ASPECTS OF THE ECOLOGY OF *IXODES* TICKS AND *BORRELIA BURGDORFERI* AT LOCH LOMOND

submitted as thesis for MSc., January 1995, to the Institute of Biomedical and Life Sciences, the University of Glasgow

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Candidates Declaration.

I declare that the work recorded in this thesis and hereby presented is entirely my own, unless otherwise stated, and that it is of my own composition. No part of this work has been submitted for any other degree.

Justin HG Williams.

January 1995

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0.2. Acknowledgements.

There are many people who have been of great help to me in the production of this thesis and with so many members of the zoology department being so helpful, I cannot thank everyone personally. Most importantly however, I must thank my supervisors, Dr Bob Furness and Dr Mike Turner for their unstinting encouragement, support, comments and criticism throughout the last two years.

The planning of research and experiment was a time demanding careful thought and also imagination. I must in particular thank Dr Roy Armstrong for some very useful interjection during this period. Dr Sarah Randolph was also very helpful with advice on mammal trapping. As the research got under way, much work was required at the Field Station and most of the research students and technicians there were very helpful, assisting me with mammal trapping and blanket dragging. Members of the Applied Ornithology Unit were also very helpful. I must once again thank Dr Bob Furness for his extensive ringing work at Tarbet and Dr Neil Metcalfe who examined his pied flycatcher population for me. David Robertson sacrificed a couple of good night's sleep to brave early morning midges and catch birds at Rowardennan. Finally I must thank Dr Norman Ratcliffe who valiantly tolerated the excitement of blanket dragging and tick counting and who later, patiently introduced me to SPSS.

Whilst members of the Applied Ornithology Unit taught a naive ecologist, members of the Laboratory of Biochemical Protozoology were confronted with a laboratory naive immunochemist. They were all very helpful but in particular I must thank once again, Dr Mike Turner and also Kate Milligan, for showing me the part of a microscope to look through and the end of a pipette to squeeze.

Perhaps one of the hardest parts is writing up and some of this has been done whilst living in Norwich. Dr John O'Brien has been most obliging with his laser-jet and my bosses have been most considerate as well. Finally I would like to especially thank Nina Dick for her support, both practical and otherwise, her comments, and encouragement.

0.3. Summary.

With increasing concern being expressed with regard to the disease, Lyme borreliosis, throughout the UK but particularly among those working in rural Scotland, this project was set up to explore the ecology of the causative bacteria, *Borrelia burgdorferi* in Scotland. The aim was to identify aspects of the ecology which would be amenable to control, thereby reducing the prevalence of the disease in Scotland. A review of the literature established that the bacteria was transmitted in the UK by the tick vector *Ixodes ricinus*. Its animal hosts remained unknown but small mammals appeared to be the most likely candidates. Deer and birds were also possible reservoir species. The prevalence of the disease would therefore depend upon the size and behaviour of the vector population and the animal reservoir. Control of either may offer control over the disease.

This study explored ways of quantifying tick populations and at factors affecting them. It showed for the first time that repeatedly dragging a blanket over the same piece of ground could lead to absolute measurements of population size. It also showed that ticks were more prevalent among the deep blaeberry bush *vaccinia myrtilum* than in the grass and leaf litter. Tick distribution was also shown to be patchy throughout the woodland. Host studies revealed large numbers of tick to be present on both birds and small mammals. These were nearly all *Ixodes ricinus*. Small mammal population sizes were measured using mark and recapture methods and bird population measurements carried out previously were used. The role of birds and mammals as tick hosts was then calculated. It was concluded that birds feed up to 10% and that small mammals feed up to 40% of the larval stage of the tick. Neither groups feed a significant number of older stages. Deer probably feed the majority of the remainder of ticks. However, it is noted that with these figures, small mammals could still be the most important animal reservoirs.

Immunofluorescence was carried out on some of the ticks. Results were equivocal but supported findings elsewhere showing that *B.burgdorferi* is ubiquitous throughout tick infested areas of the UK.

Lastly this study demonstrated the importance of ticks as potential parasites and vectors in Scotland and that for the sake of conservationists, farmers and tourists, their ecology needs further investigation.

0.4. Introduction

Lyme disease was named after the town of Lyme, Connecticut in 1975 following an outbreak of the disease. It was described as a syndrome of a characteristic skin lesion (erythema chronicum migrans or ECM), an inflammatory arthritis, peripheral neuropathy and myocarditis. As the disease became better recognised so did its spectrum of presentation.

It was recognised that the illness was related to tick bites. A bacterium was isolated from ticks by Burgdorfer et al (1982) which reacted strongly with antibodies from Lyme disease patients. This was a previously unrecognised species of spirochaete which was later named *Borrelia burgdorferi.* The illness was found to respond well to antibiotics and in particular tetracyclines and penicillins have proved most effective. Unfortunately some of the chronic sequelae in the third stage of the illness have proved less responsive.

Following the American outbreaks, the recognition of the disease and the consequent development of *B.burgdorferi* antibody assays, cases were recorded in the UK and Europe. A syndrome known as Banwarth's syndrome has been long recognised in the UK. This consists of ECM, peripheral neuropathy and white blood cell infiltration into the cerebrospinal fluid. It had been shown to be transmissible by skin graft but its cause was unknown. Guy *et al* (1993) reported results from the testing of over 15000 blood samples. This and other work have as yet found no evidence that British Lyme disease differs in any way to Banwarth's syndrome. Patients with cardiac or arthritic complications have all probably contracted their disease abroad. This does not mean necessarily that the illness is more mild in the UK and cases of meningitis and encephalomyelitis have been reported (Guy *et al* 1989).

The disease in Britain has been reported mainly from Scotland, the New Forest area of Hampshire and the Thetford forest area of East Anglia. All areas are well populated with ticks. With growing public awareness of Lyme disease, a number of countryside workers including foresters, wardens and farmers have been expressing a great deal of concern about this issue. It was recognised that measures may be called for which would reduce the incidence of the disease and this may involve either management of host reservoirs or vector management or both.

The primary aim of this thesis therefore was to review the present state of knowledge in relation to these areas of interest, with a view to identifying aspects of spirochaete,

tick or host ecology that could assist in the development of control measures. Experimental work was directed towards gaining some idea of tick population size and structure in Scottish woodland, the relevent tick hosts and the distribution of *B.burgdorferi.*

Most field work was carried out at the University Field Station at Rowardennan in Stirlingshire, Scotland. The field station stands on the banks of Loch Lomond amongst a large area of woodland. This site is described in more detail below. Most of the birds were mist netted on the other side of the loch at Tarbet in Dunbartonshire.

0.5. Rowardennan study site

These are oak woods which have probably been actively managed since at least the 13th century. From the early 1700's they were managed by coppice-with-standards management and were put on a 24 years rotation. Unlike English coppicing methods where preferred species of tree were maximised, these woods were managed to maintain the original species. The woods are therefore mainly oak *Quercus petraea* and *Q.robur* though there are small numbers of hazel *Corylus avellana* and ash *Fraxinus excelsior* . The oak woodlands are interspersed with conifer plantations throughout Loch Lomond Nature Reserve.

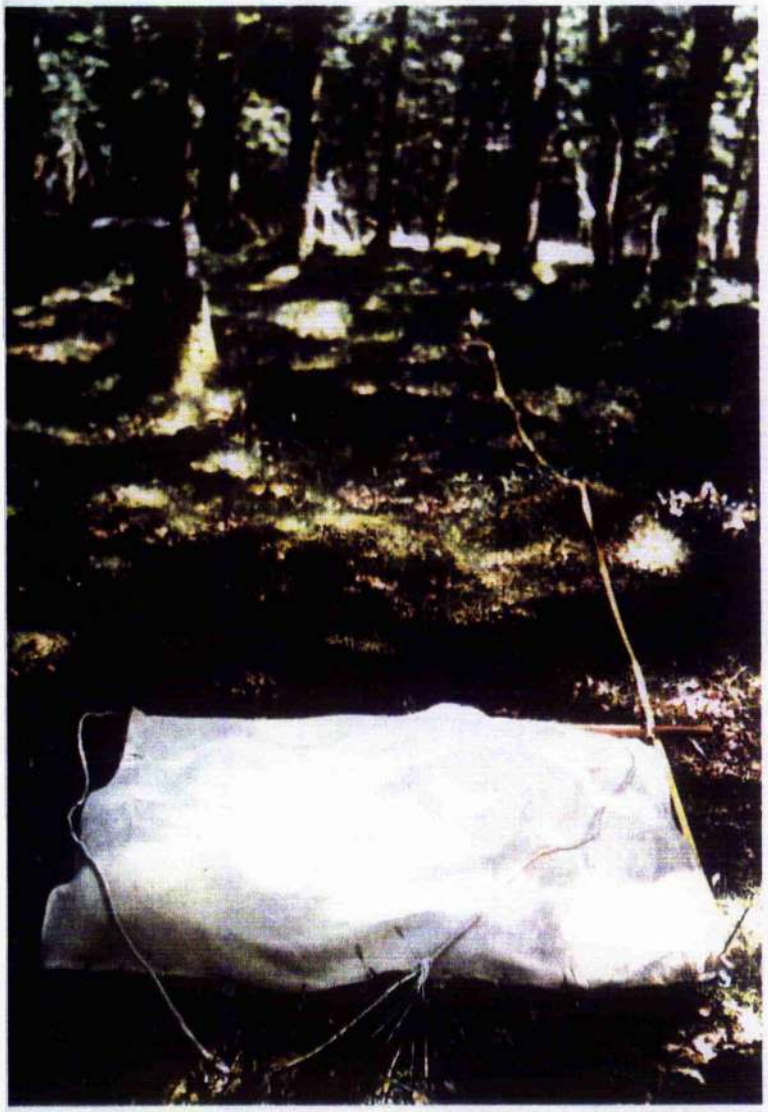
The study concentrated on a piece of land approximately 2 hectares in size. This included the single hectare covered by the small mammal traps. It was bound on the west by the main Rowardennan road, on the east and north by the field station and its track and on the south by a pylon line and associated clearing. This piece of land also constituted a small hill with north and south sides and in many places, the ground was steep. There was little flat ground, such that whilst the bedrock was mainly granite, there was generally good drainage for the plentiful rain that falls in this region.

Ground cover tended to divide the site into two types. About half of the site was covered with blaeberry bush *Vaccinia myrtilis*. This is fairly dense bush up to a metre high which dominates the ground to the expense of any other vegetation. Its base carries loosely packed leaf mould which probably offers a consistently humid environment to ticks and other invertebrates.

The rest of the site is covered in the winter with leaf litter and P*olytrichum* and S*phagnum* mosses on the exposed granite. In spring in some parts, grass (*Bromus* sp.) and wood sorrel *Oxalis acetosella* grow and this is followed later, in much of the area, by the growth of bracken *Pteridium aquilinum*.

The woodland is used by a number of vertebrate species as discussed in the relevant chapters of this thesis. A point worthy of mention is that a popular walking track runs close to the site and whilst the study site was generally free of people, the area as a whole is a popular site for tourists and Glaswegians.

A map of the site is shown in Appendix 9.



A wren *troglodytes troglodytes* mist netted at Rowardennan.

A dragging blanket at the end of a 15m measured strip in Rowardennan woods.

1.0. Biology of *Ixodes ricinus*

1.1. Introduction

Ticks are obligate blood sucking arthropods found throughout the world. There are about 850 species divided between the hard ticks, (*Ixodidae*), the soft ticks (*Argasidae*) and a third family, *Nutalliellidae* with just a single species.

There is considerable variation in life history and habitat between them. Their habitat varies from the Arctic where *Ixodes uriae* lives upon seabirds to the dry deserts and semi-desert steppes of Asia where *Hyalomma asiaticum* is found. Many are nest or burrow dwelling whilst others can be quite free ranging. Some are very host specific whilst others appear to feed upon any vertebrate that crosses their path. *Ixodes ricinus* has been recorded on over 300 species of bird, mammal and reptile (Anderson 1991) and is probably one of the most catholic of ticks.

1.2. General.

Ixodes ricinus is a hard tick of the humid and cool temperate environment ranging from the British Isles on the west to as far east as 55°E. It has been observed though it is not common, in North Africa, where it is active only in winter (McLeod 1939) and it extends northward to about 65° in mid-Sweden and Finland (Anderson 1989). Its habitat is also varied; Anderson (1989) describes it as secondary deciduous forests, shrub undergrowth and pastures. As stated it is an indiscriminate feeder.

I.ricinus as with other hard ticks has four stages. The egg hatches into the larva at the base of the vegetation. The larva then becomes active and climbs to the tip from where it quests. It then attaches to a passing host and feeds for up to seven days. After detachment it rests and moults into the nymphal stage in the vegetation and again becomes active, quests, attaches, drops off and moults into the adult. After mating the female again quests, attaches and feeds before dropping into the vegetation to begin oviposition. Males may not feed and die after mating but many questing males are also picked up and unlike some other species of prostriate ticks, they do have mouthparts capable of feeding.

1.3. Seasonal Variation and Life Cycle Timing.

The life cycle and timing have been examined in detail by many workers. The tick generally shows a bimodal seasonal distribution with a peak of activity in spring and a lesser peak in the autumn.

McLeod (1939) found this pattern upon sheep in Northumberland, Central Scotland and Western Argyllshire as did Campbell (1948) in the Borders and Argyllshire and

Arthur (1963) in Wales. Milne (1945,1946) and Milne and Lees (1951) using counts of ticks on sheep, measures of individual activity and blanket dragging confirmed these findings for the north of England. In North East Scotland a single summer peak was reported (Hendrick, Moore and Morrison 1938) similar to one found by Campell (1988) in Ayreshire. Milne (1945) also suggested that in Cumbria and Nairnshire an in-between situation arises where the two peaks are much closer together with just a small dip in June.

Gray (1987) reports a clear biannual pattern for *Ixodes ricinus* in meadows in Southern Ireland and Cerny *et al* (1974) for woodland in Czechoslovakia. Nilsson (1988) reports biannual peaks in May and November in Southern Sweden in both mature forest and sea facing meadow.

A Number of workers have looked at *I.ricinus* under controlled conditions. McLeod (1935) found it to be active only between the temperatures of 10-45^{o}C and deemed the ticks to show a preferred temperature of 15-19^{o}C, though at the higher temperatures this was more one of an excitability before lapsing into a coma before death. He attempted to measure the geotropic response and found it to start at 12^{o}C and be abolished at 30^{o}C in the dark, where in daylight the range was 14-24^{o}C. Unfortunately we have no idea as to how many ticks were used and it is difficult to see from his experimental description how the degree of activity is specific to a geotropic effect and that this is not simply a description of temperature related activity. Furthermore we have no results for nymphs or larvae. Lees (1948) was less sure of the presence of a geotropic response as the ticks aggregated equally at the top and bottom in a corked tube. He found that whilst ticks ended up at the top of his vertical glass rods they would make a number of partial descents before resting at the tip and he concluded that whilst a negative geotaxis may be of some significance, a tactile response to the tip is more important. Activity did increase with temperature and where wild caught ticks were active above 1oc and ticks previously kept at 25^{o}C were active above 5^{o}C, most activity occurred between 11-41^{o}C. There was no preferred temperature in this range. McLeod(1939) then went on to demonstrate how the seasonal activity of the tick in the UK relates to a preferred temperature range. Seasonal activity is shown to commence in the spring when the temperature rises above 7^{o}C, to persist until June when it rises above 16^{o}C and then to reappear in the autumn when temperatures fall into the appropriate range again. Milne (1945a) did not find this model very satisfactory. In Cumbria for instance, most activity occurred below the temperature threshold. He also found no autumn recrudescence upon one farm, when this was present on others two miles away in one direction and 15-20 miles the

other. He reported observing many ticks on the tops of grass stems in full sunlight. Recognising the importance of humidity to the ticks, he made the point that the longer the daylight hours the less time the ticks spent at >80% humidity. However he did not find humidity on its own correlated with the rhythm on any farm. Milne (1947) showed that in two out of 5 years the nymphal peak was later than the adult peak by 14 and 21 days. Otherwise it was concurrent. The larval peak is always several weeks later than the female peak.

Campbell (1948) examined the development of *I.ricinus* both in the field and in the laboratory and showed rates of development to be temperature related. Milne and Lees (1951) looked at individual activities of marked ticks and reviewed the literature on the subject. They suggested that wintered ticks became active after a diapause and that by the end of June most had fed. They moult or oviposit but have no time to feed before the winter diapause sets in again. Another population spends the winter engorged after an autumn feed. It moults in the summer and the next stage is ready to feed in the autumn. There would be limited crossover between these two populations.

