The Seasonal Dynamics and Host-Parasite Relationship of *Opisocrostis bruneri* (Baker), a Flea on Franklin's Ground Squirrel, *Spermophilus franklinii* (Sabine) Near Birds Hill Park, Manitoba.

by

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MASTER OF SCIENCE

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DEDICATION

I would like to dedicate this thesis to my late grandmother, Marie Carriere, who inspires me to persevere through difficult times and pursue my goals. Thanks Grandma.

ABSTRACT

The host-parasite relationship between the flea, Opisocrostis bruneri and its primary host, Franklin's ground squirrel, Spermophilus franklinii was evaluated during a 2 year study.

Adult male ground squirrels were first captured each year on 5 May. The adult males were reproductively fit during the first two weeks of May. Adult female squirrels were first captured 5-8 days after the first male capture. Mating and subsequent parturition probably occurred in early May and mid-late June, respectively. The first observed signs of above ground juvenile squirrel activity were in mid-July. Adult squirrels immerged to hibernate in early August, followed by the juvenile females and males in early to mid-September.

Thirty-six individual squirrels were involved in 95 total captures in 1982. In 1983, 40 individual squirrels were involved in 161 total captures. The recapture rate of S. franklinii was 2.6 and 4.0 in 1982 and 1983, respectively. Adult squirrels constituted approximately 75% of the total captures and this stage was most frequently recaptured in 1982 and 1983.

Eight hundred and forty-nine and 1503 O. bruneri were removed from S. franklinii in 1982 and 1983, respectively.

Adult male squirrels were most frequently infested and contributed 43% and 64% of fleas collected in 1982 and 1983, respectively.

The observed sex ratio (m/f) of fleas removed from S. franklinii was 0.70 and 0.73 in 1982 and 1983, respectively. The biweekly sex ratio (m/f) favoured female fleas in each trapping period throughout the season except during a two week period starting at the beginning of May and the end of June.

The observed prevalence of O. bruneri from all captured S. franklinii was greater than or equal to 0.75. The observed biweekly prevalence on adult male and female squirrel was always greater than or equal to 0.73 and 0.67, respectively. Juvenile male and female squirrels were always infested. Two discrete peaks of mean intensity of adult fleas were observed in early May and late August.

Female fleas containing immature ovarioles (stage 0 and 1) with or without sperm in the spermatheca were predominant during the first three trapping periods. Parous females predominated during the remaining trapping periods.

Oogenesis of O. bruneri was not stimulated by the oestrous cycle of female S. franklinii. Parous female fleas containing sperm within the spermatheca were found throughout the entire season.

O. bruneri completes at least two generations per year in Manitoba. Peaks of nulliparous female fleas were observed

in early May and early July. The shift in biweekly observed sex ratio (m/f) favouring males occurred during these periods.

Opisocrostis bruneri was generally infested with two different mites, Psyllanoetus spp. and Trichouropoda spp. The haemocoel of the flea was occasionally occupied by allantonematid nematodes. The cysticercoid stage, presumably of Hymenolepis citelli, was removed from the mid-gut of one flea and gregarine cysts were frequently found within the mid-gut of O. bruneri.

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Chapter I

INTRODUCTION

Opisocrostis bruneri (Baker) is primarily associated with Franklin's ground squirrel, Spermophilus franklinii (Sabine), but also regularly infests two secondary hosts, the thirteen-lined ground squirrel, S. tridecemlineatus (Mitchell) and Richardson's ground squirrel, S. richardsonii (Sabine) (Holland 1949, 1985). Opisocrostis bruneri has been recorded from a wide variety of accidental hosts, including Mustela, Peromyscus, Marmota, Taxidea, Vulpes, Thomomys, Sylvilagus, Canis and Felis (Smit 1983). The geographic distribution of O. bruneri overlays its primary and secondary hosts, S. franklinii, S. richardsonii and S. tridecemlineatus respectively (Holland 1952) (Figs. 1,2). In Canada, O. bruneri has been reported from Alberta, Saskatchewan, Manitoba, and within the Lake of the Woods region of Ontario (Perdue 1980, Smit 1983, Holland 1985).

Prince (1943) was the first to emphasize the potential of O. bruneri as a plague vector. Fifty per cent of fleas from S. richardsonii in Potter County, South Dakota transmitted plague bacillus to healthy animals in the laboratory. Prince remarked on the surprisingly high rate of transmission, "Thus a continuous chain of fleas capable of transmission of plague, and of hosts which have been infected extends from the Rocky Mountains and western North Dakota

Figure 1: Distribution of Opisocrostis bruneri and Spermophilus franklinii (adapted from Perdue 1980, Banfield 1981, Hall 1981, and Holland 1985).

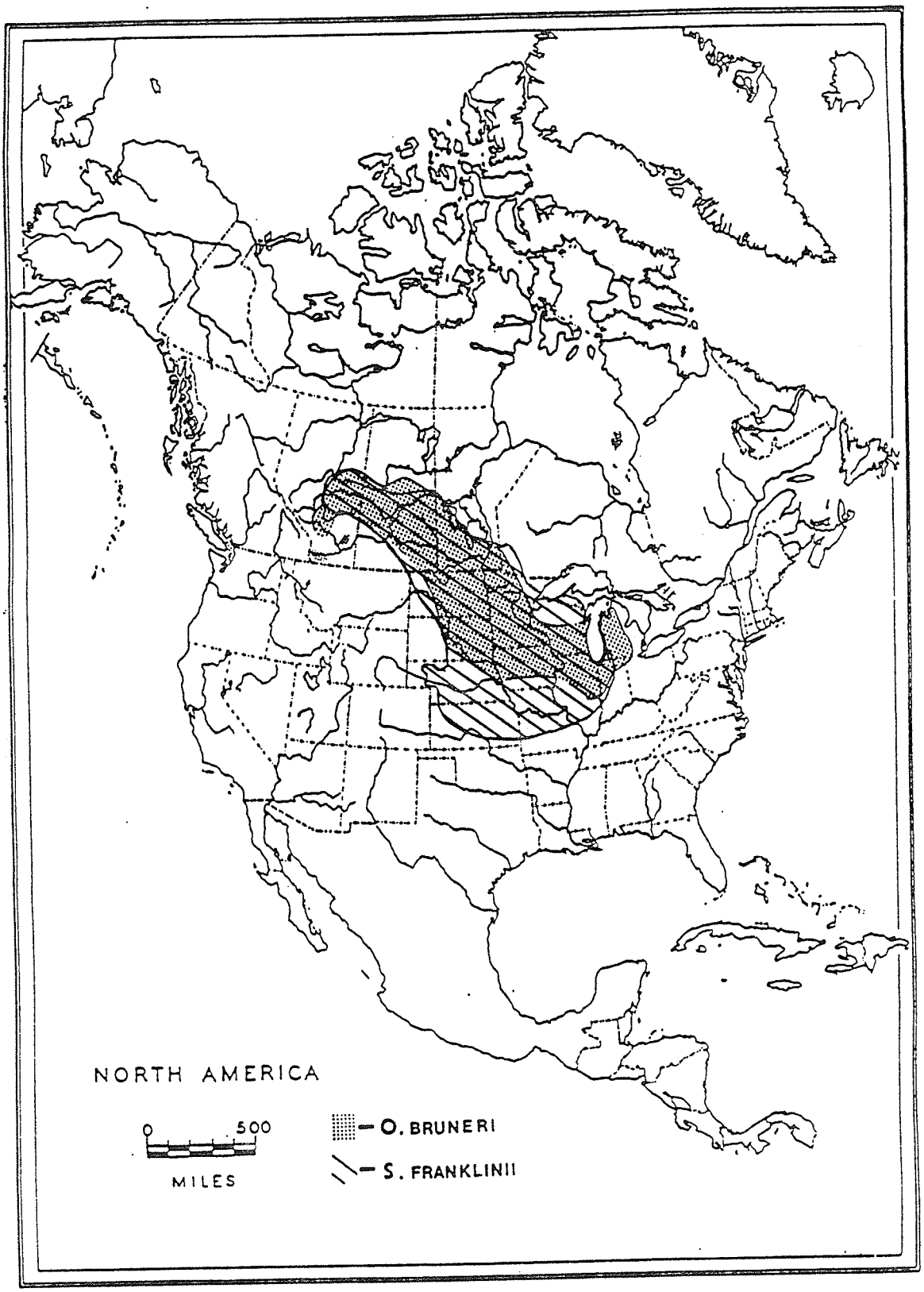
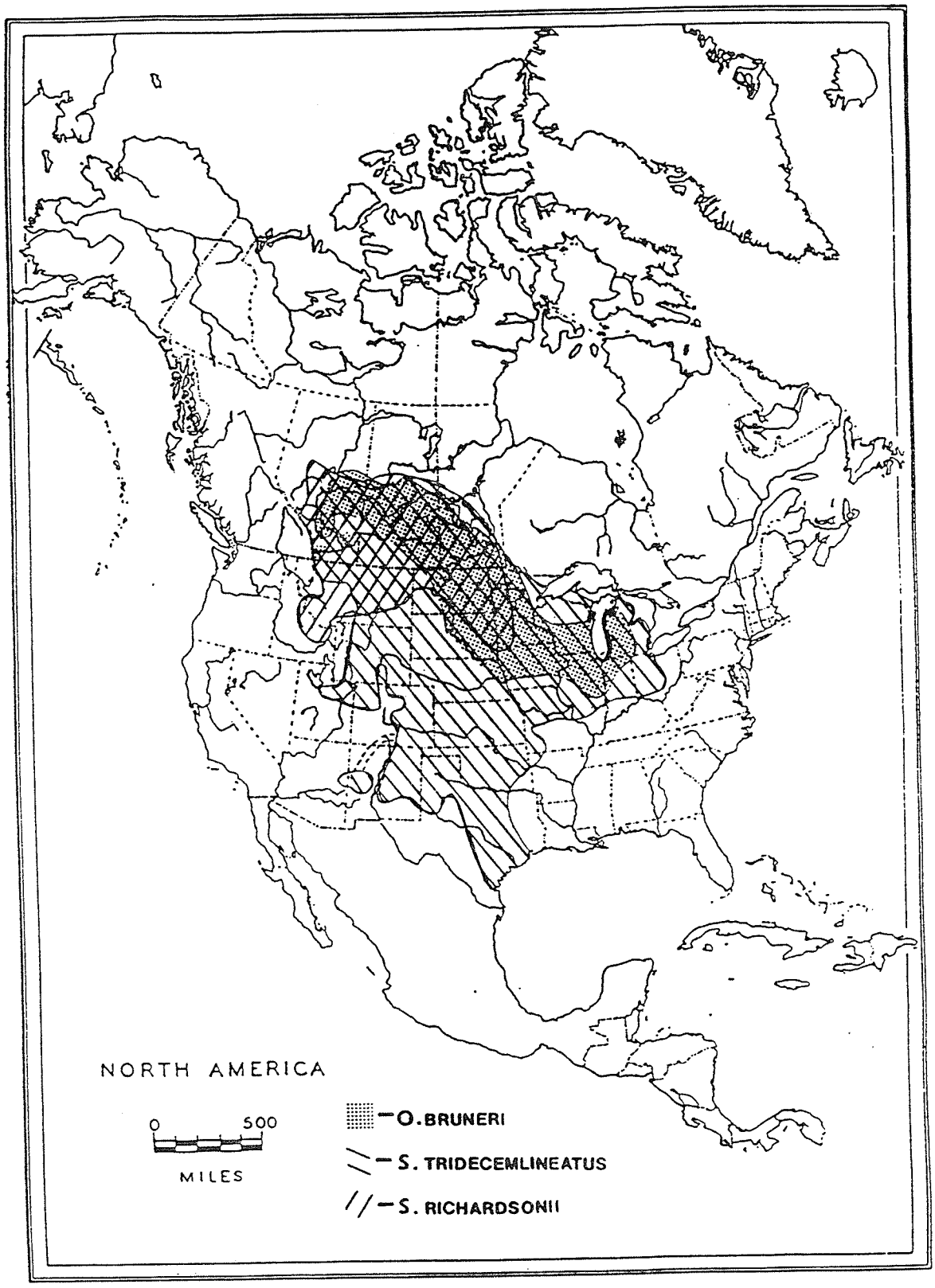

NORTH AMERICA
0
500
MILES
— O. BRUNERI
— S. FRANKLINII

Figure 2: Distribution of Opisocrostis bruneri, Spermophilus tridecemlineatus, S. richardsonii. (Dotted line represents range extension) (adapted from Perdue 1980, Banfield 1981, and Holland 1985).


NORTH AMERICA
0
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S. TRIDECEMLINEATUS
S. RICHARDSONII

in which plague prevails, to the west of the Mississippi River". Hubbard (1947) and Holland (1949) also stressed the importance of O. bruneri as a potential vector of sylvatic plague, though it is not yet established as a natural vector (Traub 1983) to man or ground squirrel.

The transfer of sylvatic plague from O. bruneri into an urban cycle depends on the opportunity of S. franklinii to maintain close contact near populated areas. Spermophilus franklinii inhabits transitional or disturbed areas; primarily oak-aspen parkland in and along the woodland-field ecotone and also along farms, suburbs, campgrounds and beach front (Banfield 1981). Transfer of fleas could occur in a transitional zone such as a sanitation dump site, between infected Spermophilus spp. and non-infected Rattus spp. The urban cycle commences when infected fleas, not necessarily O. bruneri, are transferred to uninfected Rattus spp.

Franklin's ground squirrel exhibits the lowest level of social behaviour among the six Spermophilus species in Canada (Kivett et al. 1976). Adult squirrel interaction is highest during the spring mating season. Adult and yearling males emerge from hibernation in breeding condition in late April to early May and establish breeding territories (Michener 1984). Mating commences in 1 to 2 weeks, when the monoestrus females emerge and continues 2 to 3 weeks hence (Fig. 3). Parturition occurs within approximately 28

days (Murie 1973). The young of the year begin above ground activity by the first week of July in Alberta. Adult males immerge to hibernate in late July followed by non-reproductive females and reproductive females. Juveniles hibernate in late August to early September (Banfield 1981) (Fig. 3).

The seasonal dynamics of O. bruneri on a secondary host have been investigated in two studies. Hendricks (1967) reported the infestation parameters for O. bruneri removed from S. tridecemlineatus. Two peaks of infestation (mean intensity) occurred during June and October, respectively. Kinzel and Larson (1973) investigated the relative abundance and geographic distribution of O. bruneri and Thrassis bacchi bacchi (Roth.) found on S. tridecemlineatus in North Dakota. Relative abundance was inversely related to geographic region, where T. b. bacchi was abundant in the west and O. bruneri predominant in the east. The authors suggested that the change in relative abundance across the state was due to different abiotic conditions, such as elevation and rainfall, within each region. Hendricks (1967) and Kinzel and Larson (1973), also associated abiotic conditions with changes in infestation during the year. The data from both studies were collected from several sites rather than one fixed location or host population for an entire season. Hence the conclusions from those studies may have been influenced by variation among sampling localities.

Figure 3: Schematic representation of the above ground activity of Spermophilus franklinii in Manitoba

(lined areas indicate immergence of squirrels and are separated by .5 week intervals).

