages were described from microscopic examination of clear whole mounts. Drawings were made with the aid of a camera lucida.

Results

1st Instar

This stage was only observed in larvae newly emerged from the egg. It was not recovered from lesions in the skin of the host. Following eclosion these larvae were observed to actively move along the host's hairs to which the eggs were attached. The two larvae measured were 0.5 and 0.8 mm in length and generally resembled the free-living type of flea larvae which has already been described, more closely than the later stages.

The head capsule of the first larval stage was fairly large compared with the body (Table 4.1). When Table 4.1 is compared with Table 4.2 it can be seen that the head-capsule/body proportions of the first instar larvae were closer to those of *P. hoplia* than those of the later stage of *U. tasmanica*.

Table 4.1 Head Capsule Ratios of 1st Stage of *Uropsylla* larvae.

HW/HL	1.5
HW/TW	0.612
HL/BL	0.075

HW = head width, HL = head length, TW = Width of 1st thoracic segment BL = body length. Based on 2 specimens.

The first stage was found to have preserved some of the features of cheatotaxy which are found in the free-living flea larvae (Wagner 1939, Sharif 1937, Cotton 1970). Each body segment carried a pair of ventral bristles while the last abdominal segment had two pairs of long bristles and two pairs of short bristles (Fig. 4.1 a, Plate 4.1a). The

Plate 4.1

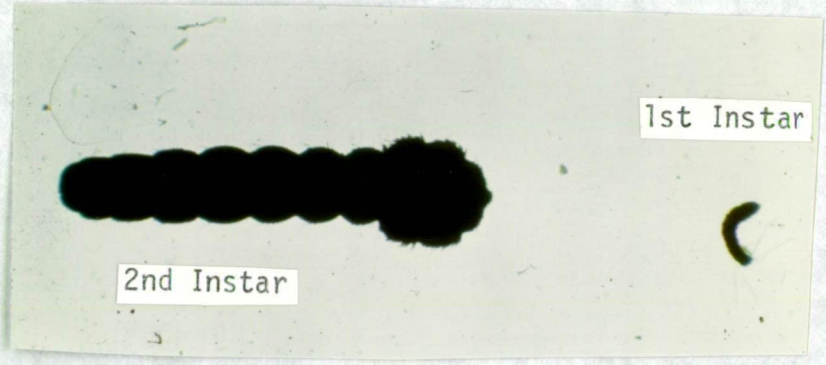
(a) First larval instar x150

(b) First and second larval instars x15

(c) Second, third and fourth larval instars x12

a

b



c

The second and third thoracic segments each carried a band of backwards-pointing denticles which were not grossly developed on the other segments. There were no fine, forward-pointing spines on the abdominal segments. Examination of this stage did not reveal any spiracles.

The head appendages appeared to be proportionally well developed in the first instar and resembled those of other species of Australian flea larvae which were examined as well as the descriptions given for other species (Cotton 1970).

2nd Instar

The second larval stage ranged in size from 1 mm to 5 mm. In this stage the four long caudal bristles which were found in the first instar were absent and the bristles were found on each segment of the first instar were only erratically present. In this stage the thoracic segments carry bands of short sharp backwards-pointing bristles and backwards-pointing denticles are present on the first seven abdominal segments.

Abdominal segments 4,5,6,7, and 8 are adorned with very fine forwards-pointing spines (Fig 4. 1.2, 4 .4C, Plate 4.7 a). Functional spiracles were observed on the 6th, 7th and 8th abdominal segments.

In this stage the head is less prominent than in the first instar and the mouth-parts and antennae appeared to be reduced in size. The thorax of this instar is expanded in proportion to the abdomen to form a knob.

3rd Instar

This stage differed grossly from the previous stage in that it lacks a thoracic knob and the abdominal segments are almost as wide as

the thoracic segments. (Fig. 4.13 Plate 4.1c). This instar has a greater degree of development of backwards-pointing bristles and denticles on the abdominal segments than the previous stage. In the third instar the delicate forward-pointing spines which were observed on the abdomen of the second instar are distributed over a reduced area and are only found on the 6th, 7th and 8th abdominal segments with some appearing towards the posterior margin of the 5th. The spiracles resemble those of the previous instar. The third stage ranges in size from 5 mm to 7 mm.

4th Instar

This instar represents the mature larva which emerges from the host. It ranges in size from 7mm to 8mm. The forward-pointing spines described for the two preceding instars were found on the 6th and 7th abdominal segments Fig. 4.14).

The moult which preceeded this stage was observed to occur during emergence from the host. Emergence is protracted and frequently takes up to one hour. It was observed that larvae which were removed from the host artificially were always coated with the sticky fluids which surrounded them in the skin lesions (Plate 4.1 c) Becuase it moults during emergence, however, the final instar is clean and easily able to crawl unimpeded through the host's fur and through the litter in the host's den.

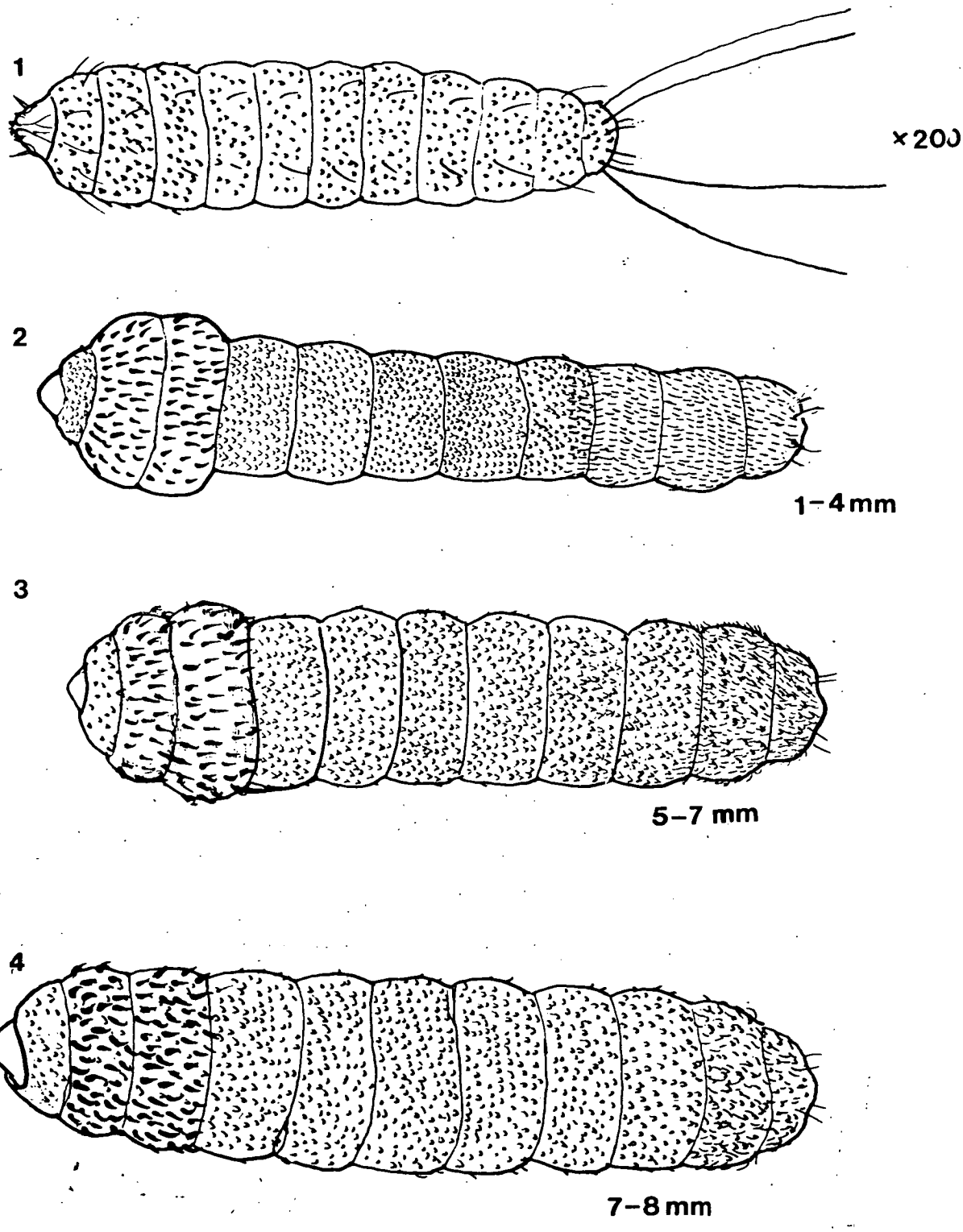


Fig. 4.1 Four Larval Instars of *Uropsylla tasmanica*.

Discussion

Because fleas normally have only three larval instars (Dunnet 1970) it is possible that one of the larval instars described in *Uropsylla* performs a special function which is related to the parasitic mode of life.

In many of the groups of protelean parasitic insects where the eggs are not deposited in the host a specialised first larval instar is found which is adapted for active movement and has certain characteristics which are not found in the later parasitic stages (Askew 1971). This phenomenon is known as hypermetamorphosis and has been described in the Neuroptera, Coleoptera, Strepsiptera, Diptera and Hymenoptera. The first instar larvae of the groups which exhibit hypermetamorphosis are generally small and fusiform. Frequently these larvae must survive exposure and in such cases are heavily sclerotised. Because these larvae are active they usually possess some body appendages such as legs in the case of the triungulin larvae of Rhipiphoridae (Coleoptera) (Linsley, MacSwain & Smith 1952) or spines as in the case of the planidium larvae of some of the Diptera. These larvae commonly possess long caudal spines which also aid in locomotion.

The first instar larvae of *Uropsylla* was not found living in the host and was observed to actively move along the hairs of the host following eclosion. Therefore it is possible that this stage represents a short lived, active and penetrative stage which has preserved those features of the free living flea larvae which facilitate locomotion. This instar also has several of the features

which have been described for the first larval instar of those protelean parasitic insect groups which exhibit hypermetamorphosis. Particularly striking is the development in *Uropsylla tasmanica* first instar larvae of the long caudal bristles and the retention of the long bristles on the body segments. (Section 4.3).

The second and third larval instars which are totally hypodermal represent two feeding and growth stages. These stages are somewhat different morphologically as the second stage has the expanded thorax which has been described in this section. It is possible that this thoracic knob is necessary to anchor the small larvae in position in the host but is less important in the later larger stage. With regard to chaetotaxy the main difference between the second and third larval stages is the distribution of the small forwards-pointing spines which are found on the last abdominal segments. These spines possibly aid in anchoring the larvae in a position in the host so that the terminal spiracles are protruding from the skin. In the small second instar larvae which has these spines extending from the 4th to the 8th abdominal segments there could be a possibility that the larvae might be enveloped in the skin lesion of the host and unable to respire. In the larger third stage larvae however this possibility is diminished as the larvae are more substantial. The third instar larvae have the forwards-pointing spines on the 6th to the eighth abdominal segments.

The final larval moult into the fourth larval instar appears to ensure that the emerging mature larva is unimpeded in its progress from the host and through the soil in the host's den.

It appears from examination of the four larval instars in *Uropsylla* that the extra instar when compared with other fleas may be the first instar which is possibly a special adaptation to the parasitic mode of life.

4.3 Comparison with Non-parasitic Flea Larvae

The aim of this section was to examine the external morphology of *U. tasmanica* larvae and to determine the special adaptations of these larvae to the parasitic mode of life by comparison with free living types of flea larvae.

Materials and Methods

For comparisons of the larvae, specimens of *U. tasmanica* and *P. hoplia* were cleared, stained lightly with azocarmine and mounted in Canada Balsam. The whole mounts were examined microscopically and drawings were made with the aid of a camera lucida. The drawings of *Uropsylla* were also compared with those of *Nosopsyllus fasciatus* published by Sharif (1937).

For comparisons of mouth parts and terminal segments specimens of *U. tasmanica* larvae, *P. hoplia* and *A. rothschildi* were subjected to electron microscope scanning. The specimens were prepared for the S.E.M. by fixing in Dubosq Brasil for at least 24 hours after which they were air dried and gold and carbon coated in a GEOL-JEE 4B Vacuum Evaporator. The specimens were then scanned in a GEOL-JXA-50A S.E.M. Micro-probe Analyser.

For comparisons of head and body measurements fixed specimens of larvae of *U. tasmanica* and *P. hoplia* were outlined with the aid of a camera lucida and measurements were taken from the drawings.

For comparisons of internal anatomy flea larvae were fixed in Dubosq Brasil, embedded, sectioned and stained according to the method described in Chapter 3 (Section 3.2).

Results

Comparison of U. tasmanica with free living flea larvae

1. Gross Morphology

When *Uropsylla* larvae were compared with *P. hoplia* and *N. fasciatus* (Sharif 1937) many striking differences were found. Firstly, in *U. tasmanica* the number of body segments is reduced from 13, found in *P. hoplia* and *N. fasciatus* to 11. Secondly, the chaetotaxy of *U. tasmanica* is modified. In *Uropsylla* the long bristles commonly found on flea larvae (Wagner 1939, Sharif 1937, Dunnet 1970) are almost absent although several very fine bristles were found on most specimens. Short backwards-pointing bristles and small backwards-pointing denticles were found to be the major external ornamentation in *Uropsylla*.

Head Capsule and Thorax

Comparison of the head capsule of *U. tasmanica* larvae and *P. hoplia* revealed that in *Uropsylla* the head is modified from the free living type described by Dunnet (1970). In *U. tasmanica* the head is wider at its base than it is long and tapers from the base into a cone shape. In *P. hoplia* the head is longer than it is wide and almost as wide proximally as it is distally. The proportion of head length to body length in *U. tasmanica* is less than half of the proportion of head length to body length in *P. hoplia*. When the width of the head capsule at its base was compared with the width of the first thoracic segment it was found that to be approximately 0.3 times as wide in *Uropsylla* but 0.7 times as wide in *P. hoplia* . These results are summarised in table 4.2.

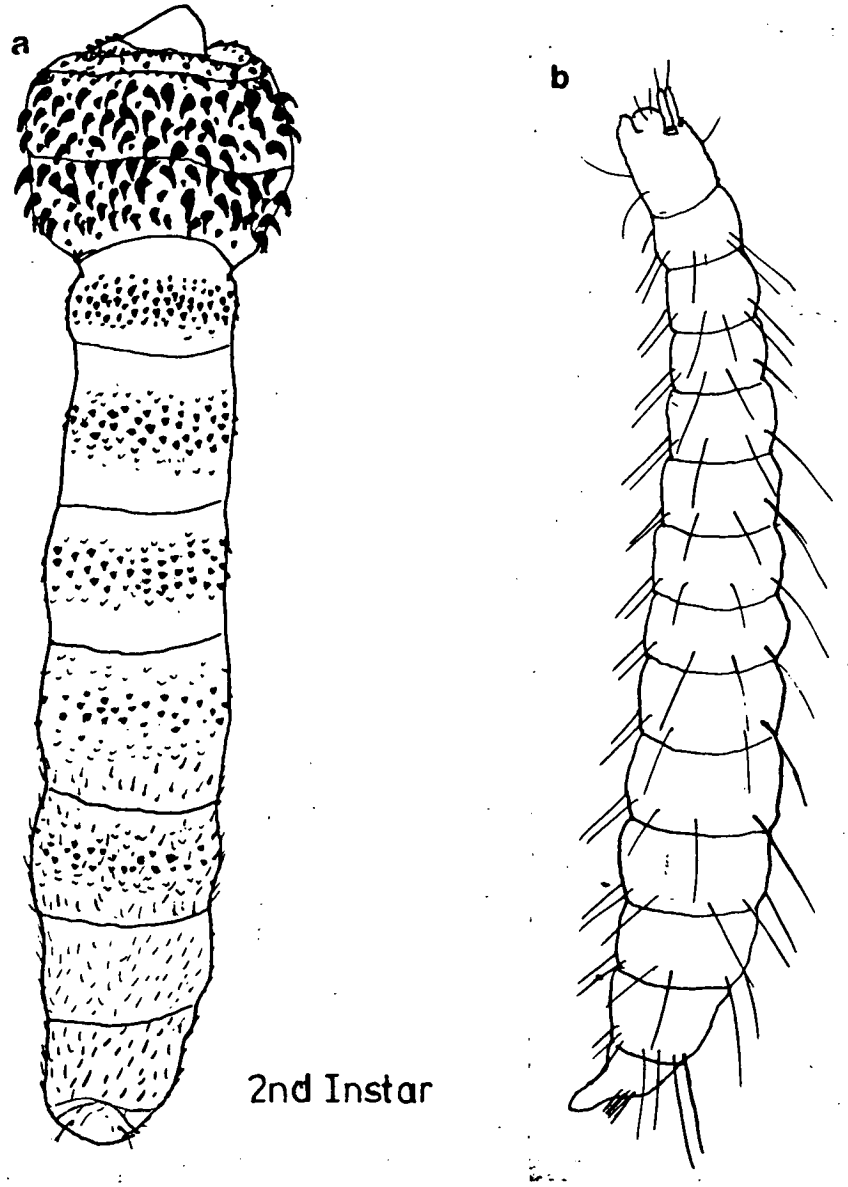


Fig. 4.2 Comparison of Gross Morphology of *U. tasmanica* (a) and (b) *N. fasciatus* (after Sharif (1937)).

Table 4.2

Comparison of measurements of head capsule of 3rd instar Larvae of *Uropsylla* and *Pygiopsylla*

	Uropsylla	*Pygiopsylla*
HW/HL	1.66 ± .02	0.83 ± .01
HW/T_{1W}	0.34 ± .03	0.73 ± .02
HL/BL	0.031 ± 0.02	0.074 ± .002
	n = 27	n = 18

HW = Head Width, HL = Head Length, T_{1W} = Width of First Thoracic segment, BL = Body Length.

The head capsule of *U. tasmanica* was found to be no more heavily sclerotised than the rest of the body. Cotton (1963) has described the head capsule of *C. nobilis* as being heavily sclerotised in comparison with the body and in *P. hoplia* and *A. rothschildi* this was also the case. A sclerotised ring was found to encircle the base of the antenna in *U. tasmanica* and this is also present in the other two species of Australian fleas studied.

Head Appendages

The antenna of the *U. tasmanica* larvae were found to be essentially similar to the descriptions given by Sharif (1937) for *N. fasciatus* and to those observed in *P. hoplia* and *A. rothschildi*, Plates (4.3, 4.5, 4.6). The main difference which was found concerned the length of the antennal shaft (as) and the sensory cones (sc) which are shorter and stouter in relation to the antennal mound (am) in *Uropsylla* than in the other species studied or in *N. fasciatus*

or *C. nobilis*. In *Uropsylla* the antennal shaft is approximately twice as long as the longest sensory cone while in *P. hoplia* and *A. rothschildi* the shaft is approximately five times longer. Sharif (1937) has not given measurement of these features in *N. fasciatus* but has drawn the antennal shaft some seven times longer than the longest sensory cone. In *P. hoplia* and *A. r. rothschildi* the antennal shaft carried one or more terminal bristles, these are absent in *Uropsylla*. In *Uropsylla* the antennal shaft is not clearly segmented as it is in *P. hoplia*.

In the electron micrographs of *U. tasmanica* and *P. hoplia* a crater (cr) was observed on the antennal mould. This was not visible in cleared whole mounts and has not been described for other species, (Plates 4.5, 4.6).

The general appearance of the antenna of *Uropsylla* larvae is one of compactness. In *Uropsylla* larvae the antenna does not protrude from the head to the same extent as it does in the other species of fleas studied.

Mouth Parts.

The mouth parts of *U. tasmanica* larvae conform to the descriptions of other species (Sharif 1937, Cotton 1963, Bacot 1914) and with those observed in *P. hoplia* and *A. rothschildi*. However, as with the head in general and the antennae, these appendages appear to be very reduced in size, particularly in relation to the width of the head at its base (Plate 4.2, b). The mouth itself is completely terminal in *Uropsylla* and slightly ventral in other species (Cotton 1963).

The labrum (lbr) conforms in shape with the description given for other fleas, (Plates 4.2b, 4.3). However, the ratio of the width of

the head at its base is approximately 0.3 in *Uropsylla* and 0.5 in *P. hoplia* and *A. rothschildi*. In both *U. tasmanica* and *P. hoplia* the ventral margin of the labrum carries a comb of sharp spines. This was not observed in *A. rothschildi* and has not been described for other species (Plate 4.3b, 4.3c). The mandibles were difficult to find in many cleared specimens of *Uropsylla* as they are small and fragile. They were found to be more prominent in the second instar than in later stages. The mandibles are roughly triangular with three teeth and appear to point anterior-lateral (Fig. 4.4). When observed in position under the labrum the mandibles appeared to be articulated so that they might be expected to swing in a lateral plane and to range from pointing inwards to forwards (see Fig. 4). The position of the mandibles agrees with Sharif's (1937) description of those of *N. fasciatus*.

The maxillae in *U. tasmanica* were similar to the descriptions given for other species (Sharif 1937, Cotton 1963, Bacot & Ridewood 1914, Oudemans 1913, Sikes 1930). However they appeared to be reduced in size to conform with the reduction of the head capsule and other mouth parts. The maxillary palps are non-segmented in *Uropsylla* (Plate 4.3 a) and short whereas segmented maxillary palps were present in both *P. hoplia* and *A. rothschildi* (Plate 4.6).

In the electron micrographs a line of articulation described by Cotton (1963) in *C. nobilis* was discernible between the cardo and stipes in *Uropsylla* but was not seen in cleared specimens (Plate 4.3b, c). This line was observed in some cleared specimens of *P. hoplia*. In both *C. nobilis* (Cotton 1963) and *Pulex irritans* (Bacot & Ridewood 1914) the maxillae have been described as being

sclerotised on the ventral region. There is no trace of such sclerotisation in *Uropsylla*.

The labium.

As with all flea larvae (Sharif 1937) the labium in *Uropsylla* is very reduced. In cleared specimens the labium could only be identified by the pair of labial palps situated near the base and between the maxillae (Fig. 4.2). In the electron micrographs the labium could be seen to be a traingular plate situated between the maxillae (Plates 4.1 b, 4.3 b). The ligula of the labium was not observed in either cleared specimens or electron micrographs of *Uropsylla*. The ligula was observed in *P. hoplia* and *A. rothschildi* is cleared specimens and can be seen in the electron micrographs of the head of the *P. hoplia* in Plate 4.5c.

The thorax.

In free living flea larvae the thorax is generally composed of segments which are slighlty smaller than the adbominal segments (Wagner 1939, Cotton 1963, Fig. 4.3). In *P. hoplia* and *A. rothschildi* the thorax conformed to the pattern described for other species of fleas.

In *Uropsylla* the thorax, particularly the third segment, is the widest portion of the body (Fig. 4.1). In the second instar the thorax is developed into a knob which bears the greatest number of the backwards-pointing bristles. In all instars the bristles are most pronounced on these segments. In other species of fleas, *P. hoplia* and *A. rothschildi* and those described in the literature, the thorax generally has similar cheatotaxy to the abdomen and the body is usually described as being undifferentiated.

Abdomen.

In *Uropsylla* the abdomen was found to have only eight segments whereas in all other described flea larvae and in *P. hoplia* and *A. rothschildi* the abdomen has ten segments. In the electron micrographs of *U. tasmanica* (Plate 4.6 a) an anal plate can be seen which may be a remnant of one of these segments. Free living flea larvae also have a pair of anal struts on the tenth abdominal segment which are used in locomotion (plate 4.6b) (Cotton, 1963, Wagner 1939). There was no indication of these structures in *Uropsylla* (Plate 4.6).

Spiracles and Tracheal System.

The arrangement of the spiracles in *U. tasmanica* larvae was different from that described for other flea larvae. (Wagner 1939, Sharif 1937). It was also different from the arrangement found in *P. hoplia* and *A. rothschildi*. Sharif (1937) has described *N. fasciatus* larvae as having a pair of functional spiracles on the second and third thorax segments with a pair on each of the first eight abdominal segments. In *Uropsylla* there were a pair of spiracles on each abdominal segment and a pair on the second and third thorax segments. It is doubtful because of their size that the third thoracic or first to fifth abdominal spiracles are functional while the second thoracic pair of spiracles appear to be completely vestigal. The largest and most obviously functional spiracles were found on the last three abdominal segments. These three pairs of spiracles were observed in sections of *U. tasmanica* larvae to be functional. Unlike other fleas, the last pair of spiracles in *Uropsylla* is terminal (see plate 4.7, Fig A 4.3a).

The position of the spiracles in each segment in *Uropsylla* differs from the other Australian species of fleas studied and from the description given by Sharif (1937) for *N. fasciatus*. In *N. fasciatus* the spiracles are found in the anterior half of each segment where they occur. In *Uropsylla* the spiracles were found near the posterior lateral edges of the segmental plates.

The tracheal system in *U. tasmanica* was similar to that described by Sharif (1937) for *N. fasciatus* with the exception of the last abdominal segments. As a result of the reduction in the abdominal segments and the terminal position of spiracles in *Uropsylla* the main lateral tracheal trunks extend the full length of the abdomen while in *N. fasciatus* the lateral trunks extend from the eighth abdominal segment forwards. The rectal sac and rectum in the two segments are supplied by posterior branches of the two main lateral trunks (Fig. 4.3a). Despite the presence of the lateral tracheal trunks in this area of the *Uropsylla* larvae however the rectal sac and rectum are served by branches of the lateral trunks which arise from the junction of the main lateral trunks and the tracheal branch coming from the seventh abdominal spiracle. This junction is found in the posterior portion of the sixth abdominal segment (Fig. 4.3 b).