Gray (1982) placed eggs and ticks in gauze tubes in two sites and observed rates of development. He then combined the findings with those from laboratory work. Larvae were most active in May and there was a resurgence in late June. Those that fed in mid-July did not go into diapause, unlike those that fed in or after the first two weeks of August. Those larvae feeding before August moulted early September and more than half were active as nymphs mid to late October. The others became active the following April and the resulting adults would be active in the autumn. He suggests that of the numbers of larvae, nymphs and adults available to feed in the autumn most fail to find a host and overwinter in the unfed state. Consequently spring fed larvae or nymphs may give rise to either spring or autumn ticks whereas autumn feeders only give rise to spring feeders. The development of diapause occurs in larvae in early August but probably depends upon the state of engorgement. Interestingly Gray (1987), found that unfed females were fertilised in different proportions through the year. 30% of ticks had mated in the early spring and by November this had risen to over 90%. He then went on to show that mating abolished the behavioural diapause in these females and reported many of the autumn questing ticks to be newly moulted and partially engorged which was in accord with previous findings. Gardiner and Gray (1986) developed a model based on temperature-related development times from Campbell (1948), for predicting the intensity of a seasonal peak dependent upon the weather. In this model a low temperature on the day of laying leads to a short egg development time and vice versa. Diapause is considered to run from August to

February but be broken by high Autumn temperatures. Predictions from this model showed reasonable agreement with field observations except that spring-laid eggs and spring-hatched larvae hatched and moulted earlier and autumn derived nymphs emerged earlier than expected.

Donnelly and MacKellar (1970) examined veterinary records from two areas over a number of years and found a close correlation between incidence of the tick-borne Babesiosis in cattle and the ambient temperature two weeks previously. This was partly due to the seasonal nature of both tick activity and temperature but the relationship also existed within the month. Rainfall showed only a minimal correlation in comparison. In Ireland, Gray (1978) also found that weekly temperature related to the incidence of the tick borne disease, Babesiosis. Interestingly air temperature was not related to overall tick activity until larvae were included. It is probably the larval stage that transmits the *Babesia* parasite (Gray 1978).

It is clear that not entering diapause may mean faster development and therefore greater reproductive rate but with a higher mortality. The fastest development time for a non-diapausing tick would be 2 years instead of 3. Gray (1982) has shown a 2/3 (69.35%) survival of overwintering fed larvae. If these were the only relevant figures, diapausing and non diapausing ticks would reproduce at the same rate. That is: 100 eggs would produce 66 adults in 2 years which means 33 adults per year. This is equal to an adult producing 100 adults over three years. A dimorphism then could be explained since reproductive rate would be equal for two different strategies. However this assumes no mortality of overwintering fed nymphs, other mortality rates to be equal and that faster development does not reduce numbers of eggs produced.

In summary then, activity and development depend largely upon temperature and so are largely confined to the period from March to October but this depends upon the conditions of the location. One can consider two populations of ticks that take three years to cycle- a spring population and an autumn population. Spring feeding adults lay eggs that hatch into larvae the following spring. These go into diapause in August and hatch into nymphs the following Spring. Similarly these enter diapause and become active as adults the following spring. The autumn feeding adults lay eggs through winter and spring that become active as larvae the following autumn. They feed but with the intervening cold winter they do not become active as nymphs until the following autumn when they feed again. The resulting adults are active the next autumn.

A number of factors complicate the picture. There is a high mortality of fed ticks over winter which would result in an ever diminishing autumn population. However many spring feeding ticks are ready to feed in the next stage in the autumn and unfed ticks in the autumn become active in the spring. There is therefore considerable cross-over between the two populations. A balanced dimorphism in life history strategies could result in the presence of the weakly developed diapause mechanism which accounts for some of the peculiarities of the tick cycle.

1.4. Water Balance

Much work has been done on water balance in ticks and is reviewed by Sonenshine (1992). All ticks have a hydrophobic lipid layer within the cuticle but the ability of this to limit loss varies immensly. *Ixodes ricinus* can tolerate a humidity of less than 80% for no more than 5 days and requires a humidity of over 90% to maintain an equilibrium under normal temperatures. This can be contrasted with *Hyalomma dromedarri* which can be found in the sand around caravansaries visited frequently by camels. Here humidity was less than 20%. Recently moulted ticks may be more susceptible to water loss than older individuals though post-moult cuticular tanning does not affect permeability. At high temperatures, at a specified critical transition temperature, the cuticle undergoes a drastic increase in permeability. This is at 32^{o}c for *I.ricinus* (Sonenshine 1992). Ticks lose a certain amount of water through respiration. This is minimized by the opening of the Spiracles. Excretion is mainly in the form of guanine, limiting water loss by this route. Ticks can actively absorb water from their environment and this seems to be done mainly by use of the salivary glands. Type I, agranular acini appear to secrete a rich, hygroscopic saline solution into the mouthparts and this acts to absorb water. During this period the mitochondria in the acinus cell can be seen to transform to a condensed state with greater intercristal space consistent with an increased metabolic rate. Disintegration of these glands during apolysis occurs some 2-3 days after the granular acini in engorged, non-diapausing, *I.ricinus* nymphs.

In effect then, *I.ricinus* is very susceptible to dessication especially during and just after moulting and when questing. It would seem likely from the strategy chosen by *I.ricinus* that rehydration by moving to the base of the vegetation uses less energy and resources than to use active sorption mechanisms or to develop a thicker cuticle. Furthermore it is likely that high humidity and rainfall are important in determining tick numbers and it would explain their notable abundance on the west coast of Scotland.

1.5. Diurnal Rhythm

Milne and Lees(1951) observed a number of ticks over a 24 hour period after Milne (1947) had collected less ticks at night than in the day by blanket dragging. It appears that during the night there is a net movement of ticks down into the vegetation. It does not appear to be clear whether ticks actually reduce questing behaviour at night as they tend to stay at the tips for two or three days at a time. It is interesting to note at this point that even though *I.ricinus* may exhibit a photoperiod regulated diapause and a diurnal rhythm it does not in fact have any eyes!(Arthur 1963). Diurnal rhythm may affect host choice or it may be that in a habitat dominated by nocturnally active hosts, the rhythm is reversed.

1.6. Habitat

As previously stated *I.ricinus* is a tick of deciduous woodland, pasture and shrub. However in comparison to the literature published on life cycle timing, that related to habitat is particularly sparse. Milne (1944) however studied it in some detail on Northumbrian sheep grazing moorland, some of the results of which are summarised in table 1. The question here was not so much as to which habitats *I.ricinus* occurs in, as to what factors in the vegetative habitat promote tick abundance. Milne first noted that *I.ricinus* was related to the rough vegetation and poor drainage. Areas of good grazing and drainage were tick free. Tick abundance was not related to soil pH, organic content (loss of weight on ignition), potassium or phosphate content or peatiness. He then looked at a valley of four hills, all fenced off from one another, that provided a "tick-gradient". The first two were heavily infested where the third was much less so and the last almost free. Bogginess, measured by depth to hard pan was significantly different between samples and the two dryer soils were the least infested two. However Milne does not claim a correlation. The land was divided into 137 tracts and relative densities of grasses and bracken measured. The hills did not differ in the proportions of common bent *Agrostis* spp., White bent *Nardus stricta,* Flying bent *Molinia caerulea* and bullfaces *Aira caespitosa* that they carried. Then, 93 hills were examined for both tick infestation (present or absent) and the dominant grass species. A X^2 test suggested white bent to be more prevalent on tick infested hills and bullfaces possibly so. No 'p' values were given. The land was then classified into types of vegetational cover and a strong relationship was apparent. Cameron's unpublished (1941) work is also shown in table 2. with similar results. The mat depth in each vegetational type was then found and a strong relationship is again apparent. There is unfortunately a paucity of statistical analysis, probably related to the absence of available methodology in 1944, but it seems that vegetational mat depth and soil drainage are the important determinants of habitat preference. Sheep density was not

mentioned for these hills but if it reflects grazing pressure it appears to be inversely related to tick numbers. This issues will be further discussed later.

Table 1 (from Milne 1944). The relationship between vegetation type and nymphal abundance.

Type of vegetation		Relative Nymphal Density
Thin vegetation layer (not 'rough')	Type 1.Short sward of hill top, or sheep lair.	1.0
Vegetation layer of intermediate thickness	Type 2.White bent(Nardus); well grazed	2.2
Thick vegetation layer	Type 3.Mixed bracken and white bent	4.6
	Type 4. Mixed herbage including rushes, wood sorrel, bilberries moss etc.	6.1
	Type 5. Very damp; ill-drained situation usually dominated by rushes	5.4

In cases where one is comparing more contrasting habitats, a number of problems are encountered. Principally the sampling method is likely to pick up different numbers of ticks between habitats simply because its efficiency alters between the two sites. For instance, a blanket dragged across flat moorland is likely to come into contact with more questing ticks than when carried through woodland where it catches on bracken, branches on the ground etc.

Tick abundance may also be measured by sampling rodents by mammal trapping and measuring infestation rates. This raises some interesting questions. If one compared the number of ticks collected per mammal trap as an index of abundance between sites, this will obviously be affected more by mammal than tick abundance. If one therefore compares ticks per, say, field vole in each site, one is more likely to get a true reflection. The closeness of the correlation then between ticks per mammal and the density of that mammal species in that site would then reflect the importance of that host in the ticks distribution. However if the ticks' host preference was determined by host frequency and the ticks preferred the most common host species, then such a

relationship would be disrupted. But, since ticks are dispersed by deer and birds, they are unlikely to become highly adapted to a local environment and so such adaptation seems unlikely. Another problem with using mammal traps to compare habitats is that the mammals microhabitat preference may be other than the habitats in the investigator's classification. Therefore if say, the rodents had a preference for bracken, when one thinks one is sampling wood litter and meadow grass one is actually sampling bracken both times. On the other hand a difference reflects a true "host's-eye view". It is the only method that will give some idea of tick populations in hidden areas such as burrows and thick shrub.

Table 2 (From Milne, unpublished data from Cameron 1941, no further details available).

Vegetational Type	% of total ticks
1.Rush	26.00
2.Nardus-Molinia	18.19
3.Bracken	15.46
4.Sheep's fescue	13.25
5.Spret	12.90
6.Burnt heather	10.15
7.Heather	2.76
8.Sheep's lair	1.31
	100.02

Hair and Bowman (1986) reviewed the behavioural ecology of *Amblyomma americanum*, commonly known as the lone star tick owing to a silvery spot on its dorsum. They compared four different types of wood and two different types of meadow. Caged adults were more active and survived longer in more humid conditions. They concluded that host density was important only secondarily to conditions in determining tick density differences between habitats. Ginsberg and Ewing (1989) compared the habitat distribution of *I.dammini* on Fire Island, New York. They used four methods: blanket dragging, mammal trapping, CO_2 traps and a

walking volunteer. The CO_2 traps were basically perforated containers of dry ice, mounted on wooden board with sloped edges, with sticky tape, sticky side up at the top of the slope. These should give an unbiased measure of habitat preference but were unfortunately poorly effective collecting only1-2 nymphs per trap on average. Collecting ticks from the volunteers clothing appeared to be an extremely simple, easily standardised means of collection. It is also very practical in that it is risk to humans that one really wants to know about. One might hypothesise that as the volunteer walks, increased perspiration may attract more ticks. This was not considered in this study and the volunteer always walked the same order through the habitats. However a significant difference was found between habitats and this was not associated with distance walked. The lowest frequency was found in the woods (0.25 adults/km), Medium frequency was in the low grass (1.1 adults/km) and highest was in the high shrub (6.04adults/km). Interestingly this pattern was different in the autumn when adults were collected at 32.3/km in woods, 12.3/km in high shrub and 0.64/km in the grass-low shrub ($P<0.001$ differences by X^2).

Nymphs and larvae were collected almost entirely by blanket dragging in the woods and there was no difference between infestation rates upon mammals between habitats or sites though numbers were small (n = 49 for two seasons and four habitats). The study suggests that adults are more likely to be found in the drier meadow because their larger volume:surface ratio makes them more resistant to dessication. A similar result was also found by Sonenshine (1968) for *Dermacentor variabilis*. Differences between sites may also be explained by other means. The nymphs and larvae remain within the leaf litter, only attaching to humans when they stir up the leaves whereas adults quest higher and get picked up by blanket drags when nymphs and larvae are left behind. The authors were unable to explain why the differences between habitats changed with season but the study did only last for 8 months.

Aeschlimann (1978) reviewed *Ixodes ricinus* biology and stated,"...But the absence of ground cover does represent an important limiting factor for the distribution of this tick. It is not found in grazed, open pasture, permanent marshland or peat-bog. It is found only rarely in intensively cultivated fields." This would probably come as somewhat of a surprise to those who spent many years looking at the tick on sheep grazing!

1.7. Mortality

Ixodes ricinus lays between 1000-2000 eggs per female depending upon the degree of engorgement (Gray 1981). When the population is at equilibrium therefore, the

mortality rate must be high. It is therefore important to know the percentage of eggs laid that are viable and what enviromental factors alter this viability. Heavy rain or frost may be factors affecting viability. Gray (1981) found over 98% of eggs hatched under his controlled field conditions.

Predation is certainly a cause of mortality in ticks as many hosts self de-tick. Milne (1949) found no ticks below the neck in 320 specimens of birds examined and Randolph (1975) describes how a collar on wood mice *Apodemus sylvaticus* prevented self deticking and led to larvae on the body where they would otherwise not be found. Helmeted guineafowl *Numida meleagris* have been shown to eat *Ixodes damminii* in the United States and to glean ticks from warthogs *Phacochoerus aethiopicus* in Africa (Duffy *et al* 1992). Furthermore a tick that has just ingested 4-5 mls of blood (Sonenshine,1992) will provide a nutritious meal and be a sought after prey item. Milne (1950) examined the matter further dropping 18 carefully labelled ticks into the vegetation. Of these 9 disappeared despite their well known quiescence, 8 were found torn open in a characteristic fashion and only one remained alive. When 11 females were put in a wire cage, bird proof but not rodent proof, 3 went missing and 4 were torn open. Further study using canister traps loaded with ticks found common shrews *Sorex araneus* to be associated with the empty tick shells. It therefore appears that predation is an important factor in *Ixodes ricinus* ecology, although if Milnes' ticks had unknowingly moved laterally it would probably be much less so. Gray (1981) suggests the cold to be important as he found that temperatures below -10^{o}C killed diapausing larvae in Ireland. However presumably this could not apply to the ticks of Finland where they would be expected to have developed a cold resistance. Likewise ticks at any latitude should then be able to tolerate most minimum winter temperatures and it may be instructive to test the cold tolerance of ticks from differing latitudes. Samsinakova *et al* (1974) described how parasitic fungi can kill off both ticks and their egg masses and Campbell (1948) describes how he painted his ticks with gentian violet or brilliant green to ward off fungal infection.

1.8. Hosts

The tick-host relationship is probably the most intriguing aspect of *I.ricinus* study. There are three main questions to be answered:

1) How does the tick find its host and how do these mechanisms result in an apparent preference for particular hosts?

2) Do ticks show a host preference in so far as they may sometimes choose not to feed on a potential host?

3) How does host density affect tick density?

1.8.1. Host finding

Although some species of tick are capable of moulting upon the host this is not usually the case. For the nest or burrow dwelling species (the nidi-colous species) re-finding the host does not represent a problem. However free ranging species such as *I.ricinus* must wait for a second host to pass by. After the hatching or moult, the larvae, nymph or adult climbs vegetation from where it exhibits questing behaviour. This involves waving its front two legs about in the air, upon which are mounted the sensory Haller's organs, universal among ticks. These detect odours such as NH_3, lactic acid or CO_2 (Sonenshine 1992). Lees (1950) has also shown the tick to respond to the odours of sheep, dog, horse, cow and rabbit hair when held at 37°c close to the tick. When a suitable host brushes the vegetation, the tick attaches. In the questing position the tick is in a potentially precarious position. It is perhaps more vulnerable to predation but more importantly it is liable to dessication. Consequently a tick such as *I.ricinus* will retreat back to the plant base in the absence of a host. Slight variations in this behaviour will result in both the questing success and choice of host. As described above, ticks have a diurnal rhythm and this will most likely cause it to attach to a daytime active host. The height in the vegetation from which the tick quests is likely to affect host choice. Ginsberg and Ewing (1989) found adult *I.dammini* up to waist height where larvae and nymphs remained in the leaf litter. Aeschlimann (1978) states but presents no evidence that female *I.ricinus* can often be found "...lurking on shrubs more than a metre off the ground", where nymphs occupy a median and larvae a lower position. Milne and Lees (1951) similarly reported without evidence, that stages were found equally at all heights.