Legend

M=mating

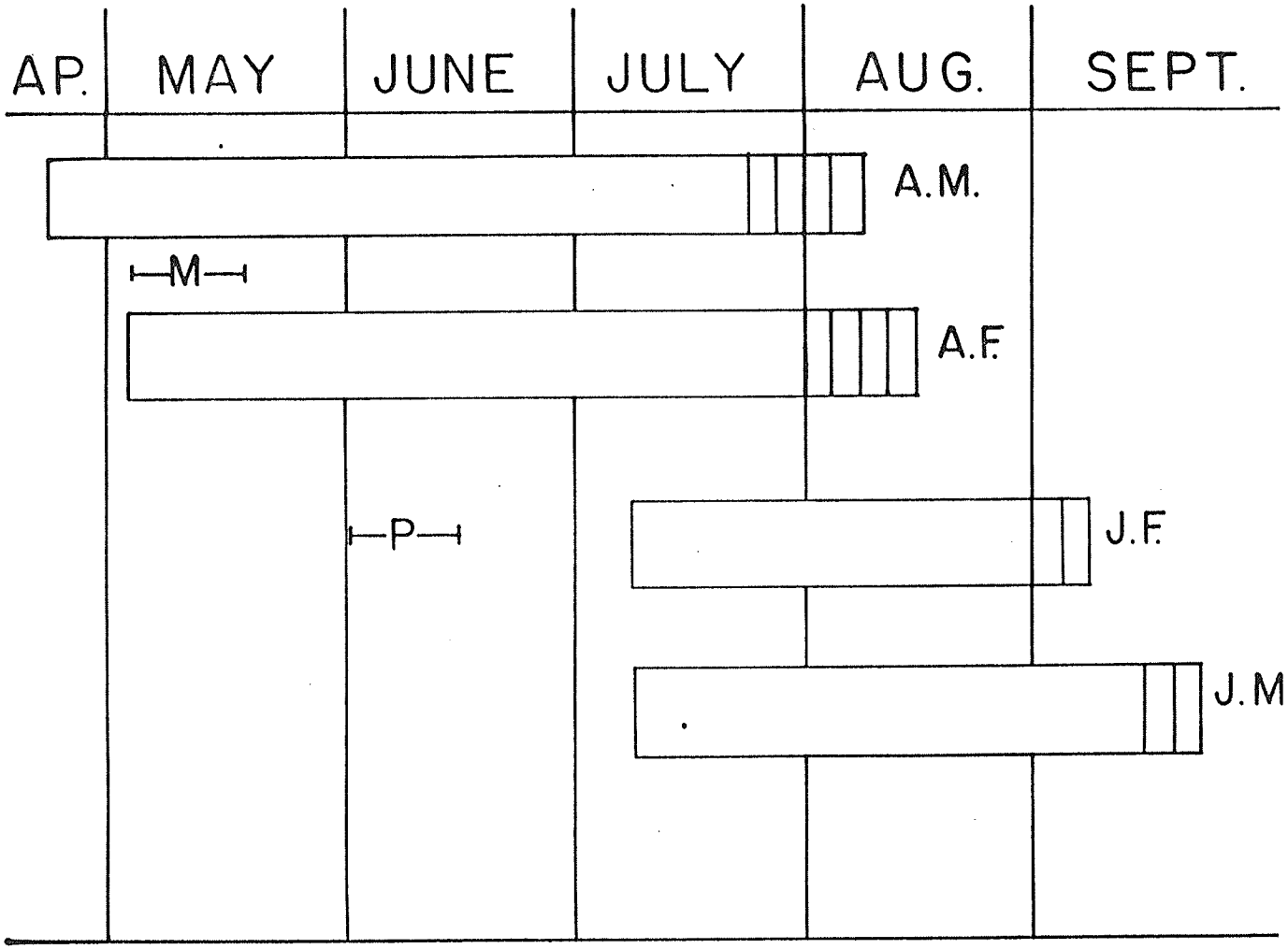
P=parturition

A.M.=adult male squirrel

A.F.=adult female squirrel

J.F.=juvenile female squirrel

J.M=juvenile male squirrel


AP.
MAY
JUNE
JULY
AUG.
SEPT.
A.M.
M
A.F.
P
J.F.
J.M

Seasonality of O. bruneri on S. franklinii was first assessed in 1979 near Birds Hill, Manitoba (Burachynsky and Galloway 1980). Adult flea populations were highest in mid-May and early September, respectively; the first peak occurred during the reproductive period of its monoestrus host, S. franklinii. The prevalence of infestation of O. bruneri on S. franklinii was $\geq$ 98% during all season. Holland (1949) reported that O. bruneri was closely associated and almost exclusively found on S. franklinii. The high degree of association may have also resulted in the dependence of reproductive cues from S. franklinii to initiate reproduction in O. bruneri. Such a relationship had been previously observed between Spilopsyllus cuniculi Dale and Oryctolagus cuniculus (L.) (Rothschild and Ford 1973).

The life history and reproductive activity of the European rabbit flea, Spilopsyllus cuniculi was the most intensive flea study undertaken. Rothschild and Ford (1973) discovered that ovarian development in S. cuniculi was initiated by circulating estrogen and prolactin in the pregnant female rabbit host, Oryctolagus cuniculus. The ovarioles of S. cuniculi were quiescent throughout the year unless a blood meal was drawn from a reproductively active doe. The reproductive condition was closely monitored by removal and inspection of ovarian tissue. Subsequently the reproductive condition of S. cuniculi and O. cuniculus were synchronized throughout the year. This intrinsic relationship

was interpreted as a highly developed host-parasite system that ensures future generations of fleas a food source, since all eggs are laid within a nest containing new born rabbit kittens.

The present study is the first attempt to identify whether O. bruneri requires reproductive stimuli from its host to initiate ovarian development, as in the observed reproductive synchrony of S. cuniculi. If ovarian development of O. bruneri is stimulated by reproductive hormones, then parous females should be present only during the host's reproductive period. The objective, therefore, was to determine seasonal dynamics, reproductive activity of female fleas, and the host-parasite relationship between a population of Opisocrostis bruneri and Spermophilus franklinii near Birds Hill Park, Manitoba.

Chapter II

REVIEW OF PERTINENT LITERATURE

2.1 FRANKLIN'S GROUND SQUIRREL

2.1.1 Historical Background

Franklin's ground squirrel was first described as Arctomys (Spermophilus) franklinii, in honour of Sir John Franklin, the commander of the Overland Expedition of 1819-1822. In 1821, Sir John Richardson first discovered S. franklinii populations near Carlton House (Prince Albert), Saskatchewan. Sir Joseph Sabine later described S. franklinii based on specimens sent from Saskatchewan. A comparative description of several spermophiles, including S. franklinii, and other quadrupeds was reported by Richardson (1829).

Franklin's ground squirrel is known by several other common names; the bushy-tailed ground squirrel, grey ground squirrel, scrub-gopher, the grey-cheeked spermophile and the whistling gopher (Seton 1909). The clear, musical call of S. franklinii was described by Seton (1909) who remarked, ". . . This is the musician of the family. Its ordinary note heard in the brushwood is in a high degree musical, resembling the voice of some of our fine bird singers".

2.1.2 Description

Franklin's ground squirrel more closely resembles a tree squirrel than other ground squirrel because of its long, bushy tail which comprises two-thirds of its total length (Jackson 1961, Woods 1980, Banfield 1981, Hazard 1982, Wooding 1982, Jones et al. 1983)(Fig. 4). The skull is relatively long, particularly the rostrum, with a narrow zygomatic process and a narrow flat cranium (Banfield 1981). The narrow skull is uncharacteristic of the sciurids.

The pelage is short and particularly wiry during the summer months. The anterior dorsal region is light grey and the remaining regions are olive brown. Each hair is speckled with one or two black bars. The cream-coloured undercoat remains thin throughout the season except during hibernation. There is a white circle around each eye. The black-tipped tail is covered with long, silver black-banded hairs. The feet are furred with grey hair and the toes have long claws. The pinnae of the ears are small and often flush with the head.

One annual molt begins in early May and lasts until mid-June (Jackson 1961, Banfield 1981, Wooding 1982). Jones et al. (1983) suggested that S. franklinii undergoes two annual molts, the first in spring after mating and the second in late summer, although no specimens of the sub-genus Poliocitellus (such as S. franklinii) were examined. Hansen

Figure 4: Adult male Spermophilus franklinii near Birds Hill Park, Manitoba.

(1954), without actually examining any specimens, stated that S. franklinii has only a single replacement of hair each year in which there exists an obvious molt line.

2.1.3 Distribution

Franklin's squirrel was originally described from a population discovered in 1822 near Carlton House (Prince Albert), Saskatchewan. Since then S. franklinii has been recorded from the following locations (Fig. 1): CANADA - in Alberta, recorded in the east-central region (Soper 1964): in Saskatchewan, found in the central region and spreading eastward from the Alberta border, excluding the short-grass prairie region, towards the borders of Manitoba and North Dakota (Woods 1980, Hall 1981); in Manitoba, primarily in the southern region and spreading northward along the west side of Lakes Manitoba and Winnipegosis up to The Pas (Sowls 1948, Hall 1981); in Ontario, recorded only from the Kenora, Rainy River and Fort Francis areas (Peterson 1966). There were unsuccessful attempts to establish a population near Georgian Bay (Seton 1909). UNITED STATES - in North and South Dakota, primarily in the eastern half except for the northern half of North Dakota (Jones et al. 1983); in Nebraska, throughout the state except in the areas of higher elevation i.e. the northwest and southwest corners (Hall 1981, Jones et al. 1983); in Kansas, S. franklinii inhabits tall and mid-grass prairies from the north-central to the eastern state edge

(Hall 1981, Jones et al. 1983); in Minnesota, found throughout the entire state except for two north-east counties (Hazard 1982), however, extensive modifications of the coniferous forest through lumbering and farming have created edge habitat available for colonization into northeastern Minnesota and Wisconsin (Robbins 1971); in Wisconsin, general range southern and north-western parts of the state (Jackson 1961, Hall 1981); in Illinois, the range includes most of the state except for the southeastern section (Hall 1981); in Indiana, S. franklinii is restricted to the northwestern corner (Lyon 1932, MacClintock 1970); in tip along Lake Michigan; in New Jersey, a pair of S. franklinii brought from Illinois escaped in May 1867 and became established in a nearby sand field (Seton 1909, Stone and Cram 1916, Howell 1938).

2.1.4. Life History

Adult male S. franklinii emerge from hibernation in a fully reproductive condition, one to two weeks prior to female ground squirrels (Sowls 1948, Banfield 1981) (Fig. 3). After the completion of the breeding season the testes ascend and become inactive (Iverson and Turner 1972). Records of first spring sightings of above ground male activity range as early as 14 April at Delta Marsh, Manitoba (Sowls 1948) to 6 May at Birds Hill, Manitoba (present study) and 11 May at Pinawa, Manitoba (Iverson and Turner 1972). The seasonal cycle of a

population near Edmonton near the northern and western range limit was similar to that observed in Pinawa, Manitoba, except all events were 2 to 4 weeks earlier (Murie 1973)

Adult male S. franklinii begin above ground activity, in Manitoba, usually two to three weeks later than S. richardsonii. In preparation for female S. franklinii, adult males establish temporary breeding territories. The appearance above ground by females marks the start of the 2 to 3 week breeding season (Banfield 1981) during which male squirrels locate and mate with sexually receptive females. Skirmishes between squirrels are common during the mating period, however, physical contact between male and female squirrels is minimal after the end of the breeding season. Mating usually takes place below ground within the female's burrow.

The gestation period of the monoestrus S. franklinii females lasts approximately 28 days (Woods 1980, Wooding 1982, Jones et al. 1983). Young squirrels are born naked and blind (Turner et al. 1976) between the last week in May and the second week of June. Parturition occurred as late as 24 June in a population near Pinawa, Manitoba (Iverson and Turner 1972).

The average litter is approximately 7.5 squirrels (Banfield 1981). Normal sex ratio (m/f) for a litter is 1:1. Although naked and blind at birth, the young soon become fully haired by Day 16 and the eyes open by Day 20 (Turner et al. 1976). The young begin foraging for solid food by the 28th

day after parturition. They do not mature sexually until the next spring.

Adult males hibernate near the end of July, followed by adult females (non-reproductive before reproductive). Reproductively active females hibernate later than non-reproductive because of the additional time required to recover from weight loss resulting from parturition. The juveniles generally remain active until the first two weeks of September. Juvenile female ground squirrels enter hibernation before juvenile males (Michener 1983). Squirrels have been observed as late as 4 October (Sowls 1948). Dispersal of juvenile male and female squirrels occurs primarily during the fall.

2.1.5 Behaviour

Spermophilus franklinii is a solitary animal and exhibits the least amount of social interaction of the North American ground dwelling sciurids (Michener 1983). Except during mating season, S. franklinii generally inhabits a solitary burrow. Only 10 per cent of daily activity is spent above ground during the summer months (Banfield 1981). Spermophilus franklinii remains underground during periods of inclement weather such as rainy, cool days.

The majority of above ground activity is spent foraging for food;animal matter comprises approximately one third of the diet (Banfield 1981, Woods 1980). Several plant species

are readily consumed such as sow thistle, chokecherry and various seeds and roots. Insects such as crickets, grasshoppers, beetles and duck eggs (Sowls 1949) comprise a portion of the animal matter diet.

Sowls (1948) reported on the life history of a S. franklinii population and its relationship to nesting ducks near Delta Marsh, Manitoba. He outlined squirrel population densities and peaks (over a 9 year period), daily activity and general diet. Of all the available duck nests, 19 per cent were destroyed by S. franklinii. Adult male S. franklinii was also observed attacking and killing young ducklings.

2.1.6 Related Research

McLeod (1933) conducted a parasitological survey of internal and external parasites of the three Spermophilus spp., including S. franklinii, found in southern Manitoba. Three external parasites, a tick, Dermacentor variabilis (Say), a flea, Opisocrostis bruneri and a louse, Linognathoides montanus were recovered from S. franklinii. Two spiurid nematodes, Rictularia citelli (McLeod) and Physaloptera spenicauda = (massino) (McLeod) and one hymenolepid cestode, Hymenolepis citelli were removed from the digestive tract of S. franklinii.

Hilton and Mahrt (1971) removed ectoparasites using a hair dissolving-floatation technique from three species of Spermophilus, including S. franklinii in Alberta.

Opisocrostis bruneri was found exclusively on S. franklinii. Three other ectoparasites were removed from S. franklinii including an ixodid tick, Dermacentor andersoni (Say), a sucking louse, Enderleinellus suturalis (Osborn) and a laelapid predaceous mite, Androlaelaps fahrenholzi (Berlese).

2.2 OPISOCROSTIS BRUNERI

2.2.1 Historical Background

Opisocrostis bruneri was originally described as Pulex bruneri in 1895 by Carl Baker. The specimens were taken on S. franklinii and S. tridecemlineatus at Lincoln, Nebraska. Jordan (1933) reclassified the genus Pulex and placed P. bruneri in a new Nearctic genus, Opisocrostis. The genus means "tassel-tail", in reference to the appendage on sternite VIII (Jellison 1947). Holland (1949, 1985) also recognized the generic status of Opisocrostis within the family Ceratophyllidae. Smit (1983) reassigned several genera, including Opisocrostis, as subgenera in the genus Oropsylla. The suppression of the genera was completed without written justification in the accompanied text, hence we continue to observe the generic status of Opisocrostis according to Holland (1985).

2.2.2 Description

Opisocrostis bruneri males are physically smaller than the females (Fig 5). Eyes and pronotal ctentidia are

Figure 5: Adult male (upper) and female (lower) Opisocrostis bruneri removed from Franklin's ground squirrels.

prominent and well developed. A complete description of O. bruneri is found in Perdue (1980) and Holland (1985).

2.2.3 Distribution

The geographic distribution of O. bruneri is restricted by the geographic ranges of its primary and secondary hosts, S. franklinii, S. tridecemlineatus and S. richardsonii. Generally, O. bruneri infests S. franklinii throughout its range and extends beyond the geographic range of S. richardsonii into the range of S. tridecemlineatus. There is no apparent host preference in regions where all three hosts are prevalent such as Birds Hill, Manitoba, since O. bruneri can be retrieved from any of the three hosts.