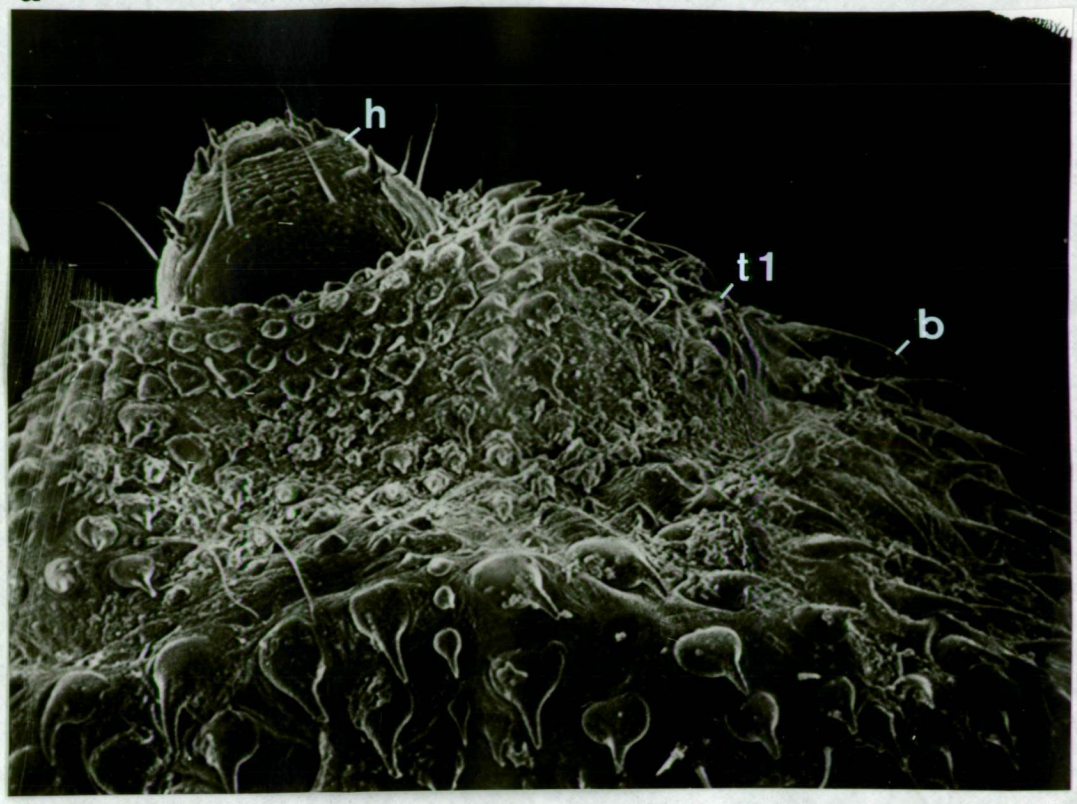
Plate 4.2

(a) Head and first thoracic segment of third instar larva of *U. tasmanica.*

(b) Head, third instar larva of *U. tasmanica.*

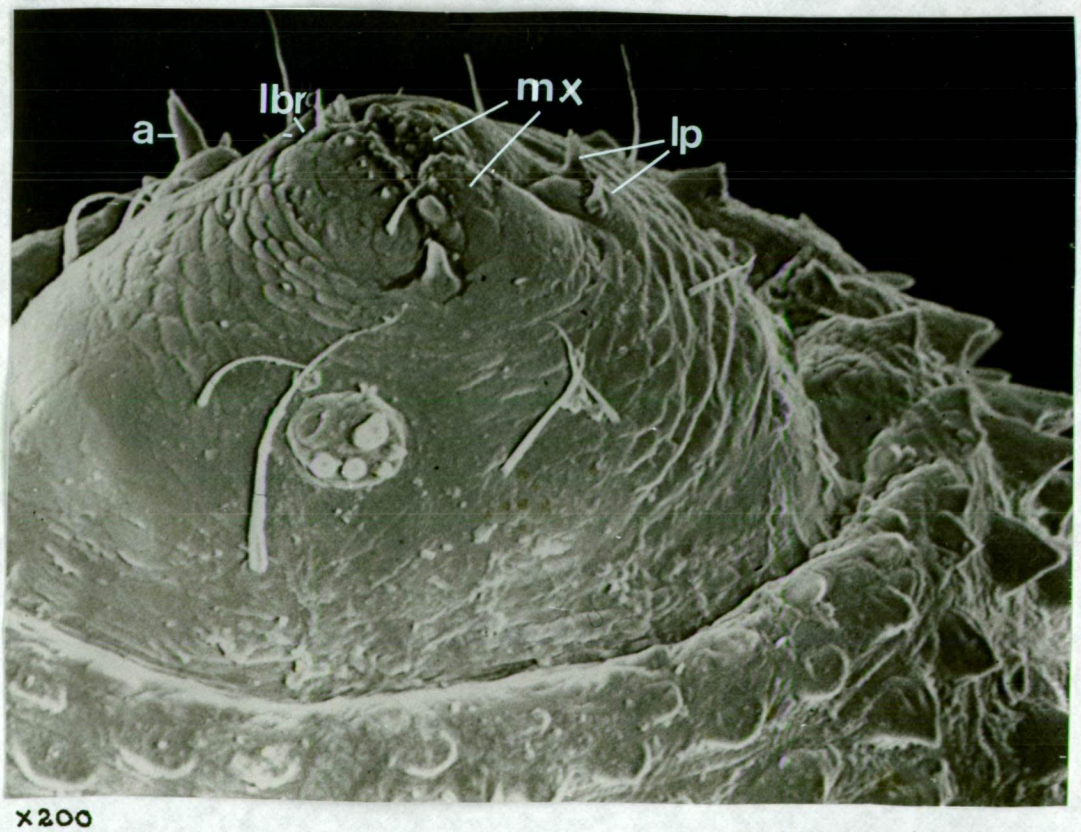
h	=	head
t_1	=	1st thoracic segment
b	=	bristle
a	=	antenna
lbr	=	labrum
mx	=	maxilla
lp	=	labial palp

a



x100

b



x200

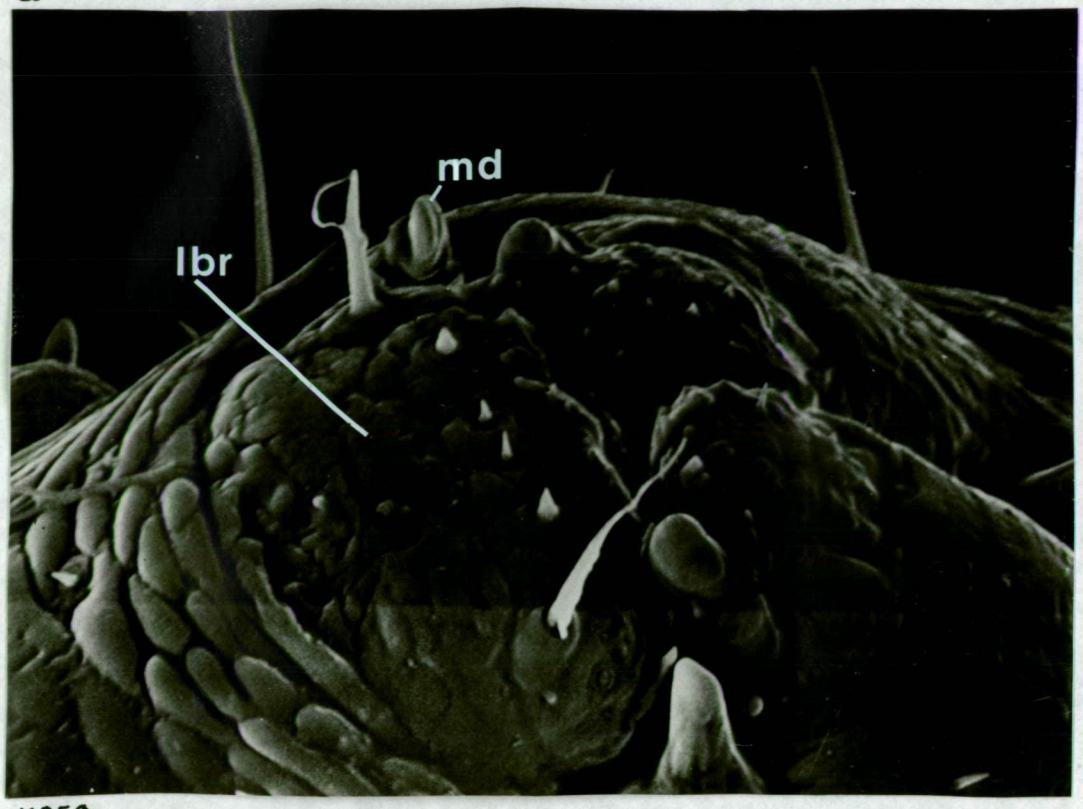
Plate 4.3

(a) Mouth of third instar larva of *U. tasmanica* showing labrum (lbr) and Mandibles (md).

(b) Dorsal view of head of third instar larva showing mandibles (md) and antennae (a). Note the row of bristles visible along the margin of the labrum (lbr).

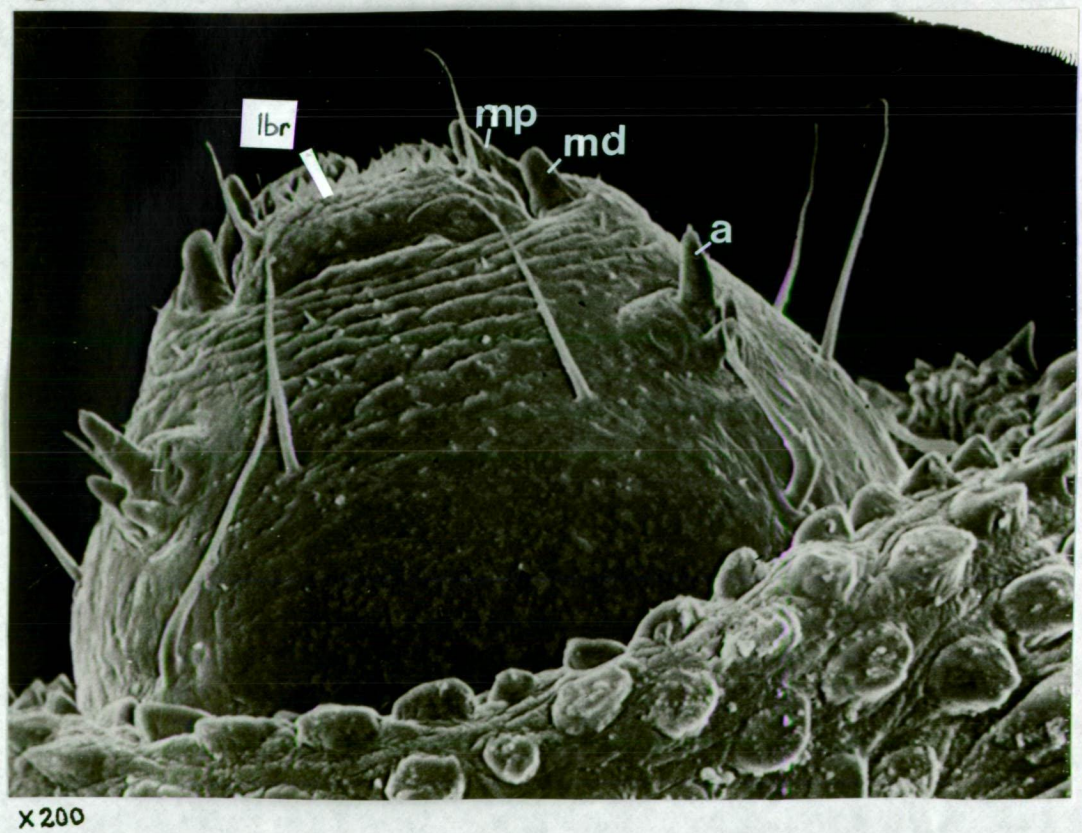
mp = maxillary palp

a



x350

b



x200

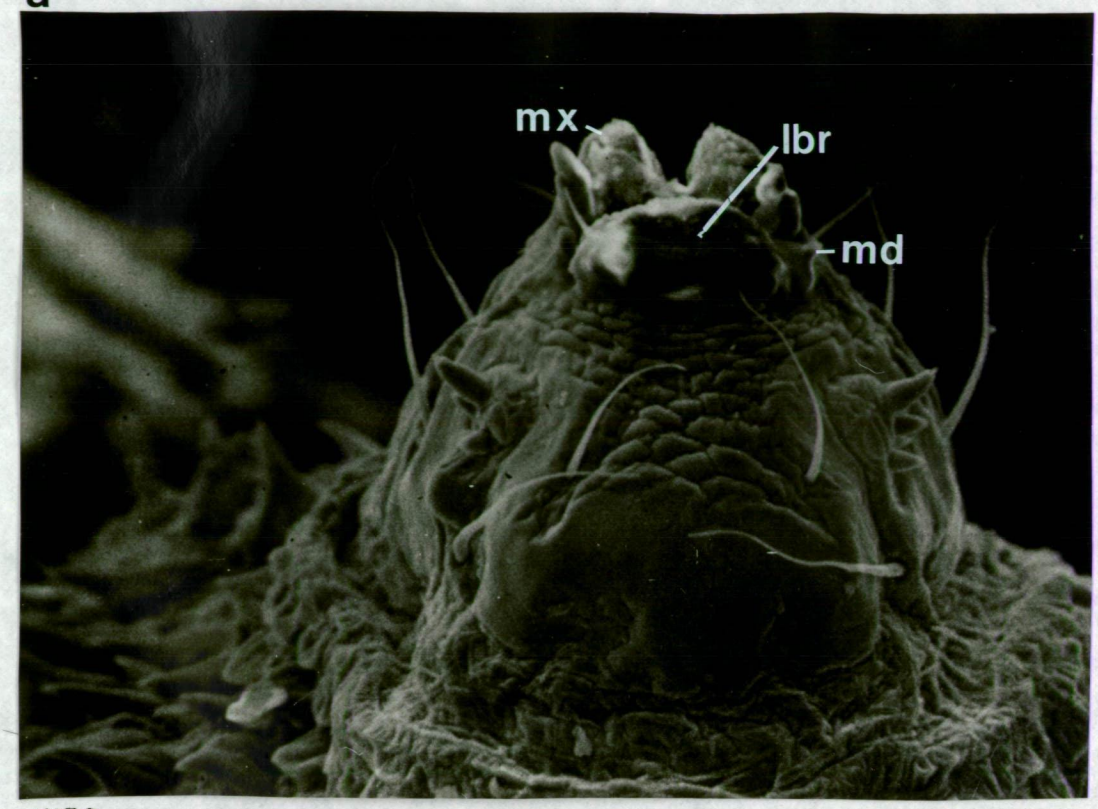
Plate 4.4

(a) Dorsal view of head of third instar larva with labrum pulled back to show the internal surface of the maxillae (mx).

(b) Ventro lateral view of head of third instar larva showing position of labium (lab) and labial palps (lbp).

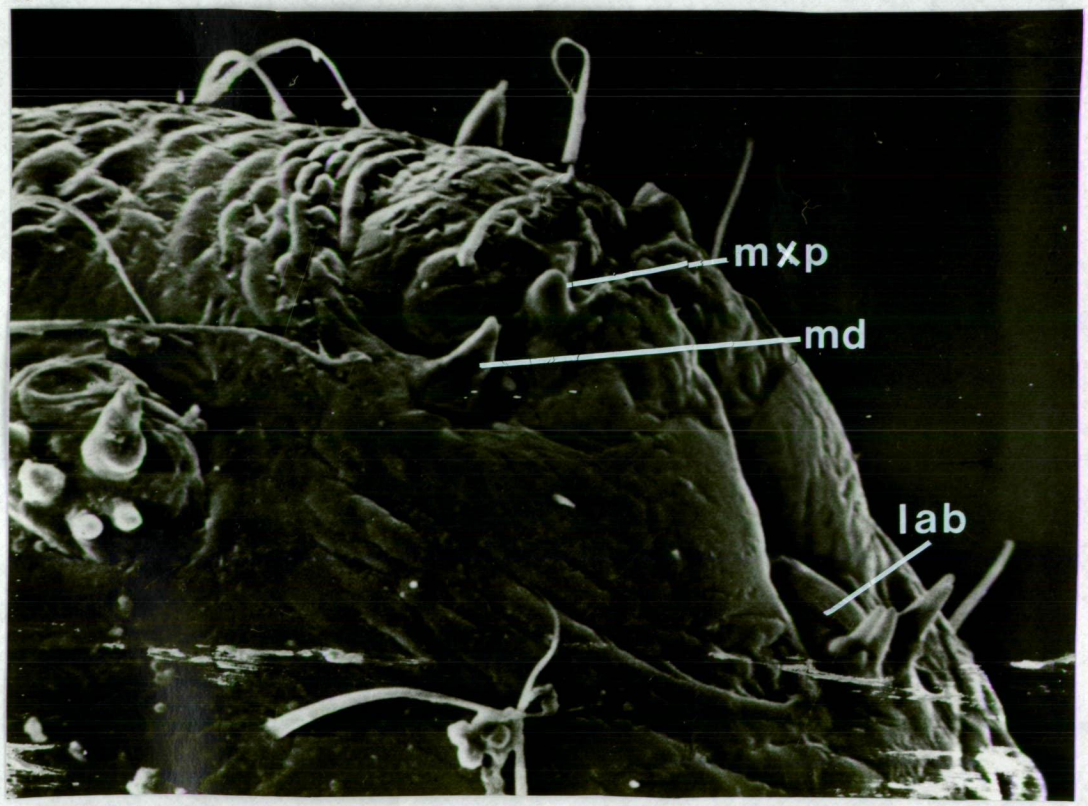
mxp = maxillary palp
md = mandible

a



X150

b



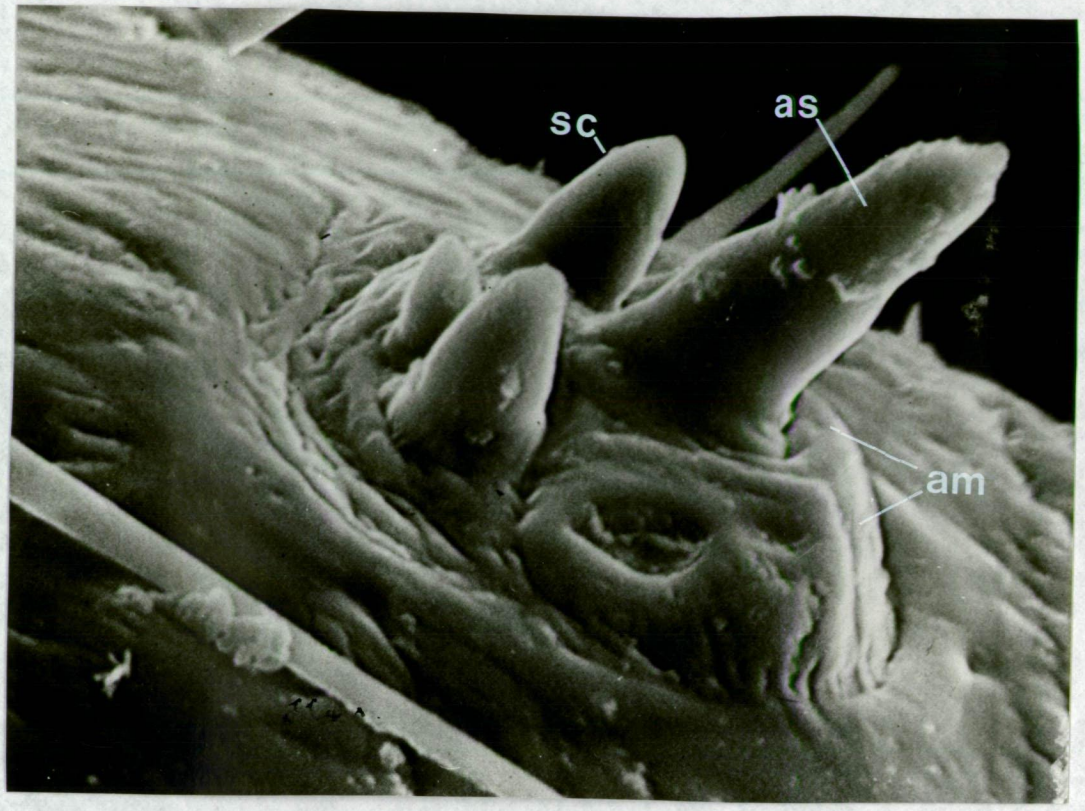
X350

Plate 4.5

(a) and (b) Antenna of third instar larva of *U. tasmanica* (×400)

sc = sensory cone
as = antennal shaft
am = antennal mound
cr = crater

a



b

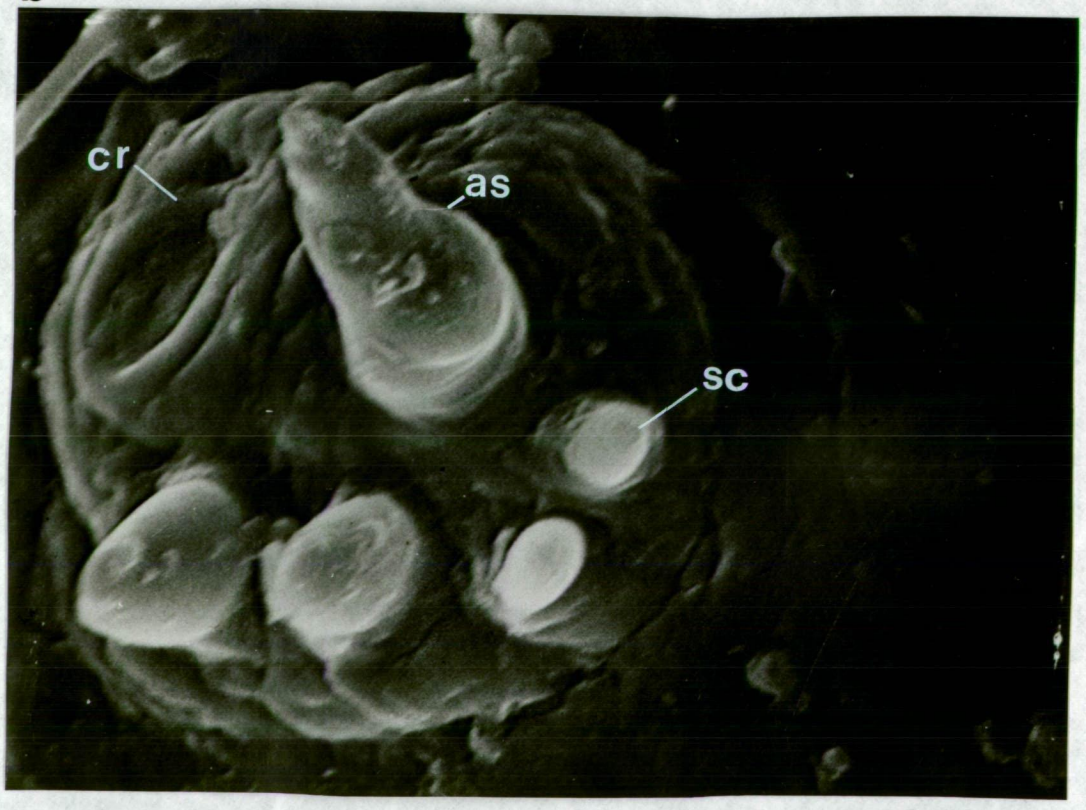


Plate 4.6

Head and Mouthparts of *P. hoplia* and *A.r. rothschildi* larvae.

(a,b) *A.r. rothschildi*

(c,d) *P. hoplia*

a = antenna

cr = crater

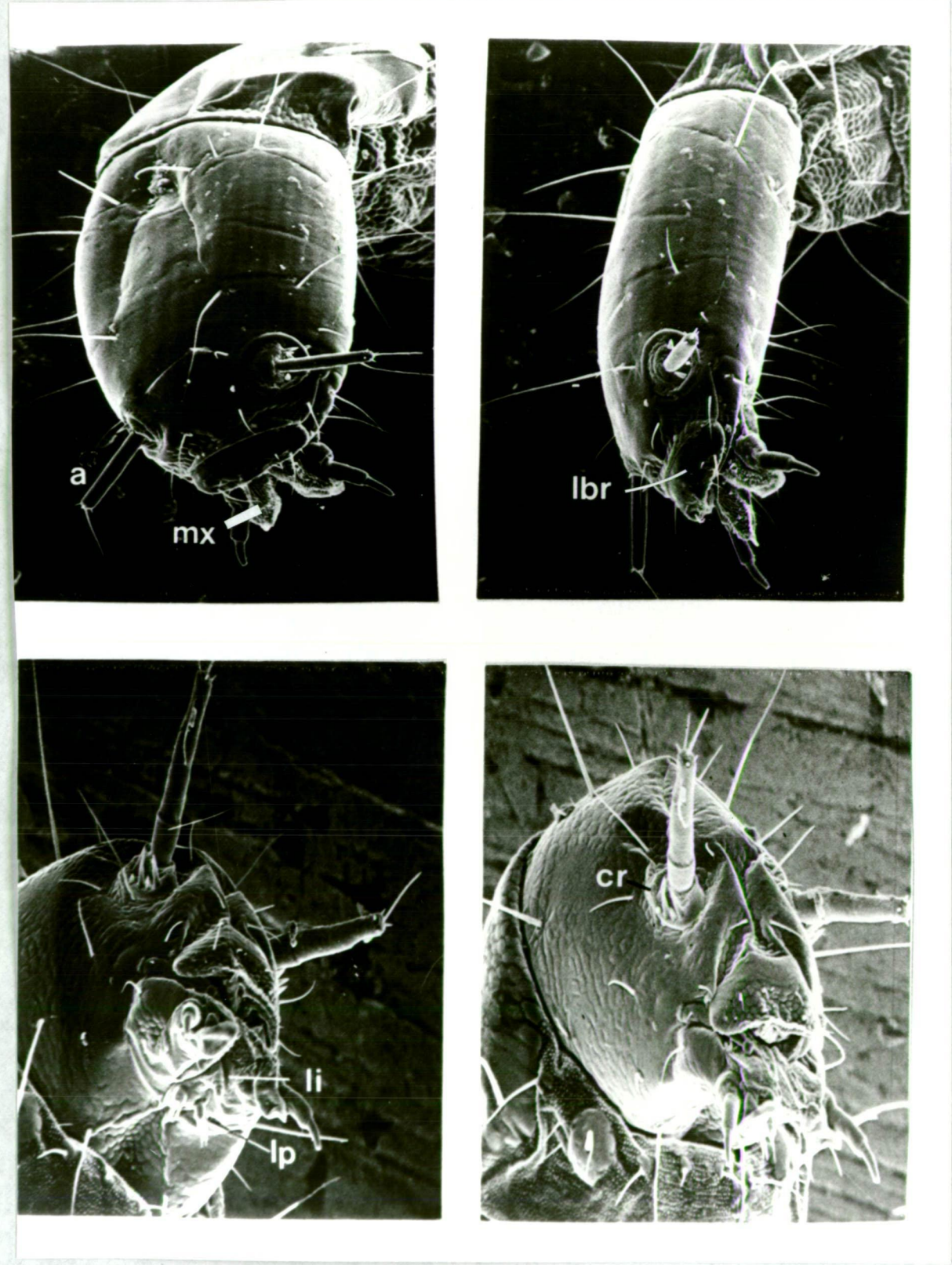
li = ligula

lp = labial palp

md = mandible

mx = maxilla

lbr = labrum



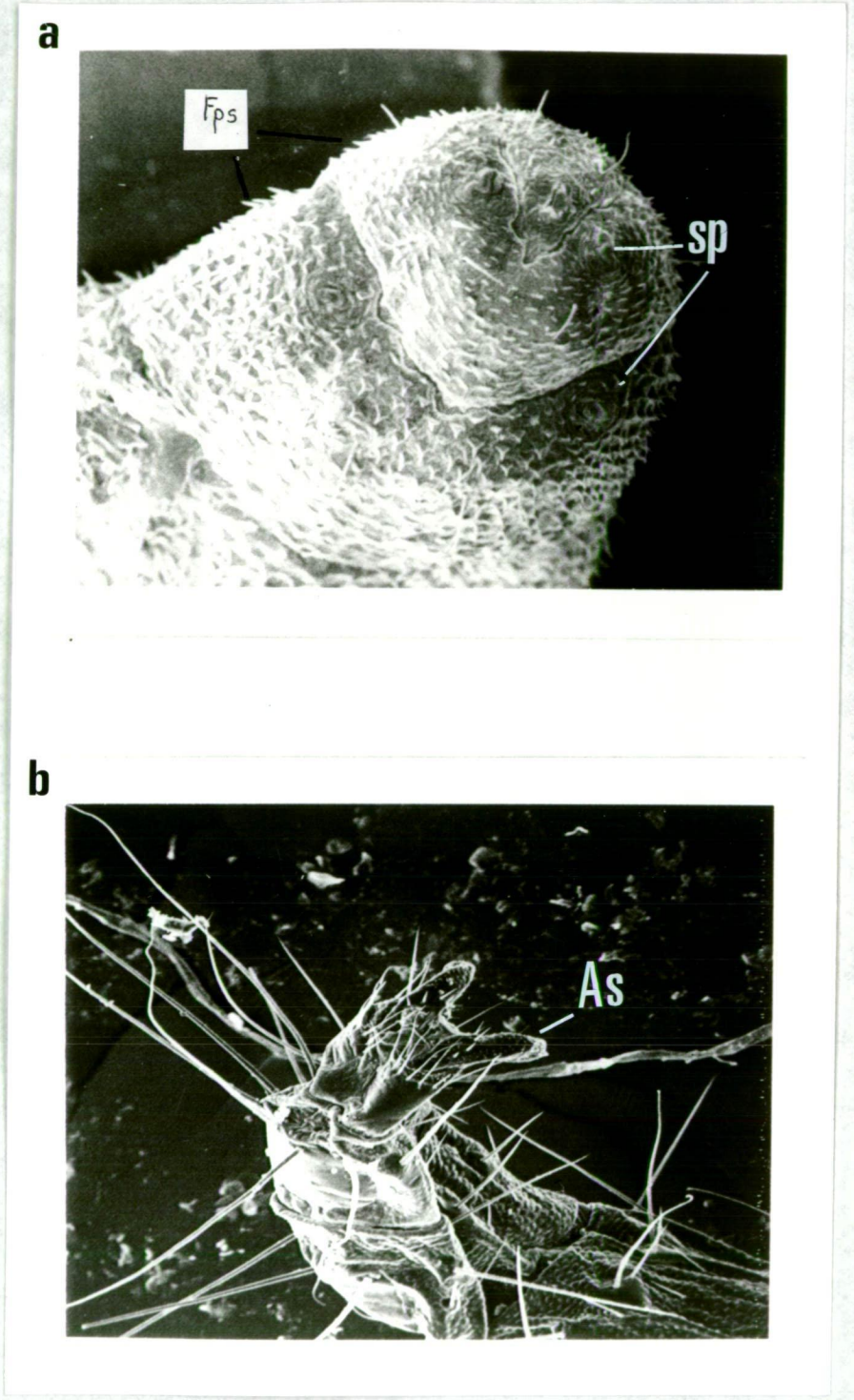
x100

Plate 4.7

(a) Seventh and eighth abdominal segments of *U. tasmanica* larva showing position of spiracles (x80).

(b) The abdominal segment of *A.r. rothschildi* showing anal struts. (x100).

Sp = Spiracle
As = anal struts
Fps = fowards pointing spines


a
Fps
sp
b
As

Comparison of Internal Anatomy of U. tasmanica with other Species of Fleas.

The aim of this section was to examine the internal morphology of *Uropsylla* larvae and to compare this with the description of the internal anatomy of *N. fasciatus* (Sharif 1937) and the internal anatomy of the larvae of *P. hoplia*. This investigation was limited to gross morphology.

Materials and Methods

Following the paper of Sharif (1937) the internal anatomy of *Uropsylla* larvae was investigated in two ways. Firstly dissections of *U. tasmanica* were done from which drawings were made with the aid of a camera lucida. Secondly larvae of *U. tasmanica* and *P. hoplia* were sectioned and stained by the method described in Chapter 3 (p.41) for adult fleas. Sections and dissections were compared with the diagrams and descriptions of the internal anatomy of *N. fasciatus* (Sharif 1937) and the terminology used in descriptions is derived from Sharif (1937).

Results

Digestive System.