1.8.2. Host Preference

Ixodes ricinus is found on practically any vertebrate that enters its habitat. In Britain 85 host species are recorded including 2 species of lizard, 49 species of bird, and 34 species of mammal (Martyn 1988). However, there is not an even distribution across or within species. Macleod (1932,1939) for instance found Black-faced sheep to be more susceptible to female tick infestation than Cheviot sheep and that susceptibility decreased in the order milk ewes, barren ewes, hoggs. Similarly, Brahman cattle in the USA support fewer ticks (*Amblyomma americanum*) than Hereford cattle (Hair and Bowman 1986). Milne (1949) also compared differences between sheep. Ewes that had recently delivered lambs were particularly susceptible. Infestation was less on coarser haired "goatskin " sheep but was not related to wool, pH, suint or greasiness. Leaner sheep were more infested than fatter sheep .

A number of workers have found differences in the degree of tick infestation of different rodent species in similar habitats. Nilsson and Lundqvist (1978) found more *I.ricinus* on *Apodemus sylvaticus* than *Clethrionomys glareolus* caught in the same traps. Matuschka (1991) similarly found more *I.ricinus* upon *Apodemus agrarius* and *A.flavicollis* than *C.glareolus*. Randolph (1975) found *I.trianguliceps* most frequently upon *Sorex minutus* followed in order by *Micromys minutus, A.sylvaticus, Sorex araneus, C.glareolus* and *Microtus agrestis*.

The differences are usually explained in terms of host habitat, size, range and periods of activity which result in an altered rate of contact with the ticks (e.g. Milne 1949, Randolph 1975). However this does not explain differences within species or indeed between domestic breeds. Nilsson and Lundqvist (1988) placed ticks upon *A.sylvaticus* and *C.glareolus*. Many on *C.glareolus* left the host without feeding whist on *A.sylvaticus*, they stayed and engorged. This would suggest that the tick in some way assesses the host before feeding and if the host is unsuitable it leaves. It also leads one to conjecture that the tick could make a choice whilst still upon the vegetation from a difference in odour production. For instance a poor conditioned animal with poor immunity perhaps produces more lactic acid and CO_2 which as mentioned are attractant to ticks.

If the tick did behave in this manner it would face a strategic dilemma at the point of questing. To what extent should a tick choose to which host it attaches? After all whilst a tick can go for many years without feeding, crawling up and down a plant consumes energy and can only be done a finite number of times. One can imagine a tick on the end of a shoot, close to dessication and with little remaining energy reserves, that must decide whether to retreat to the plant base, perhaps with insufficient energy to return, or to wait just a bit longer for a potential host to come by. And it is in this dilemma, because it failed to attach earlier to an available animal that was not the most suitable host. The same must surely apply for a tick that is upon the host that can either risk a poor feed or drop off and risk not finding another host. It may be that feeding strategy is a function of energy reserves and diminishes with their exhaustion. A study of questing behaviour and host preference may demonstrate reasons for failure of attachment as a cause of mortality, and indeed, this would be an area open to exploitation in development of suitable tick repellants.

1.8.3. Possible reasons for host preference

Host preference is likely to affect tick survival in a number of ways. The host may self de-tick though many ticks avoid this threat by feeding in such places as behind the ear

or around the eye. Animals such as rabbits are thought to be incapable of deticking (Milne 1949), because of their clumsy mouths. Hosts may offer differences in skin thickness or immunological response that make them more suitable for feeding. The tick's saliva contains a veritable array of pharmacoactive substances that must be more active against some species than others. In the white footed mouse *Peromyscus leucopus, Ixodes dammini* appears to produce immune suppressants specific to that species which prevent a response (Sonenshine 1992).

Host specificity may be important also in mate finding. Mating often occurs upon the host and if ticks are specific in their host selection they may be more likely to find a mate. In *I.ricinus* this is unlikely to be important as most females have mated upon the vegetation before feeding (Gray 1987).

A hosts range must be important to the tick. A host may wander from suitable to unsuitable habitat and this could be a disaster for the tick. However it may be that the tick does not simply drop off when replete but hangs on until it detects a safe environment e.g humidity, temperature, gases from decomposing vegetation. Matuschka *et al* (1991) attempted to address this problem in the laboratory. They used Central European *I.ricinus* and North American *I.dammini* reared in the laboratory and placed these on the candidate, caged hosts. These consisted of sand lizards *Lacerta agilis*, black striped mice *Apodemus agrarius,* yellow necked mice *Apodemus flavicollis,* bank voles *Clethrionymus glareolus*, white footed mice *Peromyscus leucopus* and red squirrels *Sciurus vulgaris.* The cage floor was divided into squares and underlying each was a cube of water. Of 350 cubes, a resting chamber covered 28 of them. The site of detachment relative to the resting chamber was then noted. With *I.dammini,* most larvae and significantly fewer nymphs detached in the resting area. However with *I.ricinus* nearly 90% of both larvae and nymphs detached in the resting chamber from *A.agrarius*. The workers were unable to induce nymphal attachment to *A.flavicollis* and *C.glareolus* but about 90% of larvae detached in the resting chamber. Lizard feeding ticks detached outside the resting chamber. These results are not easily interpreted: If nymphs are detaching in a place where the adults are not going to feed or the larvae where the nymphs refuse to attach, the tick is not going to survive. The authors suggest that *I.ricinus* is less well adapted to its host than *I.dammini* and that consequently *A.flavicollis* and *C.glareolus* may be zooprophylactic. However the paper did not state how long the hosts spent outside the resting chamber, which in view of the availability of food and the small available range, was possibly unnaturally short. Secondly 90% of ticks detached within 2 days. Larvae normally take 3-5 days to feed (Arthur 1963). Thirdly there may be other stimuli in the environment to

precipitate drop-off and these were not present here. Temperature for instance was kept unrealistically high, between 19 and 23°C. Nutall (1911) noted that *Boophilus decoloratus* 'rattled like peas on the ground' when a cow was taken from its warmed stall into the cool air of the courtyard in Cambridge. It would certainly make sense for a tick to drop off in response to a temperature drop if this reflected a move away from the burrow or nest.

It may be then that each candidate host would represent a set of advantages and disadvantages to the tick and that preference would not be advisable as they would balance. On the other hand those that were specific to their host would likely evolve a close relationship. However this is not an argument for evolving the preference in the first place. The wide range of hosts used by I.ricinus suggests that host quality varies little but that costs of failing to attach to a host are high.

1.8.4. Host Density

Density of susceptible hosts is an important factor controlling population sizes of macroparasites such as nematodes (Anderson 1979). Ticks are also forms of macroparasites and one might consider that host density should be important similarly. However one might also compare them with mosquitoes where the population is independent of host population size and more dependent on the presence of breeding sites. The fundamental difference between mosquitoes and ticks is the mobility of the former. In the presence of just a few hosts many mosquitoes can still find a feed where few ticks would get to attach and most would starve. One would expect then that host density would be important to tick numbers, determining the probability of tick-host contact and therefore the number of adults fed, numbers of eggs laid, larvae questing and so on. That is, the reproductive rate should be dependent on host density. Condition related mortality may hide this effect but logically it should be present.

One difficulty here is that many hosts reduce habitat suitability for ticks, and so tick density as they increase, by their grazing behaviour. Sheep, rabbits, cattle,and deer can all produce a very short sward at high density to the detriment of the tick population. Any study looking at host density and tick populations will have to take this effect into account.

1.8.5. Dispersal

The question of dispersal does not really appear to have been addressed in the literature. Since there is little evidence of intra-specific competition, dispersal may not be advantageous to the tick. Indeed if the tick must find a mate in the vegetation,

dispersal may present some real problems. Consequently whether or not it is advantageous to a tick to disperse may affect its choice of host. One may hypothesize that larvae feed on rodents because by dropping off in the nest they can be assured of a second host and consequently nymphs are also found on rodents. However if adults fed on the same rodents they may endanger the host and consequently the viability of their egg cluster which requires that the host has not deserted its nest 6 months later. By feeding on a different, larger host, they ensure dispersal and do not endanger their progeny.

1.8.6. Lateral Movement and Pheromones.

It has been assumed that *I.ricinus* does not move laterally apart from that which occurs incidentally to climbing up and down stems. This is based primarily upon the fact that marked ticks protected from predators do not move away from their point of placement (Milne and Lees 1951). However Gray (1982), states that unfed ticks are capable of moving some distance laterally. One would surely expect this for a tick which mates in the vegetation. Furthermore *I.ricinus*, unfed, virgin females have been shown to attract both males and females. Aqueous extracts have a similar effect though males are not attracted to males. It appears that the female releases a pheromone that has the properties of both assembly and sex pheromones (Sonenshine 1992) but this has not yet been biochemically identified. The pheromone will require lateral movement for effect.

1.9. Methodology of tick sampling

Ticks are collected by two methods principally, the blanket dragging method and by off-the-host methods. In the case of the latter the ticks may be left *in situ* and the population is not disturbed. The hosts may be trapped in the case of small mammals, shot as in the case of deer or just held as in sheep. Off-host methods offer a number of advantages. The degree of host infestation reflects tick activity over the past few days and is not so dependent upon the weather. It also reflects 'real' tick activity without the artificiality of the blanket method.

Hosts may pick up ticks from burrows and other hidden sites where the blanket never goes. However individual variation in infestation rates is enormous (see below) and so large host samples are required before inferences can reliably be drawn. Furthermore it is rarely possible to dictate the host's movements and therefore to say where the ticks have come from.

Blanket dragging is an alternative and Milne (1944) did a lot of work with this method. A standard woolly blanket is drawn over a measured piece of ground and the ticks are then counted. The result is a measure of the number of ticks active at the moment of dragging and reflects time of day and meteorological conditions. Ideally any compared drags should be carried out simultaneously. As the blanket is drawn over the ground the ticks cling to the underside. However many also fall off. The number on the blanket at 50 yards is not double that at 25 yards. However Milne with the help of RA Fisher suggested a method for estimating the ticks collected per yard if the above two measurements had been made. This assumed uniformity of the vegetation surface and raises another difficulty associated with blanket dragging in that the blanket tends to ride over taller vegetation missing ticks questing below. Bracken is the main problem here and so counts should be done before it gets too high. Blankets get less woolly through the season and Milne found that a blanket that had been used for half a season collected significantly more ticks ($P<0.01$) than a blanket used for 2 seasons.

It should be noted that some papers refer to 'flagging'. This is an American term and involves flagging a piece of cloth against the vegetation and has the advantage of not losing attached ticks. However it is also very difficult to standardize. Sometimes however the term is used interchangeably with blanket dragging.

Whatever the method of tick collection the distribution of results is unlikely to be normal and ticks tend to occur in clusters whether in vegetation or on hosts. With ticks this may be explained by the fact that the eggs are layed in clusters and so a host passing through a consequent cluster of larvae will get heavily infested. However this form of distribution is well recognized among parasites and may be the result of particularly susceptible hosts occurring in the population. It is thought to lead to a more stable host-parasite relationship (Anderson 1978).

An important point here is that when comparing some results, whether blanket counts or host counts appropriate statistical analyses are used. This was something of which Milne(1944) was well aware. RA Fisher suggested normalising the distribution by using the logarithm or the square root of the number but this didn't really change the outcome, so with a lack of better methodology he continued to treat the distribution as if it were normal. Another useful point made in this paper was that if one is comparing habitats by carrying out a number of drags on a number of occasions one is more likely therefore to get significant results by increasing the number of occasions dragged rather than the number of drags per occasion.

1.10. Tick Population Control

It may be possible now to examine the factors which determine tick abundance.

1)Conditions. Temperature extremes and dessication cause significant mortality.

2)Host factors.

a)Host Density. May determine the chance of a tick attaching and thereby avoiding starvation.

b)Host Species. May determine the likelihood of a successful feed and therefore the degree of oviposition. It may also determine likelihood of a tick being removed before completion of feed.

c)Host Immunity. Also determines the likelihood of a successful feed.

3)Tick Density.

a) Increased density leads to an increased chance of finding a partner and therefore increased mating. However, the abolition of behavioural diapause in mated females may lead to their transfer to the Autumn population where a higher mortality has a negative effect upon the population (Gray 1982).

b) Increased tick density leads to increased biting rate and so probably an increased development of host immunity, reducing feeding success and oviposition rate of later stages.

c) Increased tick density leads to an increased biting rate and therefore a likely increase in hosts' perception of the ticks presence. Hosts are then likely to increase measures taken to dispose of the ticks. Work has shown that this mechanism of density-dependent population control operates with the Triatomine bug *Triatoma infestans* that transmits Chagas' disease. (Schofield 1985). It seems that a similar situation could occur with *I.ricinus*.

2.0. The ecology of *Borrelia burgdorferi.*

2.1. Introduction

Lyme Disease is the first non-occupational bacterial zoonosis to be described (O'Neill et al 1988) and is caused by the spirochaete *Borrelia burgdorferi*. This is usually transmitted to humans by the nymphal stage of the hard tick, *I.ricinus* in Europe and *I.dammini* in North America. Other ticks have been implicated also. *Ixodes hexagonus* has been shown to be an efficient vector in the laboratory (Gern *et al* 1991), *Ixodes pacificus* appears to be important on the west coast of America and *Ixodes scapularis,* only recently differentiated from *I.dammini*, in the south eastern states of the USA. *Ixodes dentatus* feeding on cottontail rabbits *Sylvilagus floridanus* in the USA and *Ixodes persulcatus* are also competent vectors (Anderson 1989), though the strain from *I.dentatus* and the cotton-tail rabbit has been shown to be antigenically distinct (Anderson *et al.*1989).

Amblyomma americanum and *Haemaphysalis leporispalustris* may also carry the spirochaete (Anderson 1989) as does *Dermacentor variabilis* (Anderson 1983). A few individuals of *Ixodes uriae* from the Isle of May, UK (M.Harris, *pers.comm*) and from Sweden (Olsen *et al,* 1992) have been found to be positive for *B.burgdorferi.*

This review refers mainly to work done in North America where Lyme disease is most prevalent and most research has been done. However reference to work on Lyme disease in Europe, transmitted by *I.ricinus* will be made where possible.

Transovarial transmission of the tick is low but higher in *I.ricinus* than in *I.dammini*. A Massachusetts study found 2/274 i.e. <1% larvae infected whereas just under 5% of wild caught European larvae are infected (Lane *et al* 1991 and references therein). If only 20% are infected as adults this is getting on for a 25% vertical transmission rate. However larval meals will be smaller, carry a lower spirochaetal load and so will be infective much less often. Adults rarely feed on humans and so it is the nymphal stage that will transmit most of the disease. The bacteria are transmitted from tick to host perhaps by both a combination of salivation and regurgitation (Anderson 1991). Salivation is probably more important (Lane *et al* 1991).

Perhaps the most important next question to answer will address the relative involvement of species in feeding and infecting ticks. Only then can the manipulation of populations be considered in order to reduce the rate of tick infection.

2.2. Deer

Deer feed many ticks and there is strong evidence that the white-tailed deer *Odocoileus virginianus* is the primary host for *I.dammini*. Islands populated by deer have the ticks but those without, do not. Elimination of a deer herd on one island significantly reduced the tick population when it rose on an untreated island (Wilson *et al* 1988). Furthermore, Anderson *et al* (1987c) compared islands with and without deer to find that 31/51 rodents were infected with both *B.burgdorferi* and the tick transmitted protozoan *Babesia microti*, on the deer-inhabited island, where on the deer-free island both ticks and infection were absent. However Telford III *et al* (1988) looked for infected larvae from 20 white-tailed deer in an area where 23% of ground swept nymphs were infected. Only 2 of 185 derived nymphs were positive for the spirochaete and none of the blood samples grew spirochaetes. The team concluded the deer to be incompetent hosts. This is in contrast to other workers (Bosler *et al* 1983,1984 and Magnarelli *et al* 1984,1991) who have grown spirochaete and demonstrated anti-tick antibodies in deer blood. In Europe, some 50% of ticks collected from Red deer *Cervus elephus*, from the Isle of Rhum are positive by PCR for spirochaete (S.Curtin pers.com.) and some 86% of 45 roe deer from the New Forest and Thetford were antibody positive (Muhlemann and Wright 1987).