In Canada, O. bruneri has been collected in central and southern Alberta, central Saskatchewan, and within the Lake of the Woods region in North-Western Ontario (Perdue 1980, Smit 1983, Holland 1984) (Figs. 1,2). In the United States it is found primarily in the North-Central states including Montana, Wyoming, Colorado, Nebraska, North Dakota, South Dakota, Minnesota, Wisconsin, Iowa, Illinois, Michigan, Indiana and Ohio (Perdue 1980, Smit 1983, Holland 1984) (Figs. 1,2).

2.2.4 Related Research

Haas (1970) collected O. bruneri in a red fox, Vulpes vulpes (L.), den. The presence of O. bruneri was likely due to transfer of fleas from captured prey such as S. tridecemlineatus and the cottontail, Sylvilagus floridanus. Haas suggested that in studies dealing with fleas from accidental hosts, such as S. floridanus, researchers should evaluate feeding behaviour and host selection once the flea has been deprived of a host when that host was taken by a predator.

Environmental factors affecting flea abundance and geographic distribution were evaluated in North Dakota by Kinzel and Larson (1973). The relative abundance of O. bruneri and T. b. bacchi was related to the geographic and environmental regions of North Dakota, where T. b. bacchi predominated in the west and O. bruneri was abundant in the east. Geographical and climatic variation in the eastern and western regions of the state were believed to favour each respective species. The conclusions drawn from the data may be somewhat biased since each S. tridecemlineatus population was only sampled once. Increases of O. bruneri were positively affected by increased precipitation (Beasler 1976). Data was collected biweekly from S. richardsonii in North Dakota. Peaks in precipitation were followed 2 weeks later by peaks in numbers of O. bruneri. These trends were not observed with O. bruneri populations at Birds Hill Park, Manitoba.

Hendricks (1967) recorded monthly infestation parameters for O. bruneri removed from S. tridecemlineatus. Mean intensity was highest during June and October, respectively in Indiana. Female fleas were predominant during the season, except in May and August.

Chapter III

MATERIALS AND METHODS

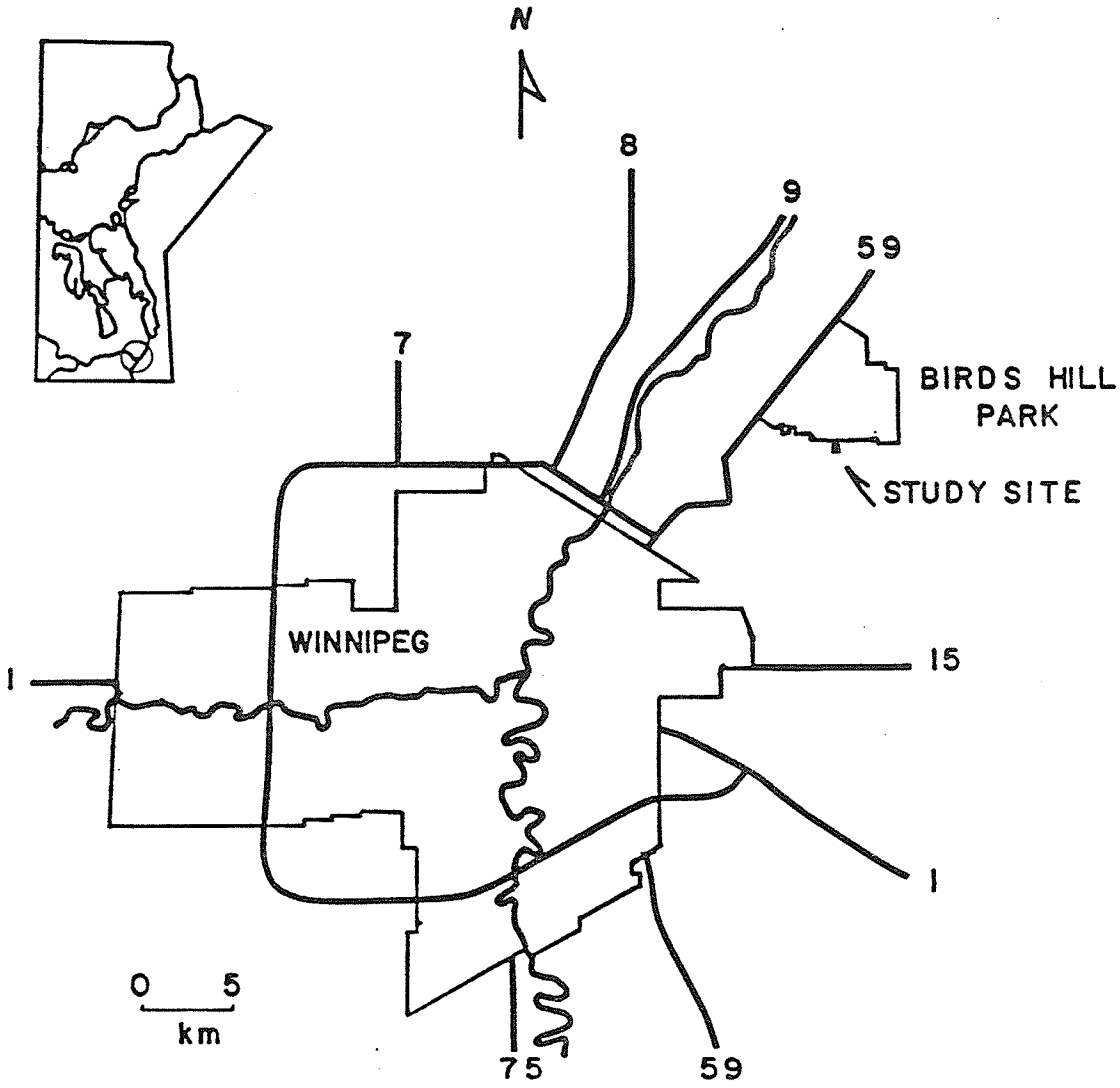
3.1 DESCRIPTION OF STUDY SITES

Two study sites were established along the southern border of Birds Hill Park, in the Rural Municipality of Springfield (Fig. 6). The official location of both sites described on the Universal Transverse Mercator grid reference sheet G2 H/15 west half for Dugald, series A743, is J21403. Rowe (1974) described the region as Aspen-Oak parkland (Fig. 7).

The dominant soil type within the study sites is a member of the Leary series and consists of well to excessively drained dark gray soils on coarse, gravel beach and outwash deposits. The thin, sandy mantle results in low water retention. The general topography is very gently sloping (Canadian-Manitoba Soil Survey 1975). The elevation for the area is 269m above sea level.

The dominant vegetation consisted of large trees such as trembling aspen (Populus tremuloides Michx.), bur oak (Quercus macrocarpus Michx.) and bushes such as saskatoon (Amalanchier alnifolia Nutt.); the understory was comprised of various grasses, wild flowers and a high density of poison ivy (Rhus radicans L.) (See Burachynsky 1982 for a list of plant spp.).

Figure 6: Location of Birds Hill research study site, in Manitoba, Canada (numbers indicate provincial highways).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7
BIRDS HILL
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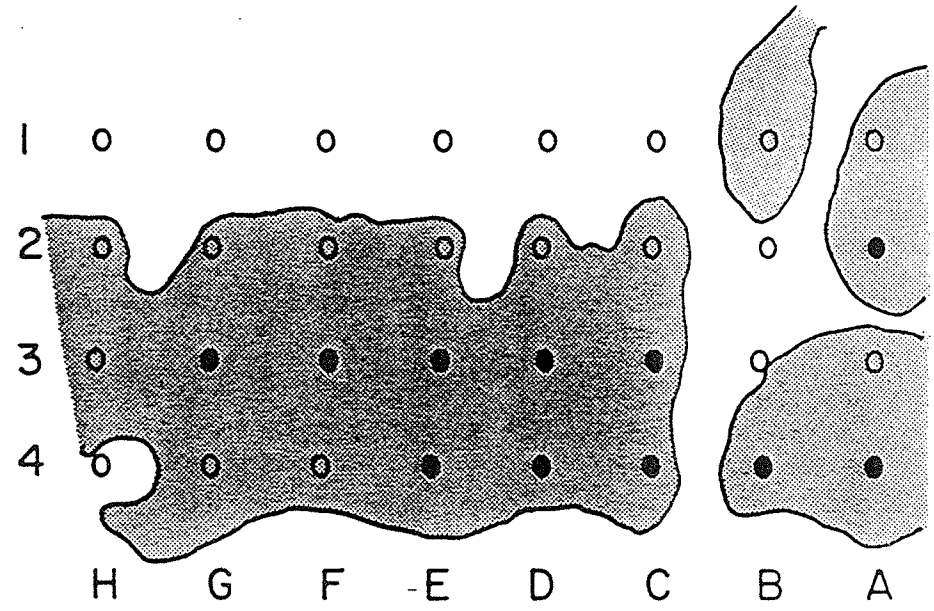
Figure 7: Representation of typical oak-aspen parkland habitat of Spermophilus franklinii at Birds Hill, Manitoba, June 1982.

3.2 TRAPPING REGIME

A 40m x 80m grid was established on two sites (1 row along the forest ecotone and 3 rows inside the forest) with traps located at 10m intervals (Fig. 8). As a result of vandalism during trapping period four in 1982, the trapping design was altered to reduce the likelihood of further incidents. Thus, only stations within the ecotone that yielded squirrels prior to vandalism were maintained for the duration of the season (darkened circles in Fig. 8). One 13cm x 13cm x 41cm wire live trap (Fig. 9) or one 7.5cm x 7.5cm x 12.5cm box trap (Tomahawk Live Trap Co., Tomahawk, Wisconsin, model nos. 202, 101 respectively) was placed within 1 metre of each station. Masonite® covers were positioned over each trap to protect the animal from sun, rain and snow (Fig. 9). Both styles of traps were used during the 1982 season. Eighteen stations were monitored weekly on plot 3 (10 stations with box traps; 8 stations with wire traps) and 19 stations monitored weekly on plot 4 (11 stations with box traps; 8 stations with wire traps). In 1983, wire traps were used exclusively at 11 and 13 stations on plots 3 and 4, respectively. Traps were set once a week on each plot between 0800-1000h. and examined 24 hours later. Captured squirrels were brought to the laboratory for further inspection and released at the capture station the following morning. Trapping regime and animal inspection procedure was followed as used by Burachynsky and Galloway (1985).

Figure 8: Trapping grid on study plots three and four near Birds Hill, Manitoba (shaded area = forest, open area = ecotone) (open circles = trapping on site for three weeks in 1982, closed circles = trapping for entire 1982 and 1983 season).

PLOT 3



PLOT 4

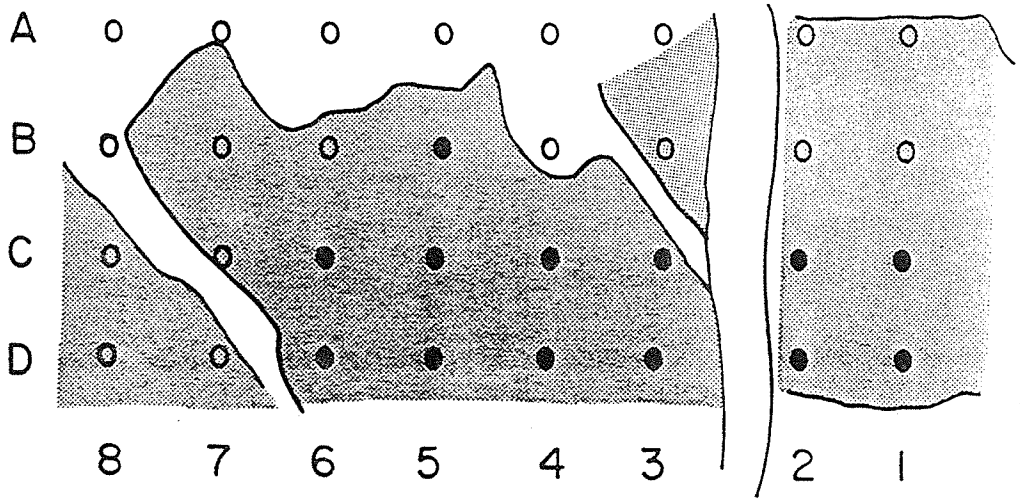


Figure 9: Tomahawk live trap model numbers 202 (centre) and 101 (right) used to capture Spermophilus franklinii with Masonite® shelters to protect animal from adverse weather conditions.

A control site, designated Plot 5, was established 1 km east of Plots 3 and 4 in 1983. Traps were set in a 40m x 70m matrix at 10m intervals. Based on 1982 data, Plot 5 was monitored for 1 to 4 consecutive days, during trapping periods of highest mean flea intensity (early May and early August) and lowest mean flea intensity (end of June). Data from Plots 3 and 4 were compared with Plot 5 to determine whether mid-season reduction in flea intensity was biased by trapping and/or flea removal activities.

The 1982 season began on 4 May and continued until 22 September. During the week of 23-29 May no squirrels were captured. Trapping ceased when no squirrels were captured during two consecutive weeks in September. The traps were baited with a mixture of Squirrel brand crunchy peanut butter and rolled oats; sliced carrots were placed beside the bait as a water source during extremely hot weather.

3.3 REMOVAL OF ECTOPARASITES

Each squirrel was assigned an identification number in the laboratory during its first capture and weighed on a Mettler P1200 scale. Reproductive condition of male and female squirrels was recorded on each capture. The pelage of each squirrel was inspected for molting characteristics such as initial molting area, area of hair loss, area of new hair growth and based on these characters, was assigned an arbitrary molting factor. Wounded animals received veterinary

assistance in the laboratory. Each squirrel was placed in a glass chamber containing ethyl ether vapour until unconscious. Each squirrel was held by the hind legs over a white plastic tray while the pelage was vigorously brushed by hand to remove any loosely attached fleas. The pelage was further examined by combing and lightly blowing on the hairs dislodging any remaining fleas. Mean intensity and prevalence were calculated for O. bruneri based on the definitions for each parameter as interpreted by Margolis et al. (1982).

Fleas were placed in stoppered glass vials and stored at -15 °C ± 1 °C. Under these conditions fleas could be stored at least 6 months with no discernable deterioration of internal tissues. On a few occasions fleas became freeze-dried and any attempt to dissect the flea would inadvertently destroy the rest of the flea's internal organs. Freeze-dried fleas were rehydrated in a cool solution of tri-sodium orthoposphate (Na_3PO_4) and physiological saline. Dissection procedures are outlined in Appendix A. Records of flea associates and parasites are described in Appendix B. The data were analyzed in two-week periods as done by Mihok (1979) and Burachynsky and Galloway (1980, 1985).

3.4 DESCRIPTION OF FEMALE REPRODUCTIVE ORGANS

Opisocrostis bruneri has paired panoistic ovarioles, each comprised of a distinct terminal filament, germarium, vitellarium and pedicel (Mead-Briggs 1962). The number of

ovarioles per ovary ranged from four to nine (avg. = 6). Ovarioles contained oocytes at various developmental stages. The stage of maturation (i.e. ovariole rating) of the largest proximal oocyte was based on parameters as outlined for S. cuniculi by Mead-Briggs (1962). Klein (1966) further subdivided the stages of ovarian development based on additional parameters such as colour of oocyte, number of eggs laid per ovariole and presence or absence of tylenchid nematodes, but these stages were not adapted here.