From dissections it was found that the intestine in *U. tasmanica* was similar to that described by Sharif for *N. fasciatus* (Fig. 4.3). In *Uropsylla* however the mesenteron was shorter than in *N. fasciatus* with the pyloris being found in the forth abdominal segment and the colon entering the rectal sac in the fifth abdominal segment. In *N. fasciatus* the pylorus is found in the seventh abdominal segment while

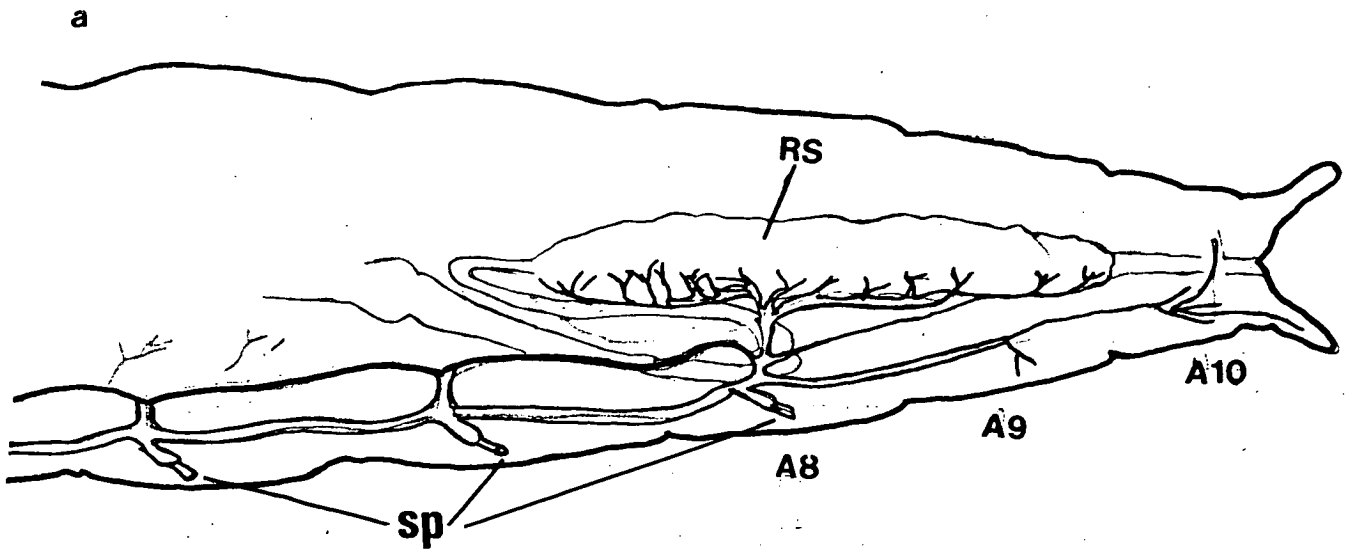
C.S.D. = Common salivary duct

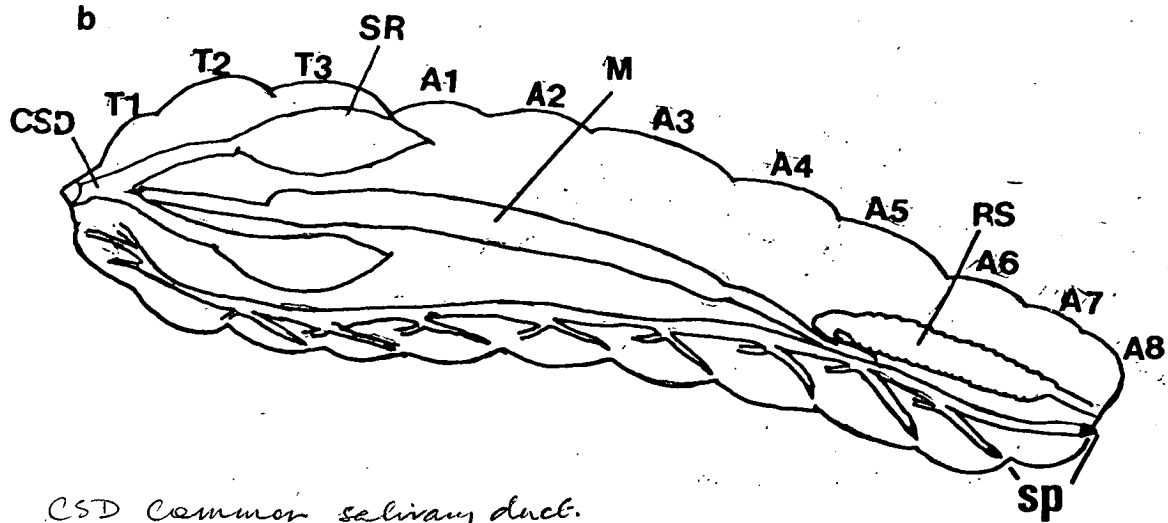
M = Mesenheron

S.R. = Salivary reservior

R.S. = Rectal sac

S.P. = Spiracle





CSD Common salivary duct.
M Mesenteron
SR Salivary reservoir
RS Rectal sac

Fig. 4.3 Comparison of Gut of

(a) *N. fasciatus* (after Sharif 1937) and

(b) *U. tasmanica*

the rectal sac extends from the seventh abdominal segment to the tenth.

The salivary glands in *Uropsylla* were tri-lobed as in Sharif's (1937) description of *N. fasciatus*. Histologically the salivary glands in *Uropsylla* (Plate 4.7B) resembled the description of *N. fasciatus* and those found in *P. hoplia*. It did appear however that the salivary glands were more extensive in *Uropsylla* than in the other species. Together with this the salivary ducts and salivary reservoir in *Uropsylla* were large. In the dissection of freshly killed larvae the salivary reservoirs occupied a large portion of the thorax (Plate 4.8).

In mature larvae which were sectioned following emergence from the host the salivary ducts, the salivary reservoirs and salivarium were found to contain a muco-polysacharide (mauve-staining) which was thought to be silk which had been produced in readiness for the building of a cocoon. This suggests that the larvae do not feed immediately prior to or following emergence.

The fat body.

In *Uropsylla*, the fat body was more extensive in all larval instars than in *P. hoplia* (Plates 4.8b, d, 4.9) or in Sharif's (1937) description of *N. fasciatus*. In particular the visceral fat body is large although the layer of parietal fat body was also thicker in *Uropsylla* than in *P. hoplia*. The fat body increased in extent throughout the larval instars and was massive in the final stage. In the final instar the fat body underwent specialisation in that two large lobes were found (running from the thorax through the abdomen) which contained a concentration of red staining globules

similar to those found scattered throughout the normal fat body (Plate 4.10 a & b). Because of the nature of the Mallory's stain which was used in the preparations it is possible that these lobes represented a specialised storage area for protein materials. The lobes were only observed in the final instar.

It is known that the fat body in insects has many functions including the storage of waste products, storage of fats, proteins and carbohydrates, detoxification of poisonous compounds and it is the site of intermediate metabolism; the fat body also stores and synthesises pigments such as ommochrome eye pigments and pterins (Wigglesworth 1972). In the case of the third larval instar of *Uropsylla* the red staining inclusions in the fat body could result from any one of these functions and clearly further work beyond the scope of this thesis is needed to explain them.

Head Capsule.

From sections through the head capsule of *U. tasmanica* and *P. hoplia* (Plate 4.8) it was seen that in *U. tasmanica* the musculature of the head was reduced in comparison with *P. hoplia* and the descriptions of *N. fasciatus* given by Sharif (1937). In *U. tasmanica* a greater portion of the head capsule was taken up by the salivarium and pharynx than in *P. hoplia* and *N. fasciatus*.

Comparison of U. tasmanica with the parasitic larvae producing myiasis in vertebrates.

The aim of this section is to compare the parasitic larvae of *U. tasmanica* with the parasitic larvae of some myiasis producing Dipterans which exploit a similar habitat to *U. tasmanica*. Comparison was carried out in order to determine, firstly, the degree of convergence between these two groups and secondly to help to interpret the adaptations found in *Uropsylla* larvae which are specifically related to the parasitic mode of life.

Materials and Methods

In comparing *U. tasmanica* with the parasitic dipteran larvae whole mounts of *U. tasmanica* and drawings of this larvae were compared with the descriptions and illustrations of Diptera given by Zumpt (1965), Askew (1971) and Keilin (1944).

Results

In general (Askew, 1971) the larvae of the protelean Diptera are adapted to living in a mass of semi-liquid food, they have a more or less cylindrical body, and reduced powers of locomotion. The cuticle is thin and the body has few obstructing projections. In all these respects *U. tasmanica* resembles these larvae.

In more detail Zumpt (1965) has described these larvae as having the head capsule and mouthparts reduced to a pseudocephalon bearing mouth hooks. In *U. tasmanica* the head is very reduced in comparison with other flea larvae but not lost as it is in the Dipterans.

Plate 4.8

(a,c) Section through head of *P. hoplia* larva. (x100)

(b,d) Sections through head and first thoracic segment of *U. tasmanica* second instar. (x100)

sal	=	salvarium
m	=	muscle
Br	=	brain
Sog	=	Sub oesophageal ganglion
Csg	=	Common salivary duct
Sg	=	Salivary gland

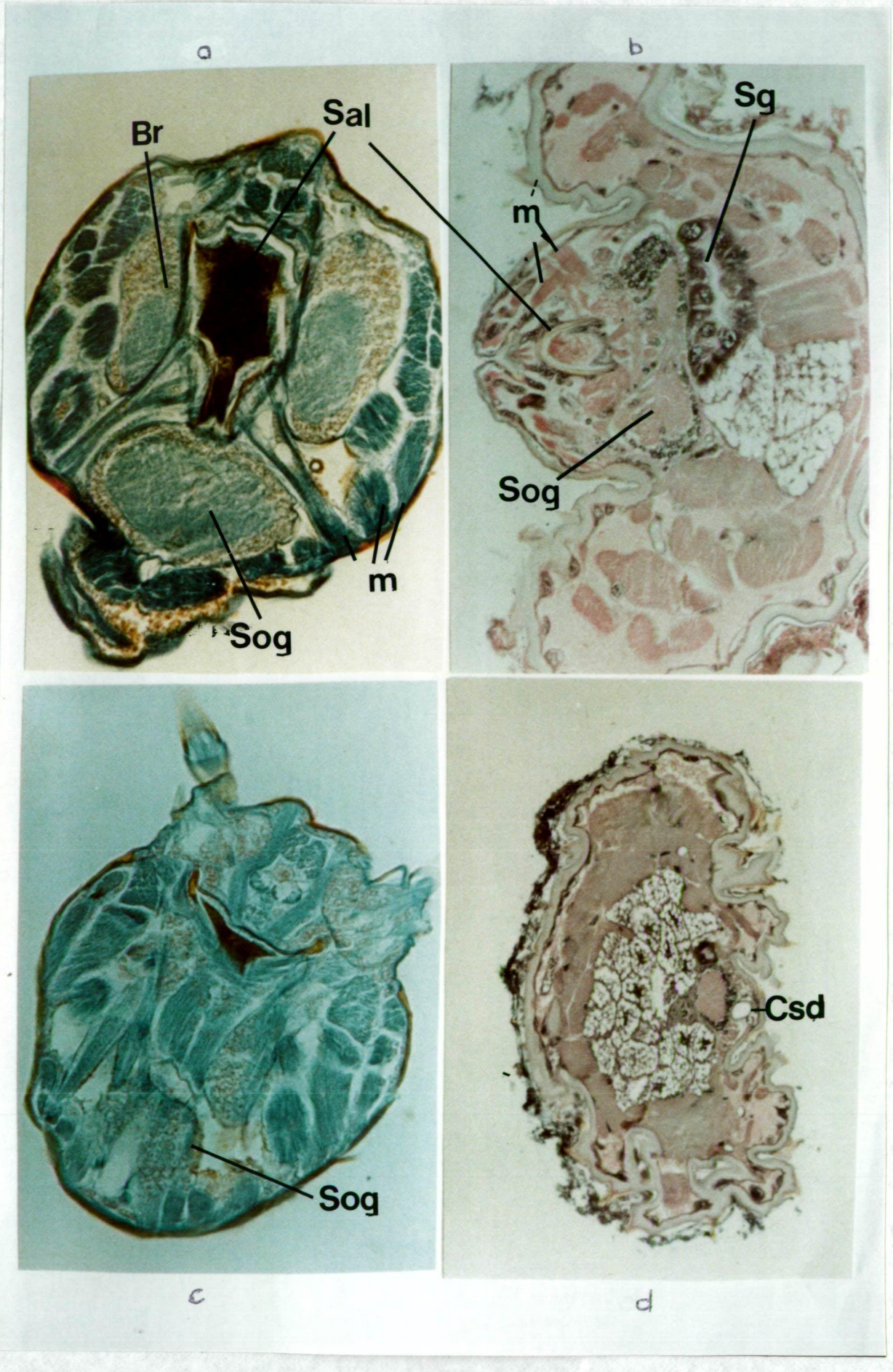

a
Br
Sal
m
Sog
b
Sg
m
Sog
c
Sog
d
Csd

Plate 4.9

(a) T.S. of abdomen of *P. hoplia* larva showing extent of fat body. (x100)

(b,c) T.S. through (b) abdomen and (c) thorax of *U. tasmanica* larvae showing extent of fat body. (x100)

v.f.b. = visceral fat body
p.f.b. = parietal fat body

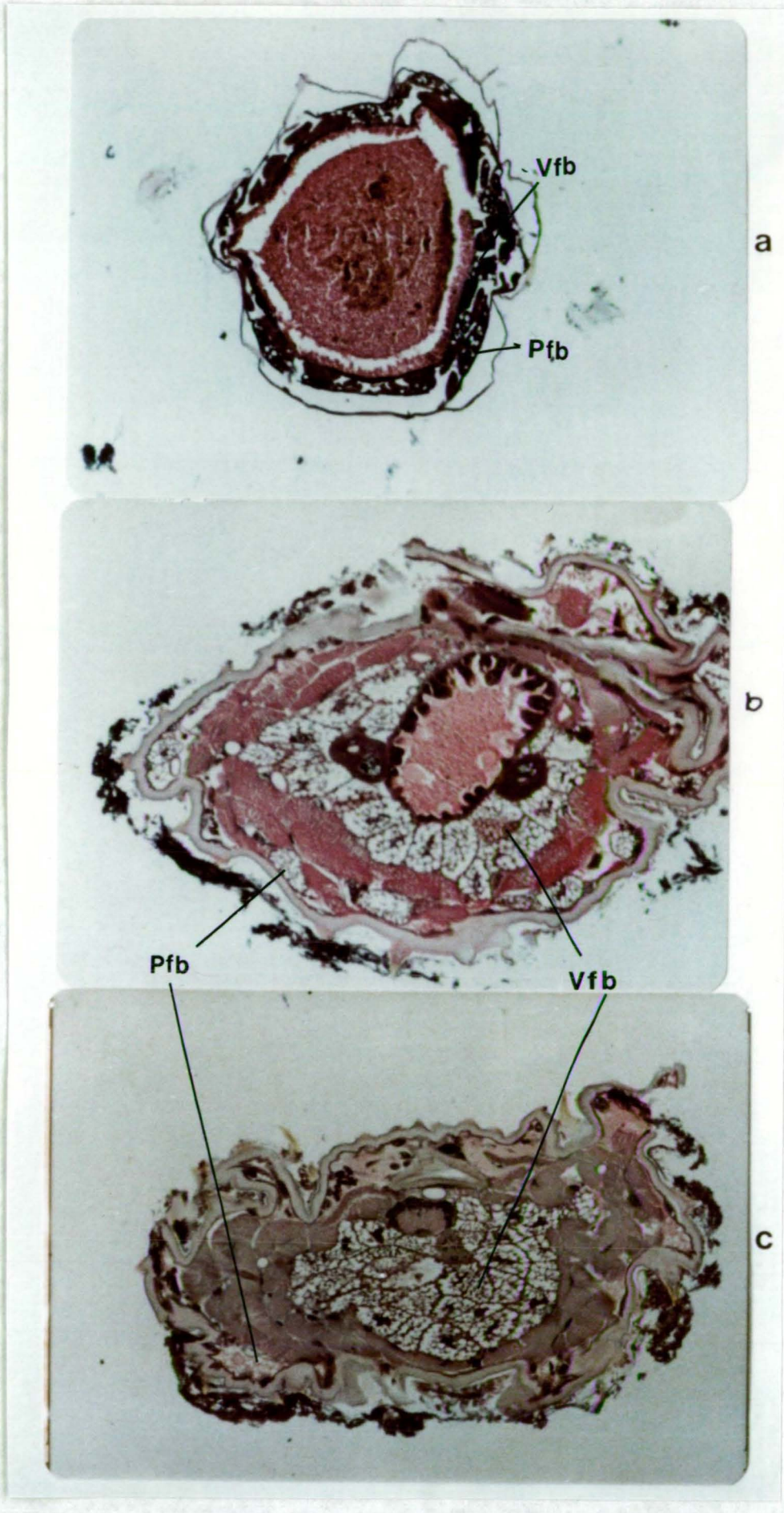

Vfb
Pfb
a
b
Pfb
Vfb
c

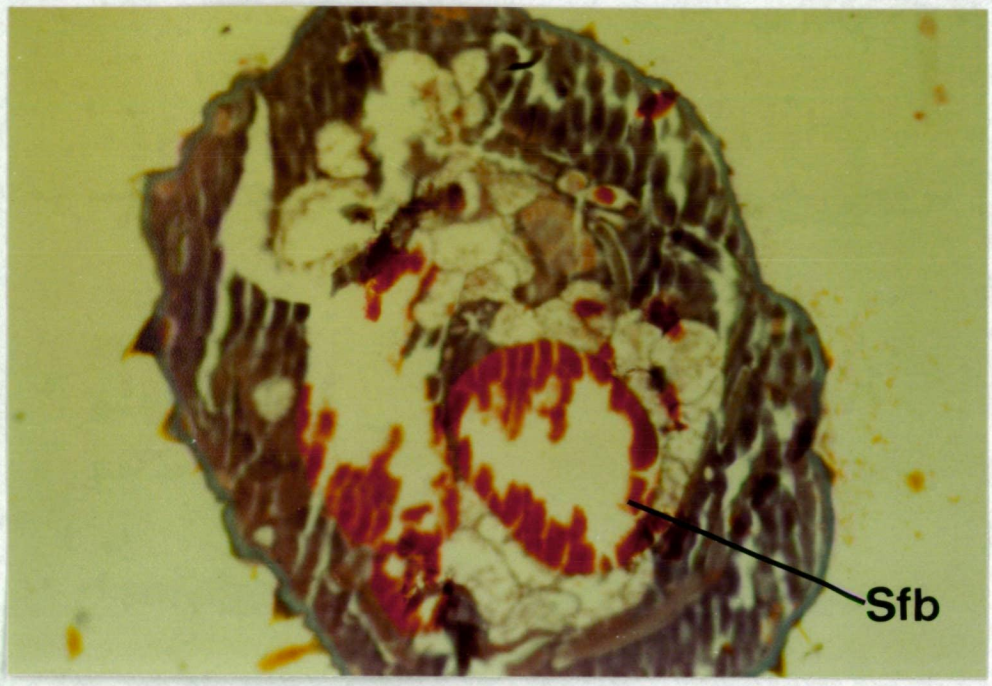
Plate 4.10

(a) T.S. abdomen *U. tasmanica* fourth instar larva showing specialised development of fat body. (x80).

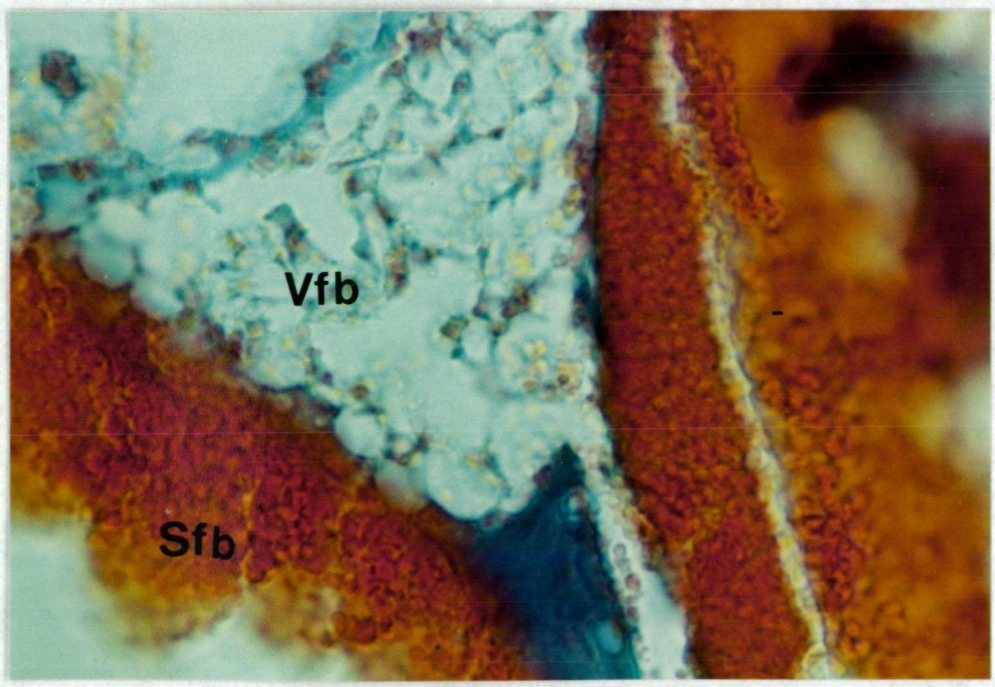
(b) Specialised fat body greatly enlarged

S.f.b. = Specialised fat body

v.f.b. = Visceral fat body



a



b

Zumpt has argued that in the Parsitic Dipterans the number of body segments has been reduced by fusion to 12 in most cases. This trend towards segment reduction has been found in *Uropsylla* larvae (Section 4.2).

The integument of the protelean Dipteran larvae is described as generally bearing denticles, spines or scales (Zumpt 1965) which is a condition which has also been observed in *Uropsylla* (Section 4.2). It should be noted here that Zumpt (1965) in illustrations of the larvae of dermal myiasis producing flies of mammals has shown in many species particularly of the genera *Cordylobia* and *Booponus* that these larvae have forward-pointing spines on the terminal segments, these were also observed in *Uropsylla*. (see fig.4.4).

Finally, Zumpt (1965) and Keilen (1944) have examined the distribution of spiracles in Dipteran larvae. It was found that myiasis producing larvae of Diptera always possess only two pairs of spiracles, one pair terminal and the other on the prothoracic segment.

The development of terminal spiracles with reduction in the size of the thoracic and anterior abdominal spiracles has already been described for *Uropsylla* larvae. This trend in *Uropsylla* parallels the reduction of spiracles and their arrangment in the parasitic Dipteran larvae. However the elaborate structure of the terminal spiracles of the Diptera (Zumpt, 1965, Keilen 1944) is not found in *Uropsylla* and reduction in the number of functional spiracles is not so complete in *Uropsylla* as it is in the Dipterans larvae.

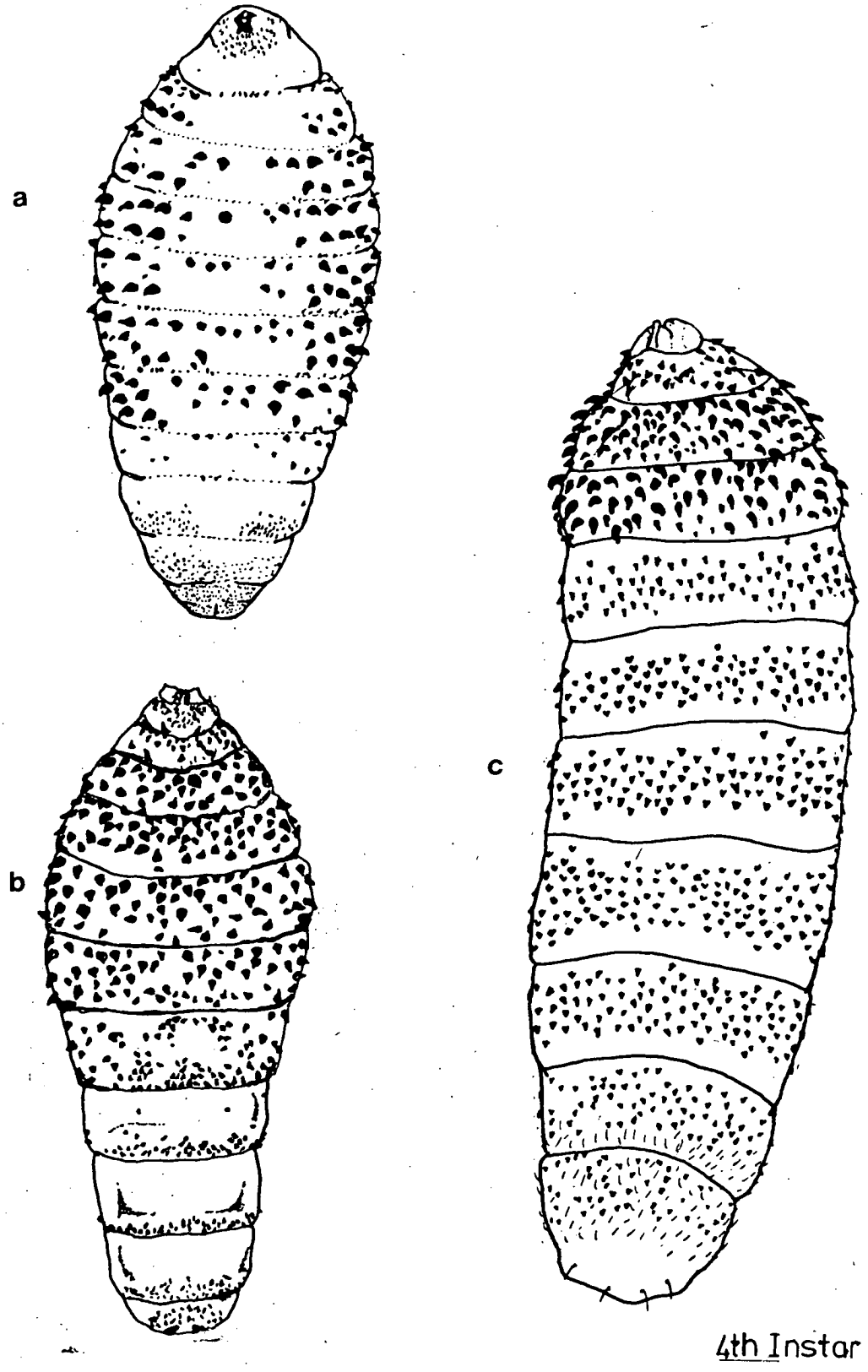


Fig. 4.4 Comparison of gross morphology of *U. tasmanica* (c) with myiasis producing dipteran larvae. (a,b) (After Zumpt 1965).

an = antenna

mp = maxillary palp

lb = labrum

lp = labial palp

lh = mouth hooks

scp = scrapers

md = mandible

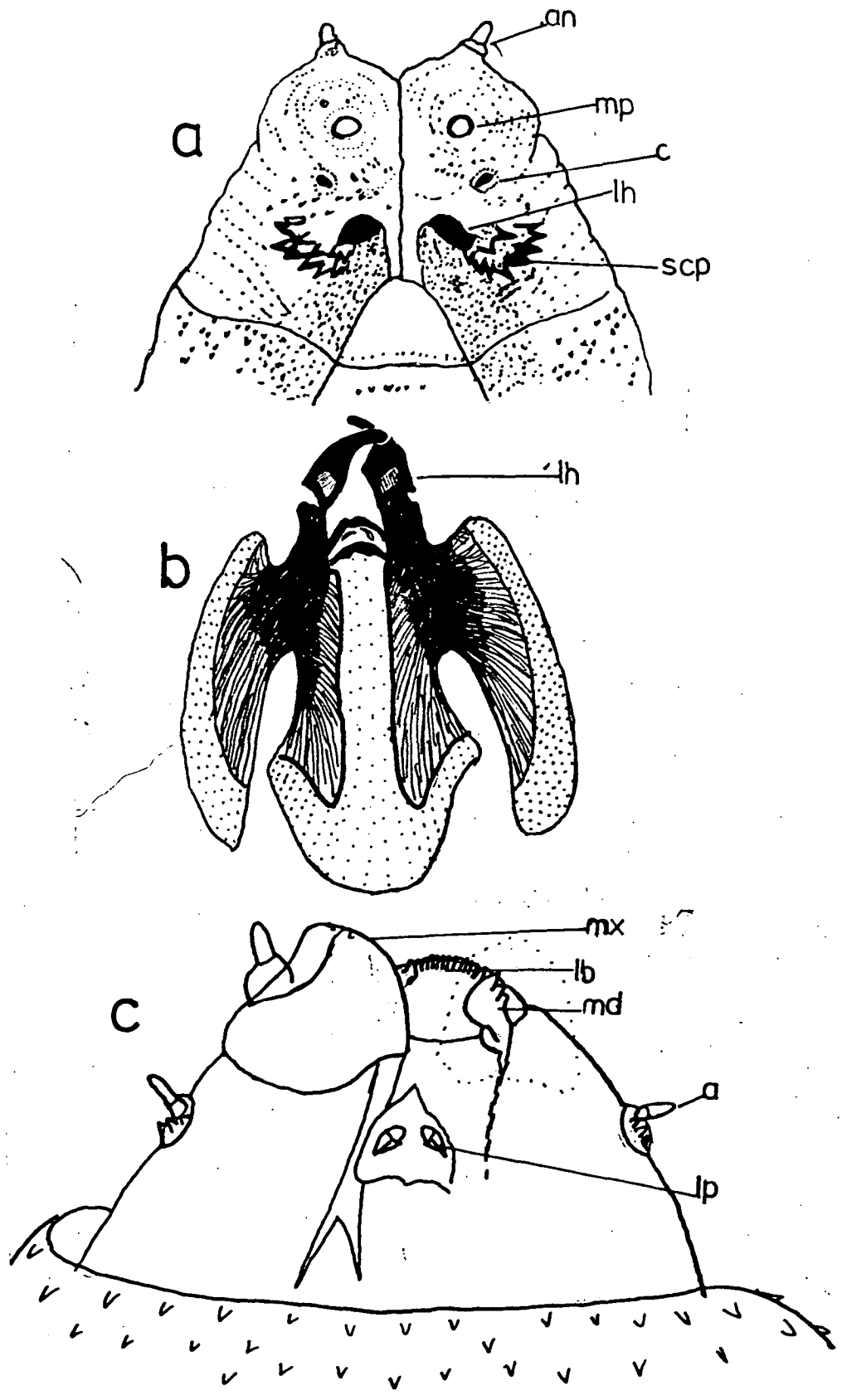


Fig. 4.5 Comparison of head and mouthparts of *U. tasmanica* (c) with myiasis producing diptera (a,b) (after Zumpt 1965).

Discussion

It was found that the modifications found in *U. tasmanica* larvae which facilitate the parasitic mode of life consist of changes to cheatotaxy, shape of the larvae, the mouth parts, and the spiracular and tracheal system. All of these modifications in *U. tasmanica* converge on the development of the parasitic larvae of the dipterans which exploit a similar environment.

It was observed that the whole body of the larvae has become shortened and thickened while protruding appendages such as spines and bristles and head appendages have been shortened. Like the larvae of dipterans, it was found that *U. tasmanica* larvae have a modified spiracular system so that stress is laid upon the terminal spiracles.