2.3. Birds

Birds may potentially play parts both as a disease reservoir and as a means of disease dispersal. Anderson and Magnarelli (1983) removed 52 infected larvae from 87 birds out of 352 larvae examined. They also grew spirochaetes from the blood of 7 of the birds. Birds commonly infected were swamp sparrows *Melospiza georgiana*, gray catbirds *Dumatella carolinensis* and common yellowthroats *Geothlypis trichas*. In further studies, Anderson *et al* (1985 and 1986 respectively) isolated spirochaetes from the liver of a veery *Catharus fuscecens* and the blood of a common yellowthroat. 2 house wrens *Troglodytes aedon* carried 6/21 larvae infected. Anderson, Magnarelli and Stafford (1990) cultured *B.burgdorferi* from 27% of larvae recovered from birds, including 7/8 of specimens from 4 American Robins *Turdus migratorius* and 14/16 specimens from one house wren. Other birds did not produce infected larvae and this included 40 from an unstated number of gray catbirds. Three nymphs moulted from larvae that had fed on American Robins produced borrelial isolates that infected 16/18 Syrian Hamsters. Analysis of strains by SDS-PAGE and Western blotting from hamsters and ticks found most of them to be indistinguishable from a reference (B31) strain. Weisbrod and Johnson (1989) looked at 9,200 birds from 99 species in a tick endemic area in Wisconsin and Minnesota and removed 235 larvae of which 49 were

infected (21%). Of these 21 were from ovenbirds *Seiurus aurocapillus* and 10 from Northern Waterthrushes *Seiurus novoboracensis*. The authors point out that nearly half of the spirochaete positive ticks were from migrating birds. Mather *et al* (1989) looked at the ability of Gray Catbirds to infect larvae. Of 28 birds only 12 larvae were removed that had blood fed and so were examined. None contained spirochaetes. The 12 catbirds were each infested with about 20 larvae. Unlike a group of mice treated similarly, none of the larvae became infected. The catbird was concluded to be an incompetent host. Burgess (1989) inoculated Mallard ducks *Anas platyrhynchos platyrhynchos* with *B.burgdorferi* and detected it in the cloacal material. The only work on birds in Europe has been by Matuschka and Spielman (1992). From two blackbirds *Turdus merulus* they collected 70 ticks, about equal numbers of nymphs and larvae. Then they placed batches of 10 infected nymphs on each of two laboratory reared blackbirds simultaneously with uninfected nymphs. This procedure was carried out at intervals over the ensuing year and after 213 nymphs and 190 larvae had fed, no infection was transmitted. Mice treated simultaneously transmitted infection as expected. The workers conclude that blackbirds do not infect ticks but also that they absorb infection from the ticks. It is suggested that this is because of the high core temperature of the blackbird. Temperatures of 43^{0}C destroy the spirochaete *in vitro*.

One must express some caution with such laboratory based infestation experiments. Here the birds are being rested and fed in a way that is very different to that in their normal habitat such that one would expect their immune systems to behave much more efficiently than in the wild.

It would seem that the only clear way to assess a hosts real infectivity would be to count the number of infected ticks that it produces in the wild. If one knows the infection rate for questing ticks and engorged ticks at each stage then the rate of infection is calculable. This is further discussed later. The main problem here is that one will require a large sample of hosts since parasite distribution is clustered so that many ticks are found upon few birds. This means that if a high proportion of ticks are infected, they may simply have clustered upon an infected bird and vice versa. Therefore, many birds of one species will be required before valid conclusions can be drawn. As for the importance of birds in general as hosts, by their sheer numbers they must be considered as potentially very important and further work is clearly required in Europe and North America to define their role both as a whole and as individual species.

2.4. Rodents

A point that is without controversy is that rodents are important hosts of *I.dammini* and carriers of spirochaetes. In the USA, the white footed mouse *Peromyscus leucopus* is a species both commonly infected and infested. Anderson (1983) for instance collected 181 larvae from 51 hosts of which 25% were infected. 46% of 339 larvae from 20 mice were collected by Mather *et al* (1989). Other American workers have come to similar conclusions and isolated the spirochaete from the tissues of *P.leucopus (*Spielman *et al.*, 1979; Piesman and Spielman 1979; Anderson and Magnarelli, 1980; Main *et al.*, 1982; Anderson, 1985; Anderson *et al*, 1987a; Bosler *et al* 1983; Levine *et al.*, 1985; Loken *et al*, 1985; Donahue *et al*, 1987) In Europe rodents have been studied mainly in the Berlin area (Matuschka *et al* 1991). Here 58.2% of 29 larvae from 22 Black striped mice *Apodemus agrarius* were infected, 25.2% of 298 larvae from 83 *Apodemus flavicollis* and 38.6% of 83 larvae from 44 *Clethrionomys glareolus*. In Sweden spirochaetes have been isolated from the tissues of *Clethrionomys glareolus* and *Apodemus sylvaticus* that reacted with B.burgdorferi specific monoclonal antibodies H5332 and H9724 and with antibodies from a patient with acrodermatitis chronica atrophicans (Hovmark *et al* 1988).

A seasonal variation in prevalence of *B.burgdorferi* has also been reported (Anderson *et al* 1987b and Piesman *et al* 1987) with infection of white-footed mice twice as high in the summer as in the winter.

P.leucopus is the only wild animal in which systemic disease has been reported. Burgess (1990) found that 16 out of 80 mice developed motor dysfunction within 47 days of being caught. This always included trembling and a reluctance to move. Other signs included circling, head tilt and an inability to stand. Clearly, if a population is being affected to this extent by a parasite, then it may be playing a significant part in its control. If the *B.burgdorferi* is not directly causing the death of the mouse it is likely to be making it highly susceptible to predation.

2.5. Other Mammals

Other mammals have also been shown to be infectious to ticks with *B.burgdorferi*. Mather *et al* (1989) compared chipmunks *Tamius striatus*, white footed mice *Peromyscus leucopus* and meadow voles *Microtus pennsylvanicus*. The mice were both more infested with ticks and infective to them than the other two species though chipmunks and meadow voles, the latter much less so, were capable of infecting ticks and therefore acting as a reservoir. *B.burgdorferi* has also been isolated from cottontail

rabbits but this isolate is antigenically distinct from isplates from other sources (Anderson *et al* 1989). Magnarelli *et al* (1984) surveyed 656 small and medium sized mammals over four years in a Lyme disease endemic area. Gray squirrels *Sciurus carolinensis*, Racoons *Procyon lotor* and Virginia Opossums *Didelphis virginiana* all produced infected ticks and all had antibodies to *B.burgdorferi.* Anderson *et al* (1984) grew the spirochaete from the blood of a woodland jumping mouse *Napaeozapus insignis.*

2.6. Modelling Lyme Disease

Lyme disease clearly has a complex ecology. It appears that the spirochaete is harboured by many avian and mammalian species and often with a high frequency. Its relationship with these animals is largely unknown but probably varies among species. The bacteria may have pathogenic effects but usually infected animals appear healthy. The period of spirochaetaemia is unknown though seasonal difference in infection rates has been demonstrated in *P.leucopus.* It has also been shown in the rate of infection of ticks from Red Deer *Cervus elaphus* (S.Curtin, *pers.comm.*). This may be a result of mortality of summer infected animals but is probably, in deer at least, due to a loss of infectivity by the arrival of winter. Whether or not immunity develops is unknown but Min Hu *et al*(1992) have shown that the antigenic profile of *B.burgdorferi* changes with passage through the tick which will clearly interfere with its development. Transmission is considered to be via tick bite. However Burgess and Patrican (1987) have shown that *P.leucopus* can be infected with an oral dose of just 400 spirochaetes. Burgess (1989) has shown spirochaetes to be detectable in the faeces of an inoculated Mallard duck *Anas platyrhynchos platyrhynchos.* All this means that faecal-oral or prey-predator transmission must be considered. An infected rodent showing motor dysfunction may be highly predisposed to predation and this could be an important means of transmission for the higher birds of prey.

Much work has aimed at differentiating "reservoir competent" species from "incompetent" ones. This competency is determined by the ability of the animal to infect ticks. Mather *et al*(1989) constructed a simple model when comparing three sites in Massachusetts. This measured the reservoir potential (R_S) of a host which was simply the product of host numbers, the degree that a host is infected and the rate at which the host infects ticks. The aim of this model was to be able to provide an estimate of the relative importance of each candidate reservoir species in the population. However, R_S for a species is not an absolute measurement and can only be calculated if all candidate species are collected at the same time in the field for population sizes and infestation rates to be measured and all have their ability to infect

ticks measured in the laboratory. Then the R_s will not be extrapolatable to other sites. Lane *et al* (1991) go on to define three criteria for a species "competence"; namely: "Most of the host population should be infected with *B.burgdorferi*, should serve as host to large numbers of larval *I.dammini* and should be extremely infectious to these ticks". If nothing else, such thinking is clearly inadequate. It dangerously assumes a host-parasite system to be at equilibrium and makes no allowance for a species that is infective at a low level for a longer period to be compared with a species highly infective for a short period. Furthermore, unless species are studied simultaneously no quantitative comparisons can be made between them and this renders the approach crude. Lyme disease is an urgent problem and control measures are needed. Crude methods which rank potential hosts in an approximate order of importance are still useful as they allow workers to focus on the most important species.

Measurements of the Reproductive rate of a parasite- Rp (Anderson and May, 1979) in association with a particular host species will give a much clearer, quantitative indication of the importance of a host to the parasite population. Furthermore the use of models based on this parameter will enable us to examine and predict the behaviour of the disease in association with the host. The simplest way to measure Rp for a particular host species is by the method of Dietz (1975) modified by Anderson (1982) where $Rp = 1 + L/A$. L is the average age of the population and A is the average age at which infection is acquired. Unfortunately this method tells us nothing about the disease dynamics. More sophisticated models will require the measurement of a number of parameters, some of which at present are impractical. However it is instructive to examine them to direct our thinking in the construction of experiments. Simply (Begon 1990 developed from Anderson and May 1979):

$$Rp = BNfl.$$

'B' is the transmission coefficient of the disease, 'N' the population size of susceptibles, 'f' the fraction that become infectious and 'l' the period over which they remain infectious. The transmission coefficient of the disease is a measure of how easily it gets from one individual to another and will therefore depend on the size of the vector population and the vector biting rate. In fact where a vector is involved: $Rp = B^2 N_v/N_h f_v f_h L_v L_h$ (Begon *et al* 1990). The important point is that Rp will be sensitive to changes in the ratio of vectors to hosts . The size of the tick population is likely to be very important for the rate of spread and therefore prevalence of the parasite in the population. Here a difficulty rises. These models are based on the assumption that the vector population size is independent of the host population size. This is true with mosquitoes whose numbers depend upon finding suitable breeding sites and whose mobility allows them to find a host and feed whatever the size of the host population.

However tick numbers quite possibly do depend upon host numbers and feeding success and this may have implications. One can imagine a host population limited in size by the parasite population which will in turn limit the vector population. If the host population develops an increased resistance and expands, so the vector population will rise at a rate dependent upon its relation to the host. The vector/host ratio may rise so that the disease can become viable once again. In other words, B may be a partial function of N. Alternatively, even though a host may be resistant to the parasite it may remain important in maintaining vector numbers when the parasite-susceptible populations are declining as a result of infection. Matuschka (1992) suggests that blackbirds *Turdus merula* may be zooprophylactic for Lyme disease as they 'absorb' spirochaetes from the ticks rendering them uninfective. However if they maintain tick populations as a result of their resistance and therefore greater abundance, they may be anything but zooprophylactic. The same will apply to the white tailed deer. Randolph (1991) raised a further confounding issue. She showed that *Babesia microti* infected hosts, fed ticks more successfully than those without infection. It was suggested that this could be by the parasite causing anti-haemostatic effects, anaemia leading to reduced viscosity or by vasodilation. It is stated that the latter is a means by which Lyme disease enhances tick feeding success and of course ECM, the hallmark of Lyme disease, is a result of vasodilation. It has also been shown that ticks can have immunosuppressive effects (Sonenshine 1992) and this raises the possibility of another vector-host-parasite interaction. Randolph makes the point that the reproductive rate of a tick-borne disease depends upon tick numbers. Therefore the main mechanism by which a tick borne parasite can increase its transmission rate will be by increasing the reproductive rate of the tick. However we must be cautious here. As Dawkins (1981) points out, a parasite is not going to go to the expense of altering its host to improve its survival when it can cheat, let the other parasites do the work, and put its energies into reproduction. Only if it is a clone is it in its interests to carry a gene which affects the host at immediate expense to itself. If a spirochaete clone in the host causes vasodilation then, this should be considered an adaptation to improve the chances of the spirochaete being picked up rather than to increase the tick's feeding success and thereby its oviposition rate. This latter mechanism could perhaps be considered adaptive when a parasite is transmitted transtadially as in the case of the *Babesia microti* studied. However all this means that a tick population will rise faster in an infected population and may indeed be in part a function of the size of the infected population.

In the simplified equation above and in Anderson and May's (1979) more elaborate model prediction of the behaviour of the disease in a population may require

knowledge of some other parameters such as the period of infection, the latent period of the disease, the duration of immunity, the natural birth and mortality rate of the host and the mortality rate of the disease or its pathogenicity. One can then predict how relative populations of susceptible, infected and immune populations will change in number and how the host population size overall will vary as a result of this parasitism. As already mentioned however, it is not yet known if the presence of antibody constitutes the development of immunity, making measurements of its rate of change at present impossible. Secondly this may be inappropriate for the study of Lyme disease if there are many host species involved and the rate of overall vector infection would be independent of the rate of host infection. A preliminary study therefore should look at the degree of correlation between the product of host infection rates (measured by the number of infected larvae removed from the host) and host population size in areas, and questing nymphal infection rates 6 months later. This should reflect the importance of that species as a reservoir of Lyme disease. However the difficulties of measuring many of the parameters still remain.

Where more than one species is involved, other models may be required and it may be useful here to make a comparison with Louping-ill. This is a viral disease, also tick-borne and involving more than one host species. It is thought to cost the Scottish game industry considerably through grouse mortality. Hudson and Dobson (1991) used Rogers (1988) two-host-one-vector model developed for African Trypanosomiasis. The difficulty arose of measuring the duration of infection but the model most usefully does not require a knowledge of the mortality rate due to the disease. Importantly they concluded that one species can maintain a disease in a community, in this case it is thought to be the sheep, such that it can remain viable in another species, despite Rp for that species being close to zero. It may be possible to extend Roger's model for any number of hosts if duration of infection can be estimated and it would then be possible to assess the impact of each individual species on an overall Rp for Lyme Disease. It may be possible to calculate this in different settings, make alterations, such as by affecting host density or vector survival rates and then predict and measure the new Rp.

3.0. Tick species abundance by Loch Lomond.

In an effort to determine the relative abundance of the different tick species of differring stages, ticks were examined using a dissecting microscope. Samples of ticks were collected from mammals, birds and by blanket dragging and examined.

Whilst the principles of identification used were based on the keys by Arthur (1963), it would clearly not have been practical to examine each tick following the keys step by step. Therefore as ticks were most commonly *Ixodes ricinus*, ticks were examined for the characteristic features of this species and passed as this if they were present. If not the tick would be examined for the features of *Ixodes trianguliceps* and if the tick was neither of these species the keys would be used.

3.1. Identifying features

Ixodes ricinus

Female: Distinctively large, orange tick. Characteristic caudally projecting spine on first coxa. Head with bilateral cornual extensions and small auriculae on ventral surface. Scutum characteristic shape, broader than long.

Male: As above but smaller and all black.

Nymph: Smaller, oval shaped tick. Black scutum contrasts with pale body. Coxal spur, cornual extensions, auriculae and scutum shape as in adult.

Larvae: Again recognised by general shape and colour. Characteristic head and scutum shape. The presence of a coxal spur however was used as the defining criteria.

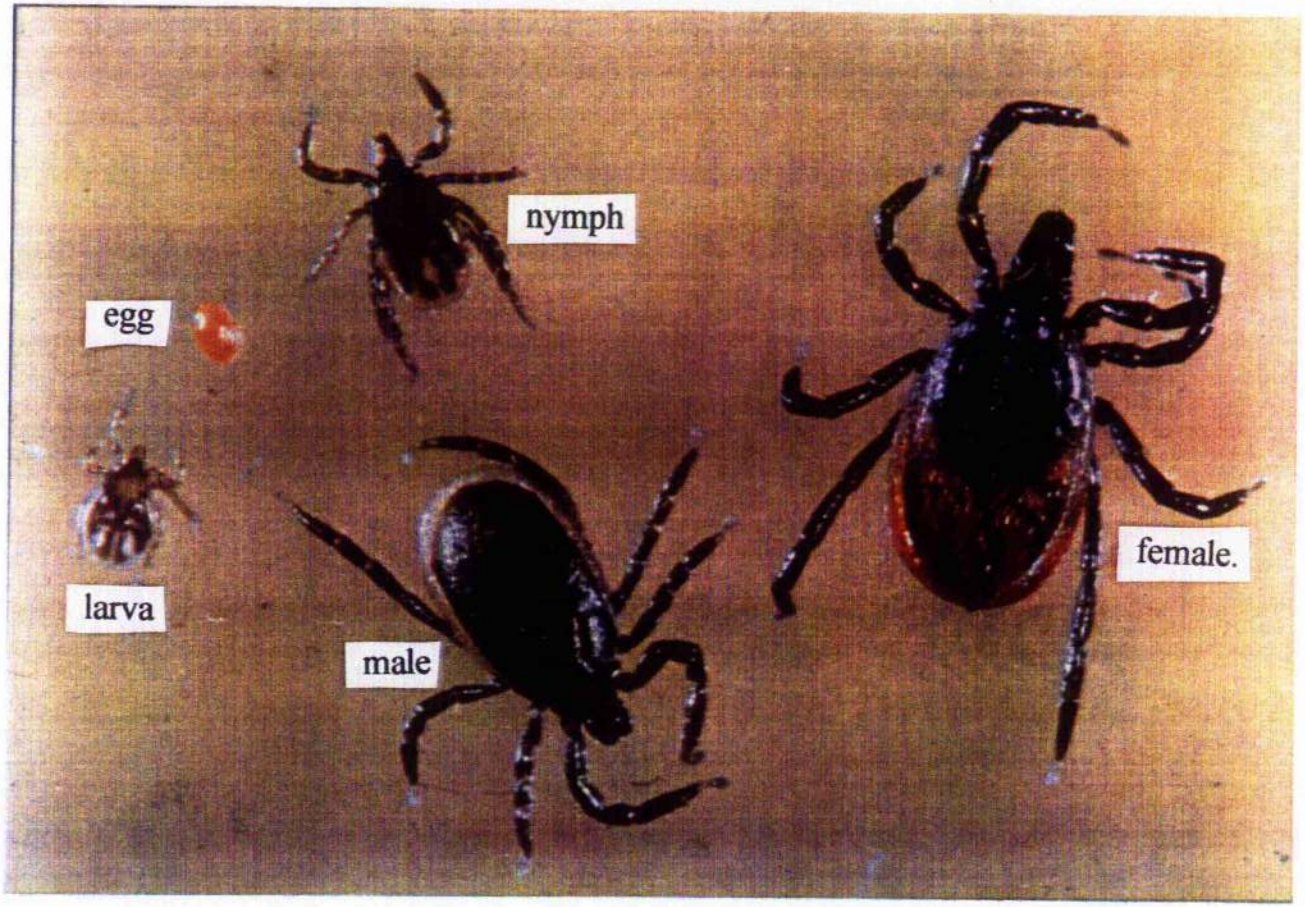
Ixodes triaguliceps

Adult: Absence of coxal spur and cuticle covering dorsal aspect of coxae. Wide base to head giving it a very triangular appearance and scutum much longer than broad.