One of the five ovarian developmental stages as defined by Mead-Briggs (1962) was assigned, based on the largest proximal ooctye within each female. The five developmental stages were later combined into three separate categories for ease of analysis: stage 0 was assigned as an undifferentiated oocyte; stages 1 and 2 were assigned to early developing oocytes; stages 3 and 4 were assigned to oocytes near or completing development (Figs. 10,11). The reproductive state of each female was based on three criteria; ovarian developmental stage, the presence or absence of sperm and the presence or absence of a corpus luteum. Females with stage 1 or 2 oocytes were combined into the "early maturing" category or stage 1 ovariole group. Similarly, females with stage 3 or 4 oocytes were combined into the "near-mature and mature" category or stage 2 ovariole group (Table 1).

The presence or absence of spermatozoa in the spermatheca was recorded for each female flea in 1982 and 1983. The

spermatheca is a storage organ for spermatozoa and is comprised of two connected compartments; the bulga and hilla. The spermatozoa are long and conspicuous and after mating were stored within both compartments. An additional criterion, the presence or absence of the corpus luteum, was used in 1983 to further evaluate the reproductive condition of each female flea. The corpus luteum was yellow and presumably a follicular epithelial relic remaining in the pedicel post-ovulation (Fig. 12).

Table 1. Description of the ovarian developmental stages based on the measurment of the largest proximal oocyte found within a female Opisocrostis bruneri removed from Spermophilus franklinii near Birds Hill Park, Manitoba 1982-1983.

Stage of Maturation	Text Figure	Germinal Vesicle	Oocyte Colour	Mean Size of Oocyte (L x W)	Amount of Yolk Depostion	Corpus Luteum	Reproductive Activity
0	-	visible	transparent	less than .15mm x .10mm	none	absent	- newly emerged
1	10 (1-2)	visible	whitish to light yellow, translucent	.15mm x .10mm to .25mm x .17mm	up to 10% of oocyte volume	absent present	- maturation has commenced within a newly emerged nulliparous female - ovulation has occured in a parous female
2	10 (3-4)	not visible	dark yellow, opaque. chorion is present	.45mm x .30mm to .67mm x .45mm	50% to 100% of oocyte volume	absent present	- maturation has commenced within a newly emerged nulliparous female - ovulation has occured in a parous female

Figure 10: Photographic representation of the ovarian ratings assigned to ovarioles developing within Opisocrostis bruneri. (Adapted from Mead-Briggs 1962).

Legend

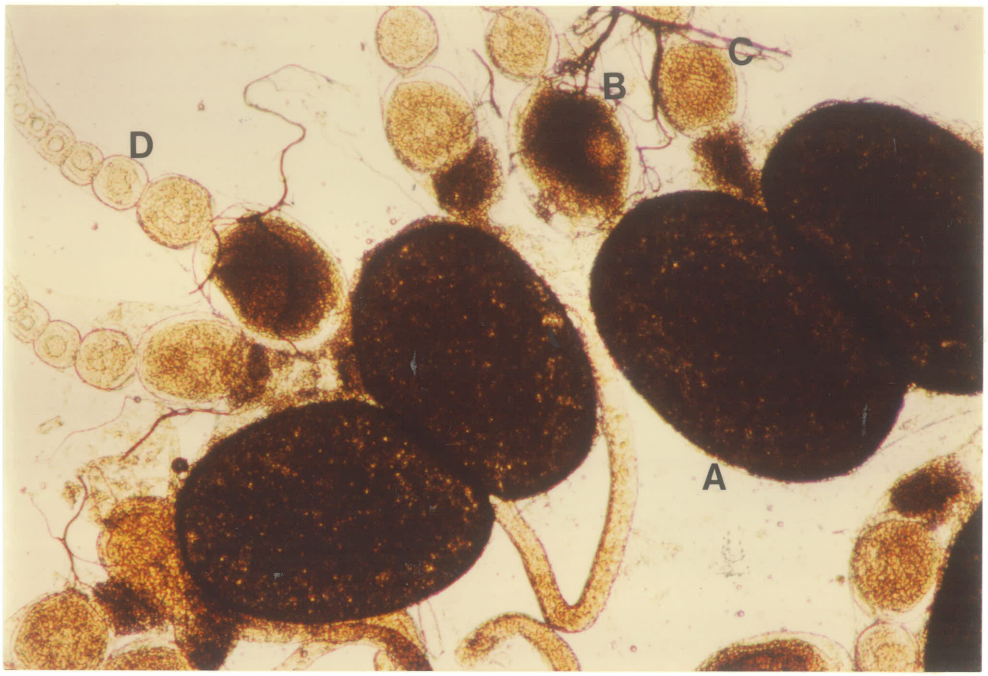
A - Stage 4 ovariole

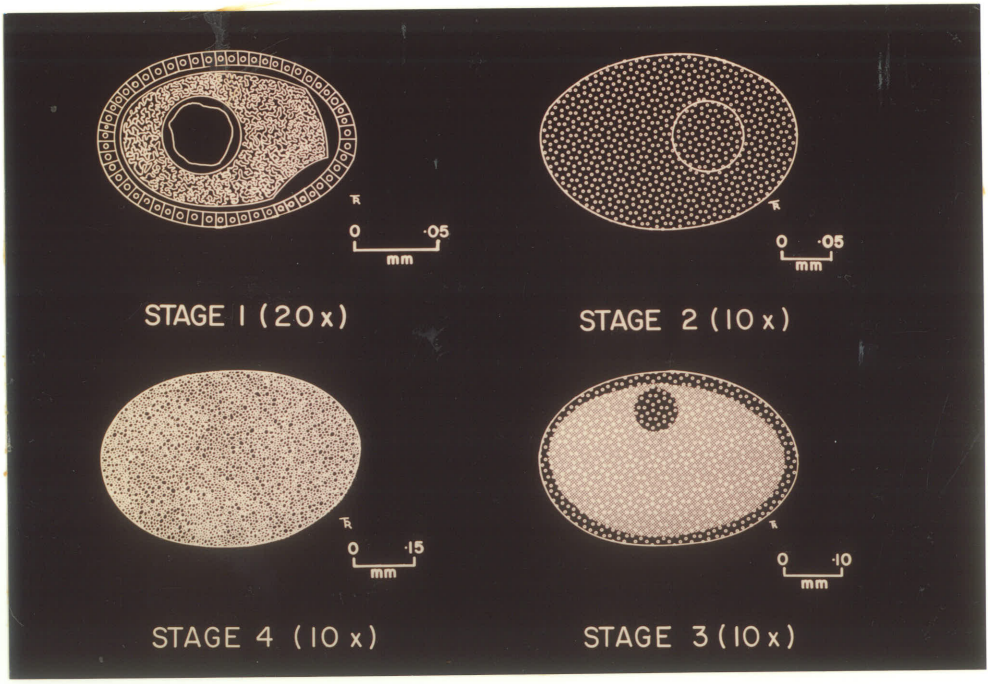
B - Stage 3 ovariole

C - Stage 2 ovariole

D - Stage 1 ovariole

Figure 11: Graphic illustration of the ovarian rating assigned to ovarioles developing within female Opisocrostis bruneri.


D
B
C
A


0 ·05
mm
STAGE 1 (20x)
0 ·05
mm
STAGE 2 (10x)
0 ·15
mm
STAGE 4 (10x)
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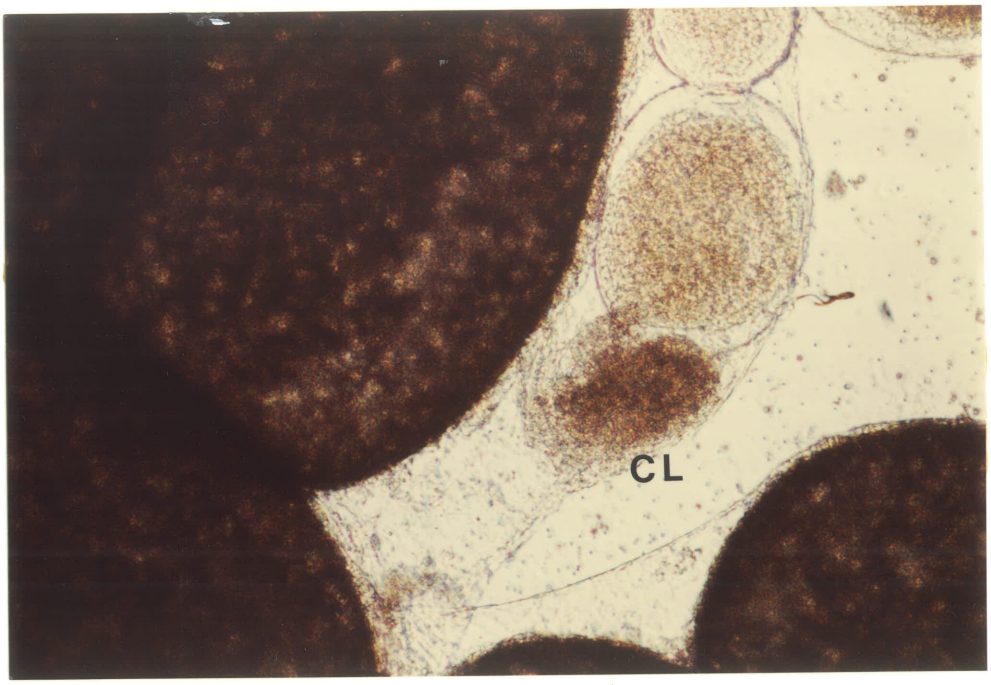
mm
STAGE 3 (10x)

Figure 12: The corpus luteum (CL), a follicular relic, located within the lateral oviduct of a female Opisocrostis bruneri.


CL

Chapter IV

RESULTS

4.1 MAMMAL CAPTURES

4.1.1 General

The first captures in each season, on 5 May, were adult male squirrels. The first adult females were captured on 13 and 10 May in 1982 and 1983 respectively. Notably pregnant female squirrels were first captured on 3 and 7 June in 1982 and 1983 respectively. The first observed signs of above ground juvenile squirrel activity occurred on 20 July, 1982 and 12 July, 1983. Generally the adult male squirrels are first to hibernate subsequently followed by adult females (non-bearing before bearing), juvenile females and finally juvenile males (Banfield 1981). Immergence in 1982 proceeded accordingly, however, the adult females preceded adult males in 1983. The last adult male was captured on 17 and 31 August in 1982 and 1983 respectively. Adult females were last captured on 25 August, 1982 and 3 August, 1983. The last capture of a juvenile female was 8 September in both years, and the last juvenile males were captured on 10 and 20 September in 1982 and 1983 respectively.

Emerging adult squirrels had long black guard hairs and a dense undercoat of short white hairs. The molting of winter pelage was observed during 15 May to 11 June. The summer pelage consisted of only long and short bristled guard hairs.

Juvenile squirrels emerged from natal burrows with a summer pelage. The fall pelage began to appear in mid-July and mid-August on adult and juvenile squirrels, respectively.

4.1.2 1982

A total of 36 individual squirrels contributed 95 total captures (recapture rate = 2.6) within 552 trap days (Table 2). Of all the captures in 1982, adult females comprised 53%, adult males 27%, juvenile females 12% and juvenile males 8%. The adult females comprised 39% of the total individuals, adult males 30%, juvenile males 17% and juvenile females 14%.

4.1.3 1983

Throughout 1983, 40 individual squirrels contributed 161 total captures (recapture rate = 4.0) within 504 trap days (Table 2). Of all the captures in 1983 adult males comprised 57%, adult females 21%, juvenile males 8% and juvenile females 8%. The adult males comprised 48% of the total individuals, juvenile females 22%, adult females 20% and juvenile males 10%.

4.2 FLEAS REMOVED FROM S. FRANKLINII

4.2.1 1982

Eight hundred and forty-nine O. bruneri were recovered from 95 squirrel captures (Table 3). The observed sex ratio (m/f) for the sampled fleas was 0.70. Of the total fleas, 59%

Table 2. Summary of *Spermophilus franklinii* captured in 1982-1983 near Birds Hill Park, Manitoba, and the number of captures (C), per cent of total captures (%), individuals (I), per cent of total individuals (%) and recapture rates (RR).

	1982					1983				
Spermophilus franklinii	C	%	I	%	RR	C	%	I	%	RR
Adult Male	26	27	11	31	2.4	92	57	19	48	4.8
Adult Female	51	54	14	39	3.6	34	21	8	20	4.3
Juv. Male	7	7	6	17	1.2	12	8	4	10	3.0
Juv. Female	11	12	5	14	2.2	23	14	9	22	2.6
Totals	95		36		2.6	161		40		4.0

were female and 41% were male. Adult male squirrels contributed 43% of the total fleas collected, adult females 36%, juvenile males 13% ,and juvenile females 8%. The observed sex ratio (m/f) on adult male squirrels was 0.83, adult females 0.64, juvenile females 0.62 and juvenile males 0.52. The biweekly sex ratio (m/f) of O. bruneri was greater than 1.00 during two periods, beginning 4 May and 1 July (Table 4).

The observed flea sex ratio (m/f) of O. bruneri on male and female squirrels, particularly during the reproductive period of S. franklinii was not significantly different (ANOVA, $P > .05$) during each 2-week trapping period. Therefore female fleas did not selectively seek reproductively active female squirrels and flea sex ratios were not biased toward squirrel sex or age.

4.2.2 1983

In 1983, 1503 O. bruneri were removed from 161 squirrel captures (Table 3). The observed sex ratio (m/f) was 0.73. Total flea captures were comprised of 58% female and 42% male. Of all the fleas removed, adult male squirrels contributed 64%, adult females 8%, juvenile females 15%, and juvenile males 14%. The observed sex ratio (m/f) on adult male squirrels was 0.78, adult females 0.79, juvenile females 0.64, juvenile males 0.57. The biweekly sex ratio of O. bruneri was

greater than 1.00 during two periods, beginning 4 May and June 26 (Table 4).

The observed sex ratio (m/f) of O. bruneri on male and female squirrels, particularly during the reproductive period of S. franklinii was not significantly different (ANOVA, $P > .05$) during each 2-week trapping period. Therefore female fleas did not selectively seek reproductively active female squirrels and flea sex ratios were not biased toward squirrel sex or age.

Two hundred and forty-four O. bruneri were removed from 24 individual squirrels on Plot 5 (Table 3). The observed sex ratio (m/f) was 0.65 for fleas on Plot 5. Male and female fleas comprised 40% and 60% respectively of all recovered fleas. Adult male squirrels contributed 47%, adult female squirrels 34%, juvenile male squirrels 17%, and juvenile female squirrels 2% of all removed fleas. The observed sex ratio (m/f) of fleas on adult female squirrels was 0.80, adult male squirrels 0.63, juvenile male squirrels 0.58, and juvenile female squirrels 0.20.

4.3 INFESTATION PARAMETERS OF O. BRUNERI

4.3.1 1982

The observed prevalence of O. bruneri was never lower than 75% (Table 5). The adult male squirrels maintained a high prevalence of fleas throughout the season (Table 6). Adult female squirrels displayed the lowest prevalence and

Table 3. Observed sex ratio (M/F), including totals (T) of male (M) and female (F) Opisocrostis bruneri removed from Spermophilus franklinii captured near Birds Hill Park, Manitoba, 1982-1983.

	Total number of removed fleas											
	1982				1983				1983 - Plot 5			
Host sex	M	F	(M/F)	T	M	F	(M/F)	T	M	F	(M/F)	T
Adult male	166	201	0.83	367	419	538	0.78	957	44	70	0.63	114
Adult female	120	188	0.64	308	50	63	0.79	113	37	46	0.80	83
Juvenile male	42	68	0.62	110	78	137	0.57	215	15	26	0.58	41
Juvenile female	22	42	0.52	64	85	133	0.64	218	1	5	0.20	6
All combined	350	499	0.70	849	632	871	0.72	1503	97	147	0.65	244

Table 4. Observed sex ratios (M/F) of Opisocrostis bruneri removed from Spermophilus franklinii captured (C) at Birds Hill Park, Manitoba, 1982-1983.