The internal anatomy of the larvae of *U. tasmanica* however, was found to have retained the characteristics of non-parasitic flea larvae except where changes to external morphology made this impossible. An example was the displacement of the first segmental ganglion from the head region into the thorax. Another major difference in structure which was observed between *U. tasmanica* larvae and those of non-parasitic fleas was the greater degree of development of the fat body in *U. tasmanica* when compared with other species. It is possible that the specialised fat body observed in *U. tasmanica* larvae aids in the survival of the flea during the extended period which it spends in the cocoon (Chapter 2, 6). Enlargement and specialised development of fat bodies in larvae is frequently found in insects which undergo a diapause at some stage prior to adult emergence (Wigglesworth 1972).

4.4 Biology of the Parasitic Larvae

This section deals with some aspects of the ecology of the parasitic larvae; feeding and food, distribution on the host; and the effect of larval infestation on the host.

Materials and Methods

Gut contents of *Uropsylla* larvae were prepared for microscopic analysis by removing the gut from live specimens which had been artifically removed from the host. Generally third instar larvae were used as these were large and easy to manipulate. The gut contents were squeezed onto glass slides and smeared. The smear preparations were stained with Giemsa.

The distribution patterns of larvae over the host were obtained from the field data, from flat study skins kept at the Queen Victoria Museum in Launceston (60 skins) and from the pelts of animals which were sacrificed in the course of other experiments carried out by other investigators (10 skins). A histogram showing the succession sites was prepared from the trapping data.

Notes were made during the trapping schedule of the condition of infested native cats. Their state of health was estimated broadly according to whether or not each animal appeared healthy, whether its normal movements were impeded in any way and the general behaviour of each animal during handling.

Results

Distribution of larvae on the host.

It was found that the degree of larval infestations (Fig. 4.7) could be divided into three grades:

Grade 1. Larvae lighly scattered over the tip of the tail; base of tail; rump; cloaca; scrotum; feet; ears. Density of larvae in affected areas approximately 4/cm^2.

Grade 2. Larvae found on the same areas as in Grade 1 but including the area between the cloaca and scrotum; middle of the tail; area around the mouth. The density of larval infestations in the tail approximately 12/cm^2.

Grade 3. Greater numbers of larvae in the previously mentioned areas but including flanks and shoulders; forelimbs; hindlimbs; back. Commonly several larvae in one lesion and larvae in open sores with extensive areas of inflamation. Density of larvae up to 40/cm^2.

The succession of infection sites on native cats from the field data is shown in Fig. 4.6 It can be seen from this figure that in all cases of larval infestation in native cats the tip of the tail was involved. The other favoured site included the base of the tail the feet, cloaca, and ears. It was generally found that once the tip of the tail was heavily infested, (in one case some 100 larvae were removed from the last 2 cm of the tail), then the infestation would spread down the middle section of the tail. In male native cats the scrotum was a heavily favoured site whereas the pouch in females was not so generally infested. In general it can be seen that the favoured sites were body extremities while the upper portion of the back, the chest and upper abdomen were not often affected. In cases where larvae were found on the least favoured sites there were heavy infestations in other areas.

Effect of Infestation on the Host.

It was found that the presence of larvae in skin lesions of the host was always accompanied by inflammation and infection. In heavily infested animals the inflammation surrounding the larvae produced large swellings and in some cases of infestation in the shoulders, the native cats were severely disabled, exhibiting partial paralysis of the forelegs. In one native cat which died, an autopsy revealed a high level of infestation in the head accompanied by a subdermal layer of pus which extended along the nostrum, around the eyes and ears and posteriorly to the base of the skull.

In no case was any native cat observed to remove any of the larvae from the skin lesions and the removal of the larvae by the investigator did not appear to cause the native cats much pain. The native cats only appeared to be sensitive to the larval infestations when large infected swellings were involved. It is possible that the removal of the larvae by the native cats would be difficult as the larvae always withdrew their terminal segments into the skin lesions when touched.

Food.

Microscopic analysis of the gut contents of larvae removed from the native cats revealed that the larvae eat a mixture of blood, pus and broken down necrotic tissues. Both erythrocytes and leucocytes were present in the gut contents but the number of leucocytes was high compared with the samples of native cat blood this indicating that they were abundant at the site of feeding. The gut contents also contained bacteria as well as unidentifiable matter which was thought

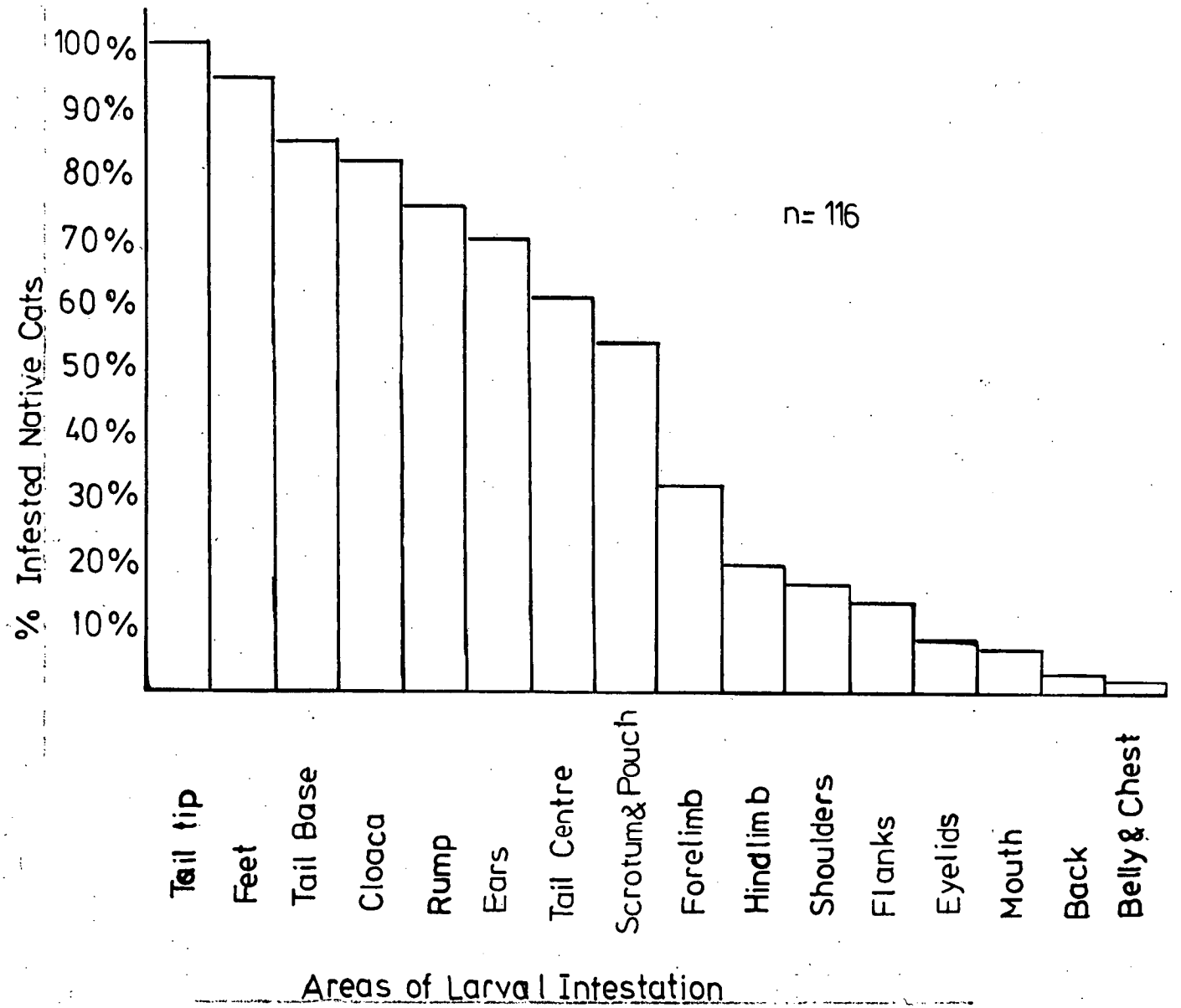


Fig 4.7 Histogram Showing Succession of Sites of Larval Infestation.

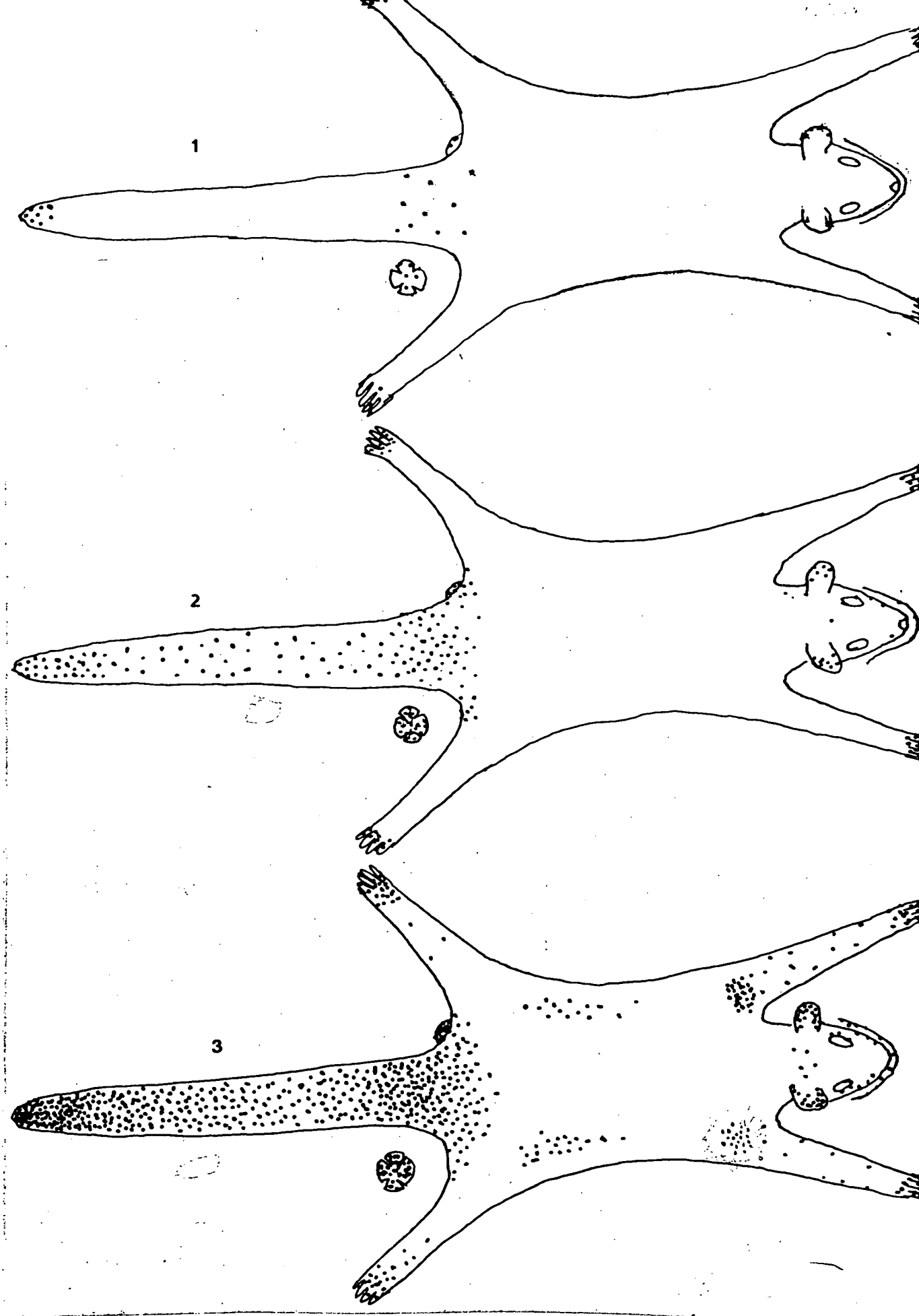


Fig. 4.7 The three grades of larval infestation in Native Cats.

1 - Light; 2 - Medium; 3 - Heavy.

to be broken down host tissues. No attempt was made to identify the bacteria which were found in the gut contents.

Discussion.

It can be seen from the results that the larvae of *Uropsylla tasmanica* prefer certain sites on the host for development. It is unknown whether site selection is carried out by the adult female in the laying of eggs or whether the larvae select the site of penetration following eclosion. However it is probable that the females deposit the eggs in the favoured areas as female fleas were frequently recovered from the tails and legs of native cats (Ch. 1 pp 6). It is however, possible that the larvae themselves may move about on the surface of the host's skin before penetration thus selecting the actual site.

The strong tendency towards site preference of larval infestation implies that certain areas on the body of the native cat are more favourable for larval development than other sites.

The preference of some sites over others is frequently encountered among dermal parasites. The stickfast fleas for example tend to attach themsleves to areas of the host which are difficult to groom thereby lessening the chance of dislodgment by the host (Rothschild and Clay 1956). However in the case of *Uropsylla* removal of the larvae by the host was not observed and the preferred sites particularly the tail are readily accessible to the host.

The fact that larval infestation is accompanied by bacterial infection suggests that the larvae may derive some benefit from the presence of these bacteria. Sharif (1948b) suggested that free living larvae of rat fleas of the genus *Xenopsylla* were aided in their development by ingesting yeasts growing on the dried food.

Pausch and Fraenkel (1966) however discounted this theory and also pointed out that these larvae had no symbiotic gut bacteria. However symbionts are found in blood sucking parasites which do not partake of any other type of food during any development stage and in insects which eat a restricted diet (Chapman 1969). According to Wigglesworth (1952) gut symbionts are found in blood sucking lice, and *Glossina* and *Nycteribidae* which are viviparous and amongst some Diptera. However symbionts are not found in fleas, blood sucking Nematocera or Tabanidea since these groups have free living larvae which eat a varied diet.

Friend *et al.* (1959) found that the larvae of *Hylemya antiqua*, the onion maggot do not derive adequate nourishment from an unvaried diet of onion but are able to utilise the bacteria which grow in the tunnels formed by the larvae. These microorganisms supply essential vitamins and other substances to the larvae's diet.

It would appear then that fleas do not have symbiotic gut parasites because of the variation in diet which normally occurs over the lifetime of a flea. However *Uropsylla* is restricted to a diet which is similar during the larval and adult stages and it is postulated that the bacteria which accompany larval infestations of *Uropsylla* are needed to supply some dietary factor which would otherwise not be obtained. On the basis of this it is further suggested that the selection of the areas of larval infestation is correlated with the probability of bacterial infection of these sites. Thus the extremities and the areas mostly to come into contact with dirt are preferred to the cleaner body surface.

Another important facet of the infestation of native cats by *Uropsylla* larvae which arises from this section is the occasional occurrence of overparasitism which may lead either directly or indirectly

to the death of the host. Larval infestation can cause death of the host through the bacterial infection which accompanies the infestation. This was only observed in the laboratory on one occasion but could be more common in the field since captive native cats were treated by the removal of most of the larvae and the application of antibiotics. Larval infestation could indirectly cause death of the host by impeding its movements and thereby reducing its ability to obtain food.

4.5 Emergence of Mature Larvae from the Host

When the flea larvae are mature they leave the host and drop into the litter of the den where they build their cocoons.

It was noticed during collection of the larvae for experiments on pupal development (Chapter 6) that the majority of the larvae tended to leave the host at approximately the same time of day during daylight hours. These observations suggested that larval emergence from the host was synchronised in some way to ensure that the larvae would leave the host at a time when they were most likely to fall into the litter in the den.

The aim of this section was to quantify emergence of the mature larvae from the native cats and to correlate their emergence with time of day.

Materials and Methods

The native cats were set up in cages with wire mesh bases raised above the floor. The cages were supplied with nesting boxes but these boxes lacked solid bases and sat on the wire mesh floor of the cages. Any flea larvae which left the host dropped through the wire mesh onto plastic sheets spread out on the floor below. Once they reached the floor the larvae did not move very quickly and could easily be found.

The cages were set up in the animal house of the Zoology Department which at that time had an 11 hour artificial daylight cycle from 8 am to 7 pm. The native cats were installed in the cages and one week was allowed for the animals to settle down and become acclimatised to the conditions in the animal house. The experiment followed this period and consisted of 8 days when larvae were collected hourly during the hours of light. The first check of the morning was made at 8 am to make sure that no larvae had left the hosts during the night. Three heavily infested male native cats were used for this experiment.

During the experiment notes were made about the behaviour of the native cats during each hourly check.

Results

The results of the experiment are shown in Table 4.3 and in figure 4.8. It can be seen from these results that the majority of the larvae emerged in the sixth hour of daylight and the least larvae emerged in the first and final hours. Notes made about the native cats during the experiment indicated that the larvae emerged from the hosts during periods of rest. If the hosts were disturbed the larvae ceased to emerge until the host had been resting for at least one hour.

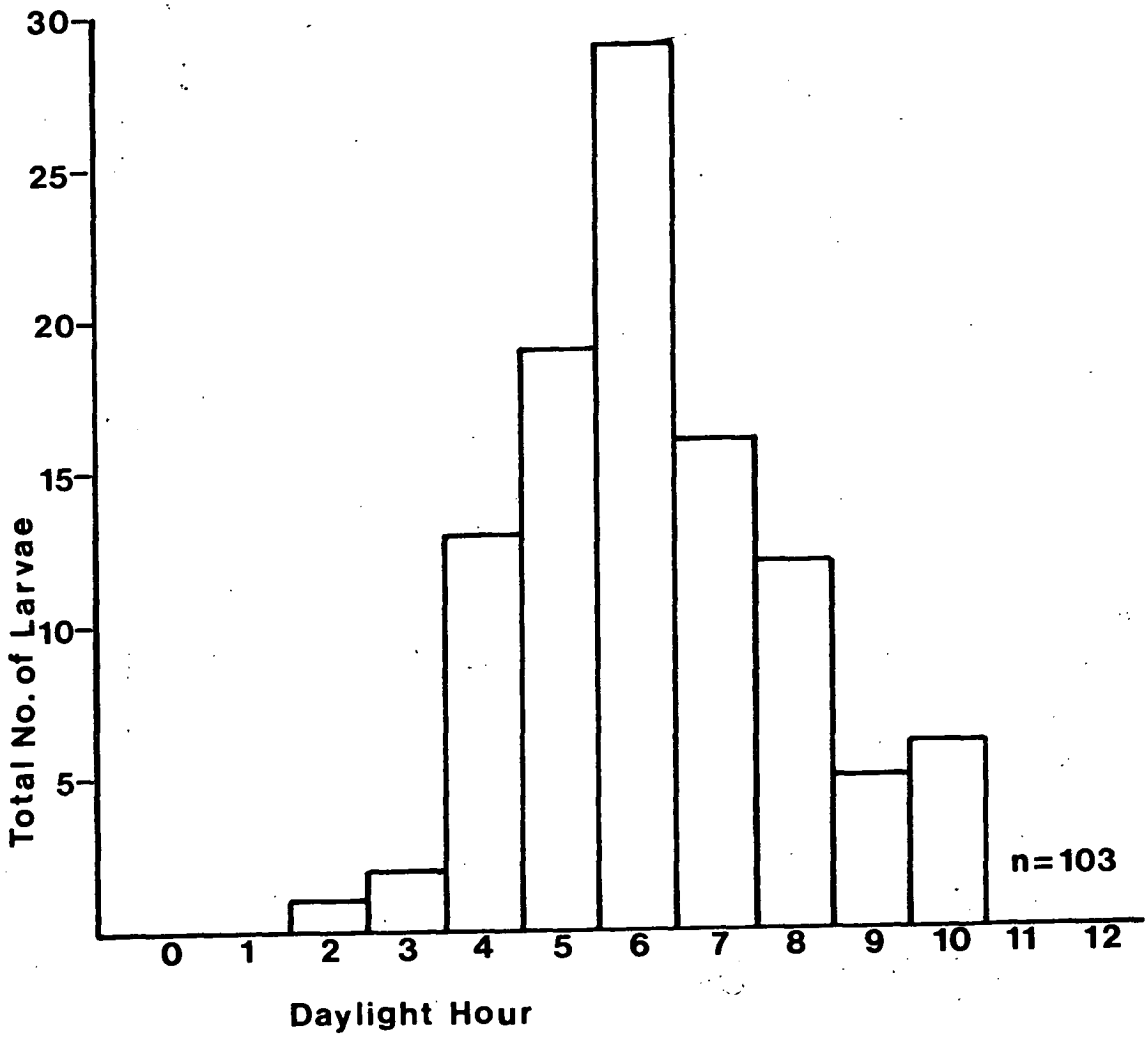


Fig. 4.8 Emergence of Mature Larvae From Native Cats During Daylight Hours.

Table 4.3

Larvae collected hourly over an 8 day period

Experiment 1.

Hour	0	1	2	3	4	5	6	7	8	9	10	11	T
Day 1	-	-	-	-	-	-	2	2	1	2	5	0	12
Day 2	-	-	-	1	2	1	3	1	-	-	-	-	8
Day 3	-	-	-	-	-	8	-	4	3	-	-	-	15
Day 4	-	-	-	1	3	-	2	-	1	-	-	-	7
Day 5	-	-	-	-	-	5	9	1	-	-	-	-	15
Day 6	-	-	-	-	4	-	1	-	1	-	-	-	6
Day 7	-	-	-	-	-	1	7	2	3	1	-	-	14
Day 8	-	-	1	2	4	3	5	6	4	1	-	-	26
Total	0	0	1	4	13	18	29	16	13	4	5	0	

Discussion

The results of the experiment indicated that the mature larvae of *U. tasmanica* were stimulated to emerge from the hosts during periods in which the hosts were at rest. Since native cats are nocturnal, the larvae would be expected to emerge during the day time. The stimulus which causes the larvae to emerge could come from external cues such as hours of daylight or could come from some physiological change which takes place in the host during sleep.

The results suggest that since the larvae leave the native cats during daylight and during the hosts' rest periods that the larvae have developed a mechanism which ensures that they leave the host at a time when they are most likely to fall into the litter of the den. By ensuring that pupation takes place in a native cat den the larvae stand a high chance of encountering a host when ready to

emerge as adults. If the larvae were to leave the hosts during the night, when the host is active and not in the den, it is unlikely that, upon emergence as adults, they would ever encounter another host and so would be unable to complete their life cycle.

Chapter 5

Development of *Uropsylla tasmanica* Larvae Following Emergence from the Host

Introduction

Warneke (pers. comm.) found that once in the cocoons *Uropsylla* larvae took some 60 days to reach the final pharate moult. It was expected that this might also be the case with the larvae collected in this study. However it was found that the larvae failed to pupate during the winter months so that larvae which left the hosts early in winter remained as larvae in the cocoons for periods which greatly exceeded 60 days. In the first year of the study it was found that those larvae permitted to build their cocoons in soil which was kept at room temperature all pupated during the month of September (Spring) regardless of their time of emergence from the host. Attempts to speed up pupation such as by warming the larvae in their cocoons had no effect and it was concluded that the development of these larvae was arrested or delayed during the months from April to September.

Delayed development of fleas during the larval stage in the cocoon or during the pupal stage has been reported many times. Bacot (1914) reported that the period of development of the rat flea varied from 7-182 days while Margalit and Shulov (1972) showed that the development of the pupae of the rat flea was retarded under various conditions of temperature and humidity. Margalit and Shulov (1972) argued that development was not arrested under these conditions but continued at a slower rate than under optimum conditions. Cotton (1970) showed that development of *Ctenopthlamus nobilis* was retarded at all stages by low temperatures. The fleas which parasitise the nests of migratory birds are known to spend long periods in the cocoon (Rothschild and Clay 1956, Askew 1971) but it is not known whether this quiescence occurs during the larval or pupal stage.

When insects encounter conditions which are unfavourable for development they may respond in one of two ways. Firstly they may respond directly to the unfavourable conditions by entering a quiescence as exemplified by insects becoming torpid in response to cold (Wigglesworth 1972). Secondly they may survive an unfavourable period by entering diapause which is a more subtle and complex response, frequently to the conditions which precede the unfavourable period and which may persist beyond the return of the favourable conditions (Lees 1955). Diapause is thought to involve inactivity of neurosecretory cells which control the activity of the prothoracic glands and the corpora allata thus causing these hormone secreting glands to cease function. The breaking of diapause involves the reactivation of the neurosecretory cells thus leading to a resumption in development.

The aim of the experiments presented in this chapter was to investigate the factors which affect the development of *Uropsylla* larvae in the cocoons andto determine the nature of the prolonged period which these larvae may spend in the cocoon.

5.2 General Procedures

The experiments were carried out in constant temperature rooms of $5^{o}C$, $10^{o}C$, $15^{o}C$ and $25^{o}C$. The temperatures in the constant temperature rooms did fluctuate ($\pm$ $0.5^{o}C$) but in all cases the larvae were insulated against these fluctuations by being placed in cupboards in the rooms. The larvae used in the experiments were mature larvae which had left the hosts and were collected from the floor below the native cat cages as described in Chapter 4 (section 4.6).

Following collection, the mature larvae were placed in petri dishes containing some 5 mm of soil which was kept moist at all times. The petri dishes were then placed in the conditions specific for each

experiment. With the exception of the experiments on the effects of photoperiod on the development of the larvae all experiments were carried out in dark conditions and the larvae were exposed to light only during the checks which were made on development and then for only a few minutes. Development was checked by opening the cocoons and removing the larvae which were then replaced in the experimental conditions in artificial cocoons which consisted of glass tubing approximately 1.5 cm long and with each end stopped with cotton wool plugs. The tubes were 2 mm internal diameter. During the course of each experiment checks on development took place at weekly intervals unless the results indicated that more or less frequent checks were necessary. During these checks four or five cocoons of each batch were opened and the occupants were then placed in artificial cocoons until eventually the larvae in each experimental group were all housed in artificial cocoons so that the development checks could be carried out visually.

During removal from the cocoons the larvae were handled with soft foil forceps in order to avoid damage. Any larvae which were damaged during handling were discarded and recorded as deaths. Dead larvae found in the cocoons were also discarded. The majority of deaths recorded during the course of the experiments appeared to be caused by invasion of the immobile larvae by dust mites. Some measures such as cleaning the areas surrounding the experiments and the use of moats were employed in an attempt to control the dust mites but to little effect. The use of miticides was considered but thought to be potentially dangerous to the larvae.

Before any experiments were carried out on the rates of development of larvae in different conditions of temperature and photoperiod it was necessary to test the effect of the use of artificial cocoons on larval

development. To this end some forty larvae were collected as they emerged from the hosts and were placed in soil in 4 petri dishes. The petri dishes were kept in room temperature for ten days to ensure that all larvae had built cocoons. After this period 38 larvae were found to have constructed cocoons while two larvae had died. At this stage ten larvae from 1 petri dish were removed from cocoons and were placed in artificial cocoons. The artificial cocoons were then put into a clean petri dish, with filter paper which was kept moist at all times.

The four petri dishes including the petri dish containing the artificial cocoons were then placed in the 25°C constant temperature room. Checks on the rate of development of the larvae in the cocoons were carried out at fortnightly and later at weekly intervals. The larvae in the artificial cocoons were checked visually while those in the natural cocoons were checked by opening two cocoons at each session and replacing the larvae in artificial cocoons. In this way the larvae were gradually transferred to artificial cocoons throughout the experiment and at all stages of development thus the rates of development of the larvae in the artificial cocoons and the natural cocoons were compared. It was thought that in this way any effects of transfer from natural to artificial cocoons at any development stage would be noticed. Once the majority of fleas in the artificial cocoons had undergone the pharate moult the remaining natural cocoons were opened.

This experiment was carried out at the same time as the experiments on the effects of different constant temperatures on larval development. All other diapause experiments were carried out the following year.

Results

At the end of the experiment there were 28 adult fleas in artificial cocoons and 7 still in natural cocoons. From this experiment three phases of development were observed. The first phase consisted of larvae which upon removal from the cocoons were capable of movement but not capable of building a new cocoon. This phase was followed by an inactive pre-pupal phase. In the pre-pupal phase larvae, when removed from the cocoons, were not capable of movement and appeared to be shorter and thicker than the larvae in the previous stage. The larvae assume a U shape in the cocoons and the larvae in the pre-pupal stage retained this shape after removal from the cocoons. The third stage is the pupal stage.

By means of the continual opening of cocoons and transfer of larvae from natural to artificial cocoons it was observed that the larvae of both groups developed at the same rate. Thus when the larvae in the artificial cocoons had entered the pre-pupal phase (25.6 ± 0.03 days) it was found that the larvae which were removed from the natural cocoons had also entered the pre-pupal phase. When the larvae in the artificial cocoons pupated (93.7 ± 0.52 days) the larvae which were removed from the natural cocoons at this time had also pupated or did so within three days. At the end of the experiment when the pupae in the artificial cocoons had undergone the pharate moult, it was found that the remaining larvae in natural cocoons had either undergone the pharate moult or did so within 4 days (25 ± 1.02 days).

It was observed that the mortality rate was higher in the larvae kept in artificial cocoons and that it was necessary to ensure that the

filter paper on which the cocoons rested was kept moist. It was necessary to moisten the filter paper every one or two days whereas the soil containing the natural cocoons needed to be moistened only every five or six days at 25°C.

5.3 Experiments

Effect of Temperature and Photoperiod on the Duration of the Free Living Period.

Following emergence from the native cats, the mature flea larvae spend several days in the litter and soil of the dens before they build a cocoon. This free living period appeared to be variable in duration thus an experiment was set up to investigate the effects of different conditions of constant temperature as well as daily cycles of photoperiod.