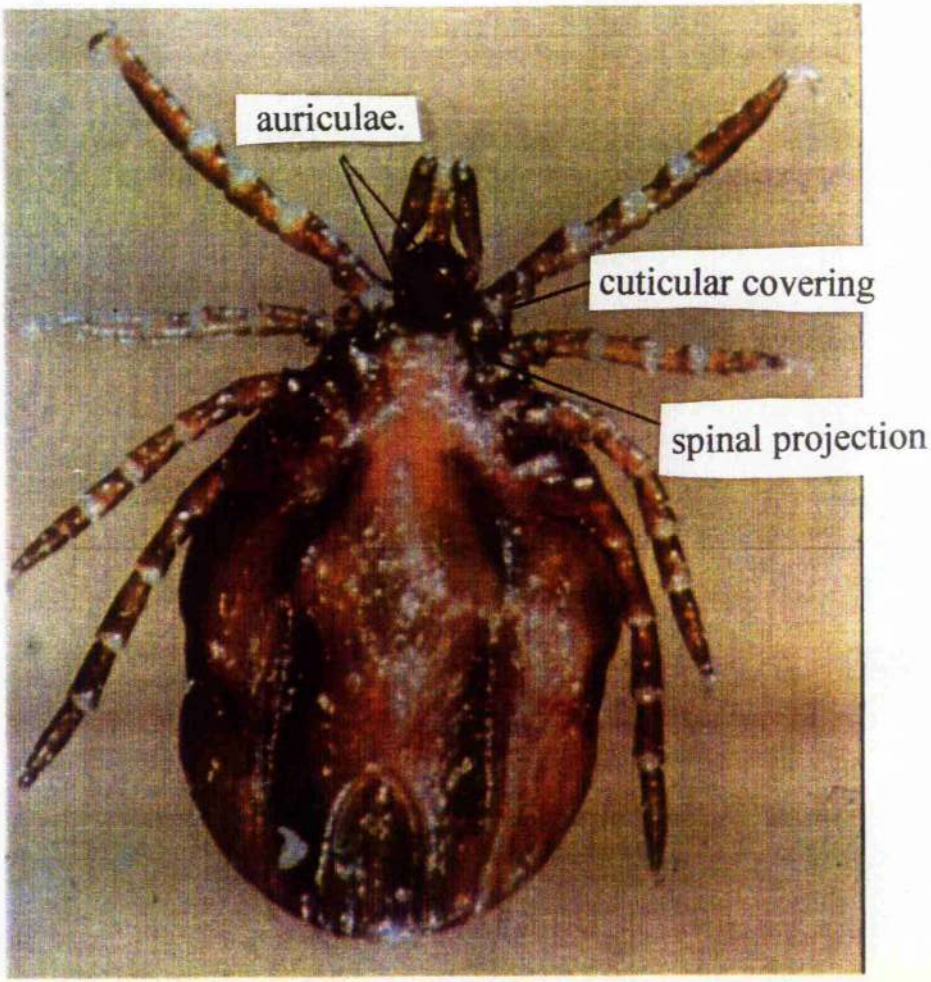
Nymph: Often very yellow appearance if not engorged. A longer narrower tick than *I.ricinus* and scutum similarly shaped. Head also very triangular and no spur on coxae.

Larvae: Also yellow with narrower scutum. Rounded first coxa.

Ixodes ricinus, all stages



Ixodes *ricinus* nymph.



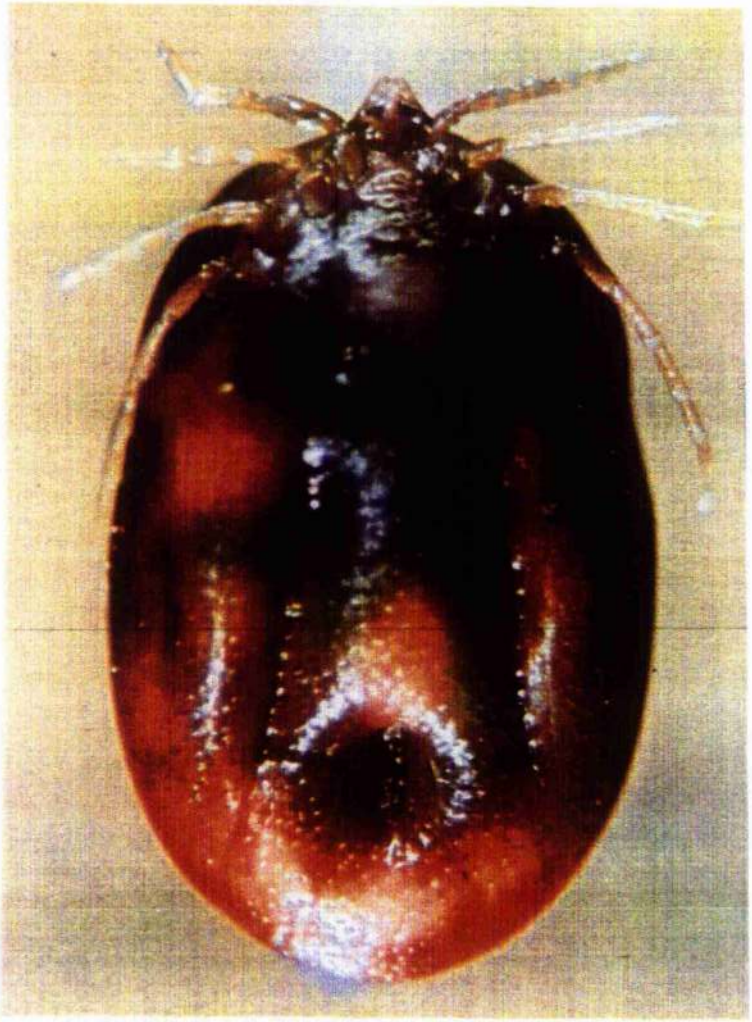
Ixodes ricinus

Female- ventral side.

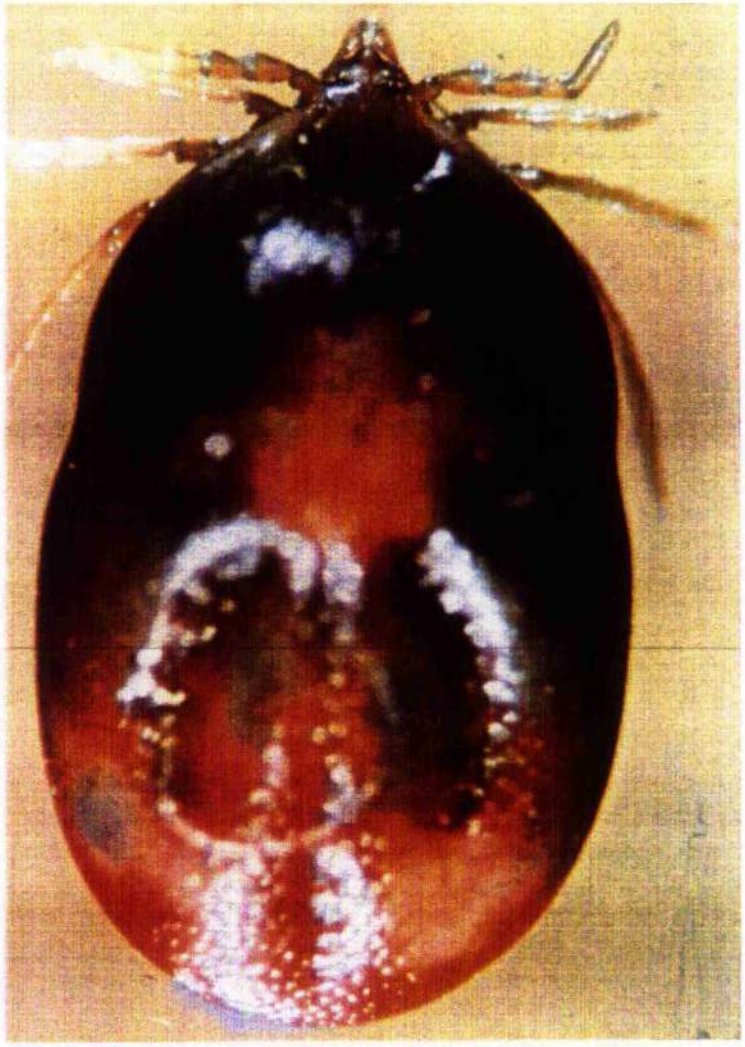
Male- dorsal side

Engorged Ixodes *triangulíceps* adult.

ventral surface

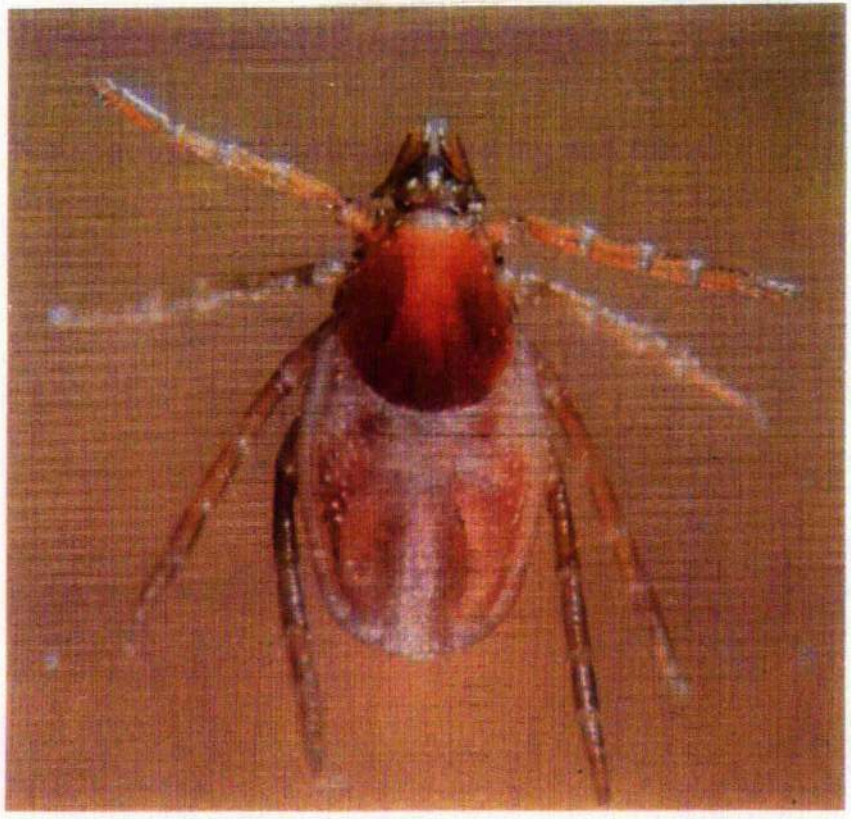


dorsal surface



Ixodes *triangulíceps* nymph.

Note: absence of cuticular covering and spinal projection on first coxa. Also absence of auriculae.



3.2Results

3.2.1. Bird Ticks.

168 ticks were examined.

Of 148 larvae, 30 were unidentifiable due to damage and all the 118 ticks identified were *I.ricinus.* Of 20 nymphs, 1 was unidentified due to damage and all 19 remaining were identified as *I.ricinus.* No adults were found on the birds. It had been thought possible that some of the nest-dwelling bird ticks such as *Ixodes frontalis* and *Ixodes arboricola* may be found on the birds as were found by Tovornik (1991) in Yugoslavia. However it appears from this study that these ticks are not frequent in this part of Scotland.

3.2.2. Mammal ticks

130 ticks were examined.

Of 122 larvae, 114 (93%) were identified as *I. ricinus.*

8 larvae were identified as *I. trianguliceps.* 3 of these came from the wood mouse *Apodemus sylvaticus* and 5 came from the bank vole *Clethrionomys glareolus.*

Of 4 Nymphs, 2 (50%) were identified as I. ricinus and 2 (50%) as *I.trianguliceps.*

All 3 adults removed were identified as *I.trianguliceps.*

1 tick was unidentified due to damage.

3.2.4. Blanket drag collected ticks.

170 Nymphs were examined and all were identified as *I. ricinus.* This was not a particularly surprising find since most species of tick are nidicolous, attaching and detaching only at the site of the host's nesting site. *I.ricinus* is an exception to this rule.

4.0. Estimating tick abundance in Rowardennan woodland.

4.1. Introduction

Estimations of tick populations, present a number of difficulties. Primarily, only ticks actively questing or feeding can be found and those dormant or moulting between stages form an invisible section of the population. Abundance must therefore usually be estimated as an index where spatial and temporal variation can then be examined. Even this approach is frought with difficulties since the efficiency of blanket dragging, the principal means of collecting ticks may well vary between environments resulting in a false picture of variation. In this section I describe work aimed at developing the blanket dragging method with a view to overcoming some of these difficulties and using this method to investigate tick abundance in vegetation.

4.2. Methods

A pale woollen blanket 1m x 1m was attached on one side to a length of 2cm diameter wooden dowling to which a dragging rope was attached. This served to deter the blanket from folding in on itself as it was dragged. Ten small (about 50g) steel weights were tied onto the trailing edge of the blanket at 10cm intervals to maintain reasonable contact between the blanket and the vegetation.

Stretches of ground of uniform vegetation type, 1m x 15m were measured out in the woodland at Rowardennan, east of Loch Lomond.. Since it was often relatively difficult to find such pieces of ground, usually because of trees or rocky ground interrupting a free run, areas could not be chosen on a strictly random basis.

Two areas were marked out for repeat dragging every month. Other dragset areas were usually marked out in pairs such that one was situated purely amongst blaeberry bush and the other was amongst the grass, moss and leaf litter. These pairs were within 10m of each other and were dragged sequentially. Another group of drags were paired to be within similar vegetation types but on opposing sides of a hill. Areas dragged repeatedly on a monthly basis were also paired with similar nearby stretches that were freshly dragged on each occassion. Thus, three variables that may affect the results from blanket dragging were addressed: vegetation type, aspect and repeated dragging, whilst weather conditions were also constant between pairs.

Each strip was dragged ten times. The blanket was dragged along the strip and then the ticks were removed at the end. Larvae were removed from the blanket by dabbing

it with a piece of sticky cellulose tape. The blanket was dragged therefore, five times in each direction. Each set of ten drags is termed a dragset.

4.3.Results

4.3.1. Abundance

54 dragsets were carried out over a six month period from April to September with the total number of ticks collected as in table 1. The ratio of males to females was 2.64:1 and the ratio of adults: nymphs: larvae was 1: 30: 142. Larvae and nymphs were both distributed in a clustered fashion with larvae much more so (fig. 10). The variance/mean ratio for nymphs was 61.6 and the equivalent value for larvae was 496. As relatively few adults were collected and the larval distribution was extremely clustered most analysis of tick distribution, either temporally or spatially, had to be confined to the nymphs.