1982			1983		
Week beginning	C	M/F	Week beginning	C	M/F
4 May	5	1.02	4 May	11	1.15
16 May	4	0.77	15 May	16	0.73
6 June	10	0.82	29 May	15	0.93
20 June	15	0.63	12 June	18	0.76
1 July	15	1.11	26 June	20	1.03
18 July	15	0.60	10 July	25	0.62
1 Aug.	16	0.54	24 July	24	1.59
15 Aug.	11	0.50	7 Aug.	15	0.63
29 Aug.	4	0.64	21 Aug.	11	0.51
12 Sep.	0	-	4 Sep.	5	0.70
			18 Sep.	1	0.79
Totals	95	0.70		161	0.72

Table 5. Infestation parameters including prevalence (P) and mean intensity (MI) for Opisocrostis bruneri removed from Spermophilus franklinii captured (C) at Birds Hill Park, Manitoba, 1982-1983.

1982				1983			
Week beginning	C	P	MI	Week beginning	C	P	MI
4 May	5	1.00	33.2	4 May	11	1.00	10.6
16 May	4	0.75	7.7	15 May	16	1.00	12.3
6 June	10	0.80	11.3	29 May	15	0.80	14.3
20 June	15	0.80	6.3	12 June	18	0.88	11.8
1 July	15	0.87	4.5	26 June	20	0.75	4.1
18 July	15	0.87	4.3	10 July	25	0.84	4.8
1 Aug.	16	0.94	12.7	24 July	24	1.00	8.2
15 Aug.	11	1.00	13.6	7 Aug.	15	1.00	12.1
29 Aug.	4	1.00	23.8	21 Aug.	11	1.00	9.9
12 Sep.	0	-	-	4 Sep.	5	1.00	30.0
				18 Sep.	1	1.00	25.0
Totals	95	0.88	10.1		161	0.91	10.2

Table 6. Infestation parameters including prevalence (P) and mean intensity (MI) for Opisocrostis bruneri removed from adult male Spermophilus franklinii captured (C) at Birds Hill Park, Manitoba, 1982-1983.

1982				1983			
Week beginning	C	P	MI	Week beginning	C	P	MI
4 May	4	1.00	40.3	4 May	9	1.00	11.6
16 May	0	-	-	15 May	11	1.00	16.1
6 June	4	1.00	14.3	29 May	9	0.89	18.8
20 June	6	1.00	7.8	12 June	12	1.00	14.8
1 July	7	0.86	7.7	26 June	11	0.73	5.3
18 July	2	1.00	1.5	10 July	19	0.84	4.6
1 Aug.	2	1.00	13.0	24 July	14	1.00	9.4
15 Aug.	1	1.00	27.0	7 Aug.	6	1.00	13.5
29 Aug.	0	-	-	21 Aug.	1	1.00	19.0
12 Sep.	0	-	-	4 Sep.	0	-	-
				18 Sep.	0	-	-
Totals	26	0.96	14.7		92	0.92	11.3

Table 7. Infestation parameters including prevalence (P) and mean intensity (MI) for Opisocrostis bruneri removed from adult female Spermophilus franklinii captured (C) at Birds Hill Park, Manitoba, 1982-1983.

1982				1983			
Week beginning	C	P	MI	Week beginning	C	P	MI
4 May	1	1.00	5.0	4 May	2	1.00	6.0
16 May	4	0.75	7.7	15 May	5	1.00	4.0
6 June	6	0.67	8.5	29 May	6	0.67	6.8
20 June	9	0.67	4.7	12 June	6	0.67	2.5
1 July	8	0.88	1.8	26 June	9	0.78	2.7
18 July	9	0.77	5.0	10 July	4	0.75	3.0
1 Aug.	10	0.90	6.6	24 July	2	1.00	8.0
15 Aug.	4	1.00	25.3	7 Aug.	0	-	-
29 Aug.	0	-	-	21 Aug.	0	-	-
12 Sep.	0	-	-	4 Sep.	0	-	-
				18 Sep.	0	-	-
Totals	51	0.80	7.5		34	0.79	4.2

Table 8. Infestation parameters including prevalence (P) and mean intensity (MI) for Opisocrostis bruneri removed from juvenile male Spermophilus franklinii captured (C) at Birds Hill Park, Manitoba, 1982-1983.

1982				1983			
Week beginning	C	P	MI	Week beginning	C	P	MI
4 May	0	-	-	4 May	0	-	-
16 May	0	-	-	15 May	0	-	-
6 June	0	-	-	29 May	0	-	-
20 June	0	-	-	12 June	0	-	-
1 July	0	-	-	26 June	0	-	-
18 July	2	1.00	2.5	10 July	1	1.00	17.0
1 Aug.	1	1.00	2.0	24 July	2	1.00	5.5
15 Aug.	1	1.00	10.0	7 Aug.	3	1.00	11.3
29 Aug.	3	1.00	31.0	21 Aug.	2	1.00	11.0
12 Sep.	0	-	-	4 Sep.	3	1.00	35.3
				18 Sep.	1	1.00	25.0
Totals	7	1.00	15.7		12	1.00	17.9

Table 9. Infestation parameters including prevalence (P) and mean intensity (MI) for Opisocrostis bruneri removed from juvenile female Spermophilus franklinii captured (C) at Birds Hill Park, Manitoba, 1982-1983.

1982				1983			
Week beginning	C	P	MI	Week beginning	C	P	MI
4 May	0	-	-	4 May	0	-	-
16 May	0	-	-	15 May	0	-	-
6 June	0	-	-	29 May	0	-	-
20 June	0	-	-	12 June	0	-	-
1 July	0	-	-	26 June	0	-	-
18 July	2	1.00	1.5	10 July	1	1.00	1.0
1 Aug.	3	1.00	15.7	24 July	6	1.00	6.3
15 Aug.	5	1.00	3.2	7 Aug.	6	1.00	11.2
29 Aug.	3	1.00	2.0	21 Aug.	8	1.00	8.5
12 Sep.	0	-	-	4 Sep.	2	1.00	22.0
				18 Sep.	0	-	-
Totals	11	1.00	6.2		23	1.00	9.5

only on two occasions were all females infected with fleas (Table 7). All captured juvenile male and female squirrels were infested with O. bruneri (Tables 8,9).

The highest levels of mean intensity, based on the total of all fleas removed from S. franklinii were observed in the spring, extending from 4 May to 15 May, and later near the end of the trapping season, extending from 29 August to 12 September (Table 5). The period of lowest mean intensity extended from 20 June to 31 July. Two peaks in mean intensity on adult males occurred from 4 May to 15 May and 15 August to 28 August respectively (Table 6), while the period of lowest intensity extended from 20 June to 31 July. Peaks in mean intensity for adult females occurred from 16 May to 19 June and 15 August to 28 August (Table 7). The period of lowest mean intensity lasted from 20 June to 17 July. The highest level of mean intensity on juvenile males occurred from 1 August to 14 August (Table 8). Mean intensity on juvenile females peaked earlier from 1 August to 14 August although based on relatively few captures (Table 9).

4.3.2 1983

The prevalence of O. bruneri on all trapped S. franklinii was 100% during the trapping season except 24 May to 23 July (Table 5). The lowest level of prevalence of O. bruneri on adult male and female squirrels was 73% and 67% respectively

(Tables 6,7). All juvenile male and female squirrels were infested (Tables 8,9).

The highest levels of mean intensity occurred during two discrete periods, from 15 May to 11 June and 4 September to 20 September, respectively (Table 5). The lowest level of mean intensity extended from 26 June to 23 July. Mean intensity of adult males was highest from 15 May to 23 June and 7 August to 3 September, respectively (Table 6). The lowest mean intensity occurred from 26 June until 23 July. The two periods of highest mean intensity observed on adult females extended from 29 May to 11 June and 24 July to 6 August respectively (Table 7). The period of lowest mean intensity extended from 12 June to 23 July. Highest mean intensity observed on juvenile males and females extended from 4 to 20 September and 4 to 17 September, respectively (Tables 8,9).

4.3.3 1983-Plot 5

Ninety per cent of all squirrels captured on control plot 5 were infested with O. bruneri. The highest level of mean intensity, based on fleas removed from all captured S. franklinii, extended from 15 to 22 May and 7 to 14 August, respectively (Table 10). Lowest level of mean intensity was observed from 26 June to 23 July. These fluctuations in mean intensity on S. franklinii were observed simultaneously on plots 3, 4 and 5. The observed mean intensity on a S. franklinii population from plots 3 and 4 (combined) compared to plot 5 during the periods starting 15 May,

Table 10. Comparison of biweekly mean intensity (MI) for Opisocrostis bruneri removed from Spermophilus franklinii captured (C) in main study site (plots 3 and 4 combined) and plot 5 near Birds Hill Park, Manitoba, 1983.

Week beginning	Week ending	Main Site C	Main Site MI	Plot 5 C	Plot 5 MI
15 May	22 May	16	12.3	4	19.8
26 June	9 July	20	4.1	10	3.3
10 July	23 July	25	4.8	2	7.5
7 Aug.	14 Aug.	15	12.1	8	15.0
Totals		76	8.3	24	10.6

26 June, 10 July and 7 August was not significantly different (using two-way factorial analysis, $P > .05$).

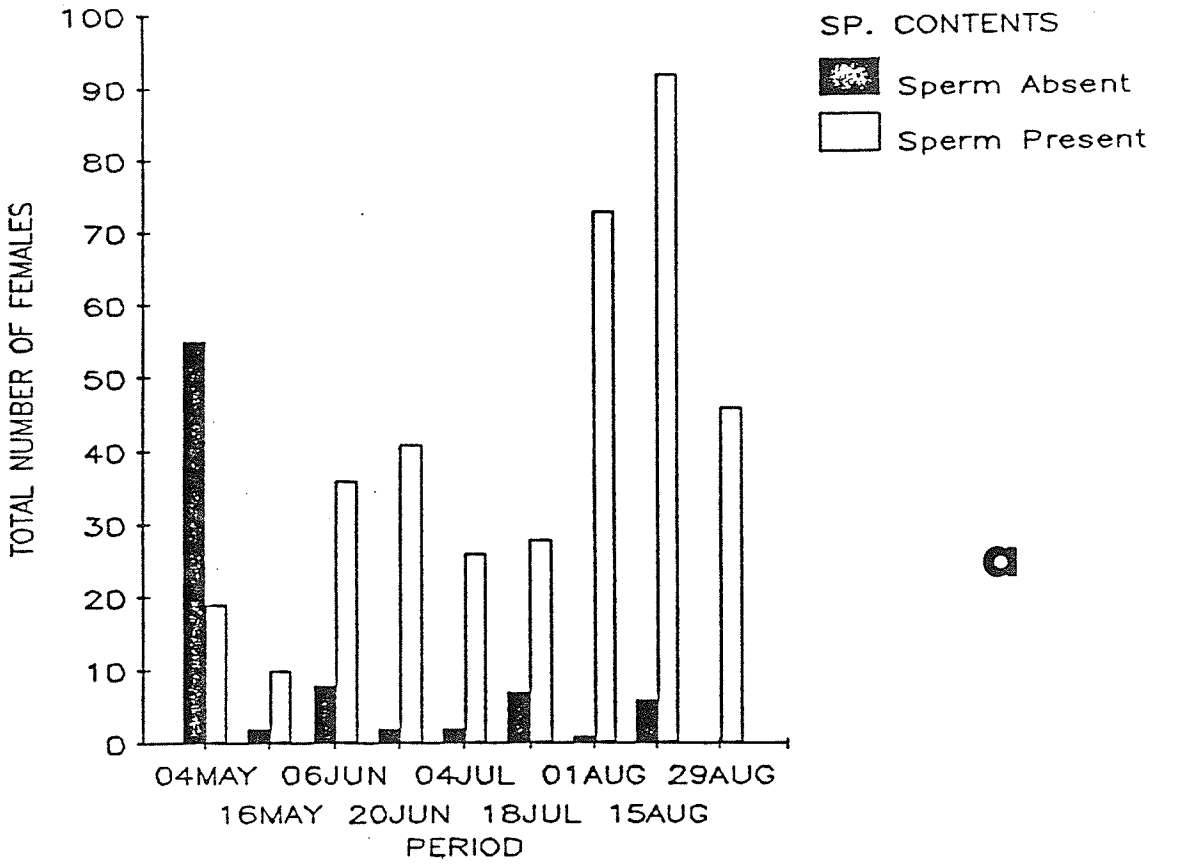
4.4 REPRODUCTIVE CONDITION OF O. BRUNERI

4.4.1 1982

Female fleas containing immature ovarioles were predominant during the first three trapping periods (Fig. 13, Table 11). Dissected females containing stage 0 and 1 oocytes comprised 91% (26% and 65% respectively) of the total fleas removed from squirrels during 4 to 15 May. Greater than 79% of the total fleas removed during 20 June to 28 August contained mature stage 2 ovarioles - the remainder was comprised of females containing stage 0 and 1 ovarioles. Females containing allantonematid nematodes were not included in further analysis since nematodes within the haemocoel suppressed ovarian development. During the last trapping period females containing either stage 1 or 2 ovarioles comprised 33% and 67% respectively, of the total captured fleas.

The highest biweekly percentage of females, with an empty spermatheca and containing stage 0 or 1 ovarioles, during trapping periods 4 and 15 May to 5 June, was 74% and 33% respectively (Fig. 13, Table 11). No less than 74% of female fleas captured from 20 June to 28 August had sperm within the spermatheca and stage 2 ovarioles with the exception of the period from 18 July to 31 July when females with an empty

Figure 13: (a) Totals of female Opisocrostis bruneri with sperm present or absent in the spermatheca (SP.), and (b) totals of ovarian ratings from Opisocrostis bruneri collected from Spermophilus franklinii near Birds Hill Park, Manitoba in 1982.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Sperm Present
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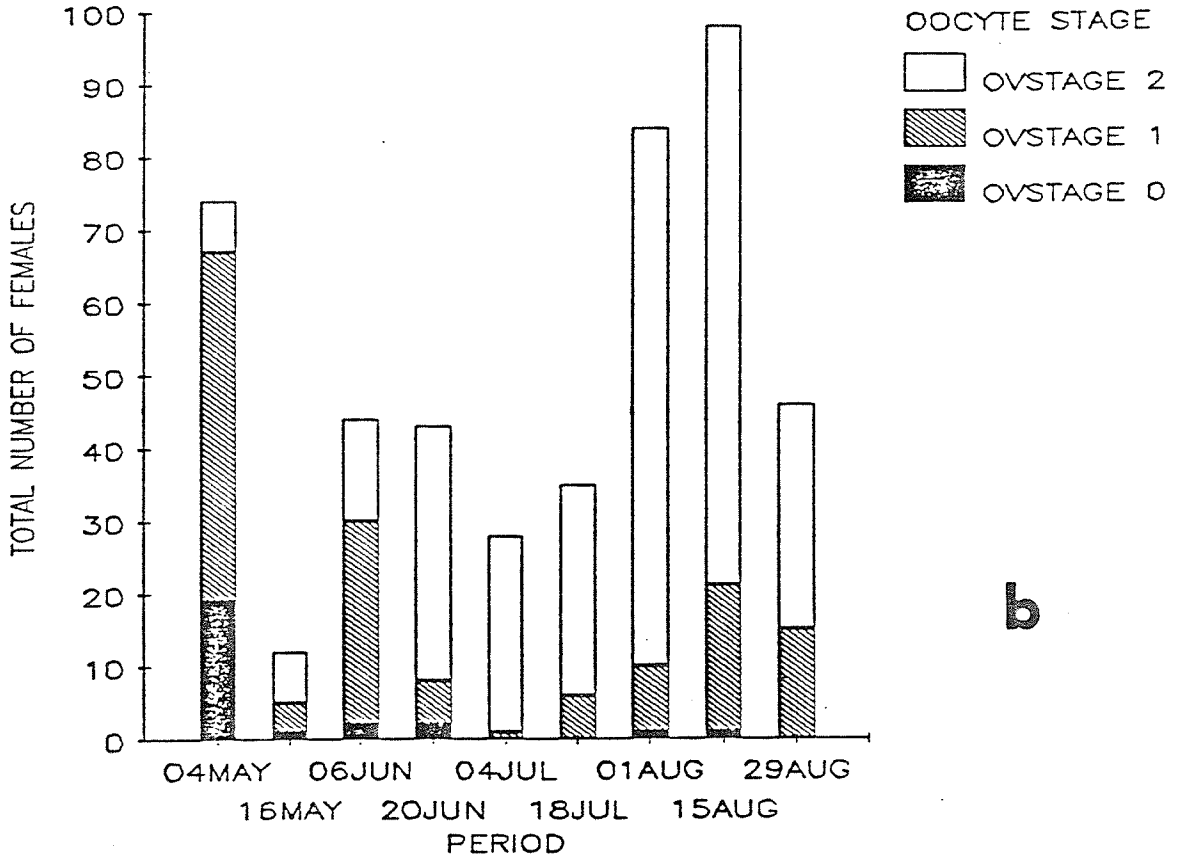
90
80
70
60
50
40
30
20
10
0
04MAY 06JUN 04JUL 01AUG 29AUG
16MAY 20JUN 18JUL 15AUG
PERIOD

a


OOCYTE STAGE
OVSTAGE 2
OVSTAGE 1
OVSTAGE 0
TOTAL NUMBER OF FEMALES
100
90
80
70
60
50
40
30
20
10
0
04MAY 06JUN 04JUL 01AUG 29AUG
16MAY 20JUN 18JUL 15AUG
PERIOD

b

Table 11. Percentage (%) of ovarian stage ratings (0-2) assessed to female Opisocrostis bruneri (with sperm present within the spermatheca) and total female fleas (T) removed from Spermophilus franklinii captured near Birds Hill Park, Manitoba, 1982.