Materials and Methods

Larvae collected on emergence from the hosts were placed in or on the soil in the petri dishes and placed in different conditions of temperature and photoperiod as follows:

	No. larvae	Light Conditions	Temperature Conditions
1.	16	Dark	Constant temp. 5 C.
2.	18	Dark	" " 10 C.
3.	15	Dark	" " 15 C.
4.	18	Dark	" " 25 C.
5.	20	16 hr. photoperiod	15 -22 C. mean temp. 19.79 C.
6.	22	14 hr. "	15 -22 C. " " 19.2 C.
7.	16	12 hr. "	15 -22 C. " " 18.6 C.
8.	23	10 hr. "	15 -22 C. " " 18 C.
9.	15	8 hr. "	15 -22 C. " " 17.4 C.
10.	18	12 hr. "	10 -17 C. " " variable

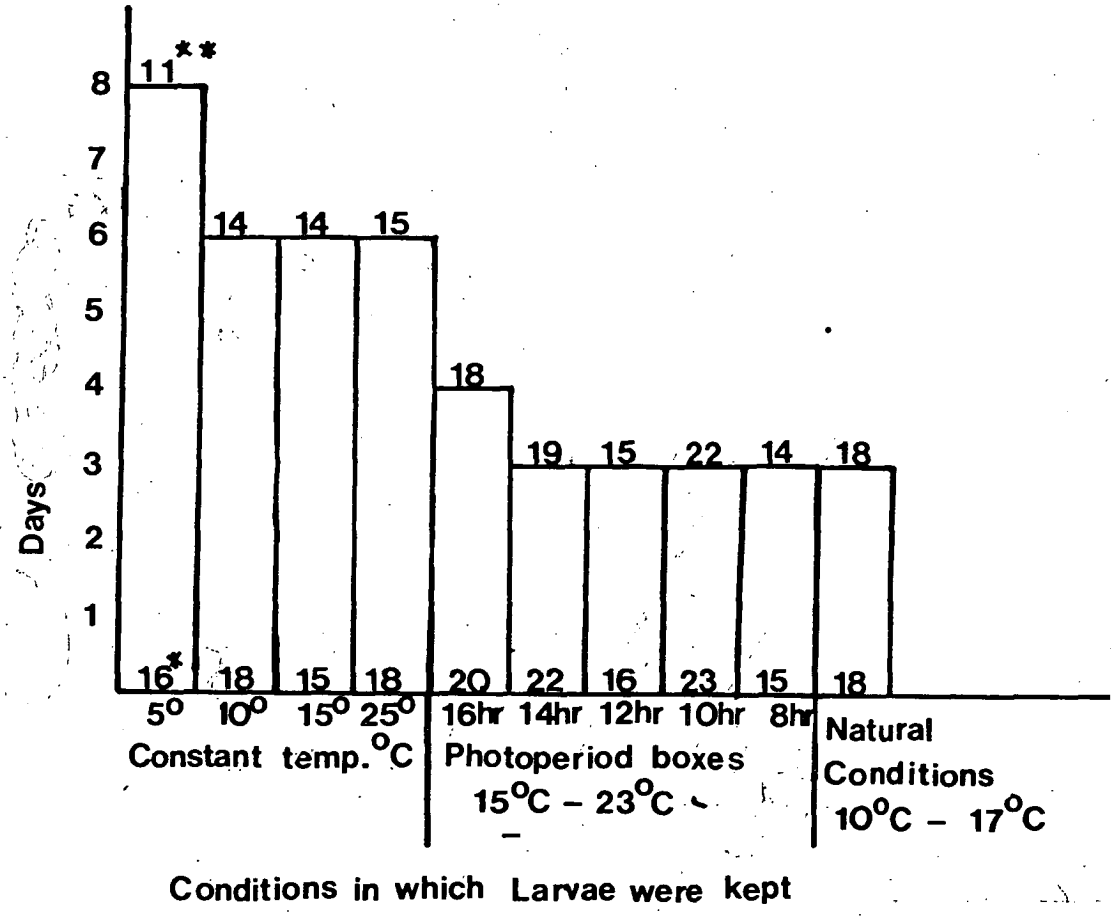


Fig. 5.1 Time taken to construct cocoons in different conditions.

* No. of larvae at commencement
** No. of surviving larvae

The photoperiod boxes were set up in the 15°C constant temperature room and during the light periods the temperature reached 22°C in the boxes. The different numbers of larvae used in the experiments depended upon the yield of mature larvae from the hosts.

Results

The results of experiment 1 are summarised in fig. 5.1. The larvae in constant temperatures took an average of six days to build cocoons at 10°, 15° and 25°C while those kept at 5°C took an average of 8 days. In the photoperiod boxes the larvae took an average of three days to build their cocoons except in the 16 hour day where an average of four days was required. Under natural conditions of daily temperature fluctuations three days was the average time taken till the cocoons were built. In most experiments some of the larvae failed to build a cocoon and died; these losses were greatest under the constant temperature conditions. The number of larvae which successfully built cocoons in each group can be seen in figure 5.1. It can be seen that while one or two larvae died in most of the groups, of the group which was kept at 5°C, five larvae failed to build cocoons.

In the 5° and 10°C constant temperature conditions several larvae left the cocoons several days after building them, these larvae failed to build a second cocoon and subsequently died. During the course of these experiments there was only one record of a larva building a second cocoon and this occurred when a larva was removed from the cocoon early in an experiment and placed in an artificial cocoon.

The Effect of Different Constant Temperatures on Larval Development

The aim of this experiment was to determine the rate of development of the fleas in the cocoons under different conditions of constant temperature.

This experiment was set up in two parts, a and b. In part a, flea larvae were collected as they emerged from the native cats and placed in soil in the petri dishes. The petri dishes were then kept at room temperature for ten days to ensure that all surviving larvae had completed their cocoons. In part b, the larvae were collected from the native cats as they emerged and placed in soil in the petri dishes. They were then placed directly into the constant temperature conditions.

The fleas from each group were checked by opening the cocoons and examining the larvae. Once removed from their cocoons the larvae were replaced into the experimental conditions in artificial glass cocoons. The cocoons were opened at such a rate that all larvae had been put into artificial cocoons by the seventh week of the experiment. Because not all of the cocoons had been opened by the time the larvae entered the pre-pupal stage the comparison of the duration of the first cocoon stage, the larval stage, is based on fewer fleas than the the comparisons of the duration of the later stages.

The experiments were set up as follows.

Part a		Part b	
5°C	40 larvae	10°C	18 larvae
10°C	40 larvae	15°C	15 larvae
15°C	34 larvae	25°C	18 larvae
25°C	38 larvae		

The discrepancies in the numbers of fleas used in the different parts of this and subsequent experiments is a result of the variable numbers of mature larvae which left the native cats during the course of this project. It was found that on any day the emergence of flea larvae could not be predicted or counted upon.

Results

The results of the experiments are shown in tables 5.1, 5.2 and 5.3.

Table 5.1. Duration of "larval" phase (including free living period).

	Part a.			Part b.		
Temp	n	$\bar{x}$ (days)	S.D.	n	$\bar{x}$ (days)	S.D.
10°	10	34.9	2.83	10	37.2	2.43
15°	10	24.8	2.415	9	26.66	2.23
25°	9	25.22	2.415	10	26.7	1.787

From these results it can be seen that the duration of the first stage was longer at 10°C than at the other two temperatures, whereas the duration of this phase was similar at 15°C and 25°C in both parts of the experiment. When the duration of the first stage at 10°C was statistically compared with the duration of this stage at 15°C and 25°C, using t tests with Bessels correction factor, the difference was found to be significant. Using the same test it was found that the differences in the duration of the larvae stage at 25°C in each part of the experiment was not significant.

The results for the group of flea larvae kept at 5°C are not shown

in Table 5.1 as these fleas had not reached the pre-pupal phase after 65 days and appeared to be torpid. This result suggests that a constant temperature of 5°C is too low for the development of the larvae in the cocoon.

Table 5.2 Duration of Pre-Pupation Period (pre-pupal and larvae)

	Part a.			Part b.	
Temp	n	$\bar{x}$ (days)	S.D.	n	$\bar{x}$ (days)	S.D.
10°C	27	134.25	4.12	16	142.6	3.96
15°C	24	119.84	3.7	11	132.51	3.87
25°C	22	120.72	3.79	14	134.74	3.88

The mean periods shown in this table represent the number of days from emergence until pupation took place. It was found, when these values were compared statistically using *t* test analysis that the period preceding pupation was significantly greater in part b of the experiment than in part a. The duration of the pre-pupal phase for each temperature in each part of the experiment was found by subtracting the mean time of the larval phase from the mean times shown in table 5.2. The approximate durations of the pre-pupal phase obtained in this way are shown in table 5.3.

Table 5.3 Duration of Pre-pupal Phase

	Part a.			Part b.	
Temp	n	Days	S.D.	n	Days	S.D.
10°C	27	94.85	1.4	16	105.4	2.09
15°C	24	95.04	1.05	11	105.63	2.1442
25°C	22	95.5	1.1001	14	109.14	2.3366

At all temperatures the duration of the pre-pupal phase was found, using *t* tests, to be significantly different between experimental groups a and b while the duration of the prepupal phase of those larvae kept at 25°C. in part b of the experiement was found to differ significantly from those of the larvae kept at 10°C. and 15°C.

The duration of the pupal phase in all the groups of fleas is shown in Table 5.4. It can be seen that pupation was of similar duration in all groups.

Table 5.4 Duration of Pupal Phase

	Part a.			Part b.		
Temp.	n	$\bar{x}$ (days)	S.D.	n	$\bar{x}$ (days)	S.D.
10°	27	26.80	1.33	16	26.4	1.41
15°	24	26.83	1.52	11	26.0	0.74
25°	22	26.19	1.44	14	25.92	1.43

It was suspected that the duration of the pre-pupal phase of the larvae in the cocoons might be variable under certain conditions as the larvae had been observed to emerge from the native cats throughout the winter months and no larvae had been found in cocoons later than October (Ch. 2). This suggests that the larvae which emerge from the native cats at the end of winter develop without undergoing long periods in the cocoons. It was decided therefore that the prolonged pre-pupal phase was possibly a diapause and to subject the larvae to conditions which are known to affect or break diapause in other insects (Lees 1955, Wigglesworth 1972).

The effect of chilling on the rate of larval development

This experiment was designed to test the effect on the rate of larval development in the cocoon of chilling the larvae for various

After 30 days the petri dishes were removed from the $5^{o}C$ constant temperature room at fortnightly intervals and were placed in the $25^{o}C$ constant temperature room.

periods of time followed by exposure to warmth. The results of the previous experiment had suggested that the larvae were unable to develop at low temperatures (at or below 5°C) but at a higher temperature the rate of development was similar throughout the range of temperatures used. At all of the constant temperatures used in the previous experiment the duration of the pre-pupal phase of development was very long, longer than would be expected in the absence of a diapause.

Materials and Methods

In this experiment mature larvae were collected from the native cats on emergence and placed into 4 petri dishes containing soil. The petri dishes, containing 14, 16, 19 and 13 larvae were kept at room temperature for ten days to allow the larvae to build their cocoons. Following this they were placed into the 5°C constant temperature room. The petri dishes were removed from the 5°C constant temperature room. After two or three days in the 25°C constant temperature room all of the cocoons of each group of fleas were opened and the larvae placed into artificial cocoons. This was done to facilitate observations of their development.

Results

The results of this experiment are summarised in table 5.5. When these results were compared using variance analysis tables and F distribution no significant differences were found. It was noticed that the values for the duration of the pre-pupal and pupal development stages were similar to those of group A of the previous experiment. The values for the duration of larval stage are smaller in this experiment than in the previous experiment because they were recorded as the time taken to reach the pre-pupal stage following transfer from 5°C to 25°C.

Table 5.5

	Group 1	Group 2	Group 3	Group 4
Time in 5°C	30 days	44 days	58 days	72 days
n at transfer	12	13	15	11
Mean Duration of Larval Phase at 25°C (days)	13.16	13.15	12.66	12.94
S.D.	2.14	2.98	2.63	2.49
n	12	13	15	11
Mean Duration of Pre-pupal Phase at 25°C (days)	94.67	95.76	95.13	96.09
S.D.	4.56	4.6	5.51	5.0
n	8	9	12	9
Mean Duration of Pupation (days) at 25°C	27.5	26.125	25.26	26.65
S.D.	3.58	3.6	4.15	4.11
n	8	8	12	9

The 10 days in which the larvae were kept at room temperature as well as the period in which they were kept in the 5°C condition were omitted from the calculations of the durations of the larval phase as this did simplify the data and had no effect on the inter-group comparisons.

Effect of Different Photoperiods on Larval Development

This experiment was set up to test the effect of photoperiod on the rate of development of the larvae in the cocoons. In the previous experiment, which tested the effect of chilling followed by exposure to high temperatures it was found that the length of the pre-pupal phase was not shortened by any of the treatments. It was known from observations of the native cats in the enclosure that these animals sleep in dens which may be below the soil surface. Also the cocoons are constructed in the soil and litter in the native cat dens and are themselves coated with soil particles. While it seemed improbable that light would reach the larvae in the cocoons under these circumstances there was a possibility that this would occur.

Materials and Methods

As they emerged from the native cats mature larvae were collected and placed into 5 petri dishes containing soil. The petri dishes immediately were placed into photoperiod boxes of 8, 10, 12, 14 and 16 hours of light per day. The petri dishes contained, 11, 12, 11, 12 and 10 larvae respectively. After six days in the photoperiod boxes the cocoons which had been built by the surviving larvae were all opened and the larvae were placed in artifical cocoons so that their rates of development could be easily checked. The larvae were checked every second day or daily. The temperature regimes of these photoperiod conditions are shown in the table on p.111

Table 5.6

Effect of Different Photoperiods on Larval Development

	Photoperiod, Hours of Light/24 hours.				
	8	10	12	14	16
N	11	12	11	12	10
Mean Larval Phase (Days)		15.89	15.89	16	16.125
S.D.		1.16	1.36	1.35	1.25
n	0 *	9	9	12	8
Mean Pre-Pupal Phase (Days)		94.85	65.33	10	9.6
S.D.		3.13	2.42	1.63	1.02
n	0 *	7	6	7	5
Mean Pupal Phase (Days)		27.14	26.16	26.85	27.4
S.D.		1.35	1.83	1.57	1.95
n	0 *	7	6	7	5

* All animals kept at 8 hour photoperiod died.

Results

It can be seen from the results (Table 5.6) that the larvae completed the larval phase of development in a shorter time than was found in the larvae which were placed in constant temperature conditions in Experiment 1 part b (Table 5.1). In this experiment the mean duration of the larval phase was 15.97 ± 0.2 days compared with 26.68 ± 0.44. The larval phase in this experiment was only compared with the experimental groups of part b of the first experiment which were kept at or above 15° C since the photoperiod boxes were set up in the 15° C constant temperature room therefore this was the lowest temperature to which the larvae were subjected. The highest temperature to which the fleas were subjected was 23° C. The difference between the duration of the larval phase in the two experiments was found to be significant when *t* test using Bessel correction was applied. There was no significant difference between the mean duration of the larval phase of any of the groups subjected to the different photoperiods.

When the duration of the pre-pupal phases of the different groups of larvae were compared it was found that the group subjected to a 16 hour photoperiod took only 9.6 ± 0.456 days while those larvae subjected to a 14 hour photoperiod took only 10 ± 0.616 days. Those larvae subjected to a 12 hour photoperiod remained as pre-pupae for 65.33 ± .987 days and those subjected to a 10 hour photoperiod took 94.85 ± 1.183 days to complete the pre-pupal phase. The larvae which were kept in an 8 hour photoperiod all died, apparently from infestation by mites, during the larval phase and no comparisons could be made with these. When these results were compared statistically using variance analysis and F distribution tables the differences were found to be highly significant.

Itwas found that the mean time for pupation was similar in all of the experimental groups, with a mean of 27.12 ± 0.31 days which did

not differ significantly from the means for the duration of pupation of any of the previous experimental groups.

Effect of Different Thermoperiods on the Development of Larvae

It was noticed during the photoperiod experiments that the temperature in the photoperiod boxes fluctuated in daily cycles as a result of the light being switched on or off. It was decided therefore to examine the effect of the daily temperature cycles, thermoperiod, on the development of the larvae.

Materials and Methods

This experiment was set up in the same way as the previous experiment with the exception that petri dishes containing the larvae were covered with several layers of dense black material thus excluding light from reaching the larvae. Once again the larvae were removed from their cocoons six days after emergence from the native cats and placed in artificial cocoons. The only light to which the larvae were exposed was during their removal from the cocoons and during the daily checks which were made on their development. This experiment was begun at the end of August when the numbers of larvae found in the native cats were small as this time of year coincides with the disappearance of *U. tasmanica* from the native cat population. For this reason the numbers of larvae used in the experiments were small and there was no chance of repeating the experiment in the following year.

The petri dishes were placed in 8, 10, 12, 14, and 16 hour photoperiod boxes and contained 5, 5, 6, 6, and 5 larvae.

Results

The results are shown in Table 5.7.

Table 5.7 Thermoperiod, hours of warmths/24 hours.

	8	10	12	14	16
N	5	5	6	6	5
Mean Larval Phase (Days)	16	16	15.75	15.8	15.33
n	3	3	4	5	3
Mean Pre-pupal Phase (days	115	97.67	62.66	9.25	8.67
n	1	3	3	4	2
Mean Pupal Phase (days)	27	25.33	26.33	26.24	26.50
n	1	3	3	4	2

It can be seen from Table 5.7 that the number of surviving fleas was small 1-4 and therefore statistical analysis of the results was impossible. However the results which were obtained were very similar to those obtained in the photoperiod experiment with short prepupal times for those fleas kept at high thermoperiods, 14 and 16 hours while the pre-pupal phases of the fleas kept in short thermoperiods were long and comparable with the results of the early experiments where the larvae were kept in constant temperatures. The surviving flea from the group which was kept at an 8 hour thermoperiod took 115 days to complete its pre-pupal development which is similar to the duration of pre-pupal development of the group of fleas used in part b of the first experiment where the fleas were exposed to constant temperatures from the emergence of the larvae from the host (Table 5.2).

Effect of Thermoperiod on Larval Development at the Pre-Pupal Stage

The final experiment to be carried out in the series was designed to test whether the application of different thermoperiods affected the rate of development of the larvae once they had reached the pre-pupal phase under conditions of constant temperature. By the time this experiment was carried out (October), there were no larvae available from the native cats however there were some larvae still torpid in the 5°C room which had not developed since being placed there in May. Therefore these larvae were taken out of the 5°C constant temperature room and allowed to reach the pre-pupal stage at 15°C constant temperature. During this period they were all removed from their cocoon and placed in artificial cocoons. The larvae were checked each day and three groups of 12, 14 and 13 larvae which were judged to have entered the pre-pupal

phase on the same day for each group were wrapped in dense black cloth and placed in photoperiod boxes of 10, 12 and 16 hours of light per day. These groups of fleas were then checked every one or two days until pupation took place.

Results

The results of this experiment are shown in Table 5.8. It can be seen from the table that in all cases the duration of the pre-pupal phase was long and was longer in the 10 hour thermoperiod than in the 16 hour thermoperiod. It was found using variance analysis and F distribution tables that the differences were signficiant between the 16 and 10 hour thermoperiods. When the duration of the pre-pupal phase of this group of fleas was compared with that of the larvae used in Experiment 1, Table 5.3, it was found that the duration of this phase was shorter in the larvae used in this experiment than it was in the first experiment. The mean time of duration of the fleas used in this experiment was 81.45 ± 0.54 days compared with 95.27 ± 0.32.

It could be seen that the application of different thermoperiods did not greatly shorten the duration of the pre-pupal phase once the fleas had completed the larval phase. However the duration of the pre-pupal phase was slightly shorter in these larvae than it had been in the larvae which had been kept at constant temperatures and this difference was significant using t test $\geq$ 0.5%.

Table 5.8. Thermoperiod, Hours of Warmth/24 hours

	10	12	16
N	12	14	13
Mean Pre-pupal Phase (Days)	86.6	86.166	74.7
S.D.	2.633	3.0	3.465
n	10	12	10

Observations on Larval Development in the Native Cat Enclosure

During the course of the experiments which were carried out during the winter of 1978 checks were made at fortnightly intervals on the development of the larvae in cocoons in the dens of the captive native cats. Each fortnight a total of five cocoons were opened and the state of development of the larvae was recorded. If any of the cocoons contained dead larvae more cocoons were opened until five observations were recorded. These observations were carried out from June to November.

Results

The results are shown in figure 5.2. It can be seen from this figure that larvae and pre -pupae were present in the cocoons from June till September. During September pupae were found as well as some pre-pupae. Pupae and adults were found in October and only adult fleas were found in November.

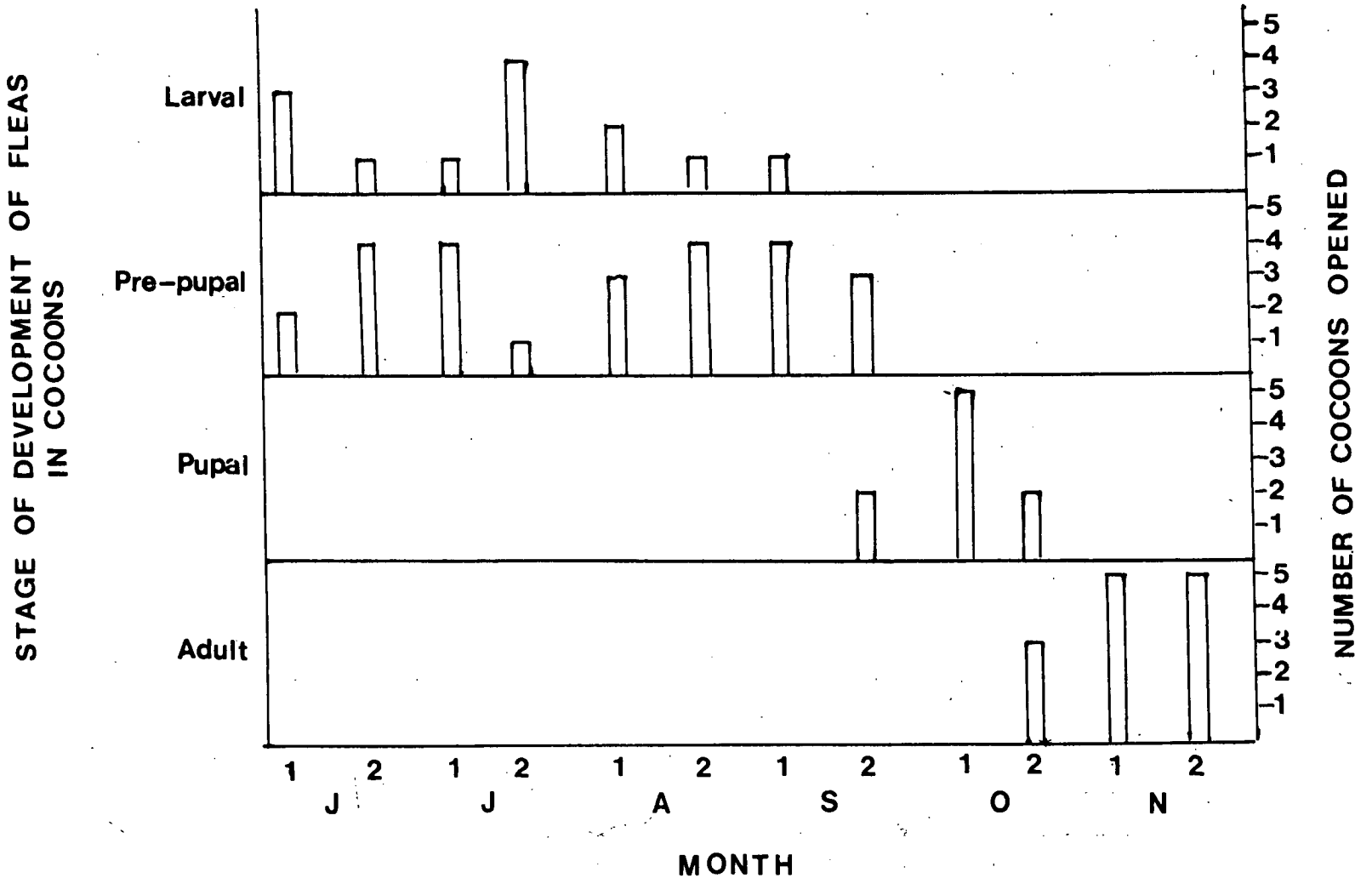


Fig. 5.2 Fortnightly observations of development of fleas in cocoons in the den litter of outdoor enclosures.

5.4 Discussion

The results of the experiments suggested that *U. tasmanica* larvae, under certain conditions, enter diapause prior to pupation. This possibility was supported by the observations on larvae in cocoons removed from the dens of the native cats in the outdoor enclosures. Here pupation of all larvae took place within a short period of time at the end of winter (September) regardless of when the larvae emerged from their hosts. It appeared then that those larvae which emerged early in winter failed to pupate until the end of winter while those larvae which emerged towards the end of winter pupated almost immediately.

Examination of the larvae in the cocoons revealed three phases of development, an active phase, an inactive, pre-pupal phase and the pupal phase. It appeared from the initial experiments that diapause took place during the pre-pupal phase as it was this stage which lasted the longest time.

When the factors which control the diapause were investigated, it was found that chilling the larvae, at 5°C, had the effect of preventing further development until the larvae were once again exposed to warmth (25°C). However, chilling the larvae stopped them entering the pre-pupal phase and did not affect the duration of the pre-pupal stage once the larvae had been placed in warm constant temperature conditions.

When the larvae were exposed to different photoperiods it was found that the duration of the pre-pupal phase could be reduced to approximately 10 days (Table 5.6) under the influence of a long day-length (16 hours day light). The duration of the pre-pupal stage appeared to not be shortened by exposure of the larvae to a short day-length (10 hours). These results suggested that the duration of diapause might be under the

control of photoperiod.

In a further experiment the larvae were subjected to the same temperature conditions as in the photoperiod experiment with the exception that they were kept in the dark. In this experiment the only external stimuli allowed to reach the larvae were the fluctuations in temperature, thermoperiods caused by the turning on and off of the light in the photoperiod boxes. The results, although the numbers of fleas were very limited, were similar to those of the photoperiod experiment. Thus, similar results were obtained in the absence of light. This suggests that the duration of the diapause might be controlled by temperature cycles where periods of warmth and cold were needed in order that diapause development might be completed.

In the thermoperiod experiment the temperature range was 15°C to 22°C and the mean temperatures were 17.4, 18.0, 18.6, 19.2 and 19.79°C for the 8, 10, 12, 14 and 16 hours of warmth thermoperiods. It can be seen that these mean temperatures were within the range of temperatures used in the constant temperature experiments where no shortening of duration of the pre-pupal phase occurred. (Table 5.2, 5.3).

It must be stressed that the apparent effect of different thermoperiods is based on only a few results and the experiment was not able to be repeated because of lack of time. Also, the experiment was carried out at the end of the larval season and the possibility of an effect of some factor associated with this cannot be excluded. It has been found (Chapman 1969) in some insects that the age or condition of the mother can influence the onset of diapause in the progeny as can the external conditions experienced by the mother prior to the deposition of eggs (Saunders 1976). Also the diet of the larvae towards the end of the

season may not have been of the same quality as earlier in the season. The quality of diet has been known to affect the induction of diapause in insects such as the pink bollworm, *Pectinaphora glossypiella* (Adkisson 1961, Bull and Adkisson 1960, 1962, Adkisson et al 1963) where it is thought that the change in oil content of the ripening cotton seeds on which the bollworms feed affect their response to daylength. It has also been found (Vinogradova and Zinovjeva 1972) that the condition of the mother can modify the reaction of the parasitoid *Aphaerata minuta* to photoperiod. However since the results of the photoperiod experiments were similar to those of the thermoperiod experiments and since the larvae used in these experiments emerged from the native cats early in winter it is thought that factors such as have been described are unlikely to have influenced the results of the thermoperiod experiments.

The influence of thermoperiod on the induction of diapause has not been studied as extensively as has the effect of photoperiod. There are however some well documented examples of thermoperiod induced diapause in the absence of light.

A daily thermoperiod cycle has been found to induce diapause in the larvae of *Ostrinia nubilalis* (Beck 1962b) in the absence of light whereas in constant temperatures (within the limits of the thermoperiod temperatures) diapause occured in only a few specimens. A similar result was obtained by Menaker and Gross (1965) working with *Pectinophora gossyiella* in constant darkness. In experiments with the parasitic wasp *Nasonia vitripennis* Saunders (1973) raised females from the egg stage in the dark and then subjected the adults to various thermoperiodic cycles. It was found that females exposed to daily temperature cycles of less than 13 hours warmth produced mostly diapausing larvae whereas females which were subjected to longer periods of warmth produced non-diapausing larvae.

Goryshin and Kozlova (1967) (unseen, from Saunders 1976) have reported thermoperiod induced diapause in several species.

The effects of thermoperiods on diapausing insects have also been studies in conjunction with photoperiod. (Lees 1953, Beck 1962, Goryshin 1964, Pittendrigh and Minis 1971). It has generally been found that periods of cold in a daily temperature cycle play an important role in diapause. Meats and Khoo (1976) have investigated oocyte development in *Dacus tyroni*. In this species egg development is arrested or reversed in adverse conditions. It was found that the flies developed in a way not related to mean temperature but to what proportion of the day was above a certain threshold which was necessary for development. Development did not take place in constant cold conditions (below the threshold) but could take place in conditions with a fluctuating temperature where the mean temperature was below the threshold level.

Retardation of oocyte development in such conditions fits into the definition of diapause of Andrewartha (1952).

In view of these examples, it is possible that the rate of development in *U. tasmanica* larvae is controlled by the daily temperature regime which undergoes subtle changes throughout the winter months.