Table 3. Numbers of ticks collected by blanket-drag sets at Rowardennan.

		total	mean/dragset	SD
All (n = 54)*	larvae	5715	132.9	256.9
	nymphs	1189	22.0	36.8
	males	11	0.20	0.60
	females	41	0.55	0.91
Blaeberry (n = 20)*	larvae (n = 16)*	517	32.3	62.7
	nymphs	625	31.2	58.6
	males	6	0.30	0.80
	females	17	0.85	1.14
Leaf litter (n = 34)*	larvae (n = 27)*	5198	222.2	340.0
	nymphs	564	16.6	11.5
	males	10	0.29	0.93
	females	24	0.69	2.1

*number of dragsets performed

As an area is dragged repeatedly the number of ticks collected on each extra drag diminishes, such that if a graph is drawn of the cumulative total of collected ticks, a curve forms which leads towards an asymtote. In this study the 54 dragsets were analysed to give the mean number of nymphs collected for all the dragsets and then blaeberry and leaf litter separately at each drag within the dragset (appendix 1). Cumulative frequency curves were then drawn (Fig. 9). The curves can be seen to be

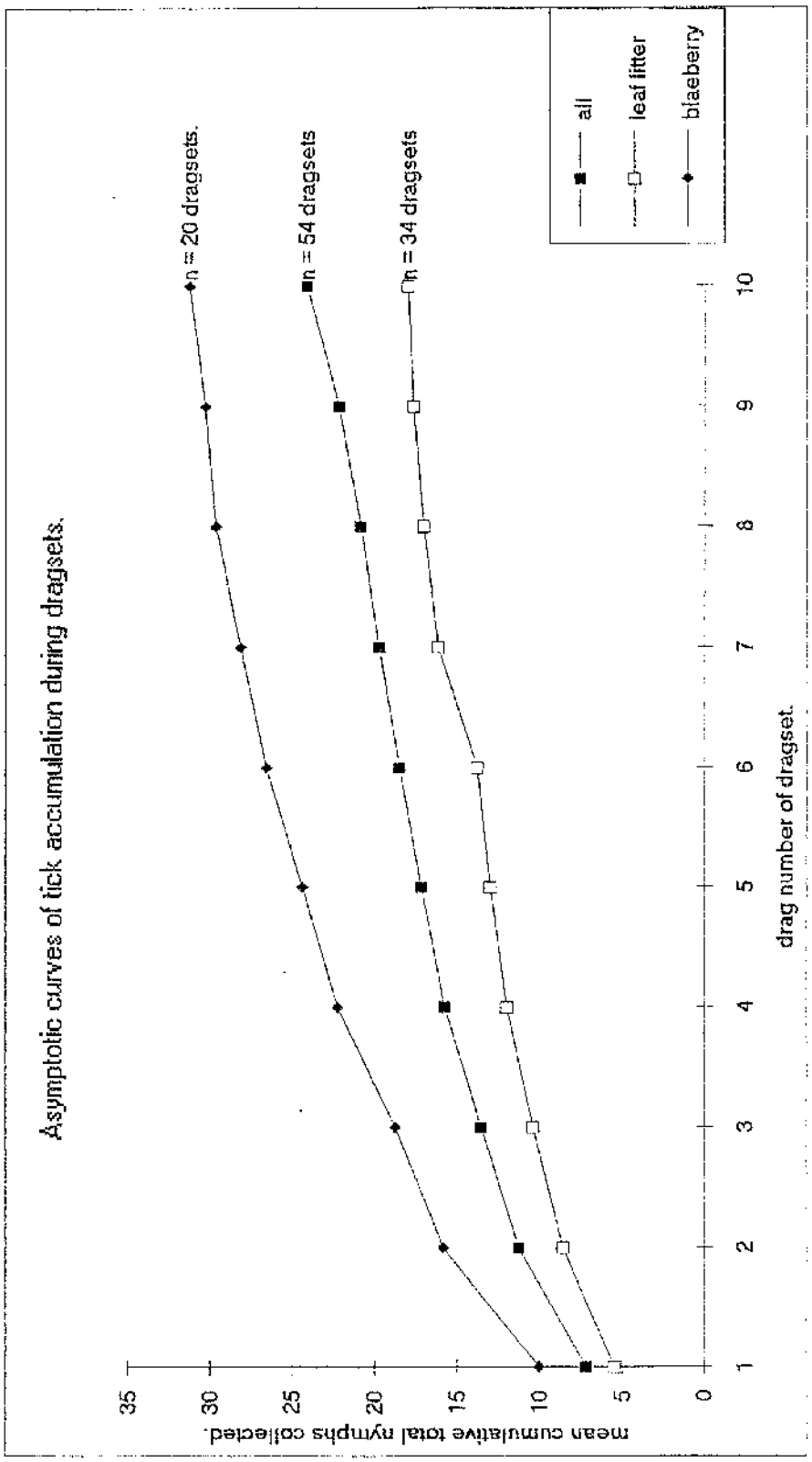


Figure 9. Graph showing tick accumulation during dragset.

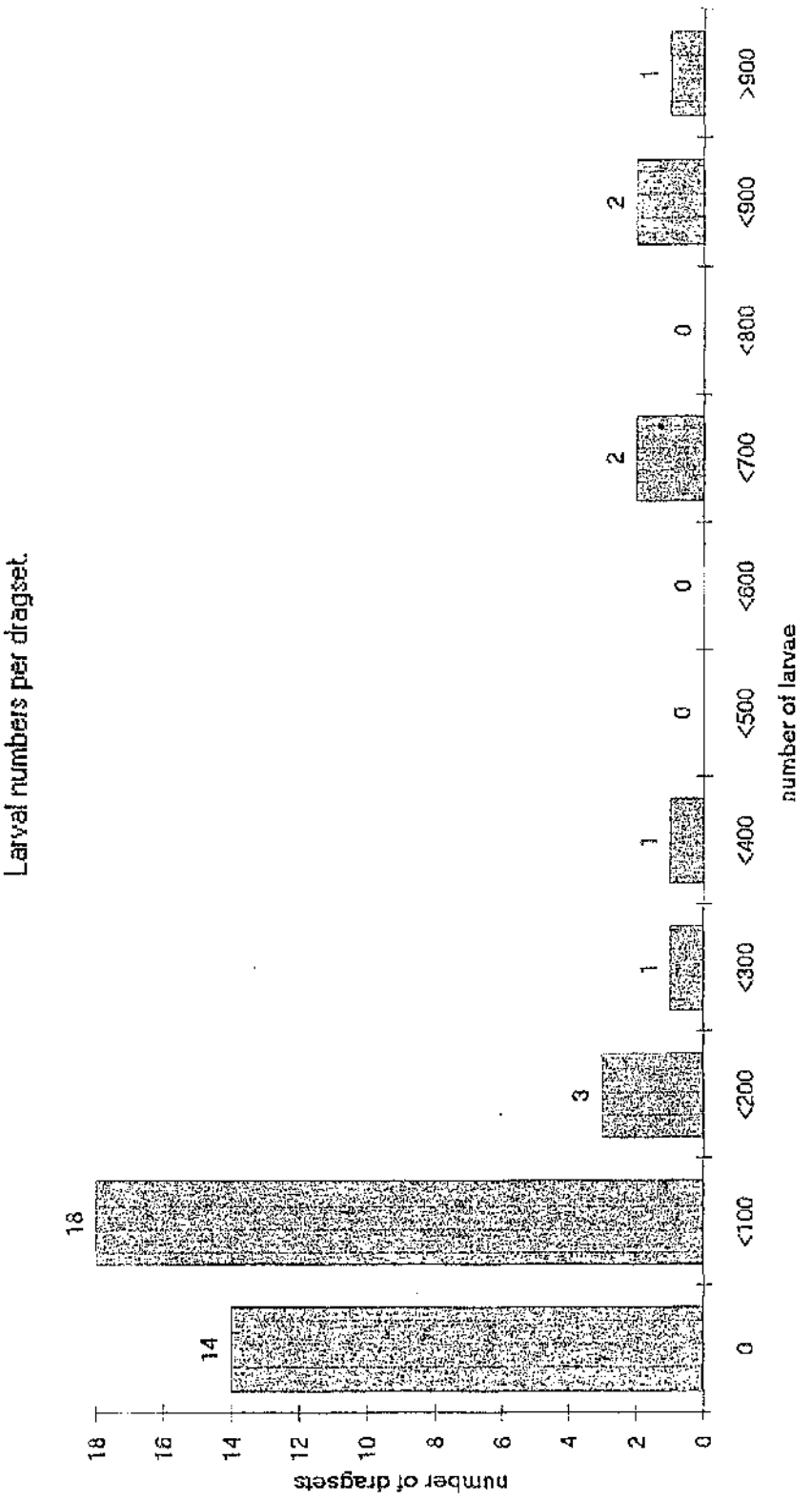


Figure 10. Histogram of larval numbers per dragset.

reaching an asymptote and empirically it is reasonable to assume that the asymptotic figure will reflect the true total of ticks questing at the time of the dragset. It appears that in the case of blaeberry, 98% of ticks are collected and in the leaf litter 95% are collected (see also appendix 2). Since 1189 nymphs were collected in 54 dragsets, the mean nymph count over the six month period was 1.468/m²/dragset. This reflects the abundance over 6 months in all areas.

Table 4. A comparison of numbers of ticks collected in repeatedly dragged areas with other matched areas dragged only once.

dragset pair	primary* dragset	secondary* dragset		log (primary)	log (secondary)
DA2/DI	21	31		1.32	1.49
DA3/D2E	31	23		1.49	1.36
DA4/D3A	46	12		1.66	1.08
DA5/D4C	37	37		1.57	1.57
DA6/D5A	22	18		1.34	1.26
DB3/D2H	26	20		1.41	1.30
DB4/D3B	24	9		1.38	0.95
DB5/D4D	20	11		1.30	1.04
DB6/D5B	7	3		0.85	0.48
means				1.36	1.17
variances				0.053	0.11
t=2.58	**P (2-tailed)**	**= 0.033**			

*primary dragsets were the monthly repeated dragsets and secondary ones were those performed in parrallel, matched for date, vegetation type, aspect of slope and weather conditions

The two areas that were repeatedly dragged on a monthly basis (areas A and B) totalled 173 nymphs and 107 nymphs respectively, each over 6 dragsets. This amounts to an average of 1.922/m², peaking at 3.067/m² in May on dragsite A, and 1.189/m² peaking at 1.733/m² in May on site B. In areas A and B therefore, the minimum number of nymphs coming out of winter dormancy to quest in this season was 9.333/m². Nine of these drags on areas A and B were paired at the time of dragging with areas of close proximity (table 4). As they collected more ticks than those that

they were paired with, it is clear that the numbers of ticks collected on one dragset is not diminished as a result of the collection the previous month.

4.3.2. Vegetation Type

In order that paired dragsets could be compared by means of a two-tailed, paired t-test, nymph counts were first logarithmically transformed. The resulting pairs (table 5.) showed a significantly greater number of nymphs in blaeberry bush compared to other vegetation which was a mixture of leaf litter, grass and moss (T=2.79, n =10 pairs, P=0.019,). This outcome is supported by the differing patterns of nymphal accumulation during dragsets (fig 1 and appendix 1).

Table 5. A comparison of tick numbers collected in Blaeberry and leaf litter.

Dragset pairs	Nymph total in blaeberry	Nymph total in leaf litter	log (blaeberry count)	log (leaf litter count)
D2E\D2F	26	33	1.41	1.52
D2C\D2F	8	10	0.90	1
DK\DL	6	2	0.78	0.30
DG\DH	8	8	0.90	0.90
DI\DJ	31	27	1.49	1.43
D2P\D2N	8	5	0.90	0.70
D2J\D2K	53	26	1.72	1.42
D3C\D3D	10	9	1	0.95
D3E\D3F	77	31	1.89	1.49
D4A\D4B	14	7	1.15	0.85
D5D\D5C	8	2	0.90	0.30
t = 2.79	**p(two tail) = 0.019**		1.19	0.99
			0.15	0.197

Site.

Of five pairs of dragsets carried out during April and May, all among oak woodland with blaeberry bush ground cover, a highly significant difference was found with ticks much more abundant on the south compared to the north side of a hill (T=5.31, n=7 pairs, P=0.0018 on log-transformed nymph counts, Table 6.). Unfortunately there were no other areas in the wood where north and south sides of a hill could be compared since most of the northerly facing wood is planted with conifers.

However on other south facing slopes in other parts of the wood there was not an abundance correspondingly large to that found on the south side of the hill under more intensive study.

Table 6. A comparison of tick numbers collected on two sides of a hill.

Dragset pair	North aspect	South aspect	Log (N)	Log (S)
D2D/D2C	8	26	0.90	1.41
D2E/D2F	10	23	1	1.36
DE/DF	8	38	0.90	1.58
DG/DJ	8	27	0.90	1.43
DH/DI	8	31	0.90	1.49
D3D/D3E	29	77	1.46	1.89
D3C/D3F	10	31	1	1
means			1.01	1.45
variances			0.04	0.07
t = 5.31	**P = 0.0018**			

4.3.4. Seasonal variation.

Seasonal variation in tick numbers is shown in fig 11 and 12 and appendix 3. Whilst sample size by month are small and because of the clustered tick distribution, seasonal variation is likely to be subject to sampling error. However it appears that adults become abundant earlier in the year and peak from April to May.

Overall dragset means suggest that nymphal numbers peak in June. However dragsites A and B, where the same site was dragged each month suggest, in agreement, that nymphal numbers peak in May. In these latter sites since the same area is being studied each month sampling area should not be expected to be a compounding factor. Larval numbers appear to peak just a month or so later in June.

4.3.5. Temperature.

It was considered that some of the variation in tick activity may be due to variation in ambient air temperature and so regression analyses were conducted (figs. 13 and 14). Air temperature recorded during dragging at Rowardennan correlated highly significantly with both the maximum air temperature ($r = 0.879$, $n = 36$, $p<0.01$) and the earth temperature ($r = 0.639$, $n = 50$, $p<0.01$) measured at the weather station at Ardentinney in Argyll. However, tick activity showed no significant correlation with either the Rowardennan temperature or the more accurate Ardentinney measurements.

The seasonal variation in tick abundance as measured by blanket dragging at Rowardennan in 1993

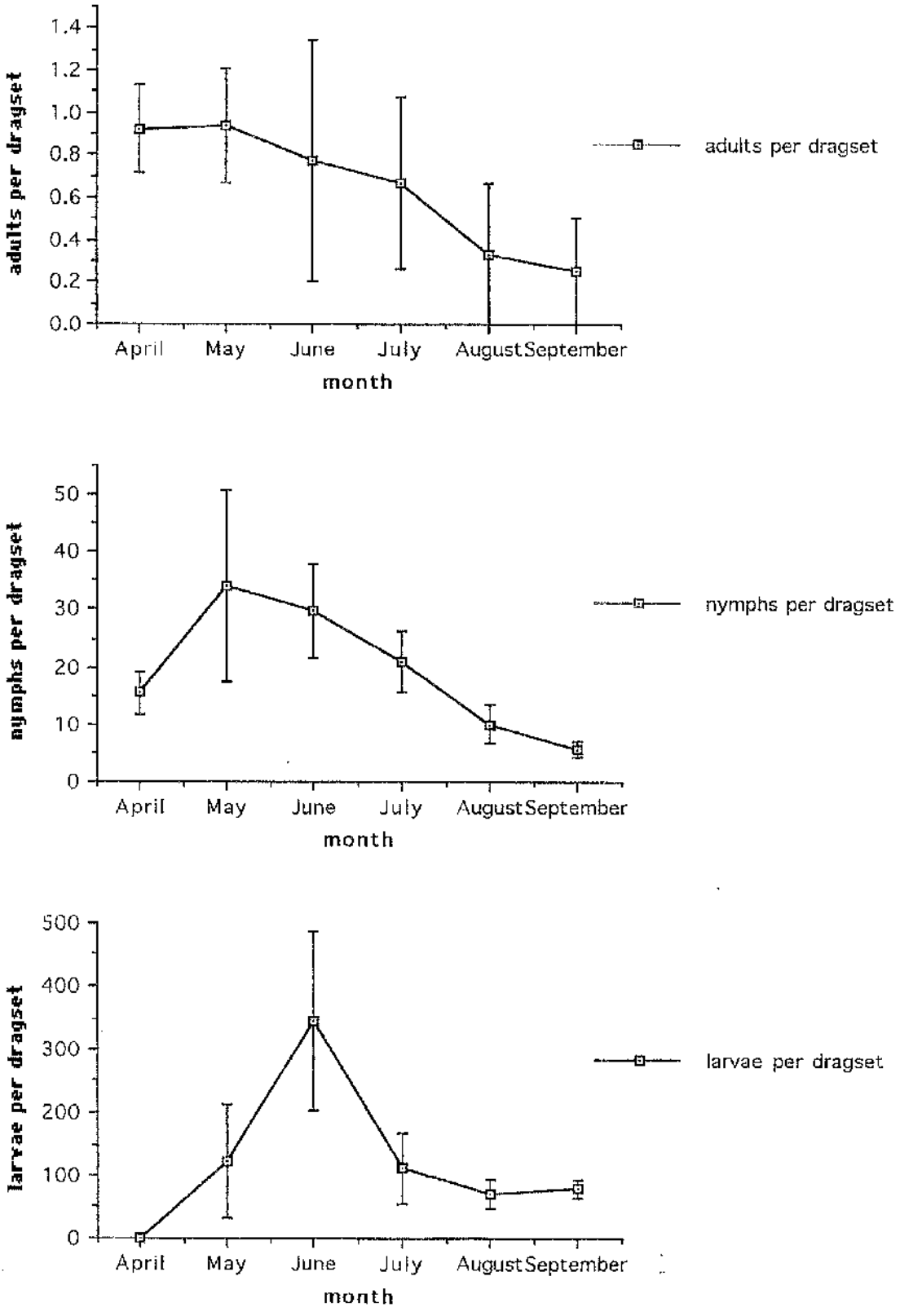


Figure 11. Graphs showing pattern of seasonal variation in tick numbers collected by blanket dragging.