Week beginning	Ovarian Stage 0 %	0 T	1 %	1 T	2 %	2 T	Total
4 May	4	19	12	48	9	7	74
16 May	8	1	33	4	59	7	12
6 June	5	2	50	28	27	14	44
20 June	5	2	12	6	79	35	43
1 July	0	0	0	1	92	27	28
18 July	0	0	6	6	74	29	35
1 Aug.	0	0	11	9	88	74	83
15 Aug.	1	1	16	20	76	77	98
29 Aug.	0	0	33	15	67	31	46
12 Sep.	0	0	0	0	0	0	0
Totals		25		137		301	463

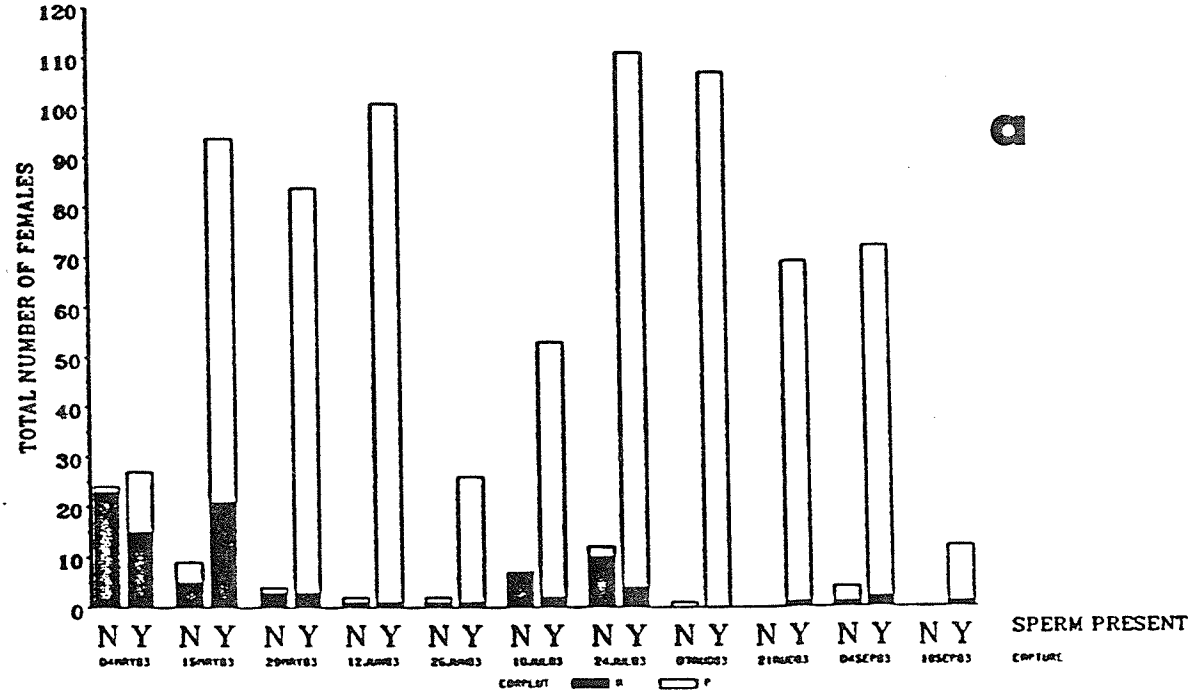
spermatheca and either stage 1 or 2 ovarioles comprised 11% and 9% of the total captured fleas.

4.4.2 1983

Female fleas with undeveloped ovarioles were dominant throughout the first three trapping periods. Female fleas with stage 0 and stage 1 ovarioles comprised 72% (1% and 71% respectively) of the total from 4 May to 14 May (Fig. 14, Table 12). Females containing stage 2 ovarioles comprised no less than 72% of total captured fleas from 15 May to 17 September. During the last trapping period stage 1 and stage 2 comprised 41% and 59%, respectively, of the total ovarioles from dissected female fleas.

The highest occurrence of females with an empty spermatheca was 44% during the first trapping period; of these females, 42% contained stage 1 ovarioles and no corpus luteum while the remaining 2% contained stage 2 ovarioles without a corpus luteum (Fig. 14, Table 12). Females containing sperm within the spermatheca, and with either stage 1 or 2 ovarioles and a corpus luteum comprised no less than 83% of the females captured from 29 May to 22 September. Nulliparous fleas (containing stage 1 ovarioles without a corpus luteum) with or without sperm comprised 8% and 5% respectively, of all captured fleas during 10 July to 6 August. Three nulliparous females were recovered between 4 and 22 September.

Figure 14: (a) Totals of female Opisocrostis bruneri with (Y) and without sperm (N) within the spermatheca (corpus luteum present (P) or absent (A)), and (b) totals of ovarian ratings from Opisocrostis bruneri collected from Spermophilus franklinii near Birds Hill Park, Manitoba, 1983.


TOTAL NUMBER OF FEMALES
120
110
100
90
80
70
60
50
40
30
20
10
0
a
N Y N Y N Y N Y N Y N Y N Y N Y N Y N Y N Y
SPERM PRESENT

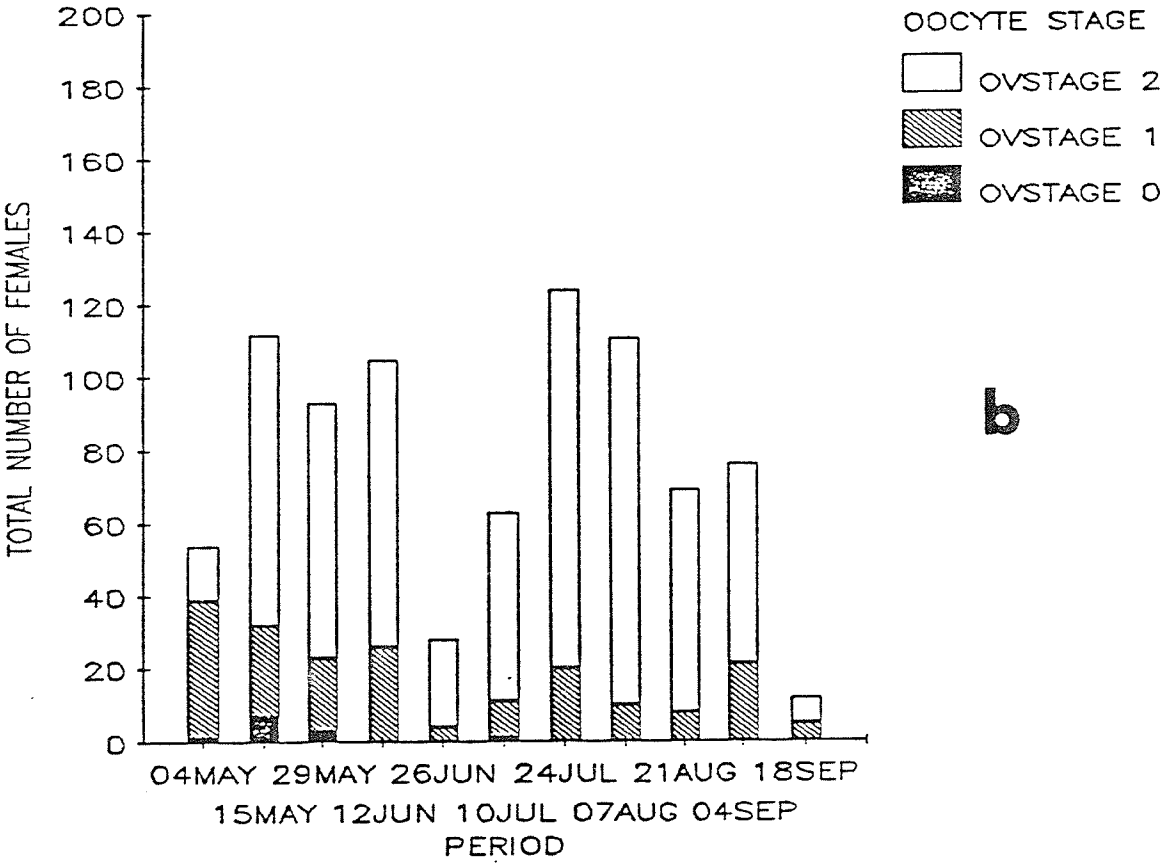

TOTAL NUMBER OF FEMALES
200
180
160
140
120
100
80
60
40
20
0
OOCYTE STAGE
OVSTAGE 2
OVSTAGE 1
OVSTAGE 0
b
04MAY 29MAY 26JUN 24JUL 21AUG 18SEP
15MAY 12JUN 10JUL 07AUG 04SEP
PERIOD

Table 12. Biweekly summary of ovarian stage ratings (0-2), including total fleas removed (T) and percentage of fleas with the same reproductive status (%), assigned to parous (corpus luteum present) female Opisocrostis bruneri with (Present) and without (Absent) sperm in the spermatheca removed from Spermophilus franklinii captured near Birds Hill Park, Manitoba, 1983.

Week beginning	Ovarian Stage 0				Ovarian Stage 1				Ovarian Stage 2				Total Number of fleas removed
	Present		Absent		Present		Absent		Present		Absent		
	%	T	%	T	%	T	%	T	%	T	%	T	
4 May	0	1	-	-	10	15	0	22	13	12	2	2	52
15 May	0	3	0	3	7	22	0	2	62	75	4	4	109
29 May	0	3	-	-	14	17	0	3	74	69	1	1	93
12 June	-	-	-	-	23	25	0	1	74	77	1	1	104
26 June	-	-	-	-	7	3	0	1	82	23	4	1	28
10 July	0	1	-	-	3	5	0	5	79	50	0	2	63
24 July	-	-	-	-	4	10	0	10	82	102	2	2	124
7 August	-	-	-	-	8	9	1	1	91	100	-	-	110
21 August	-	-	-	-	10	7	0	1	88	61	-	-	69
4 September	-	-	-	-	21	18	3	3	71	54	1	1	76
18 September	-	-	-	-	33	5	-	-	58	7	-	-	12
Totals		8		3		136		49		630		14	840

Chapter V

DISCUSSION

5.1 Host-Parasite Relationship

There were more captures of adult female squirrels and adult male squirrels in 1982 and 1983 respectively. These differences were probably due, in part, to trap exclusion caused by the smaller Sherman - style traps used in 1982. The likelihood of trap exclusion is supported, in part by the increased captures of heavier male squirrels in wire traps as observed in 1983. Since S. franklinii populations are susceptible to dramatic fluctuation (Sowls 1948, Banfield 1981) the observed change between the two trapping seasons was not an unusual deviation in populations and structure. The increased numbers of individual juvenile females in 1983 may have resulted from high mortality in overwintering adult female squirrels. There may also be newborn mortality as a result of separating female squirrels from their litter for up to 24 hours. The unguarded squirrels are susceptible to starvation, and possible predation from other animals including male S. franklinii (Sowls 1948). The relative proportion of captured individual adult and juvenile squirrels within the population, did not significantly change in 1983 i.e. the total number of fleas contributed by adult and juvenile squirrels was similar. The increase in adult male captures more than compensated for the reduction in adult female captures. The adult male

squirrels began above ground activity in spring 1-2 weeks prior to emergence of adult females. Adult males were first captured 5-10 days earlier than the first females. This time is spent establishing new breeding territories and searching for receptive females. In searching for optimal breeding sites, male S. franklinii may change residency three of four times after leaving the hibernation burrow (Haberman and Fleharty 1972). Male and to a lesser extent female Spermophilus disperse up to one month after mating. The host population is comprised of adult squirrels at the beginning of the season. Above ground activity of juvenile squirrels begins during the first week of July. During this time the young begin to forage and disperse to establish new homes (Banfield 1981).

Adult squirrels immerge during the period of late July to mid-August as observed near Birds Hill Park. The host population will be comprised of juvenile squirrels, for the duration of the season. The adult squirrels' departure reduces the remaining number of potential O. bruneri hosts.

The proportion of captured fleas for each year from adult (~76%) and juvenile (~24%) squirrels remained the same each trapping season despite fluctuations within the host population in 1983. This may be, in part, a result of adults spending more time above ground and being available earlier in the season. The mean intensity of O. bruneri on male squirrels was higher than on female squirrels and provided a larger component of the total number of captured fleas. Infestation bias

favouring male fleas was also observed with Thrassis bacchi gladiolis (Jordan) and Hoplopsyllus anomalus (Baker) removed from Spermophilus leucurus (Merriam) (Parker 1958). Male squirrels tend to occupy larger home ranges particularly in spring prior to mating. Flea populations within nests and on juvenile squirrels prior to emergence may also effect the flea sex ratios on male and female S. franklinii but this could only be determined by excavating active burrows and observing flea feeding activity on 2-3 week old squirrels.

The flea infestations on captured S. franklinii may be indicative of the flea sex ratio within the squirrel's nest since female S. cuniculi, in seeking an actively reproducing host, tend to be more active then male fleas (Rothschild and Ford 1973). Pregnant O. cuniculus harbour more female S. cuniculi than male O. cuniculus (Rothschild and Ford 1973) because female fleas seek and remain on pregnant rabbits which contain hormones that stimulate ovarian development in S. cuniculi.

Spermophilus franklinii completed one annual molt during the summer season. Molting of the thick winter pelage commenced soon after the 3rd week of May and was completed within one month. There was no evidence of a molt line as observed on other Spermophilus spp. (Hansen 1954); instead hair loss occurred in patches. This may have been a result of rigorously brushing each squirrel during flea removal. The adult squirrels bear a summer pelage for approximately 6 weeks.

During the middle of the period juvenile squirrels emerged and began above ground activity. The fall pelage appears on adult squirrels by late July, prior to hibernation. The fall pelage appeared on juvenile squirrels by early August.