In the first experiment two groups of fleas were placed in constant temperatures (Section 5.3). One group was kept at room temperature for 10 days and the other group was placed directly into the constant temperature conditions. The results of the experiment showed that the duration of the pre-pupal phase was significantly longer in the group which was placed directly into the constant temperature conditions. This suggests that the fleas which were kept at room temperature, with daily fluctuations in temperature (estimated to range between 10°C to 20°C) for ten days entered a shorter diapause than the other group. In the final experiment larvae were used which had been kept, firstly at 5°C

in a torpid state and then at 15°C constant temperature conditions until the pre-pupal stage was attained. After this the larvae were placed in different thermoperiod conditions. It was found in the group of larvae placed in the 16 hr. thermoperiod that the duration of the pre-pupal phase was significantly shorter than in the other two groups. The duration of the pre-pupal phase in all groups was slightly but significantly shorter than it was in the comparable groups of larvae used in the first experiment. This, together with the results of the thermoperiod experiment, suggests that exposure to a specific temperature regime during the early stages (the free living and active larval stage) determines the induction of diapause. The results of the experiments of section 5.3 suggest that the duration of the diapause was shortened by placing the larvae in thermoperiods with long periods of warm.

In insects diapause is an adaptive phenomenon which usually affects one stage of development of the insect and enables this stage to survive adverse conditions (Lees 1955). The larvae of *U. tasmanica* are found as parasites in the native cats during the winter months. During the parasitic phase these larvae develop in an environment where they are protected from cold or desiccation. Following emergence from the host however, the larvae enter the soil and litter in the hosts' dens. Here the temperatures in winter are possibly quite low, and unsuitable for further development. It is possible that by entering diapause, those larvae which emerge from the hosts early in winter are able to survive the adverse conditions in the dens until spring. A daily regime of temperature fluctuation has been found to occur in the litter of native cat dens in the enclosure (Appendix II p.182) The temperature cycle is controlled by the ambient air temperature and the presence of native cats

in the dens. It was found that the daily temperatures peaked during daylight probably in response to both den occupation by the native cats and high daytime temperature. The temperature of the litter in the native cat dens was also influenced by the number of native cats occupying the den, increasing as the number of native cats increased. It is possible that further research into the changes in daily temperature cycles of native cat dens and further experiments with *U. tasmanica* larvae could explain the unusual diapause which appears to occur in this flea.

Chapter 6

The Cytology of *Uropsylla tasmanica*

6.1 Introduction

This chapter includes an attempt to clarify, by means of chromosomal analysis, the position of *Uropsylla tasmanica* with regard to its phylogeny and evolution. At present, *Uropsylla* is included in the monotypic sub-family Uropsyllinae, in the Family Pygiopsyllidae (Dunnet and Mardon 1974). The Pygiopsyllidae represent a major radiation of Australian fleas and include the monotypic sub-families Uropsyllinae, Lycopsyllinae and the Pygiopsyllinae. The Pygiopsyllinae contain eleven genera including *Pygiopsylla, Bradiopsylla, Acanthopsylla* and *Choristopsylla.*

Uropsylla was first described by Rothschild (1905) and at that time there was insufficient knowledge of Australian species for an evaluation of its relationships. The present position of *Uropsylla* in the family Pygiopsyllidae is accepted by Dunnet and Mardon (1974) following the work of Jordan (1947). Oudemans (1909) raised the Uropsyllinae to the family level while Wagner (1939) included it in his family Certaophyllidae but did not include it in his sub-family Pygiopsyllinae. According to Jordan (1947) and Dunnet and Mardon (1974), *Uropsylla* resembles *Lycopsylla* and *Bradiopsylla* in several features, particularly of the male genitalia, and in the possession of an anterior tentorial arch which is found in these three taxa as well as in the *Choristopsylla.*

The determination of chromosome number and comparisons of chromosome morphology have been widely used as a tool in phylogenetic studies of plants and animals (White 1973). The usefulness of comparative cytology in phylogenetic studies depends upon the variation of chromosome number and morphology in the group being studied (White 1973).

The karyotypes of fleas have not been extensively studied although Bayreuther (1969) in his investigations of *N. fasciatus* has demonstrated

a multiple sex determining mechanism and chromosomal polymorphism in this species. Other karyotype determinations of fleas have included *Leptopsylla musculi* (2n = 22) (Karnkowska 1932), *Ctenocephalidus canix* (2n = 14) (Kichijo 1941), *Xenopsylla cheopis* (2n = 14+xxy ♂) (14+xxxxo ♀) (Bayreuther & Brauning 1971) and *Leptopsylla segnis* (2n = 20) (Bayreuther 1969).

In studies of karyotype of other groups it has been found (White 1973) that it is difficult to distringuish clearly defined type numbers at a high level in the systematic hierarchy. Between the lower categories however (families, genera etc) estimations of karyotype usually do not represent a random sample thereby affording a means of intra-group comparison.

Comparison of karyotype between groups involves comparing both the number and morphology of chromosomes. Since evolution of karyotype may involve decrease or increase in chromosome number, it is often difficult to ascribe a "primitive number" to any group. Possible exceptions include the Odonata where n=13 is the most common chromosome numbers in the sub order Anisoptera, the second most common number in the Zygoptera also occurs in the Anisozygoptera, which is the most primitive sub order (White 1973). It has also been found that very few species of Odonata vary to any great extent from the n=13 chromosome number (Kiauta 1967).

In other insect orders e.g. Orthoptera it is possible to assign a typical chromosome number to groups within the order such as the Acridoidea (White 1973).

In view of this and the fact that so few species of fleas and no Australian species of fleas have been studies cytologically an attempt

was made to investigate the chromosomes of three Australian species of fleas, *Uropsylla tasmanica*, *Pygiopsylla hoplia* and *Lycopsylla nova*. These three species were then compared with each other and with those fleas which have already been studied.

6.2 Materials and Methods

The chromosomal preparations of *U. tasmanica* were prepared from pupae while those of *P. hoplia* and *L. nova* were prepared from eggs and adult fleas. The *Uropsylla* had pupated following their emergence as larvae from the native cats while the *P. hoplia* were removed from the native cats. The *L. nova* were collected from wombats. Wombats are sturdy creatures, difficult to catch and even more difficult to restrain. Some 14 wombats were examined during the course of the project and of these only two had any fleas. Traub (1972c) has suggested that the wombat fleas might be nest fleas and therefore it is not surprising that so few wombats were found carrying fleas. For this reason the chromosome preparation was eventually prepared from 21 eggs which were laid in glass tubes by two female *L. nova* following capture.

Preparation of chromosomes for slides

The cells were prepared in the following way.

1. The tissues, eggs, pupae or adults, were macerated in 0.04% colchicine in insect ringer solution, and allowed to stand for one to two hours at room temperature in the solution.
2. This solution containing suspended cells was centrifuged at 800 rpm for 10 minutes.

3. The supernatant was discarded and the pellet was resuspended in 0.53% KCl for ½ an hour at room temperature.
4. After ½ an hour had lapsed an equal amount of fixative was added to the suspension and left to stand for ½ an hour.
5. After ½ an hour the suspension was centrifuged at 800 rpm for 10 minutes.
6. The supernatant was discarded and fresh fixative was added to the cells.
7. After ½ an hour the suspension was centrifuged at 800 rpm for 10 minutes.
8. The supernatant was discarded and the pellet was again resuspended in fresh fixative.

The fixative consisted of 1:3 glacial acetic acid: methanol 100%.

Preparation of Slides

The slides were prepared by adding two or three drops of the cell suspension to clean glass slides. To this was added double the number of drops of 60% acetic acid solution. The slides were then allowed to dry on a slide warming tray. When dry the slides were immersed in 100% methanol for 30 - 60 seconds and then allowed to dry.

Staining

The slides were stained with Giemsa solution in ph 6.9 Phosphate Buffer.

6.3 Results

The results of the chromosomal investigations of *P. hoplia*, *L. nova* and *U. tasmanica* are shown in figs. 6.1, 6.2, 6.3. Figure 6.1 shows the range of chromosome numbers found in the chromosomal preparations for the three species. It can be seen in *P. hoplia* and *L. nova* that the most common chromosome number (including sex chromosomes) is 2n = 20. In *U. tasmanica* however the chromosomal preparations yielded no clear cut chromosome number and it was found that the diploid number of chromosomes (including sex chromosomes) varied from 8 to 17 with the most commonly occurring numbers being 12, 13 and 14.

Figures 6.2, 6.3 and 6.4 show the idiograms of the three species of fleas. Because of the inconsistency of chromosome numbers in the cell preparations of *U. tasmanica*, the idiogram shown in figure 6.2 is a composite one, showing 2n = 14. Examination of those cells with 11, 12 or 13 chromosomes revealed variations in the chromosomes which were present while the chromosome complement of those cells with 14 chromosomes appeared to be constant. In many (25) of the *U. tasmanica* preparations there appeared to be two unpaired chromosomes and these are shown in the idiograms as possible sex chromosomes.

Examination of the cell preparations of *P. hoplia* indicated a sex determination mechanism which involves a non-homologous pair in one sex (Fig. 6.3). In *L. nova* the chromosomes were constant and no possible sex determining mechanism was observed (Fig. 6.4a).

Comparison of the idiograms indicated that *L. nova* and *P. hoplia* had the same chromosome number and fundamental number (number of chromosome arms) and that the chromosomes were all metacentric or slightly submetacentric. The chromosomes themselves however were not similar in

morphology as in *L. nova*, Pairs 2 and 7 (Fig. 6.4) had heterochromatic segments which were not observed in *P. hoplia*.

In *U. tasmanica* the chromosomes differed in both number and morphology from those of *L. nova* and *P. hoplia*. In this species the chromosomes were largely submetacentric and appeared to be larger than those of the other two species although fewer in number.

6.4 Discussion

Because of the variation in chromosome numbers observed in the cell preparations of *U. tasmanica*, the idiogram which was derived from this species must be regarded as tentative. The results did indicate however that *U. tasmanica* has fewer chromosomes than either *P. hoplia* or *L. nova* and a smaller fundamental number than either of these species.

Since all three species studied displayed full complements of metacentric chromosomes it is difficult to postulate a simple pathway in terms of dislocation and fusions by which *U. tasmanica* with 2n = 14 could have arisen from an ancestor with a karyotype similar to either *P. hoplia* or *L. nova*. Similarly it would be difficult to derive the karyotype of *P. hoplia* or *L. nova* from an ancestor similar to *U. tasmanica* which could be more distantly related to either *L. nova* or *P. hoplia* than either of these two species are to each other. It is possible however that the similarities between the chromosomes of *P. hoplia* and *L. nova* are more apparent than real.

When the karyotype of the three species studied in this chapter are compared with other flea karyotypes it can be seen that the chromosome numbers of the Australian species fall within the range of chromosome numbers so far published (2n = 22 - 2n = 14) (Karnkowska 1932,

Kichyo 1941, Bayreuther 1954, 1969 and Bayreuther & Brauning 1971). The Australian fleas also resemble the other fleas studied in having high numbers of metacentric and submetacentric chromosomes.

Further analysis of the karyotypes of the Australian fleas including investigation of the derivation of the karyotypes should, as has been predicted by Rothschild (1975a) prove interesting and illuminating.

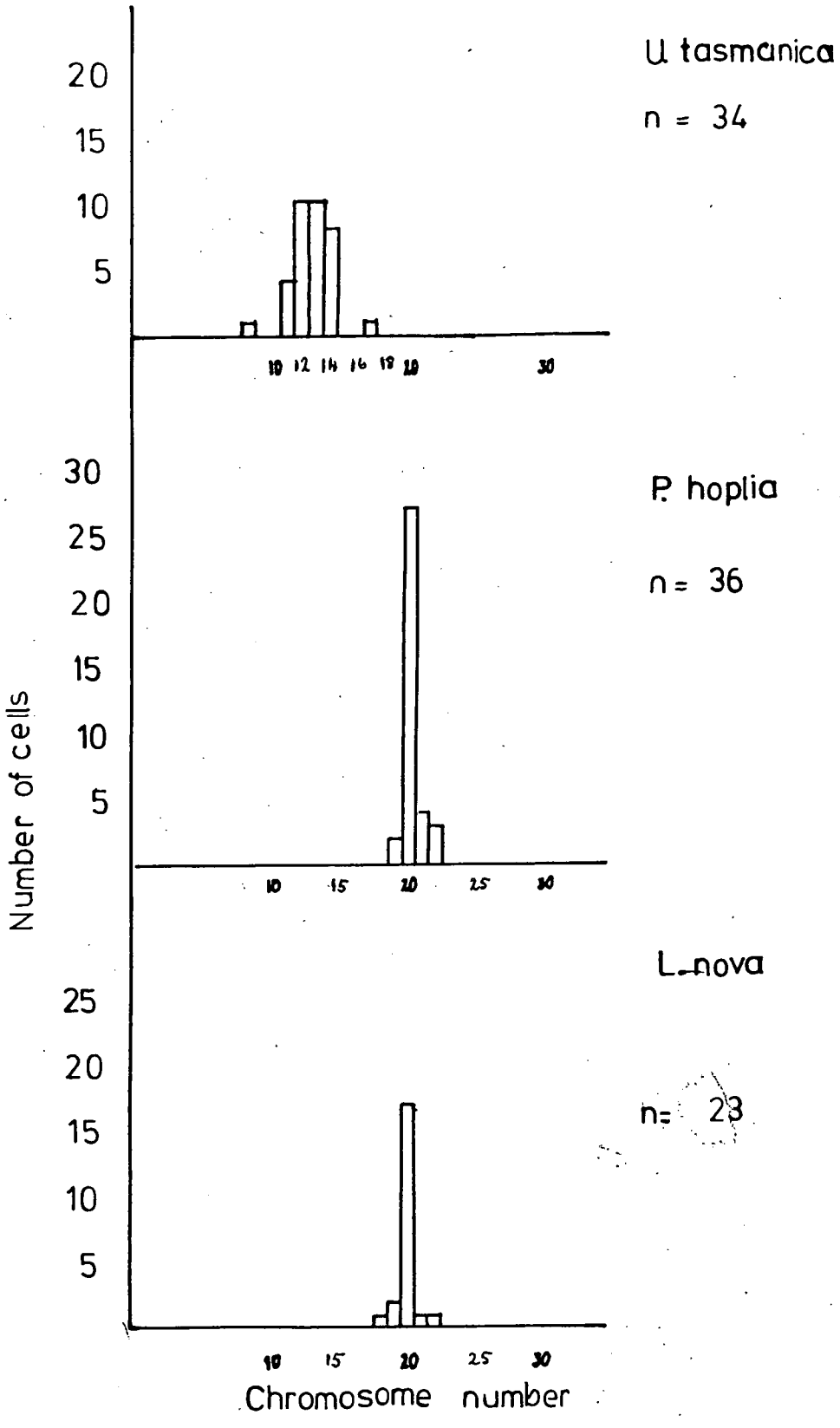


Fig 6.1 Frequency of chromosome numbers in cell preparations.

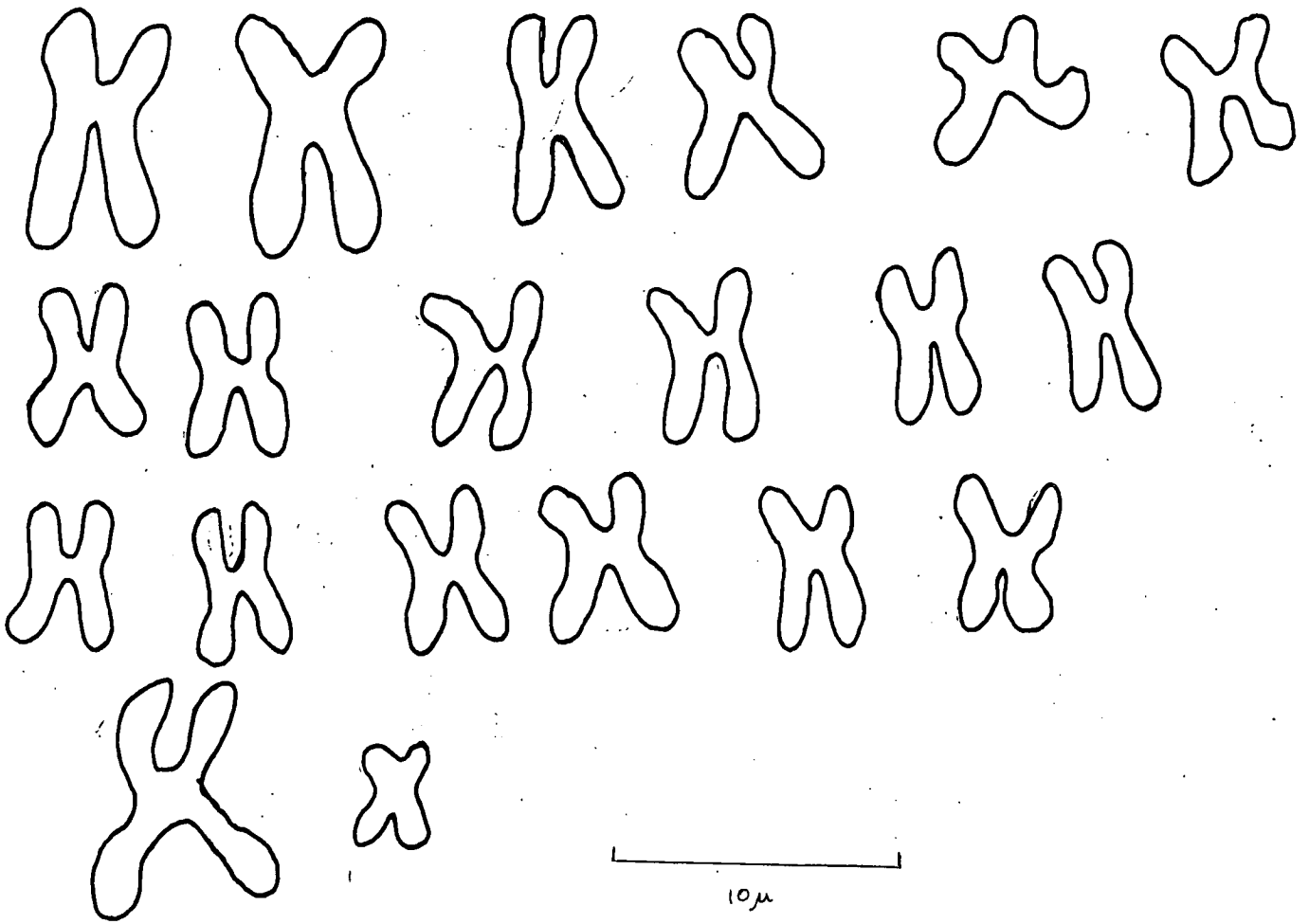


Fig. 6.3 Idiogram of Chromosomes of *Pygiopsylla hoplia*.

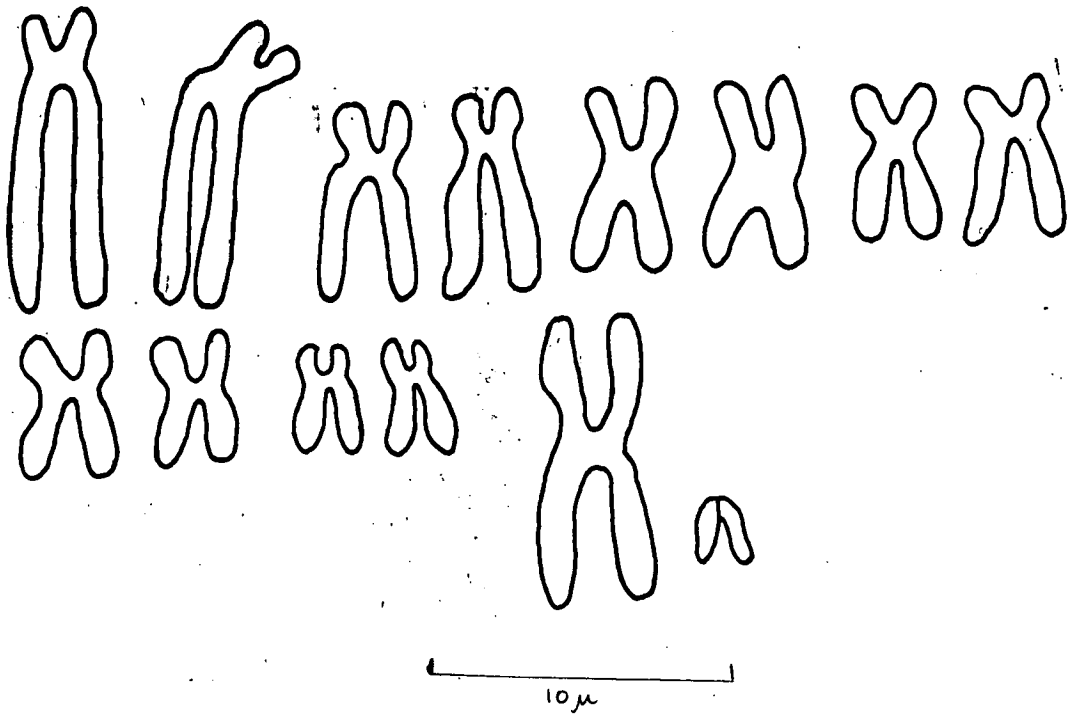


Fig. 6.2 Idiogram of Chromosomes of *Uropsylla tasmanica*.

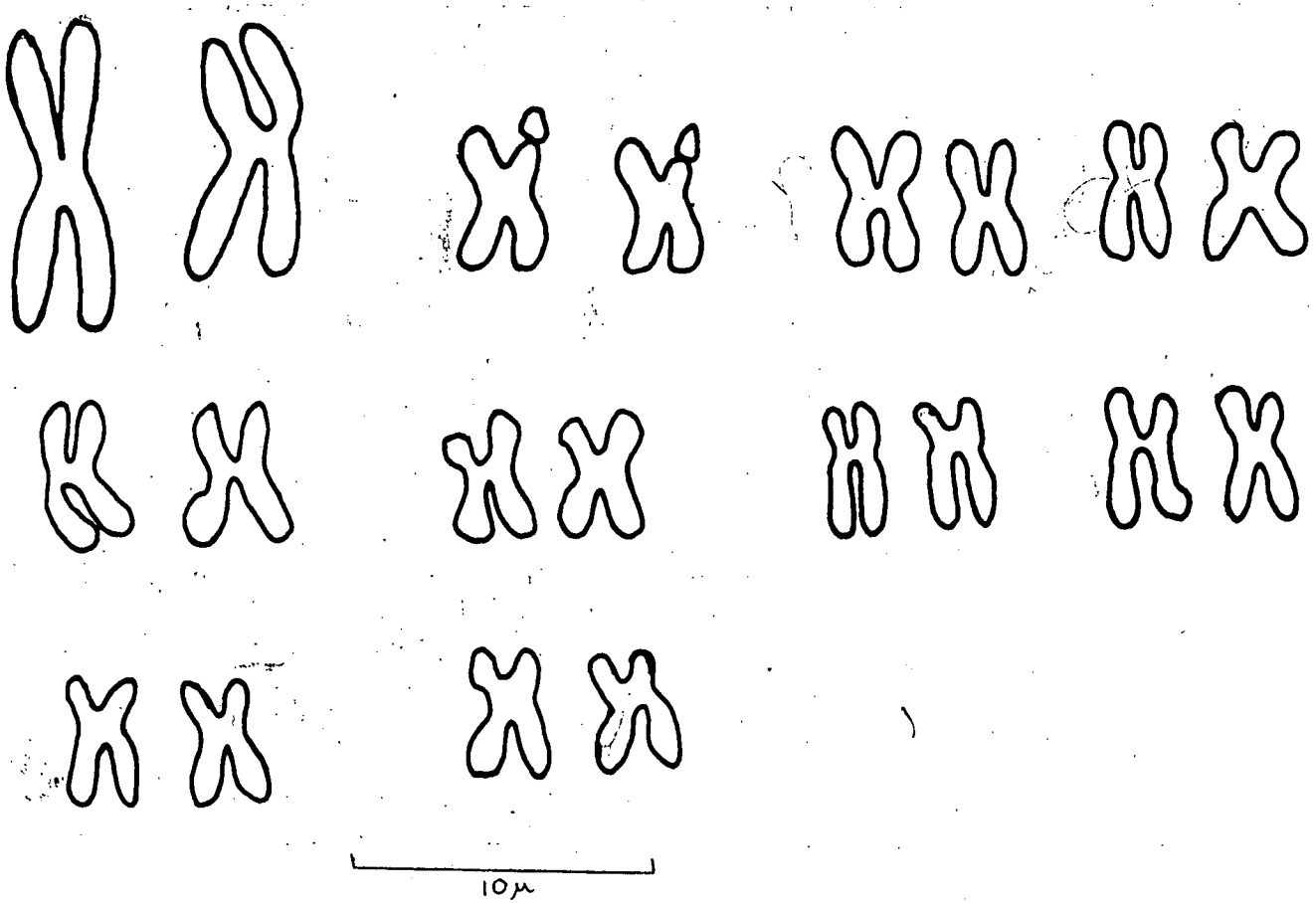


Fig. 6.4 Idiogram of Chromosomes of *Lycopsylla nova*.

Chapter 7

General Discussion

7.1 The Annual Cycle of *Uropsylla tasmanica*

The phenology of *Uropsylla tasmanica* was revealed to some extent by analysis of the trapping data (Chapter 1). It was found that *U. tasmanica*, like many other extensively studied species of fleas (Bibicova etal. 1963, Bibicova and Gerasimova 1967, Amin 1966, Darskaya, 1963, 1970, Humphries 1967b, Cotton 1970, Volyanskiy 1972, Ulmanen and Myllymaki 1971, Brink-Lindroth 1971, Rykman 1971b and many others) exhibits a season pattern of population growth and reproduction. It was found that adult *U. tasmanica* were only present on the hosts from March to September and that larval infestation of the native cats most intense during June and July. It could also be seen that occurence of *U. tasmanica* coincided with the breeding phase of the host and that the adult and larval fleas disappeared from the native cats in September when the newborn native cats were weaned and first appeared in traps in the field. It was noted that the incidence of *U. tasmanica* on native cats caught in the field study increased sharply during March and declined sharply towards the end of August. These results suggested that the period during which the adults *U. tasmanica* were able to live and reproduce on the host was rigidly controlled.

In an attempt to investigate the emergence of the adult *U. tasmanica* into the native cat population experiments were set up which were described in Chapter 2. The results of these experiments indicated that the adult fleas remained quiescent in their cocoons until emergence was stimulated by mechanical disturbance (and possibly slight changes in temperature (Cotton 1970)) but that their responsiveness to these disturbances increased with the passage of time. It was further found that in dry conditions the fleas became responsive to mechanical disturbance in less time than they did in moist conditions. It was

therefore postulated that the fleas' emergence was controlled by their responsiveness to mechanical stimulation and that this responsiveness was increased by the passage of time. It was further suggested that the fleas' response to desiccation was a type of safety device which ensured that some fleas survived particularly dry summers.

From the data presented in Chapter 1 it could be seen that the initial appearance of adult *U. tasmanica* on the native cats in March was synchronised. In other words the numbers of fleas of this species built up very quickly, indicating that the fleas were ready to emerge in response to mechanical stimulation at the same time. Now whilst it was suggested that readiness to emerge was a function of time, in order for the fleas to emerge synchronously it would be necessary for them to spend the same period of time quiescent as adults in their cocoons. This could be accounted for by the fleas all pupating at the same time since the duration of pupation was found (Ch. 5) to be fairly constant under different conditions.

When post parasitic development of the larvae of *U. tasmanica* was investigated it was found (Chapter 5) that the larvae did in fact all pupate during early spring regardless of when, during the winter months, they left the hosts and constructed a cocoon. It was found that the fleas underwent a diapause prior to pupation and this diapause effectively prevented larvae from pupating before September (spring). It was also found that larvae which left their hosts towards the end of the flea's breeding season (at the end of winter) did not enter a diapause but pupated almost immediately.

The diapause which occurs in cocooned larvae prior to pupation appears to serve the dual purpose of ensuring the survival of the next generation of fleas during the adverse conditions of winter and of synchronising the emergence of this generation of fleas into the native cat population.

Wigglesworth (1972) has described the build up of a massive fat body in the pre-diapause stage of diapausing insects. It was found (Ch. 4 pp 88) that the final larval instar of *U. tasmanica* did amass a huge fat body in comparison to the final instar larvae of other Australian fleas.

As to the reason for the seasonality exhibited by *U. tasmanica* there are several possibilities. Firstly and most obviously, there is the possibility, based on the experiments presented in Chapter 3 that *U. tasmanica* is unable to reproduce at any time other than during the reproductive phase of its host.

Another possibility can be found in an interpretation of the trapping data (Chapter 1) which shows that during the period when the numbers of *U. tasmanica* peaked fleas of other species found on the native cats were very few in number. It is possible that the presence of other fleas and other ectoparasites such as ticks in some way interferes with the development of the parasitic larvae of *U. tasmanica*.

Thirdly, it is possible that some aspect of the hosts' behaviour during the period outside its breeding phase prevents *U. tasmanica* from effectively carrying out some part of its breeding cycle. An example of this would be the possible differences in patterns of den occupancy by native cats at different times of year (Chapter 1 pp22). If, as was indicated by the observations on captive native cats, these animals do change dens before and after the breeding season such changes could jeopardise the fleas' chances of finding a host if they were to emerge into dens which are uninhabited during summer. It was found (Chapter 2 pp26) that *U. tasmanica* adults in the absence of a host do not survive for long periods at relative humidities lower than 80%.

This type of life cycle with a short active reproductive phase in the adult and an extended cocoon life is reminiscent of the life cycle of fleas of many migratory birds. (Rothschild and Clay 1957). Generally, in the bird fleas the breeding period of the fleas must be timed to coincide with the brief period which is spent on the nest. In the hen flea *Ceratophylus gallinae* breeding is restricted to spring despite the fact that modern hens in the domestic situation may be on the nest all year round. Mammal fleas, generally speaking normally breed all year round although the survival or development of immature stages may be retarded in unfavourable seasons. The long period of inactivity of fleas in the cocoons as a normal part of the life cycle is found more frequently in bird nest fleas than in mammal fleas and is necessitated by lack of an available host.