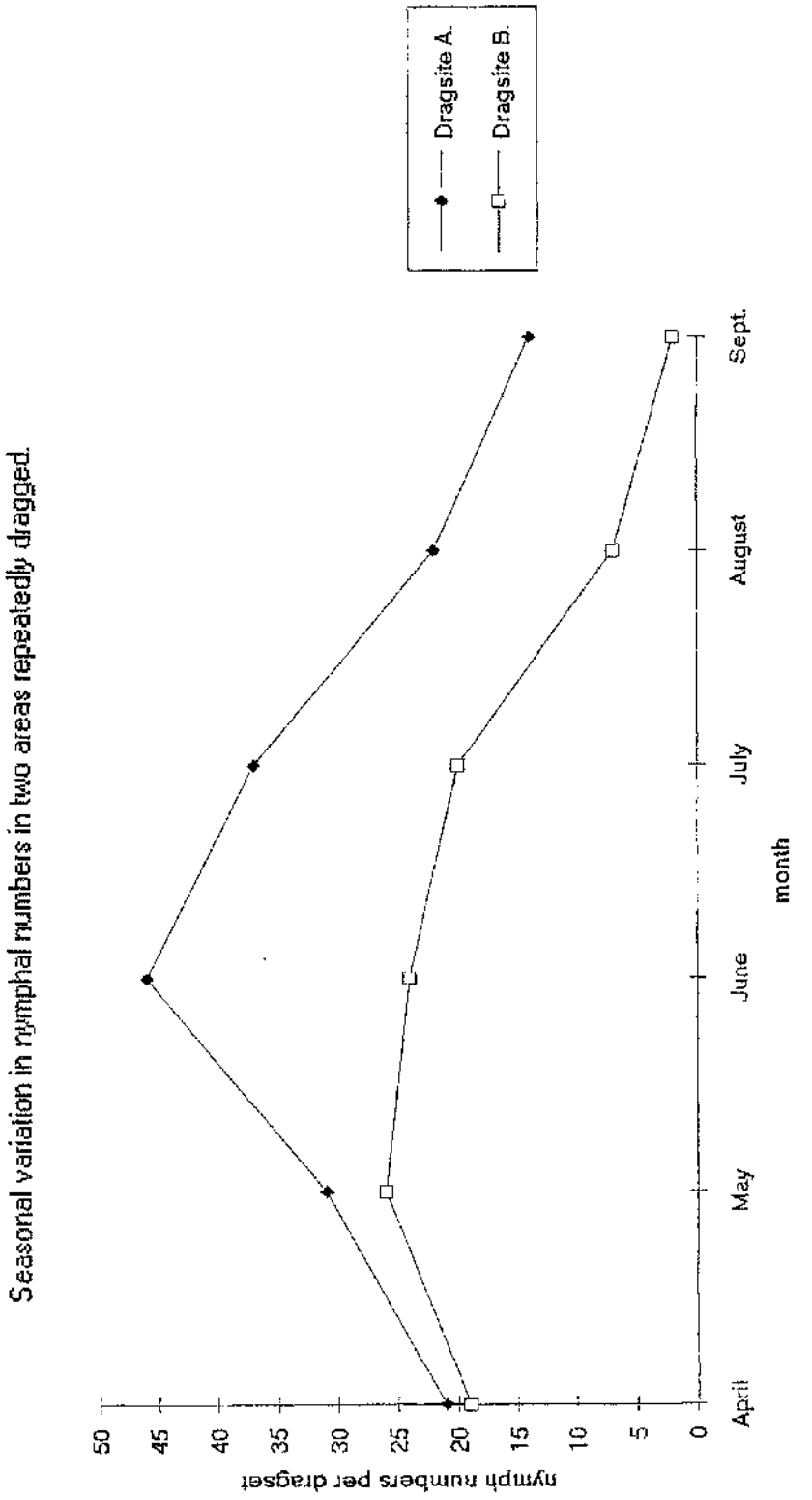
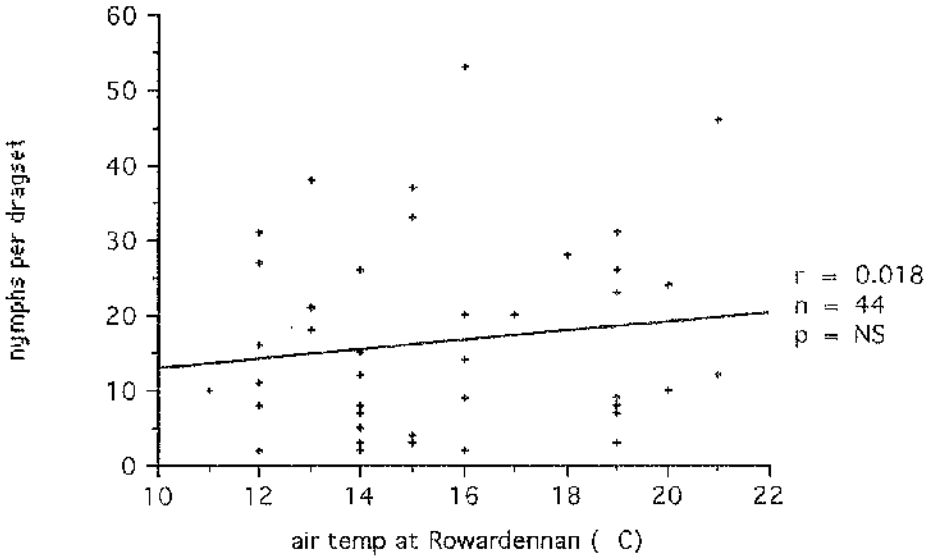


Figure 12. Seasonal variation in nymphal abundance in two areas repeatedly dragged.

The relationship between nymphal activity and air temperature.



Relationship between temperature at Ardentinney and nymphal activity.

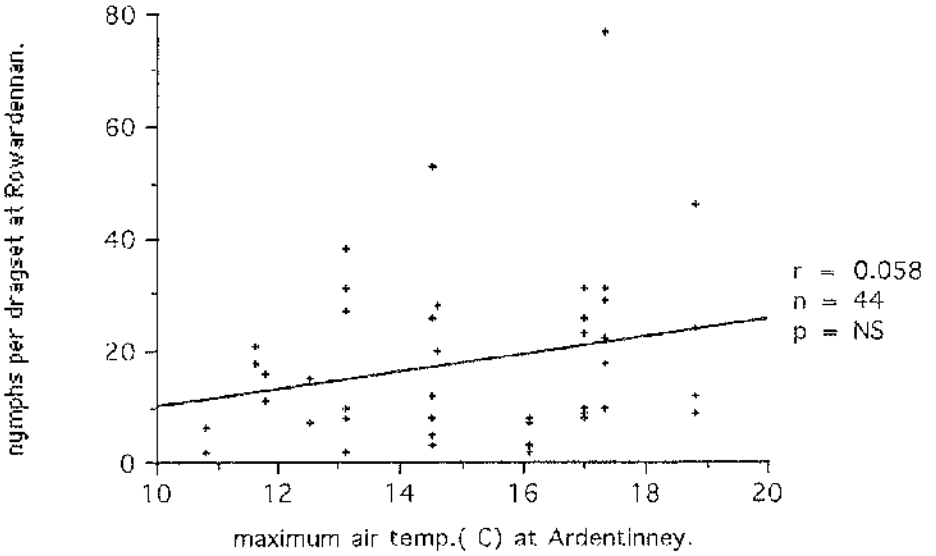
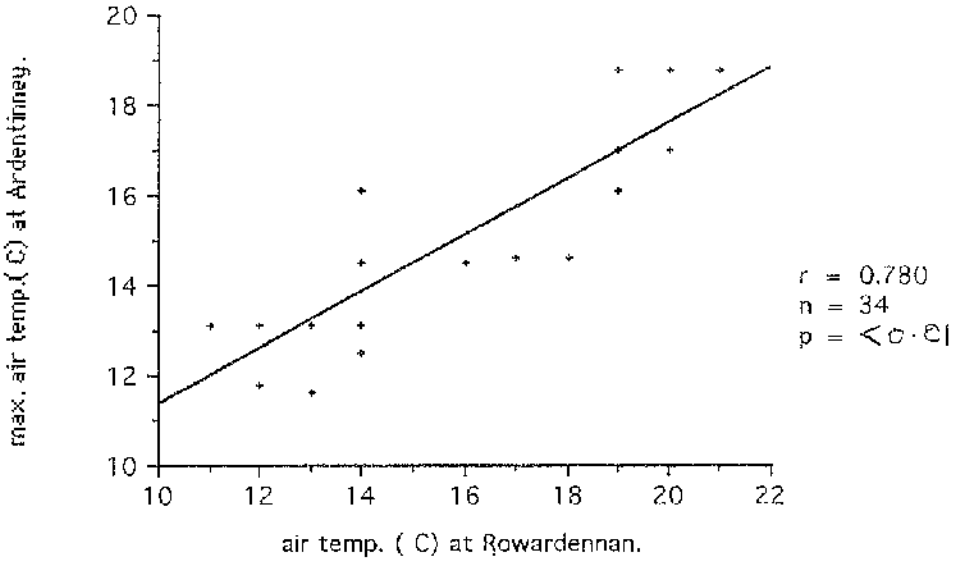


Figure 13. Scattergraphs exploring relationship between temperature and nymphal activity.

The relationship between temperature at Rowardennan and Ardentinney.



Relationship between temperature at Rowardennan and Ardentinney

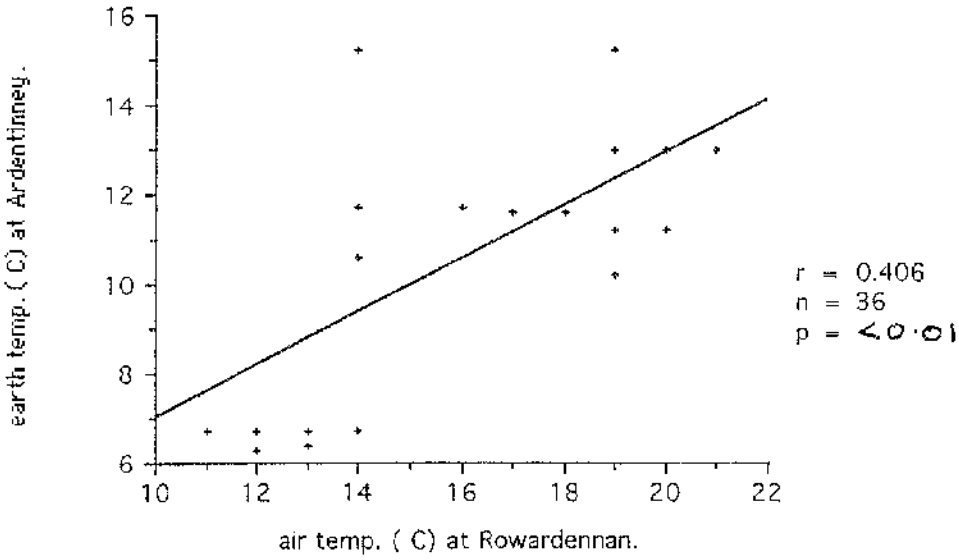


Figure 14. Scattergraphs exploring relationship between temperatures at Rowardennan and Ardentinney.

4.4. Discussion.

4.4.1. Validity of the blanket dragging method for assessment of tick abundance.

Fig 1 and appendices 1 and 2 demonstrate that cumulative total curves for a tick collection dragset of ten drags begin to reach an asymtote by ten drags, supporting the idea that ten drags picks up nearly all the ticks questing at the time of collection and can therefore be used to estimate the absolute population of questing ticks in an area at any one instant. The extrapolated graphs suggest that collection in the blaeberry picked up 94% and collection in leaf litter picked up 97% of questing ticks.

Another reason for carrying out repeat dragging was that it was considered that efficiency of collection may vary between habitats. Appendix 2. shows no significant differences in the percentages of the total number of ticks caught by each stage of the dragset between the two different habitat types and indeed suggests that total questing populations could be estimated in one drag since one could assume that it caught 30% of the total. However such estimates would at present be considered less reliable.

4.4.2. Abundance.

In the study sites A and B, the number of nymphs that quested in a season amount to a minimum of 9.33/m^2. Since dragging was done on a monthly basis, if the questing life of a tick is longer than a month, any tick becoming active after a dragset would be expected to be picked up the following month and therefore this total would be a reasonable estimate of the total number of nymphs in an area in a season. However if this were the case then one would expect the numbers in a previously dragged area to be lower than the numbers in a fresh area. In fact the opposite was found to be the case. If the questing life is shorter than a month, either because questing ticks are removed by hosts or because they die before finding a host, then some ticks would be missed in an area and the estimate provides only a minimum estimate of actual abundance. It is difficult to explain why the intensively studied sites should produce more ticks than other sites since there was no apparent reason why the $30m^2$ should be any different to those in their matched pairs. These results indicate that caution should be expressed in extrapolating the estimates for these sites to the rest of the study area, though if one multiplies the mean dragset total by six (sites A and B were dragged six times at monthly intervals), the equivalent figure for the rest of the area is not so different at 8.8 nymphs/ m^2 .

It is clear that whilst this experiment is not constructed with sufficient power to tell us the average length of the questing life of a nymph, it could be extended to do so. If the interval between dragsets on a single site was gradually increased until abundance matched that on freshly dragged sites, the implication would be that this time interval would equal the questing life of the nymph. Any shorter time interval would lead to a lesser number of ticks upon the repetitively dragged site. Unfortunately, in this study there was insufficient space within the study area to carry out the requisite number of drags. The questing life of the nymphal stage of *Ixodes ricinus* in the wild does not appear to have previously been studied. Lees and Milne (1951) looked at the questing life of adults and estimated it at 30 days. This varied from 1-123 days. One would expect that adults, being larger with greater energy reserves and thicker cuticles would survive for a greater length of time than nymphs. Lord (1993) looked at mortality rates of nymphal *I.dammini* in protected field enclosures and estimated it at 4% per day in 1989 and 4.8% in 1990 with a 90% mortality after 46days. However the ticks were wild and so had already aged by an indeterminate amount by the time of the experiment. Moreover both the species and the conditions of Westchester County, N.Y. state are different from those in the present study.

Therefore taking all into consideration it would seem unlikely that nymphs at Rowardennan survive for more than a month and so an estimate of 8 nymphs/m^2 as a measure of abundance is an acceptable minimum. A considerable number however are likely to have become active and died or been removed by hosts however inbetween drags. If drags were every 30 days then the mean age of a questing tick would be 15 days. If the mortality rate was 4%/day then this would mean that 54% would have died between beginning questing and the dragging occasion. Judging by the number of adults found, the number likely to have fed on hosts will be low and so total population is unlikely to be greater than 16 nymphs/m^2.

4.4.3. Life History.

The ratios of different tick stages provides some idea of the mortality rates of the ticks between stages. The larvae: nymph: female ratio of 197: 41: 1 suggests that if an adult female lays 1500 eggs (Gray 1981), 13% of eggs survive to be larvae. Of these, 20% feed and survive to be nymphs and of these a further 2.4% survive to be females and therefore presumingly, a further 2.4% survive to be males. However these figures assume that the tick population has held a constant size and structure over the last three years. The unequal numbers of males and females collected (male: female ratio was 2.64:1) most likely reflects the fact that males often mate in the vegetation before feeding and therefore die without questing.