The reduction in mean intensity of O. bruneri on S. franklinii during late June and early July coincided with two major changes within the host population, the appearance of juvenile squirrels and adult squirrels molting into the summer pelage. The host population increased with the introduction of juvenile squirrels, thus the potential number of O. bruneri hosts increased dependent on the actual number of new squirrels. There are 2-3 squirrels per hectare and the average litter size is 7.5 squirrels per female (Banfield 1981). The potential squirrel population could increase from 2 squirrels per hectare to 8 squirrels per hectare for up to 6 weeks. The prevalence of O. bruneri dropped below 100 per cent soon after molting commenced in adult squirrels. The condition of the host's pelage greatly affects flea infestation (Marshall 1981). The thick undercoat, found in the spring and fall, increased the likelihood of maintenance on the host by increasing the potential number of attachment sites. The sparse, wiry haired summer pelage does not enable the flea to remain on the host because there are fewer hairs available to contact and therefore the flea is more likely to be groomed out by the squirrel.

The reduction in mean intensity, during late June to late July, was not a sampling artifact. Changes in mean intensity observed on plot 5 were not significantly different from mean intensity on plots 3 and 4 during the same time periods. These characteristic changes in mean intensity were observed on the same site for three consecutive years (1981-1983) and also by Burachynsky and Galloway (1980) during (1979-1980), despite removing 2-3 times more fleas in each subsequent year.

5.2 Seasonal Dynamics of O. bruneri

The sex ratio (m/f) of O. bruneri favoured males in early May during adult emergence and in late June during juvenile emergence. Female O. bruneri predominated for the remainder of the year. The temporary imbalance could result from the emergence of new males. Male fleas, excluding S. cuniculi, are smaller and generally more active and more susceptible to separation from their host (Marshall 1981). Klein (1966) observed that 75% of the newly emerged Synopsyllus fonquerniei Wagner and Rothschild were males, but only 39% were mature fleas since mortality was higher in older male than female S. fonquerniei. Female fleas live longer than males, and hence the proportion increases over time and females may dominate until males are contributed from another generation (Bibikova and Zhovtyi 1979).

The peaks in mean intensity in May and late August separated by periods of low intensity, appear to coincide with the

emergence of a new flea generation. Similar spring and fall increases in flea populations on Spermophilus spp. in temperate regions have been attributed to emergence of adults from overwintering autumnal pupal and from emergence of the first generation imagoes (Zhovtyi 1974). Abiotic conditions such as precipitation and relative humidity have caused shifts in flea populations, hence one set of conditions favors one species over another (Parker 1958, Ryckman 1971).

Monitoring the reproductive state of adult female fleas provided conclusive evidence regarding the onset of new generations. In previous studies such as Hendricks (1967), Kinzel and Larson (1973) the reproductive status of female fleas was not investigated and ,therefore, changes within a flea population may have been misinterpreted as being effected by outside factors.

Generally, female fleas in early May contain undeveloped ovarioles and no corpus luteum within the oviducts and represented a newly emerged overwintering population. The presence of corpora lutea and of spermatozoa in the spermatheca is regarded as satisfactory evidence that eggs have been laid (Mead-Briggs 1962). There was also a small component of mated females with maturing ovarioles but most had not oviposited based on the absence of corpus luteum. Flea emergence from overwintering cocoons is perhaps stimulated by vibrations caused by an emerging ground squirrel. Furthermore, reproductively active females that had not oviposited were

recovered from the earliest caught squirrel which indicates flea emergence is initiated by stimuli from the squirrel within the overwintering burrow. Silverman and Rust (1985) stimulated emergence of Ctenocephalides felis (Bouche) by increasing ambient temperature and applying direct pressure on the cocoon. Variable response to host emergence may be an intrinsic behaviourial adaptation to variation within a host population or to differential emergence dates between alternate host species i.e. secondary hosts.

Two weeks after the first squirrels emerged, female fleas with mature ovarioles, corpora lutea present at the base of the largest ovariole and sperm in the spermatheca were predominant for the duration of the season. The observed state of reproductive activity for O. bruneri (Figs. 13,14) is contrary to the original hypothesis (Fig. 15). Ovarian development in S. cuniculi occurs in a relatively short time (~28-30 days) and after oviposition the ovarioles are quiescent until the onset of the next breeding season (Rothschild and Ford 1973). The proportion of parous and multi-parous females cannot be determined during each trapping period without knowing the longevity of female O. bruneri. Silverman and Rust (1983) and Silverman et al. (1981) influenced the survival and emergence of C. felis adults from cocoons by changing ambient temperature and relative humidity. The abiotic conditions within the burrow remain relatively stable based on the soil type and soil temperature data (Krpan 1982). The longevity of

males appears to be less than 8 weeks, based on the shift in sex ratio in early July. The longevity of females cannot be clearly defined based on the presented data. Spilopsyllus cuniculi females have been reported to live longer than males (Rothschild and Ford 1973). Further, there was no shift in flea sex ratio in the fall, hence it is likely that female O. bruneri outlive male fleas. The large proportion of females in the later part of the season may be comprised, in part, of late emerging females. The female fleas continue to oviposit until the end of the season. The eggs develop within the summer burrow and the subsequent third-instar larvae, pupae or adults overwinter in cocoons.

The large proportion of nulliparous females (no corpus luteum in the oviduct and with or without sperm in the spermatheca) during the first and second sampling periods represented newly emerged fleas according to criteria by Klein (1966). The subsequent small peak in July and to a lesser extent in September represent the emergence of a first and possibly second generation. Thus, oviposition and mating continues throughout the season irrespective of the reproductive condition of female S. franklinii.

5.3 Life History of O. bruneri

The original hypothesis was that oogenesis in O. bruneri was stimulated by increasing circulating reproductive

hormones within female S. franklinii. The proposed interdependence was based on the occurrence of two peaks in adult O. bruneri activity (Burachynsky and Galloway 1980, Galloway and Reichardt, Winnipeg, unpublished), and the monoestrus reproductive cycle of S. franklinii. If oogenesis in O. bruneri was stimulated by reproductive hormones, then the following criteria would be observed: 1) there would be only one generation of O. bruneri each year; 2) ovarian development would begin in late May concurrent with reproductive activity of S. franklinii; 3) female fleas would harbour mature ovarioles only during parturition of S. franklinii; 4) flea mating would occur for a maximum of 8 days post-partum and would not re-commence until the following year; 5) female fleas would remain non-reproductive for the duration of the season, i.e. ovarioles would remain quiescent, corpus luteum not present at the base of each ovariole and sperm is not present within the spermatheca. These five criteria are illustrated in Figure 15.

Adult flea host-seeking behaviour during the squirrel reproductive period should alter such that female and male fleas are attracted almost exclusively to pregnant female squirrels. Pregnant female S. franklinii, prior to parturition, would harbour the highest proportion of female fleas, as observed with S. cuniculi (Rothschild and Ford 1973). Opisocrostis bruneri infestation parameters on male and female squirrels would reestablish to levels prior to parturition.

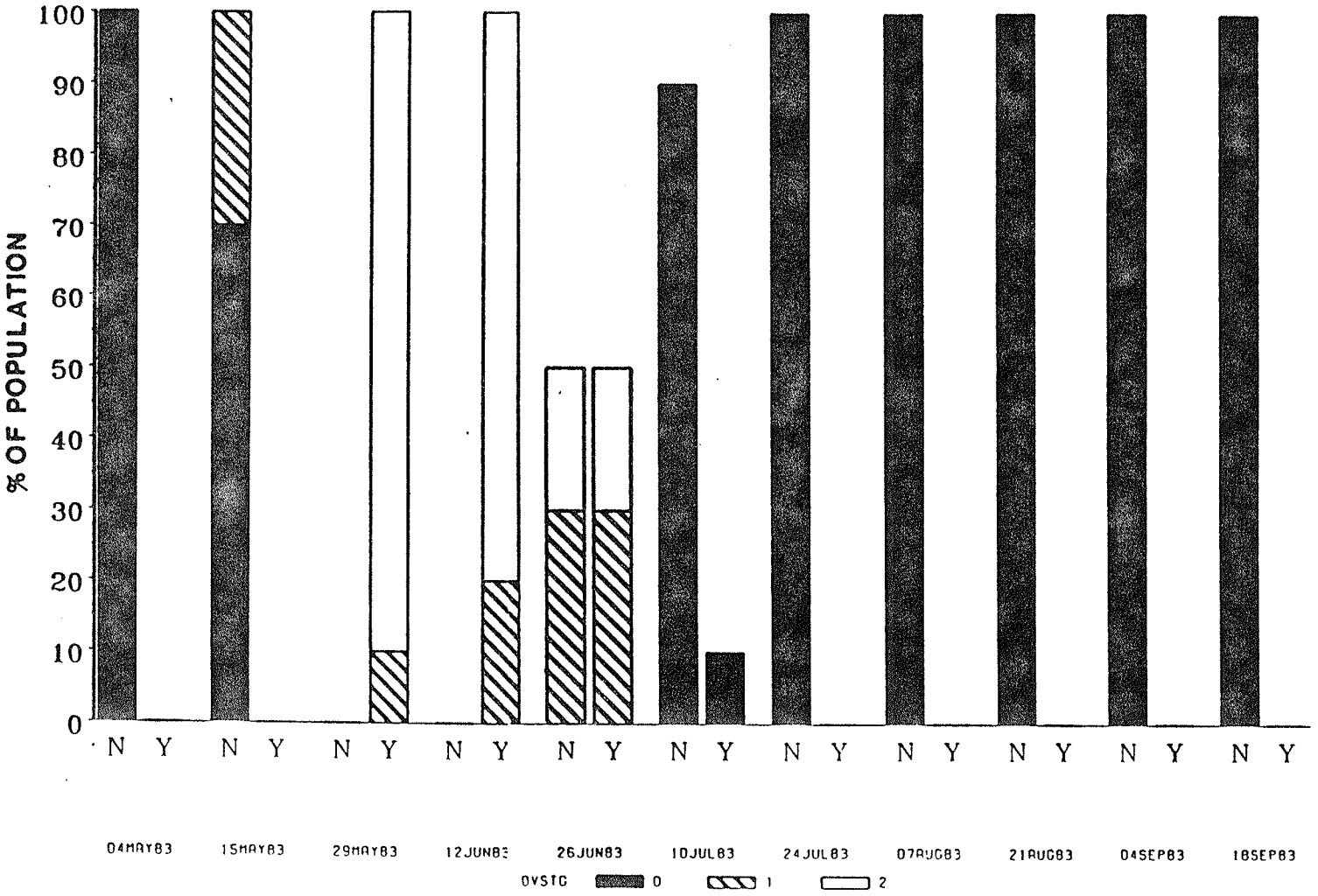
Figure 15: Profile of Opisocrostis bruneri development as predicted by the relationship observed between Spilopsyllus cuniculi and Orctolagus cuniculus (Rothschild and Ford 1973).

Legend

N - sperm absent in spermatheca

Y - sperm present in spermatheca

ovstg - observed ovarian developmental stage


% OF POPULATION
100
90
80
70
60
50
40
30
20
10
0
N Y N Y N Y N Y N Y N Y N Y N Y N Y N Y N Y
04MAY83
15MAY83
29MAY83
12JUN83
26JUN83
10JUL83
24JUL83
07AUG83
21AUG83
04SEP83
18SEP83
OVSTG 0 1 2

Opisocrostis bruneri were found on newly emerged male ground squirrels in the spring. The reproductive condition of the new fleas may represent adult fleas that overwinter and emerged with S. franklinii and subsequent oogenesis commenced after S. franklinii emerged from hibernation. The resumption of feeding and oviposition may also represent newly emerged fleas from cocoons overwintering in the squirrel's summer burrow. Flea emergence may commence after S. franklinii completed the last bout of torpor and re-established residence in the summer burrow. The actual time spent active below ground prior to emergence is not known, but it is possible that a squirrel would first prepare the new summer burrow. Male squirrels harbour heavy flea loads and frequent several burrows prior to the mating season. Hence, while searching other burrows for receptive females, males can introduce new fleas and stimulate emergence of new adult fleas from overwintering cocoons. New fleas are also introduced into the burrow during mating. Rothschild and Ford (1973) observed S. cuniculi transfer between host rabbits during mating. Furthermore, transfer was common during mutual grooming and other cohabitational activities. There is minimal contact between male and female S. franklinii except during mating (Banfield 1981). Soon after mating adult males disperse away from their mating sites. Overwintering O. bruneri appear to disperse in the spring on adult male S. franklinii. Zhovtyi (1974) suggested this type

of overwintering system was typical for fleas of Siberian Citellus spp.

Marshall (1981) observed Archaeopsylla erinacei (Rothschild) on hibernating hedgehogs, Erinaceus europaeus (L.), clumped around the tail or grouped on the side which the hedgehog was lying. The fleas did not feed or reproduce during the winter but feeding and oviposition quickly resumed once the hedgehog had emerged from hibernation.

The eggs deposited by the newly emerged females develop into the summer generation inside the summer burrows. Burrow environment remains stable and may attain temperatures of 18 °C (Krpan 1982). Orchopeas howardi (Baker) completed development from egg deposition to adult emergence under conditions of 16 °C and 80% r.h., in approximately 65 days (Sikes 1930). There are approximately 60 days between the first and second peak of non-reproductively active females, hence there would be sufficient time to complete development if the incidence of non-reproductive females collected in July represents a new generation. The low number of recovered fleas may be due to innate behaviourial responses that limit or reduce host seeking activity and keep fleas in the nest until mating has been completed as observed with Ceratophyllus gallinae (Schrank) (Humphries 1968). Newly emerged fleas tend to feed less frequently, but imbibe more blood each time than older fleas (Novokreshchenova 1966). The change in observed

sex ratio represents the increase in males which is associated with emergence of a new generation (Bibikova and Zhovtyi 1980).

Juvenile squirrels emerge from natal burrows and begin above ground activity during the emergence of new fleas. The juvenile squirrels disperse from natal burrows within one month after emergence (Holekamp 1984). Reproductively viable fleas disperse with the juveniles in the fall.

The summer generation immediately commences egg laying and continues into September. The eggs oviposited as late as mid-September could develop to pupae or teneral adults and overwinter within a cocoon because soil temperature within the summer burrow will not drop below 15 °C till the end of October (Krpan 1982). The third instar larvae, pupae or teneral adults remain quiescent until the following spring.

The few nulliparous females recovered in the fall may represent a secondary outlying portion of the population capable of completing a third summer generation in the southern limits of O. bruneri distribution. Hendricks (1967) removed O. bruneri from S. tridecemlineatus in mid-October in Indiana. The extended S. tridecemlineatus and S. richardsonii season would provide the environment for a third generation. The few nulliparous females may represent the outlying proportion of a population that emerges to begin the third generation. However, the majority should remain as pupae within a cocoon until the next spring.

The reproductive cycle of O. bruneri was not synchronized to the oestrous cycle of S. franklinii. Opisocrostis bruneri appears to complete one generation each summer and at least a second generation that may overlap into the following spring. The first generation starts and completes development in May and early June, respectively. First generation adult females oviposit and the eggs immediately begin development and represent the second generation. The third instar larvae, second generation pupae and teneral adults may overwinter in cocoons within the squirrel's summer burrow. The spring and fall peaks of O.bruneri mean intensity resulted from changes within the squirrel population; namely spring emergence of adult squirrels and adult immergence in combination with fall juvenile dispersal, respectively. The introduction of juvenile squirrels and the summer molt on adult squirrels combined to reduce O. bruneri mean intensity on S. franklinii from early June to early August.