Following the descriptions given by Traub (1972c) of the differences between nest fleas and fur fleas, *Uropsylla* is undoubtedly a fur flea. The pronotal ctenidium consists of 14 spines on either side, this is reduced in nest fleas. It also exhibits great development of the pleural arch and is a powerful jumper. The eye is large and the body of the flea carries numerous rows of bristles with marginal spinelets on the terga as well as the already mentioned spines of the ctenidial comb. In nest fleas the chaetotaxy is reduced, the thorax and pleural arch and hence the jumping ability are reduced, and the eyes are small or absent. Traub has also described nest fleas as tending towards diminution in size and as tending towards the loss of sclerotisation of the caudal margins of the abdominal segments. This leads to a striped appearance of the abdomen of nest fleas. Nest fleas frequently develop specialised features such as, lengthening of stylets and labial palpi sometimes with an increase in the number of segments; development of broadened sections of antennal segments or of lobes of the coxae, or of straight or angled anterior margins of some thoracic sterna; elongation

of the legs at times accompanied by the development of a fringe of bristles on the male hind tarsus. *Uropsylla* adults are large fleas strongly sclerotised and showing none of these features of nest fleas. In fact *Uropsylla* is unique among fleas so far as is known for the large amoun of time it does spend on the host firstly as the adult and also during larval development. Despite all this however its reproductive cycle and subsequent development still resembles most that of the fleas of birds with a short annual breeding cycle.

This resemblance has come about as a result of the seasonality exhibited by the flea which coincides closely with the breeding cycle of its host. This is a case where a mammal flea has become tied to the breeding cycle of a monestrous host. I could find no other examples of this in the literature. A summary of the life cycle of *U. tasmanica* and its host is presented in figure 1.7 (Chapter 1).

7.2 The Parasitic Larvae of *U. tasmanica*

The special anatomical and behavioural adaptations of the larvae of *U. tasmanica* are described in Chapter 4. It was found that while maintaining many of the basic structures of non-parasitic flea larvae *U. tasmanica* larvae have undergone some fundamental changes, particularly with regard to chaetotaxy, shape, segmentation, and position of the spiracles. The modifications evident in this larva appear to converge on the development of the larvae of the myiasis-causing dipterans. *U. tasmanica* larvae also exhibit similar habits to the larvae of many of the dipterans which cause dermal myiasis in mammals. It is not surprising then that these larvae should resemble at least externally, the larvae of an unrelated group which has evolved to exploit a similar environment. It is interesting to speculate on the pathway taken by *U. tasmanica* in developing the parasitic larvae.

Zumpt (1965) has traced two possible pathways for the development of parasitic larvae in Dipterans. The first of these is the Saprophagus Root. This line is represented by some members of the Calliphoridae. Some species e.g. *Lucilia sericata* normally breed in carcasses or sometimes faeces. They are sometimes deposited by the female in wounds where the larvae feed on dead tissue and normally are restricted to this dead tissue. Other Calliphorid larvae however do not remain restricted to the dead tissue of wounds and may start to invade healthy tissue. These larvae are called facultative parasites and an example is *Lucilia cuprina*. In the final step towards obligatory parasitism the larvae can no longer breed in decaying matter and are completely dependant upon living animals. The path of evolution in this case is from feeding on decaying animal matter through to feeding on decaying animal matter while at the same time making use of living matter to the final phase of obligatory parasitism on a living host.

The second pathway to obligatory parasitism which was described by Zumpt is the Sanguinivorous Root of the myiasis producing flies. In this case larvae which feed saprophagously in the nests of vertebrates but which later develop predatory habits e.g. *Muscina stabulans* could, since they possess biting mouthparts, attack the vertebrate occupant of the nest thereby acquiring a blood meal. Such larvae could then develop into ectoparasites of the host vertebrate. Examples of facultative ectoparasitic dipteran larvae include *Neottiophilum Passeromyia* and *Protocalliphora* in birds' nests and *Pachychoeromyia* and *Auchmeromyia* in mammalian dens. In order that a dipteran larva could develop into an obligatory ectoparasite of a vertebrate host via a sanguiniverous pathway it would be necessary for the larva to have undergone sustained and close proximity with an immobile host such as bird nestlings or immature mammals deposited in the maternal den.

In considerations of the evolution of the obligatory endoparasitism in *U. tasmanica* several important facts need to be remembered. The diet of flea larvae of other species and their dependance upon the blood contained in the fecal pellets of adult fleas is variable. *Pulex irritans* is able to develop on a variety of organic materials (Askew 1971) as are the larvae of *Xenopsylla cheopis* (Pausch and Frankel 1966). In the rabbit flea *S. cuniculi* (Rothschild and Ford 1966b) the larvae feed on the faecal pellets from the adults and in this species the defecation rate of the adults increases before the eggs are laid. However Rothschild (1975a) has reported that the larvae of *S. cuniculi* have been found feeding on the tissues of dead rabbit nestlings. *Nosopsyllus fasciatus* actively seeks out adult fleas and ingests blood as it passes from the anus (Molyneux 1967). According to Molyneux the larvae of *N. fasciatus* attach themselves to the pygidial region of the adult thereby stimulating defaecation. The larvae of *Holopsyllus glacialis* have been found living as ectoparasites in the fur of the host, the arctic hare (Freeman & Madsen 1949) Finally there have been cases of the larvae of *Tunga* and *Dasypsyllus* living as facultative ectoparasites on the host. (Rothschild 1975a).

In the examples given above there are some which indicate that the larvae could develop towards obligatory endoparasitism by a saprophagous pathway, e.g. the larvae of *S. cuniculi* and others which could develop towards obligatory endoparasitism by a sanguinivorous route such as *N. fasciatus*. Unfortunately, Freeman & Madsen (1949) did not describe the diet of *Holopsyllus glacialus* larvae therefore it is not known whether these flea larvae actually bite the host or whether they feed on debris such as adult fecal pellets, in the fur of the host.

It was observed, (Chapter 4 section 4.4) that the larvae of *U. tasmanica* ingest blood and pus as well as bacteria and broken down host tissues. This diet and the fact that the larvae preferentially infest

areas of the host which are most likely to be infected with bacteria suggests that the endoparasitic larvae could have arisen by a saprophagous route. It is possible that the larval ancestors of this flea began to feed on open wounds of the host. There is possibly some support for this theory in the coincidence of the breeding period of *U. tasmanica* with both the breeding period of *D. viverrinus* and also the autumn and winter months. During winter and partly as a result of breeding behaviour, the native cats do suffer more physical injury than in summer. Native cats are not frequently found with infected open sores during summer but are frequently injured in this way in winter. It is possible however that *U. tasmanica* larvae were among those larvae which, like *N. fasciatus*, were especially dependant upon a blood diet and could therefore have evolved along a sanguinivorous pathway particularly since flea larvae with the exceptions of *U. tasmanica* and *H. glacialis* do feed and develop in the nests of and therefore in close proximity to their hosts.

7.3 Phylogeny and Evolution of *Uropsylla tasmanica*

The determination of the relationships between different groups of fleas has always been difficult and there are two reasons for this. Firstly there is no fossil record for the order and it is recognised that extant fleas represent only a sample of the many different types of fleas which must have developed during the mammalian radiation and of which many types have since become extinct. (Jordan 1947, Holland 1964). Secondly it is now recognised (Jordan 1947, Holland 1964, Hopkins and Rothschild 1953-71) that many of the external features of fleas upon which taxonomy is traditionally based have, in the past, been misinterpreted, particularly with reference to which features are ancestral and which are specific. Further to this Holland (1964) has pointed out that fleas are undoubtedly an ancient group, older than the mammals, which having developed with mammals have undergone numerous host changes, and host specificity in general is not sharply defined. Holland also points out

that many examples of convergent and parallel evolution occur in fleas thus making the task of the taxonomist even more complicated.

In his original description of *Uropsylla tasmanica*, Rothschild (1905) did not include the new genus Uropsylla in any known group of fleas. Jordan (1947) assigned the genus to the family Pygiopsyllidae as the Uropsyllinae largely on the basis of the presence of a fourth link plate between the mesothorax and the basal abdominal sternum. This characteristic is found in all of the Pygiopsyllidae although it also occurs in some genera of the Neopsyllinae. Beyond this *Uropsylla* is generally thought (Jordan 1947, Hopkins and Rothschild 1953 - 1971 and Dunnet and Mardon 1974) to be most closely related to *Lycopsyllinae*. However it is clear from the literature that the contemporary view of the taxonomic position of *Uropsylla* has not been reconsidered since the determination of Jordan (1947). In his paper on phylogenetic problems within the fleas Jordan (1947) made the assumption that the marsupial fleas could be expected to have retained some of the primitive characteristics of fleas because of their isolation and development on a "primitive" mammal group.

Those features considered primitive by Jordan and which are found in *Uropsylla* are many and are frequently similar to characteristics of *Rhopalopsyllus*, another "primitive" group of fleas to which *Uropsylla* is only distantly related. The primitive features of *Uropsylla* are thought by Jordan to have been modified to various degrees in other members of the Pygiopsyllidae. This implies that *Uropsylla* has been separated from the main stem of the Pygiopsyllia radiation for a considerable time.

Chapter 6 of this thesis includes an attempt to determine the chromosome number of *Uropsylla* and to compare this with the chromosome numbers of *Lycopsylla nova* and *Pygiopsylla hoplia*. On the basis of the actual numbers of chromosomes found it appeared that *Lycopsylla* was closer to *Pygiopsylla* than either were to *Uropsylla*. Examination of the morphology of the chromosomes suggested that the similarity suggested by the equal chromosome numbers of *L. nova* and *P. hoplia* might be more

apparent than real. Examination of the morphology of the chromosomes of the three species indicated that *U. tasmanica* was further removed from the other two species than was suggested by comparison of chromosome number alone.

The other point which must be remembered in discussion of the evolution of *Uropsylla tasmanica* is the extreme specialisation of this flea. It is the only known flea whith an endoparasitic larval stage and it is to be expected therefore that some of the external features of the adult flea have arisen in connection with this. Such features possibly include the extensions of the eighth tergites into terminal palps which are used by the female in the deposition of eggs and by the male during copulation. Female *Uropsylla tasmanica* glue their eggs onto the host's fur and during egg deposition the hosts hairs are grasped between the terminal palps. During copulation (observed in the maturation experiments presented in Chapter 3) the male fleas position their terminal palps between those of the female thereby possibly facilitating access to the vagina. This indicates that the terminal palps are an adaptive characteristic of both sexes and not merely a remnant feature.

The absence of antepygidial bristles and the female stylet in *Uropsylla* could also be associated with the peculiar egg laying habit of *Uropsylla*. However absence of these features is considered by Jordan (1947) to be a primitive condition of fleas. In the case of *Uropsylla* (since antepygidial bristles and the female stylet are also absent in several other unrelated genera and in *Lycopsylla* which do not glue eggs to host's fur) this condition may have facilitated the development of the parasitic larvae as their presence could interfere mechanically with egg deposition.

Another feature of the adult fleas which is possibly related to its specialisation is the retention of the rows of combs which has been mentioned by Jordan (1947) as one of the primitive features of *Uropsylla*.

The adults of *Uropsylla* spend most if not all of their time on the host and exhibit the characteristics of fur fleas to a great extent. These characteristics include adornment by bristles and spines which hinder the removal of fleas from the host's fur. (Traub 1972c).

One feature of *Uropsylla* which may on further investigation prove to be of taxonomic importance was the observation (Appendix II Plates 1-3) of the peculiar female reproductive tract in this flea. It was found in *U. tasmanica* that the vagina is long and shaped like a 7. (Appendix II Plate 3A Fig. 1). Vaginae of this shape and complexity have not been described in any of the species examined by Rothschild and Traub (1971), although a vaginal clamp had been described by them in the Histrichopsyllidea formed by an outgrowth of the vaginal floor. This was a knob like projection which articulated with a socket directly anterior to the duplicatora vaginalis.

The use of cement during the deposition of eggs was found on investigation (Appendix II Fig. 2) to be common in other species of Australian fleas. Cement was not used by *Lycopsylla nova*, a nest flea (Traub 1972c), but was used by *P. hoplia* and *S. dasyurus*. It is possible that the use of cement is a primitive feature of the Australian flea fauna and if this is so would certainly have facilitated the development of the parasitic larvae.

A further observation (Appendix II pp180) concerning the host specificity of *U. tasmanica* indicated that this flea is more rigidly bound to the marsupial genus *Dasyurus* than might be suggested by data previously available (Dunnet and Mardon 1974).

Taken overall the data suggests that *Uropsylla tasmanica* has developed from a primitive ancestral flea which also gave rise to the

Pygiopsylla, *Bradiopsylla* and *Lycopsylla*. The ancestral flea probably had certain features of morphology and chaetotaxy which were retained by *Uropsylla* and modified in the other groups. It is also possible that the ancestral flea employed cement during egg deposition, a feature which is still apparent in some Pygiopsyllids and which was exploited by *Uropsylla* in its unique development.

It is perhaps of special importance that *Uropsylla* has developed on marsupials of the genus *Dasyurus*. The Dasyuroidea which contains *Dasyurus* is thought (Tate 1947, Ride 1962) to be very old and to have diverged from the main stem of the Australian marsupials early in the radiation. It is possible that *Uropsylla* has been isolated and has developed in isolation for a very long period of time, as Traub (1972c) states "Fleas are more specialised for their particular mode of life than had been realised a few years ago, not only with respect to their behaviour and physiological responses but also concerning their morphology. All of these phenomena are intimately related to corresponding features of their host and bespeak of an ancient and intimate association between parasite and host, even to the point of evolving together ...".

Summary of Main Points

Chapter 1.

Uropsylla tasmanica adults and larvae occur on their hosts only during the Autumn and Winter months at which time the other species of fleas found on native cats decline in numbers. Larval infestation of native cats is greatest during June and July (100% of all animals caught had larval infestations of which 67% and 61% were classified as heavy) but drops sharply in September (40% of all animals caught had larval infestations and all of these were light). There was no significant difference in the incidence or intensity of larval infestation between male or female, young or old native cats. Native cats mate in May and young native cats are caught in the field in September to October. Testes development in native cats is apparent in February and subsides in September. The native cats are in various phases of their reproductive cycle from February to September and this period coincides with the incidence of *U. tasmanica* infestation.

Chapter 2.

Pharate *U. tasmanica* remain quiescent in their cocoons over summer having pupated in Spring. Emergence from the cocoons is in response to mechanical stimulation. In the absence of any disturbance the fleas remain in the cocoons and eventually die. "Readiness" to emerge increases with the passage of time and with the degree of desiccation experienced by the fleas. At 80% R.H. fleas emerged (in response to mechanical stimulation) some 71 days later than fleas kept in conditions of 20% R.H. After emergence, and in the absence of a host, fleas survive for longer periods in conditions of high relative humidity (32 days at 80% R.H. compared with 13 days at 20% R.H.).

Chapter 3.

Adult fleas removed from the cocoons during the months of December and January were found to be sexually immature. In male fleas the sperm bundles were judged to be at stage 3-4 of development as described by Rothschild et al (1971) for the rabbit flea. Following a blood meal these fleas were found to be sexually mature after three days. Experimental results suggest that *U. tasmanica* may, to some extent, depend upon its host's hormones in order to achieve complete sexual maturation. The evidence for this is threefold. Firstly, in feeding trials (which took place in January) the fleas showed a marked preference for sub-adult native cats over adult native cats (23% of available feeding time was used by fleas on sub-adult hosts compared to 0.8% of available feeding time used on adults). Secondly, fleas were attracted although not to the extent of feeding, to an adult native cat which had been rubbed with urine from the sub-adult animals and sexual behaviour was greatest following feeding trials on sub-adult native cats and the adult native cat carrying the sub-adult urine. Thirdly, in experiments using mammalian hormones it was found that successful copulation with sperm transfer took place only in the fleas which were treated with hydrocortisone + prolactin or with prolactin. Yolk deposition occured in females which had been treated with prolactin, either alone or with hydrocortisone while chorionated eggs were found in one female flea which had been treated with hydrocortisone + prolactin. The coefficient of variation for egg size in this female was 101% compared with 41.97% in females of the control group.

Chapter 4.

The parasitic larvae of *U. tasmanica* differ from the free living larvae of other species of fleas in a number of ways: *U. tasmanica* has

four larval instars compared with three in other species; *U. tasmanica* has 11 body segments compared with 13 in other species, it appears that *U. tasmanica* has lost the last two abdominal segments as it has terminal spiracles and no anal struts; the long bristles found on other species of flea larvae are not found on the last 3 instars of *U. tasmanica* larvae which are adorned with backwards pointing denticles and spines; the head and mouthparts of *U. tasmanica* larvae are greatly reduced in comparison to those of other species; the number of functional spiracles is reduced in *U. tasmanica* larvae. In gross morphology and chaetotaxy *U. tasmanica* larvae resemble the larvae of myiasis producing dipterans.

Following emergence from the host *U. tasmanica* larvae may enter a pre-pupal diapause. Induction of the diapause is possibly controlled by the daily thermoperiod cycle experienced by the larvae following emergence from the host (larvae kept at 15 C and 25 C constant temperature took 95.04 and 95.5 days respectively to undergo the prepupal phase while larvae kept in thermoperiod conditions of 10 hrs. warmth and 16 hrs. warmth (15 C - 23 C) took 97.67 and 9.25 days respectively.

When the chromosomes of *U. tasmanica* were studied and compared with those of *P. hoplia* and *L. nova* it was found that *U. tasmanica* had 2n = 14 chromsomes while *P. hoplia* and *L. nova* both had 2n = 20. It is possible that *U. tasmanica* is more distantly related to either *L. nova* and *P. hoplia* than these species are to each other.

Appendix 1

Raw Trapping Data

Year	Location	Sex	Age	No. of fleas	Species of fleas	Larval Infestation	Remarks
1979	Buckland	F	2	4	4 x *P. hoplia*	Nil	
		F	2	3	3 x *P. hoplia*	"	HEALTHY,
		F	1	2	1 x *P. hoplia* & 1 x A. Spp.	"	TICKS IN TAILS
		F	2	6	4 x *P. hoplia* & 2 A. Spp.	"	AND EARS
		M	1	0			
		M	3	2	2 x A. Spp		
1979	Bermuda	F	1	3	3 x *S. dasyurus*		
		F	2	4	3 x *S. dasyurus* & 1 *P. hoplia*		
		F	1	1	1 x *S. dasyurus*		
		M	1	2	2 x *S. dasyurus*		
		M	1	0	-		
		M	3	5	3 x *S. dasyurus* & 1 A. Spp.		

Year	Location	Sex	Age	No. of fleas	Species of Fleas	Larval Infestation	Remarks
1979	Buckland	F	1	2	2 x *P. hoplia*	Nil	
		F	1	4	2 x *P. hoplia* & 2 A. Spp.	"	
		F	2	-	-	"	HEALTHY WITH
		M	3	7	4 x *P. hoplia* & 3 A. Spp.	"	
		M	1	5	5 x *P. hoplia*	"	TICKS IN
		M	3	4	4 x A. spp.	"	
		M	3	6	4 x *P. hoplia* & 2 A. Spp.	"	TAILS AND
*1979	Cradoc	-	-	6	1 x *U. tasmanica* & 5 x *S. dasyurus*	" "	EARS
		-	-	12	12 x *S. dasyurus*	"	
1979	Bermuda	M	1	2	2 x *S. dasyurus*	"	
		M	1	6	5 x *S. dasyurus* & 1 x *P. hoplia*	"	
		M	3	3	2 x *P. hoplia* & 1 x *S. dasyurus*	"	
		F	1	-			
		F	2	2	2 x *S. dasyurus*.		

* Supplied by J. Godsell from her trapping area close to Bermuda.

Year	Location	Sex	Age	No. of fleas	Species of Fleas	Larval Infestation	Remarks
1977	Buckland	M	1	4	4 x *P. hoplia*	Nil	
		F	3	2	2 x *P. hoplia*	"	
		F	2	7	6 x *P. hoplia* & 1 A. Spp.	"	
		F	1	9	6 x A. Spp. & 3 x *P. hoplia*	"	
		M	3	7	4 x *P. hoplia* & 3 x A. Spp.	"	
		M	3	4	4 x *P. hoplia*	"	
		M	3	12	7 x *P. hoplia* & 5 A. Spp.	"	
1978	Buckland	F	1	11	2 x *U. tasmanica* & 9 x *P. hoplia*	"	
		F	2	2	2 x *S. dasyurus*	"	
		M	3	4	4 x *P. hoplia*	"	
1978	Bermuda	M	2	4	4 x *S. dasyurus*	"	
		M	2	7	1 x *P. hoplia* & 6 *S. dasyurus*	"	
		F	2	9	9 x *S. dasyurus*	"	
		F	1	6	4 x *S. dasyurus* & 2 x A. Spp.	"	
		M	3	11	10 x *S. dasyurus* & 1 x *M. hercules*	"	
1979	Bermuda	M	2	15	2 x *U. tasmanica* & 13 x *S. dasyurus*	Light	TICKS AND LARVAE IN TAIL, EARS AND SCROTUM

Year	Location	Sex	Age	No. of fleas	Species of fleas	Larval Infestation	Remarks
1977	Buckland	F	1	7	2 x *U. tasmanica* & 5 x A. Spp.	Light	1 ONLY
		M	3	6	3 x *U. tasmanica* & 2 x *P. hoplia* & 1 A. Spp.	Nil	
		M	2	16	10 x *U. tasmanica* & 3 x A. Spp. & 1 x *P. hoplia* & 2 x *S. dasyurus*	Heavy	
		F	1	12	6 x *U. tasmanica* & 3 x *S. dasyurus* & 2 x A. Spp. & 1 x *P. hoplia*	Medium	
		F	2	7	2 x *U. tasmanica* & 4 x *P. hoplia* & 2 A. Spp.	Medium	
		M	3	27	20 x *U. tasmanica* & 4 x A. Spp. & 1 x *P. hoplia* & 2 x *S. dasyurus*	Very heavy	ALMOST PARALYSED VERY OLD ILL MALE
1978	Buckland	M	3	14	11 x *U. tasmanica* & 3 x A. Spp.	Medium	
		M	2	6	2 x *U. tasmanica* & 3 x *P. hoplia* & 1 A.Spp.	Light	
		M	3	7	7 x *U. tasmanica*	Light	
		F	1	12	7 x *U. tasmanica* & 5 x *P. hoplia*	-	

Cont../

April cont..

Year	Location	Sex	Age	No. of fleas	Species of Fleas	Larval Infestation	Remarks
1978	*Brown Mtn.	M	3	15	13 x *U. tasmanica* & 2 x *S. dasyurus*	Heavy	
		F	1	16	2 x *U. tasmanica* & 14 x *S. dasyurus*	Light	
	Bermuda	M	3	10	6 x *U. tasmanica* & 4 x *S. dasyurus*	Medium	
		F	2	1	1 x *S. dasyurus*	-	
		F	1	4	2 x *U. tasmanica* & 1 x *P. hoplia*	Light	

*Native cats caught by V. Moss in Honours Project.

Year	Location	Sex	Age	No. of fleas	Species of fleas	Larval Infestation	Remarks
1977	Bermuda	M	3	16	9 x *U. tasmanica* & 7 x *S. dasyurus*	Heavy	
"	"	"	2	13	6 x *U. tasmanica* & 3 x *S. dasyurus* & 4 x *P. hoplia*	"	
1977	Buckland	M	3	-	-	Heavy	
"	"	"	2	6	2 x *U. tasmanica* & 4 x *P. hoplia*	Medium	
		F	2	9	6 x *U. tasmanica* & 2 x *P. hoplia* & 1 C. ff.	Light	FEMALE NATIVE CATS FREQUENTLY SHOW SIGNS OF HAVING MATED ANIMALS NOT IN AS GOOD CONDITION AS IN SUMMER
1978	Bermuda	F	2	12	11 x *U. tasmanica* & 1 *S. dasyurus*	Heavy	
"	"	F	2	7	7 x *U. tasmanica*	Heavy	
"	"	M	3	13	9 x *U. tasmanica* &	Heavy	
					3 x *P. hoplia* & 1 A.spp	Heavy	
1978	Brown Mt.	M	3	6	4 x *U. tasmanica* & 2 x *S. dasyurus*	Heavy	
		M	2	13	9 x *U. tasmanica* & 4 x *S. dasyurus*	Heavy	
1979	Bermuda	F	1	7	3 x *U. tasmanica* & 4 x *S. dasyurus*	Light	
"	"	F	1	4	1 *S. dasyurus*	V. light	
"	"	F	2	10	3 x *U. tasmanica* & 7 x *S. dasyurus*	Medium	

cont.../

May cont...

Year	Location	Sex	Age	No. of fleas	Species of Fleas	Larval Infestation	Remarks
1979	Bermuda	F	3	5	5 x *U. tasmanica*	Heavy	FEMALE NATIVE
"	"	M	2	17	5 x *U. tasmanica* & 6 x *P. hoplia* & 6 x *S. dasyurus*	Heavy	CATS FREQUENTLY SHOW SIGNS OF HAVING MATED
"	"	M	2	3	3 x *S. dasyurus*	Light	
"	"	M	3	6	3 x *S. dasyurus* & 2 Ac. sp. & 1 *P. hoplia*	Medium	ANIMALS NOT IN AS GOOD CONDITION AS IN SUMMER
"	"	M	3	-			
"	"	M	3	6	6 x *S. dasyurus*	Light	
"	"	M	3	9	4 x *U. tasmanica* & 5 x *S. dasyurus*	Light	
"	"	M	3	-			

Year	Location	Sex	Age	No. of fleas	Species of fleas	Larval Infestation	Remarks
1977	Buckland	M	3	10	10 x *U. tasmanica*	Heavy	
		M	3	4	4 x *U. tasmanica*	Heavy	
		M	3	16	12 x *U. tasmanica* & 4 Ac. Spp.	Heavy	
		M	2	11	11 x *U. tasmanica*	Heavy	
		M	3	6	4 x *U. tasmanica* & 2 Ac. Spp.	Heavy	
		F	2	5	5 x *U. tasmanica*	Medium	SOME FEMALES
1977	Bermuda	M	3	6	*U. tasmanica*	Medium	
		M	3	6	*U. tasmanica*	Medium	HAVE NEW BORN
1978	Bermuda	M	3	11	*U. tasmanica*	Heavy	POUCHED YOUNG
		M	3	4	*U. tasmanica*	Heavy	
		F	2	9	*U. tasmanica*	Medium	
		F	2	2	*U. tasmanica*	Heavy	
1978	Buckland	M	3	8	8 x *U. tasmanica*	Heavy	
		M	1	4	4 x *U. tasmanica*	Heavy	
		F	2	5	4 x *U. tasmanica* & 1 *P. hoplia*	Heavy	
		M	3	5	5 x *U. tasmanica*	Medium	
		M	2	6	5 x *U. tasmanica* & 1 Ac. Spp.	Medium	
		F	1	7	7 x *U. tasmanica*	Heavy	

Year	Location	Sex	Age	No. of fleas	Species of fleas	Larval Infestation	Remarks
1977	Bermuda	M	3	7	*U. tasmanica*	Heavy	
		M	3	5	*U. tasmanica*	Medium	
		F	2	9	*U. tasmanica*	Heavy	
	Buckland	M	-	-	-	Heavy	
		M	-	-	-	"	
		F	-	-	-	Medium	MOST FEMALES
		F	-	-	-	Heavy	
		F	-	-	-	Medium	HAVE POUCHED YOUNG
1978	Bermuda	M	3	4	*U. tasmanica*	Heavy	
		M	3	3	*U. tasmanica*	Heavy	
		M	3	6	*U. tasmanica*	Heavy	
		F	2	8	*U. tasmanica*	Medium	
	Buckland	M	3+	12	*U. tasmanica*	Heavy	
		M	2	-	-	Medium	
		M	2	3	*U. tasmanica*	Medium	
		M	3	6	*U. tasmanica*	Medium	
		F	2	1	*U. tasmanica*	Light	
		F	1	10	*U. tasmanica*	Heavy	SICK ANIMAL

August

Year	Location	Sex	Age	No. of fleas	Species of fleas	Larval Infestation	Remarks
1977	Bermuda	M	2	4	*U. tasmanica*	Medium	
		M	3	3	*U. tasmanica*	Heavy	
	Buckland	M	2	2	*U. tasmanica*	Light	
		M	3	1	*U. tasmanica*	Light	POUCHED YOUNG
		F	1-2	1	*U. tasmanica*	Medium	NO LONGER PRESENT
1978	Bermuda	M	3	6	*U. tasmanica*	Heavy	
		M	2	4	*U. tasmanica* & 1 *S. dasyurus*	Heavy	IN FEMALE POUCHES
		F	1	-	-	-	NOT CAUGHT IN FIELD
		F	4	1	*U. tasmanica*		
		F	2	5	3 x *U. tasmanica* & 2 x *S. dasyurus*	V. light	
	Buckland	M	2	1	1 x *U. tasmanica*	Medium	
		M	3	7	5 x *U. tasmanica* & 2 x *P. hoplia*	Light	
		F	2	3	3 x *U. tasmanica*	Medium	
		F	1	-	-	Light	
		M	1	4	4 x *U. tasmanica*	Heavy	

Year	Location	Sex	Age	No. of fleas	Species of Fleas	Larval Infestation	Remarks
1977	Buckland	M	2	4	3 x *P. hoplia* & 1 x A. Spp.	Light	
		F	3	5	2 x *P. hoplia* & 3 x A. Spp.	Nil	
1978	Buckland	M	J*	3	2 x *P. hoplia* & 1 x *U. tasmanica*	Light	INFESTATIONS V. LIGHT WITH SOME DEAD LARVAE IN LESIONS
		M	1	4	4 x *P. hoplia*	-	
		F	2	3	1 x *P. hoplia* & 2 x A. Spp.	Light	
		F	1	3	3 A. Spp.	-	
		M	3	5	4 x *P. hoplia* & 1 A. Spp.	-	
		F	J	2	2 x A. Spp.	-	
1977	Bermuda	M	3	4	4 x *S. dasyurus*	Light	
		M	3	2	1 x *S. dasyurus* & 1 x A. Spp.	-	
		F	2	2	2 x *S. dasyurus*	-	
1978	Bermuda	M	1	4	2 x *S. dasyurus* & 1 x *U. tasmanica*	-	
		M	2	2	2 x *P. hoplia*	Light	
		M	3	6	3 x *S. dasyurus* 2 x A. Spp. & 1 x *P. hoplia*	-	
		F	2	5	3 x *S. dasyurus* & 1 x *P. hoplia* & 1 x *U. tasmanica*	-	
		F	1	3	1 x *S. dasyurus*	-	

* J = Juvenile

October

Year	Location	Sex	Age	No. of fleas	Species of fleas	Larval Infestation	Remarks
1977	Buckland	M	3	5	4 x A. Spp. & 1 x *P. hoplia*	Light	
		M	J	4	4 x *P. hoplia*	-	
		M	J	7	6 x *P. hoplia* & 1 A. Spp.	-	
		M	2	3	2 A. Spp. & 1 *P. hoplia*	-	SOME OF THE
		F	2	6	6 x *P. hoplia*		
		F	2	3	2 x *P. hoplia* & 1 A. Spp.	Light	ADULT NATIVE
		F	3	9	6 A. Spp. & 3 x *P. hoplia*	-	
		F	J	1	1 x *P. hoplia*	-	CATS WERE IN
		F	3	5	3 x *P. hoplia* & 2 A. Spp.		
		F	3	7	1 *U. tasmanica* & 6 x *P. hoplia*	-	POOR CONDITION
1978	Bermuda	M	3	4	4 x *S. dasyurus*	Light	
		M	3	6	3 x *S. dasyurus* & 3 A. Spp.	-	
		F	2	4	4 x *S. dasyurus*	-	
		F	J	5	5 x *S. dasyurus*	-	
		F	J	3	3 x *S. dasyurus*	-	

Year	Location	Sex	Age	No. of fleas	Species of Fleas	Larval Infestation	Remarks
1978	Buckland	F	5	6	4 x *P. hoplia* & 2 x A. Spp.	Nil	
		M	3	4	4 x *P. hoplia*		
		M	3	3	3 x *P. hoplia*		
		M	3	5	2 x *P. hoplia* & 3 x A. Spp.		
		M	3	8	3 x A. Spp. & 3 x *P. hoplia* and 2 x *S. dasyurus*		
		F	3	5	2 x *P. hoplia* & 3 x A. Spp.		
		F	3	3	3 x A. Spp.		
	Bermuda	F	J	3	3 x *S. dasyurus*		
		M	3	18	16 x *S. dasyurus* & 2 x A. Spp.		
		M	J	7	4 x A. Spp. & 3 x *S. dasyurus*		
		M	3	4	4 x *S. dasyurus*		

Year	Location	Sex	Age	No. of fleas	Species of Fleas	Larval Infestation	Remarks
1978	Buckland	F	2	-	-	Nil	
		M	3	12	8 x *P. hoplia* & 4 x A. Spp.	"	
		F	J	4	3 x *P. hoplia*	"	
		M	J	6	5 x A. Spp. & 1 *P. hoplia*	"	
							CONDITION
	Bermuda	F	J	5	5 x *S. dasyurus*	Nil	
		F	J	-	5 x *S. dasyurus*	"	
		F	2	3	5 x *S. dasyurus*	"	GOOD
		M	J	4	5 x *S. dasyurus*	"	
		M	J	2	5 x *S. dasyurus*	"	

Appendix II

Further Observations

Female Reproductive Tract of *U. tasmanica*

The reproductive tract of female *U. tasmanica* was observed from the sections of the fleas used for the experiments described in Chapter 4. It was found that the shape of the vagina of *U. tasmanica*, (observed in cleared whole mounts as well as in median sections) differed from the descriptions of flea vaginae compiled by Rothschild and Traub (1971).

The vagina of *U. tasmanica* was long and shaped like a 7 (Plate 3A Fig. 1). The vagina was lined with cuticle to beyond the bend after which it was lined with epithelial cells.(Plates 1, 6). The ventral wall of the vagina was also lined with hair like bristles. At the turn of the vagina an invagination was observed in the ventral wall which was also lined with bristles. In some sections of the fleas the ventral flap formed by this invagination appeared to be in apposition to a flap formed by the dorsal wall of the vagina between the duplicatora vaginalis and the duct of the bursa copulatrix. These two flaps in apposition appeared to form a valve by which the vagina could be closed. The entire portion of the lower vagina which was situated at the bend appeared to fit into the curve of the upper wall just anterior to the two flaps. (Plates 3, 4).

In *Uropsylla*, Hopkins organ was not recognised although a cuticular thickening was found posterior to the glandula vaginalis in some specimens. The duct of the bursa copulatrix was found to be heavily chitonised and the perula was cup shaped. The glandular cells surrounding the duct of the bursa copulatrix extended into the lobe formed by the junction of the duct and the duplicatora vaginalis.

Plate 1

(A) L.S. through female *U. tasmanica* showing vagina and oviducts. (x 200)

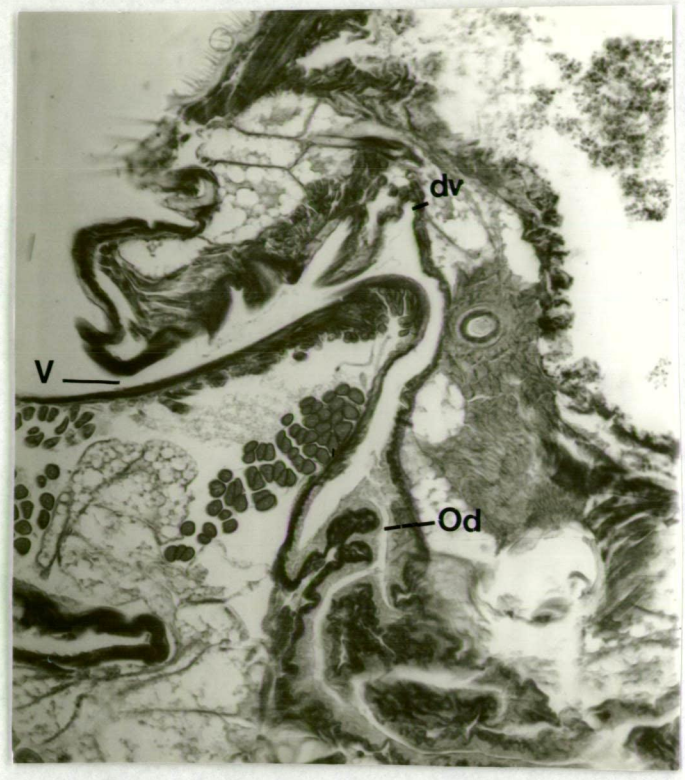
(B) Section of floor of vagina in the region of the duplicatora vaginalis showing knob and socket. (x 300)

v = vagina

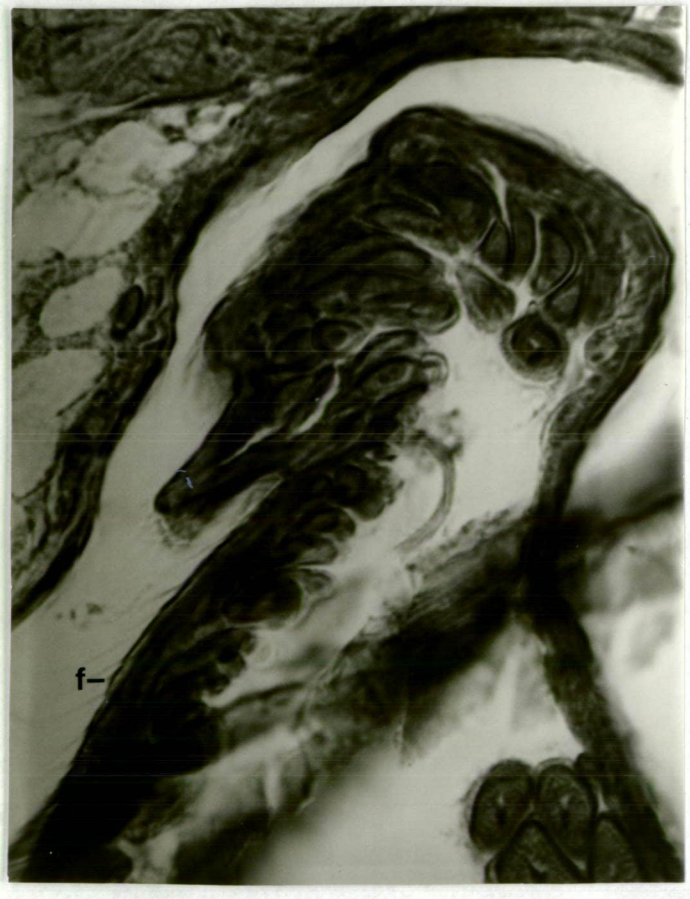
Od = oviduct

dv = duplicatora vaginalis

f = floor of vagina



a



b

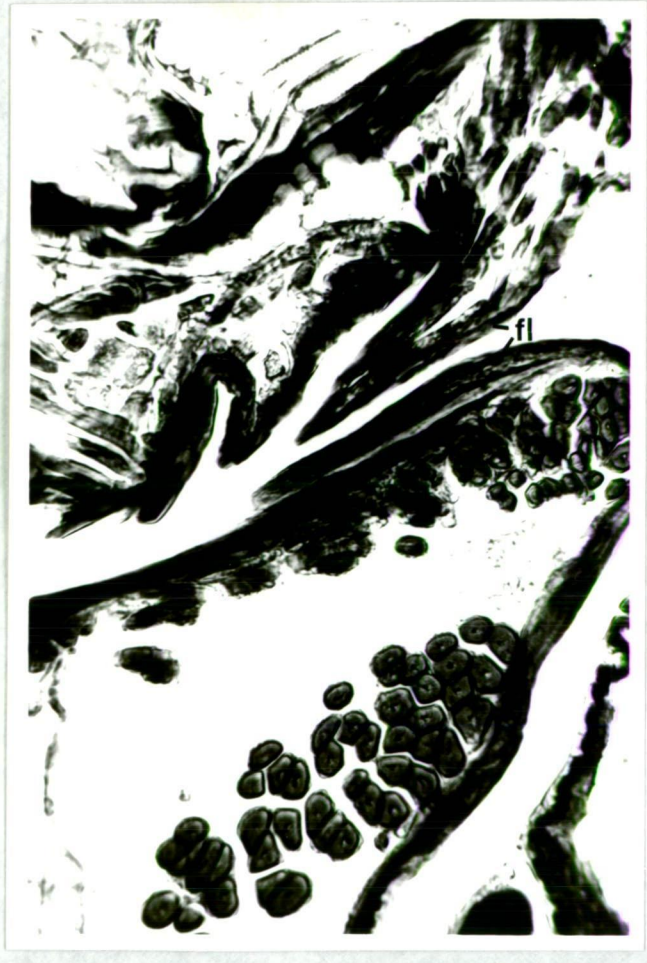
Plate 2

(A, B) Sections through vagina showing apposing flaps formed in the posterior vagina.

fl = flaps

a

x 200



b

x 300

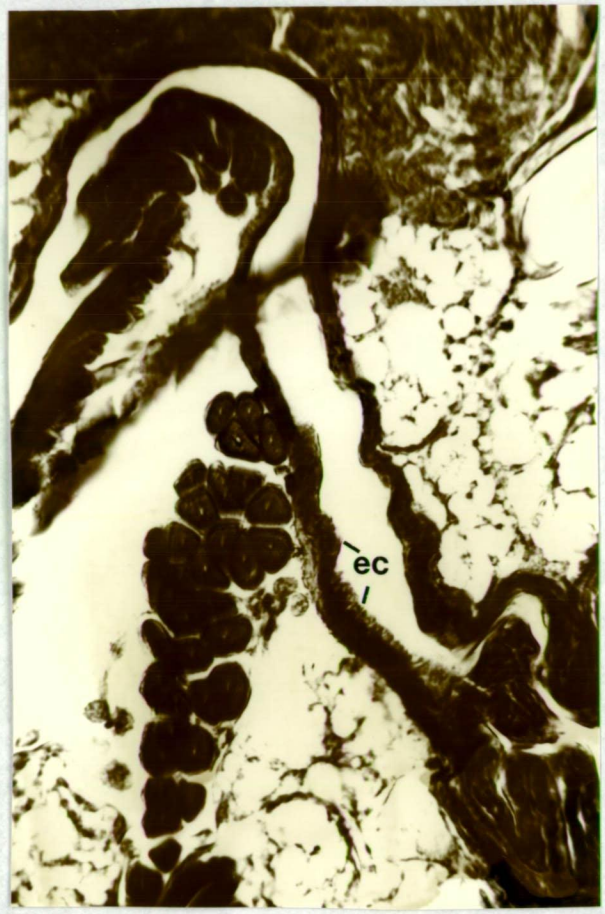
Plate 3

(A) Section of vagina showing the extent of the epithelial lining of the anterior vagina.

(B) Section (greatly magnified) of the floor of the posterior vagina showing the thin cuticular hairs lining the vagina.

ec = epithelial cells

a



×300

b

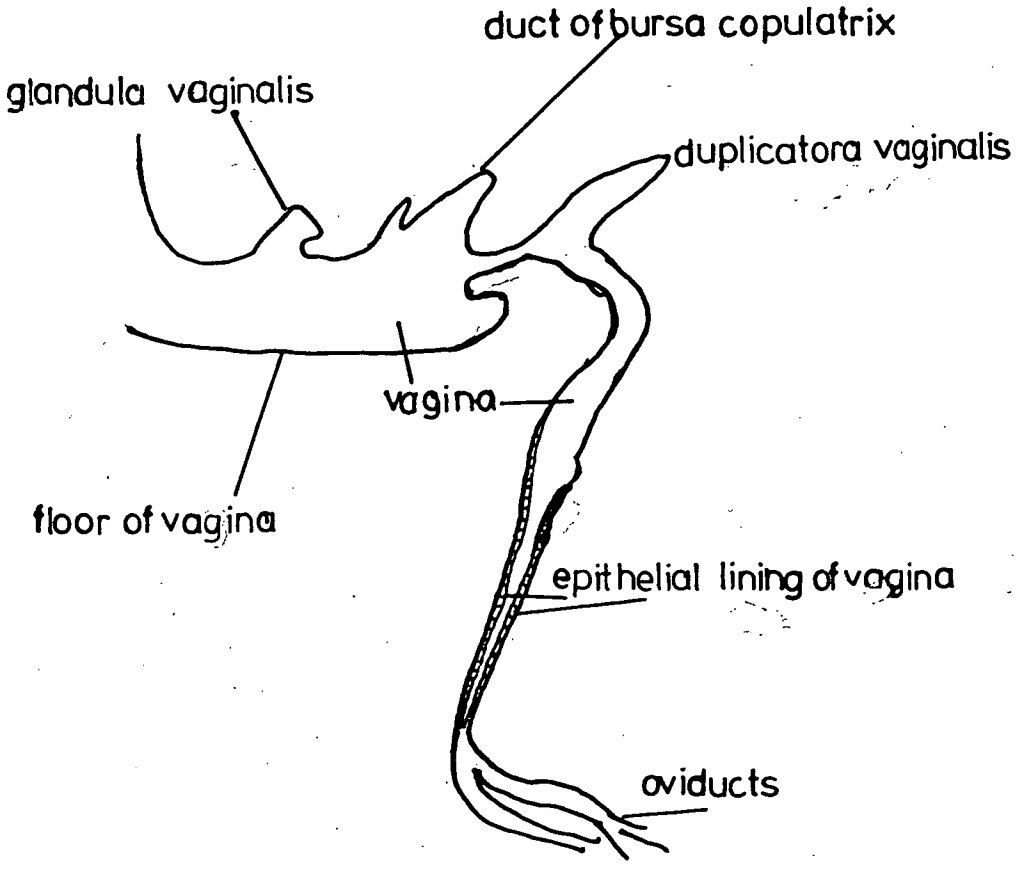


Fig. 1. Schematic representation of vagina of *U. tasmanica*. (Terminology after Rothschild and Traub 1971).

Use of Cement During Egg Laying

Figure 2 shows the extent of the use of cement during the egg deposition in four species of Australian fleas.

The drawings were made from eggs laid by female fleas in tubes containing den litter with the exception of the *U. tasmanica* egg which was observed on the hairs of a native cat.

It can be seen from these drawings that *P. hoplia* lays its eggs in batches. These eggs are cemented to the substrate as well as to each other. *S. dasyurus* eggs are deposited singly but are cemented to the substrate. It was found that these eggs frequently broke during attempts to dislodge them. The eggs of *L. nova* are laid singly and are not cemented to the substrate. *U. tasmanica* eggs are glued to one or more host's hairs and are laid singly.

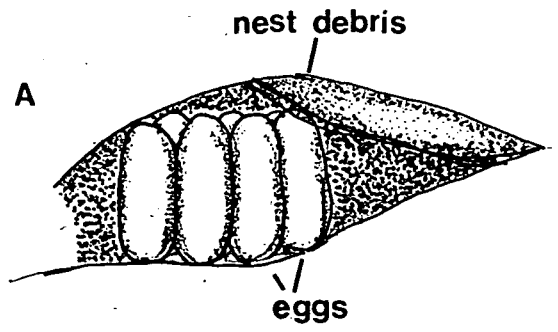
Fig. 2. The use of cement in egg laying in four species of Australian fleas.

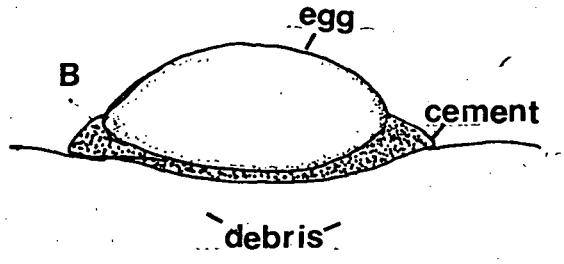
(A) *Pygiopsylla hoplia* (x 65)

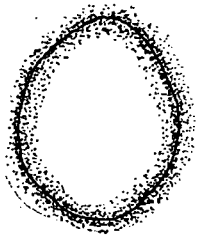
(B) *Stephanocircus dasyurus* (x 60)

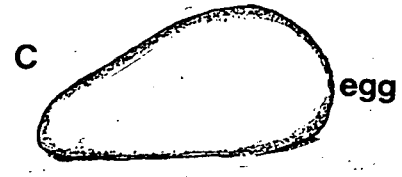
(C) *Lycopsylla nova* (x 60)

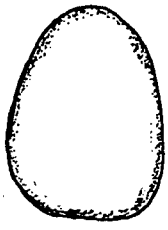
(D) *Uropsylla tasmanica* (x 60)

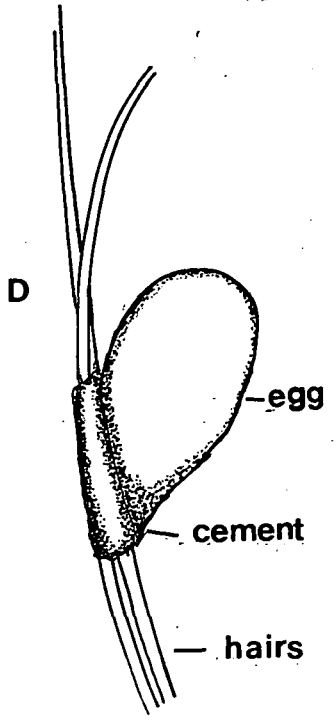

nest debris
A
eggs


egg
B
cement
debris




C
egg




D
egg
cement
hairs

Host Specificity of *U. tasmanica*

The extent of host specificity in *U. tasmanica* was investigated in three ways.

Firstly, all other Dasyurids accidentally caught during the field programme, *Sarcophilis harrisii, Dasyurus maculatus,* were checked for *U. tasmanica* adults and larvae. Secondly, an examination was made of all study skins of *S. harrisii,* and *D. maculatus* kept at the Queen Victoria Museum at Launceston.

The possibility of Uropsylla larvae being preserved on Thylacine *(Thylacinis cyanocephalus)* study skins was investigated by examining flat skins held at the following Australian Museums -

Queen Victoria Museum, Launceston	-	2 pelts
Tasmanian Museum, Hobart	-	2 pelts (incomplete)
Australian Museum, Sydney	-	6 pelts
National Museum, Melbourne	-	3 pelts
South Australian Museum, Adelaide	-	2 pelts

Thirdly, an experiment was set up in which adult fleas collected in the field were introduced into what were considered escape proof aquaria containing (a) a pair of kowaris *(Dasyuroides byrnii)* and (b) a marsupial mouse *(Antechinus minimum)*. Ten adult *U. tasmanica* were used for each species of marsupial and in each case the subjects were checked at two day intervals for *U. tasmanica* or indications of larval infestation. Neither the kowaris nor the antechinus had carried any fleas prior to the introduction of *U. tasmanica* into their aquaria.

Results

1. Other Dasyurids

Sarcophilus harrisii

Although adult *Uropsylla tasmanica* have been recorded from the Tasmanian devil (Dunnet and Mardon 1974) none were found on any trapped animals. In fact all 11 devils which were caught were remarkably free of ectoparasites. The examination of pelts at the Queen Victoria Museum did not reveal any larvae of *Uropsylla*.

Dasyurus maculatus

The tiger cat has been recorded (Warneke unpublished, Dunnet 1970) as carrying both adult *Uropsylla* and infestations of larvae.

Thylacinus cyanocephalus

The pelts from the Tasmanian Museum and the National Museum revealed traces of scars of the type left by *Uropsylla* or by ticks. The pelts from the Australian Museum and the South Australian Museum also bore traces of some endodermal parasitism and two larvae were obtained from these collections. These larvae resembled the parasitic dipteran larvae in form and chaetotaxy but not in the number of spiracles which were found and they resembled *Uropsylla* larvae particularly with regard to the head but not with regard to chaetotaxy.

So far these larvae have not been able to be identified to the order level. One of the specimens was damaged, the other was fairly intact but further specimens are needed before a definite appraisal can be given. They were however definitely not any of the larval instars of *Uropsylla tasmanica*.

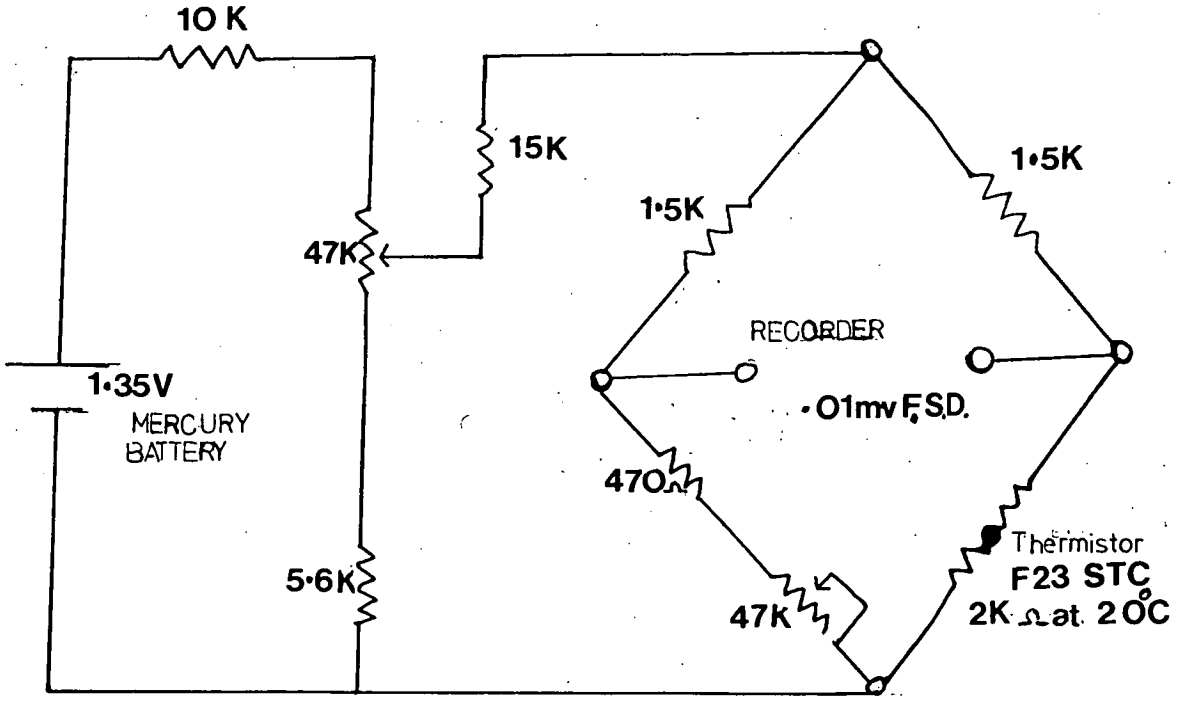
Antechinus minimumus and Dasyuroides byrnii

It was found that the *U. tasmanica* quickly disappeared from the aquaria and it is thought that they were caught and eaten by the kowaris and the antechinus. There were no signs of larval infestation at any time although some fleas survived for at least four days on the kowaris.

Daily Temperature Cycles in the Litter of the Native Cats Den

The daily temperature cycles of the litter in the main native cats den in the outdoors enclosure were recorded over several months. A continuous record of temperature fluctuation in the den was made by means of a thermistor connected to a pen recorder.

Fig. 3 Diagram of Thermistor, Pen Recorder Circuit.



The pen recorder was calibrated by means of immersing the thermistor into water of varying known temperatures. An average per hour daily cycle was calculated for each month of the recording.

Results.

The results are shown in fig. 4. It can be seen that daily temperature cycles were fairly constant during January and March while large hourly fluctuations occured during April where the standard errors of the means were greater than in any other month. During October the hourly temperatures fluctuated during the night hours but were fairly constant during the hours of daylight.

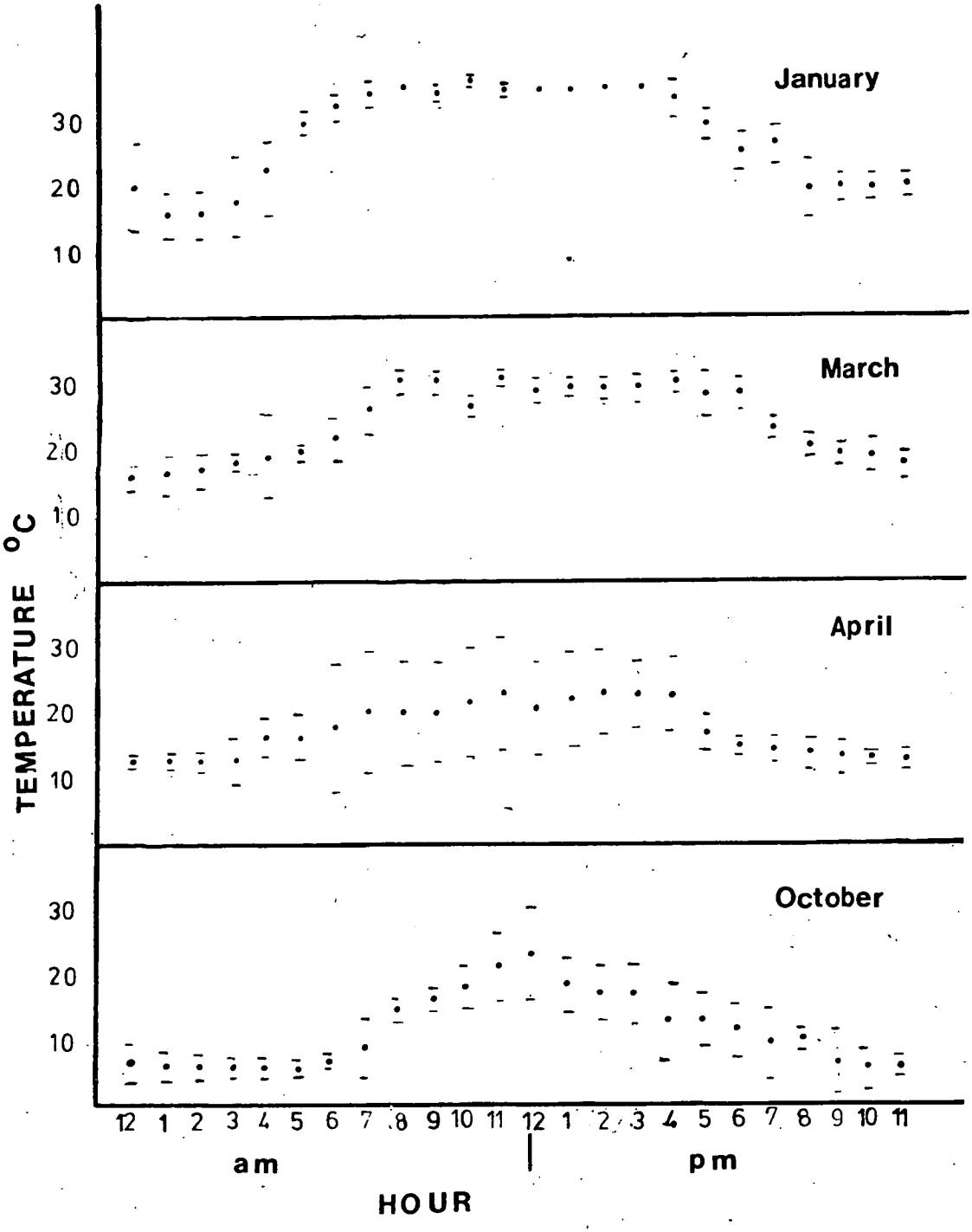


Fig. 4 Average Daily Cycle of Temperature Fluctuations in Native Cat Den Litter for the months of January, March, April and October. Horizontal bars represent standard errors of the mean.

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