The life history of O. bruneri was based on fleas removed from S. franklinii. The actual length of the life cycle, and the overwintering stages of O. bruneri could not be determined due to two deficiencies. Firstly, O. bruneri could not be reared in the laboratory and was not easily recoverable or readily available from active S. franklinii burrows. In addition, the start of spring flea development and the actual time S. franklinii spent active below ground prior to spring emergence were unknown. Neither the actual age or time since

emergence from the cocoon could be determined for fleas removed from S. franklinii, particularly the first spring captures. Traps were set out as early as possible each season to ensure capture of the earliest emerging squirrel. The length of the reproductive cycle and mating habits of O. bruneri were also unknown. These shortcomings could possibly be overcome by successfully rearing O. bruneri on S. franklinii in an artificial burrow system. The possibility of overlapping generations could be investigated by observing flea development over an 18 month period (Spring-Summer-Fall-Winter-Spring-Summer-Fall). The life cycle and reproductive condition of laboratory cultured fleas at various times during the season could be compared to field populations. Further, the reproductive cycle and mating habits such as mating frequency could also be determined in a cultured laboratory population.

Chapter VI

CONCLUSIONS

1. Opisocrostis bruneri populations complete at least two generations per year in Manitoba. The emergence of new adults coincides with the discrete peaks of mean intensity in early May and late August. Small numbers of nulliparous females and a shift in biweekly sex ratio favouring males were observed in early May and early July, indicating the appearance of newly emerged adult fleas.
2. Young S. franklinii are born in early June. Parous female O. bruneri predominated from early June till September. Flea reproduction continued throughout the season, suggesting oogenesis of O. bruneri continues independently and was not stimulated by the oestrous cycle of female S. franklinii.
3. The lowest mean intensity values were on S. franklinii observed from the last week in June to early August, coinciding with the emergence of juvenile squirrels and the immergence to hibernation of adult squirrels. The annual molt on adult squirrels beginning in mid-May, reduces the amount of hair on the body surface and promotes easier removal of fleas by host grooming. The low mean intensity values resulted from an increased potential number of hosts for O. bruneri

and the presence of the summer pelage on S. franklinii.

4. Fleas disperse to and from the flea natal site depending on squirrel activity. The spring dispersal was on adult male squirrels and the summer dispersal on juvenile squirrels. Mean intensity values in early May were 2-3 times higher on adult male squirrels than adult female squirrels. Juvenile squirrels were always infested with O. bruneri but the highest infestations occurred at the end of August and early September during the fall squirrel dispersal.

5. O. bruneri overwintered in the burrows of S. franklinii either as a third-instar larvae, a teneral adult or pupae within a cocoon. Spring emergence of S. franklinii stimulated either the final pupal molt and/or adult emergence from the cocoon.

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Appendix A

APPENDIX A

A.1 DISSECTION OF OPISOCROSTIS BRUNERI

A.1.1 Preparation

The flea was placed on a glass slide and immersed in 3 to 4 drops of physiological saline to prevent desiccation of reproductive organs. The exterior of each flea was examined for mites and hypopal stalks.

A.1.2 Procedure for the Dissection of Male Fleas

The abdomen was severed from the body by cutting the pleural membrane between the first and the second abdominal segments. The mid- and hind-guts were removed from the haemocoel and examined along with the haemocoel, and the endoparasites were classified and recorded.

A.1.3 Procedure for the Dissection Female Fleas

The procedure to expose the haemocoel was outlined in section A.1.1. The spermatheca was then removed by an incision through line A-A (Fig. 16) which provided an opening to pull out the organ. Pressure applied with fine forceps, initially at tergum VII and gradually moved anteriorly, forced the ovarioles through the opening at the second abdominal segment. The spermatheca and ovarioles were cleaned of all extraneous tissues and placed under

Figure 16: Schematic diagram of female genitalia in Opisocrostis bruneri.

Legend

A-A = dissection line

as = antepygidial setae

se = sensilium, pygidium

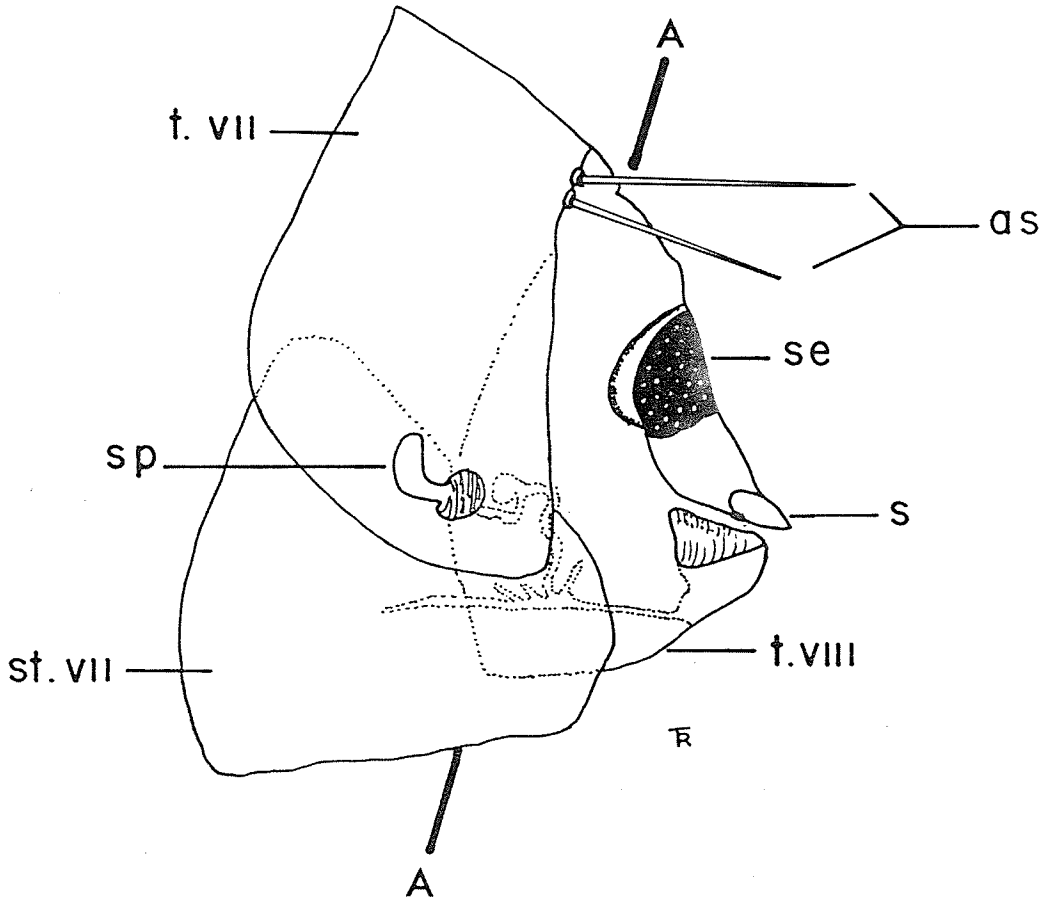
s = anal stylet

sp = spermatheca

st. VII = sternum VII

t. VII = tergum VII

t. VIII = tergum VIII


A
t. VII
as
se
sp
s
t. VIII
st. VII
R
A

a coverslip. A few extra drops of physiological saline were added under the coverslip to reduce pressure exerted on the ovarioles and to maintain consistency of shape. The spermatheca was examined for the presence of spermatozoa and the largest proximal oocyte was measured and assessed an ovariole rating. Both spermatheca and ovarioles were viewed under a Wilde phase-contrast microscope.

Appendix B

APPENDIX B

B.1 PARASITES AND ASSOCIATES OF O.bruneri

B.1.1 External Parasites

Opisocrostis bruneri was occasionally infested with two different types of mite : 1) Uropodid mite, genus Trichuropoda. The mites were in the hypopal (dispersal) stage and attached to the exoskeleton via a stalk. Several have reported on the occurrence of phoretic mites via a stalk of the flea's integument (Schwan and Corwin 1987). 2) Anoetid mite, genus Psyllanoetus (Fig. 17). This species was located under the tergal and sternal plates. The presence of either mite species did not appear to affect the reproductive state of female O. bruneri.

B.1.2 Internal Parasites

The haemocoel of O. bruneri was occasionally occupied by allantonematid nematodes (Fig. 18). Generally the haemocoel would contain one to six large female nematodes with several L1 to L3 stage nematodes. Host ovaries were completely undeveloped when nematodes were found within the haemocoel, except in one flea. Nematodes were never recovered from ovarian tissue. The presence of allantonematid nematodes within the haemocoel inhibits oogenesis in Spilopsyllus

Figure 17 : An anoetid mite under the sternite of *Opisocrostis bruneri*.

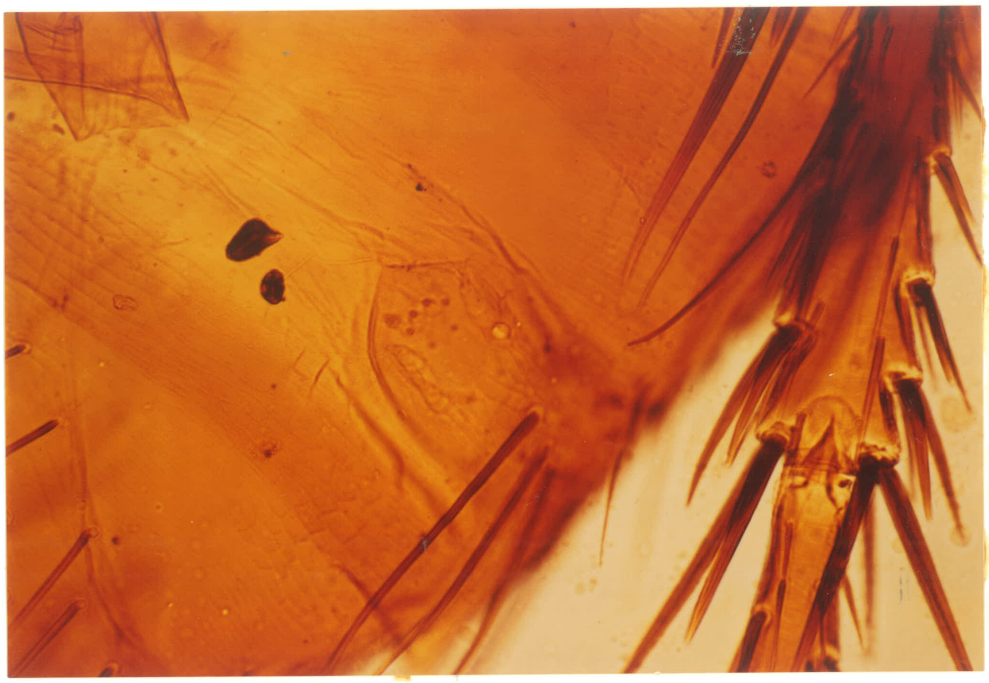
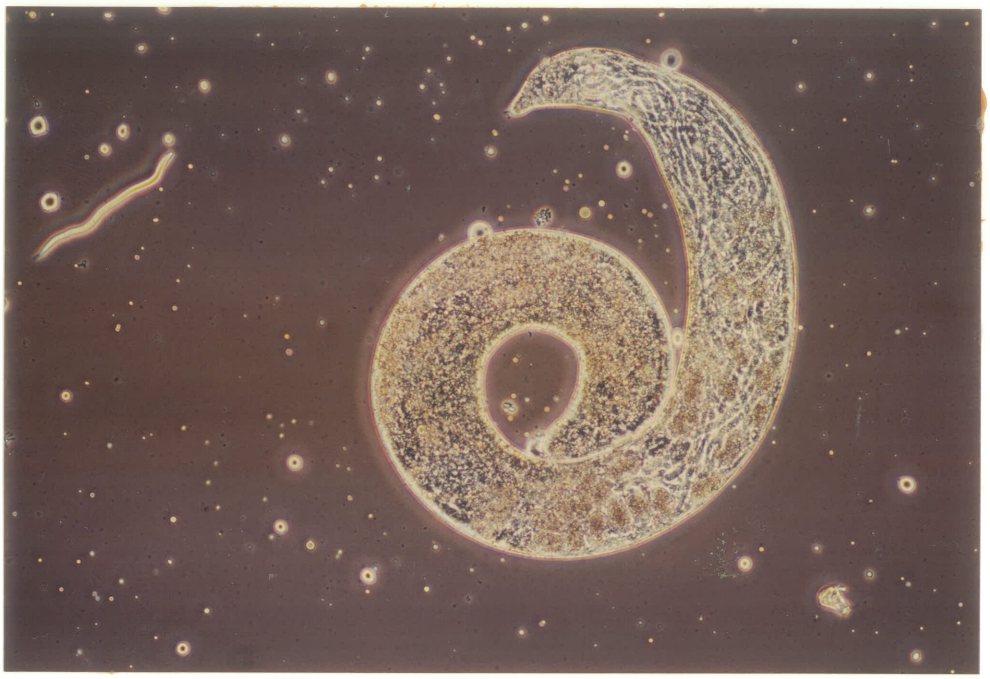
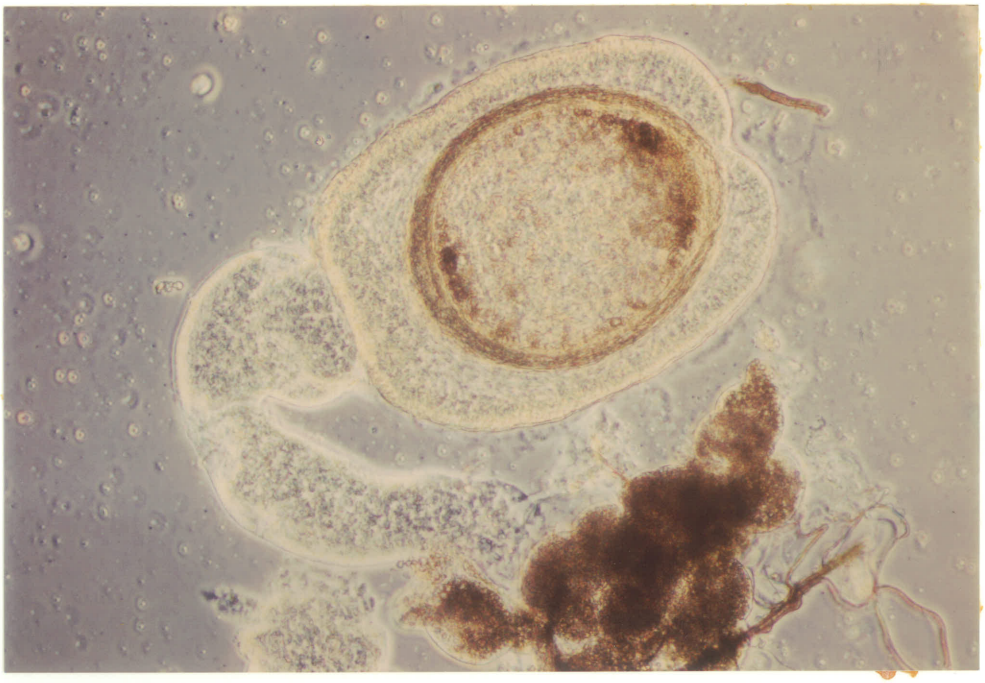


Figure 18 : An adult female allantonematid nematode removed from the haemocoel of a female Opisocrostis bruneri.

Figure 19 : The cysticercoid stage, presumably, of Hymenolepis citelli removed from the mid-gut of Opisocrostis bruneri.





cuniculi (Launay and Deunff 1984). Nematodes recovered from within the haemocoel of four rodent flea species, including Monopsyllus wagneri (Baker) caused castration in male fleas (Poinar and Nelson 1973).

The cysticercoid stage, presumably of Hymenolepis citelli was recovered from the mid-gut of one O. bruneri (Fig. 19). Gregarine cysts were also discovered within the mid-gut of several O. bruneri during each sampling period.