4.4.4. Tick Habitat.

Tick habitat has been discussed earlier (see literature review) where it was concluded that the important feature determining abundance on sheep grazing land was drainage. This determines the thickness of the vegetation and the humidity of the tick microhabitat. Dessication is considered to be a major factor in tick mortality (Gray 1981). In this study there were low numbers of ticks on the northerly side of the hill which was mostly blaeberry bush but greater numbers in blaeberry compared to grass and leaf litter. The base of the blaeberry vegetation carries thick and rather loosely packed leaf litter with therefore a higher humidity than the more open ground of the grass. This offers one explanation for this difference in abundance but does not explain why one side of the hill carried so much greater a number of ticks than the other. The greater abundance on this south slope was not reflected on other south facing slopes in the woodland. It may therefore simply be that rather than the north side of the hill carrying a low number of ticks, the south side of the hill was for some reason particularly highly infested perhaps for instance that it was an area well grazed by deer. This idea that tick abundance may be partly determined by host populations will be further explored later.

4.4.5. Seasonal Variation.

These results suggest that around Loch Lomond the adult numbers peak between April and May and larval numbers peaked in June. The dragsets that were repeated on the same strip showed that nymphs peaked in May whilst the dragset average suggest a June peak. Intuitively one would expect the dragsets on the same plot to reflect seasonal variation more accurately than on a number of plots where there is variation between dragsets and similarly one must be cautious when interpreting results for adults and larvae.

However the seasonal pattern found in this study is in agreement with that found in Northumberland by Milne and by others in the UK as discussed earlier where the adults peaked first in April/May followed by nymphs and then by larvae in June.

4.4.6. Temperature.

As tick numbers increase as the season progresses and gets warmer, it is perhaps surprising that there is no correlation between abundance and temperature. This may be explained by a decline in numbers from June to September. Although tick activity may be shown to relate to temperature in the laboratory, from this study there is no evidence that it is an important factor in the field.

5.0. Small mammal populations and tick infestation at Rowardennan.

5.1. Introduction

The abundance of a tick population can reasonably be assumed to depend, at least in part on the size of the host population. Furthermore the potential of an animal as a disease reservoir will be considerably affected by the size of its population both absolutely and in relation to its vector population. In this study it was thought that wood mice *Apodemus sylvaticus* and bank voles *Clethrionomys glareolus* may play an important part as hosts to the tick *I.ricinus* and may also act as a reservoir for *B.burgdorferi*, the causative agent of Lyme disease. By measuring the size and dynamics of the small mammal populations therefore, and the infestation rate of the mammals by ticks, it should be possible to estimate the contribution made by the small mammals to supporting the tick population.

5.2. Method

The study site was the Rowardennan woodland as previously described. The small mammal population was measured by a Longworth trap, mark and release programme. A grid was set up of 50 traps at 25 points, each one 20m from the next, covering a total of 10,000m^2 . Traps were baited with a mixture of seeds. At the request of Scottish Natural Heritage, one of the licensing bodies for this study, all longworth mammal traps had 12mm circular holes drilled into one side. This was done to allow the escape of shrews which would otherwise be inclined to die in the traps. Sub-adult mice and voles will also have escaped through these holes and thus will not have been sampled.

Potential grid sites were first assessed by placing 5 clusters of 10 traps over three days at 5 different sites about the woodland. Two clusters which were particularly successful were about 100m apart and determined the perimeter of the grid. This meant that at the beginning of the study about half of the traps were situated in open woodland and about half amongst blaeberry bush. After the first two months the grid was moved about 30m in a north-westerly direction (see appendix 11), increasing the proportion in the cover of the blaeberry bush in an attempt to increase captures.

Trapping was carried out monthly from April to September 1993. Sessions lasted from five days at first, down to four and then three days when it was found that new animals were caught only rarely after three days. Trap rounds took place twice daily as required by the trapping license.

Bank Voles *Clethrionomys glareolii.*

For the first three months of the study mammals were examined at the site of capture. They were sexed, weighed and their ears were carefully examined for ticks which were removed with a pair of fine watchmaker's forceps. Other obvious ticks would also be removed. The mammals were marked at this stage by fur-clipping. Fur was clipped on either side of the rump, the shoulder, the flank or the groin, with care taken to avoid the nipples of lactating females. The mammals were then released and ticks stored in 'eppendorf' tubes for later examination.

In the last three months of the study, closed mammal traps were collected and removed to the field station. Mammals were weighed and then placed in a screw topped jar. This contained a tissue pad soaked in methoxyfluorane, lying under a square of metal gauze. Once anaesthetised, the mammal was removed. It was marked by toe-clipping as described by Begon (1979) and a blood sample was taken from its tail and/or its toe wound. The animal was examined for ticks and then placed in a cage to recover. Once all mammals had recovered they were taken to a spot in the centre of the grid and released.

5.3. Results

Between April and September 1993, a total of 75 small mammals were trapped on 248 occasions. These consisted of 29 wood mice *Apodemus sylvaticus* and 46 bank voles *Clethrionomys glareolus*. 30 mammals were caught in more than one month. A mean of 5.83 + 2.49 (1 SD) mammals were caught per 12 hour trapping session. During the study 5 mammls were killed. 2 bank voles died in the traps for unknown reasons and 3 wood mice as result of getting stuck in the shrew escape holes. Since numbers of captures and especially recaptures were relatively small, it was not faesible to analyse the data seperately for the two species and it by combining the data chance effects were minimalised.

5.4. Population estimates

There are a number of methods available for estimating population size from capture-mark-recapture (CMR) data and a number of methods were employed to estimate the mammal population at Rowardennan.

5.4.1.MNA method.

The simplest method is that of Krebs (1966) who described the minimum number alive (MNA) method. This simply counts those animals caught in a session and adds on

those caught before and after but not during it. This will always underestimate the size of the population and does not allow the calculation of a standard error of estimate. However it reflects patterns of fluctuation clearly and is not subject to the errors introduced into the estimates generated by other, more complex methods, where the vagaries of animal behaviour cause them to violate the inherent assumptions of the method.

In this study, only one small mammal was trapped either side but not during a month's session (in August) so table 7. principally reflects number of mammals caught.

Table 7. Estimated small mammal population size by MNA method.

Month	April	May	June	July	August	Septem-ber	Total
MNA	21	12	20	15	22	16	106

5.4.2. Haynes, trap-out method

Another simple model estimates the size of the population by making the assumption that all animals are equally trappable and that therefore by chance one will trap the vast majority a few times and a few very often, the number trapped decaying exponentially as the trapping number increases. The data from this experiment were analysed for the six month period as in fig 16 and appendix 5.

It can be seen that when the line is extrapolated back to zero, it can be estimated that 59 small mammals were trapped no times in the six months. This means that during the study period there were 132 mammals in the study area and that 56% of small mammals were trapped. As has been mentioned the main assumption that has been made here is that all animals are equally trappable. This is clearly not the case. It is well known that mammals show considerable variation in their individual prediliction for traps, with some showing strong avoidance behaviour and others what has been called an addiction. The addiction was apparent in this study where three mammals were caught 11, 15 and 28 times. However the shape of the above curve and therefore the point to which it extrapolates has been mainly determined by the numbers of animals caught 1,2 or 3 times and differences here may not be so subject to behavioural influence.

5.4.3. Peterson Estimate

The best known model of population estimation is the Peterson estimate where the proportion of recaptured marked animals out of a total capture is assumed to be equal

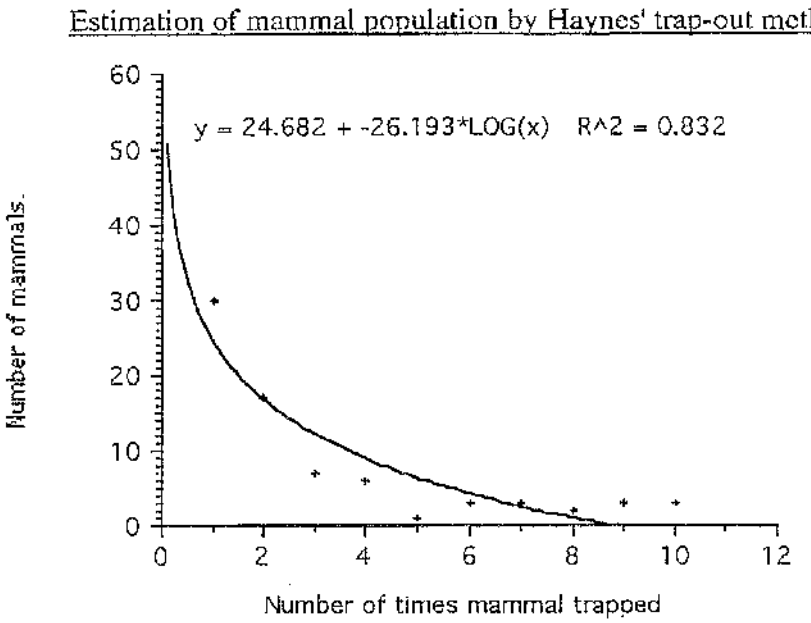


Figure 16. Estimation of mammal population by Haynes' trap-out method.

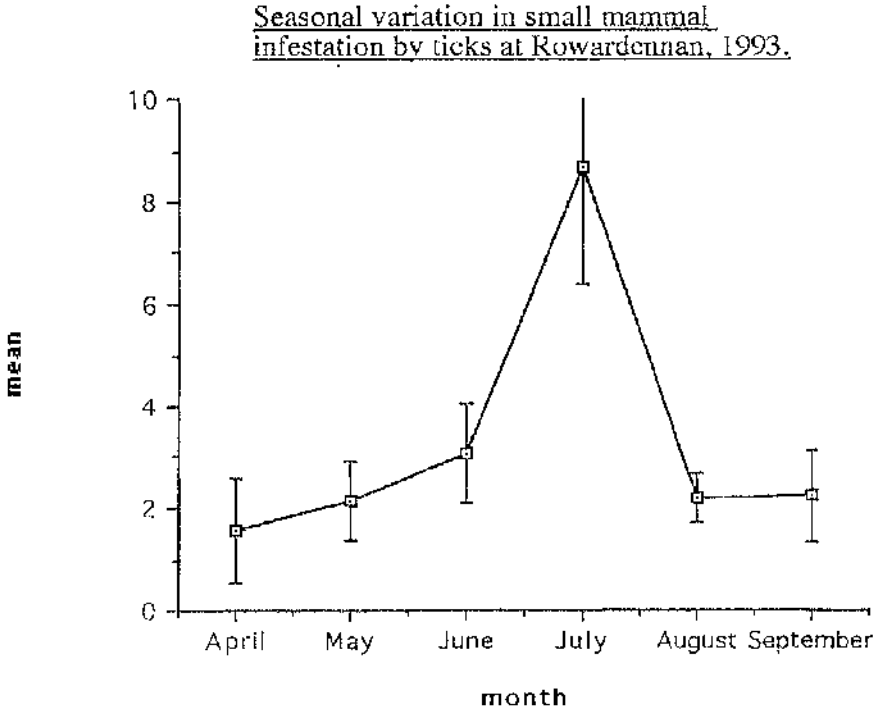


Figure 17. Graph showing seasonal variation in mammal infestation rates.

to the proportion of marked animals in the population. The model makes a number of assumptions which are easily violated in the field situation. These are considered in relation to this study.

1. Permanence of marks. In the first part of this study, marking was by fur clipping, allowing recaptures to be reliably identified day-to-day. Within a few days it was clear whether marks had been produced by scissors or other causes but between months when some hair had regrown this was much less obvious and it was felt that inter-month reliability of the marks was poor.

2.Effect of Capture. The models assume that capture has no effect upon the likelihood of recapture. As has been mentioned this is unlikely as some mammals seem to find the experience of capture sufficiently rewarding for them to return frequently. It is also assumed that capture doesn't increase the risk to an individual of death or affect its chances of emigration. In this study a few mammals died in the traps though this can be taken into account in the models.

3.That all mammals are equally susceptible to capture. This assumption will be violated for a number of reasons. As has been shown by Montgomery (1987), mammals vary in their trappability according to sex and age and external factors such as weather conditions. In this study where the area was relatively small (one hectare), there will also be edge effects which increases the capture rate of those mammals at the periphery. This is because the probability of a trap capturing an individual is reduced by its proximity to other traps. At the edge this effect is reduced. Another problem may arise if traps are spaced further apart than the mammals range such that some traps are more likely to lie within a territory than others. In this study the 20m spacing ensured that the site was saturated with traps.

4.Constant population. Closed models such as the Peterson Estimate require that there are no births, deaths, immigration or emigration from the population. That is, the population is closed. This is clearly going to be an invalid assumption for inter month estimates but may be acceptable when studying a population over a five-day period (Montgomery 1987).

5.Short sampling period. In open models that assume a fluctuating population, it is assumed that there is no change immediately before and after sampling and that therefore sampling takes an insignificant length of time. In closed models it is assumed that sampling has no significant effect upon the population size whilst the population is

being sampled. In this study sampling is effectively being carried out for the full four days of a trapping session. This is a short but significant length of time for inter-month studies. For day-to-day studies a closed population will have to be assumed. Clearly if the study has marked 56% of mammals the samplable population will diminish significantly as a session progresses and it seems likely that about 25% or even 50% of animals are caught during a trapping period. This will raise the question as to whether the estimates produced are for the population before or after sampling.

The design of a mammal trapping study will clearly be very difficult if one is to avoid a violation of the above assumptions and clearly it will not be possible to escape the vagaries of animal behaviour. One needs to minimise the sampling time whilst maximising numbers. The larger the numbers and area studied, the more accurate the figures will be and the less time required, so trap number is the most important factor. Unfortunately, this study was limited by trap availability, so that numbers of mammals caught are relatively low. However, the data have been analysed using CMR models, such that the resulting estimates can be compared.

5.4.4. Weighted Mean

This method is very similar to the Peterson estimate but uses the data from a number of days instead of just two occasions. It assumes that the population is closed and makes the other assumptions described. It was used to look at the population on a monthly basis, at the captures during one, four day session. Estimates were as in table 8. It is important to note that the standard error is dependent only upon the numbers of captures and recaptures and does not reflect any possible error due to a violation of assumptions.

Table 8. Estimates of small mamml population by weighted mean method.

Month	estimated mice & voles/hectare	S.E
April	23.00	6.47
May	13.60	3.78
June	24.20	4.50
July	18.50	5.33
August	36.58	9.14
September	17.70	3.54

5.4.5. Fisher-Ford Method

This is a much more complex model which allows a population to be fluctuant and which allows the calculation of parameters such as rate of gain, rate of loss and survival rate. It can therefore be used to look at the small mammal population over the six month period and is considered probably the most suitable of the open population models when numbers are low. In this study it is the numbers of recaptures that are particularly low.

Table 9. Numbers of small mammals captured and released at Rowardennan from April to September.

Month (i)	Captures (n_i)	Released (r_i)	month of release of marks m_{ij}					
			1	2	3	4	5	6
April	_	14	14					
May	10	10	2	8				
June	17	15	1	5	10			
July	15	13	2	0	0	11		
August	21	18	2	0	0	3	13	
September	16	-	1	0	0	1	9	5

From the above table the 'total months survived' is calculated as (2x1)+(5x1)+(1x2)+(2x3)+(2x4)+(3x1)+(1x5)+(1x2)+(9x1) = 42.

The next step is to calculate a survival rate for the population that will give a total months survived of 42. Three survival rates were postulated and the resultant marks that would be at risk in the population from the known release figures were calculated. From this it was possible to calculate the average age of the marks in the population at each month. The sum of the products of average age and number of marks caught in a month gave a prediction of the total number of months that would be survived at the input survival rate. Results were as in table 10.

Table 10. Calculated months survived by the population for three hypothesised survival rates.

Survival rate	Total months survived
0.55	42.66
0.6	44.48
0.7	48.31

These results formed a straight line graph, which was used to predict that the survival rate for a population with a total of 42 months survived would have a survival rate of 0.535 per month. A new table can now be constructed (table 11).

Table 11. Estimated small mammal population at Rowardennan in 1993 by the Fisher - Ford method.

Month	n_i+1	m_i+1	M_i	N
April	15	1	0	-
May	11	3	7.49	27.5
June	18	7	9.36	24.1
July	16	3	13.03	69.5
August	22	6	13.93	51.1
September	17	12	17.08	24.2

M_i is an estimation of the number of marks in the population at risk of capture just before month *i*. N is en estimate of the total population size. on the This method assumes a constant survival rate and this assumption can be tested by comparing the predicted months survived with those observed and performing a chi-squared test (table 12).

Table 12. Observed and predicted small mammal survival rates at Rowardennan in 1993.

months survived	May	June	July	August	September
predicted	2	8.58	3.1	8.8	9.47
observed	2	2	6	9	16

$X^2 = 12.26$ and df = 4. The difference is just significant at the $p<0.05$ level so that whilst the assumption does not hold true, it is unlikely to be a major source of error.

5.4.6. Triple-capture method.

A final method used to analyse the population data is the triple catch method. This was used on the captures for July, August and September when marking was most reliable and recaptures at their most numerous whilst making relatively few assumptions. It does not require that ingress and egress rates should be constant in particular. These data gave an estimate for the August population of 33.1 + 9.69 (1 SE), a survival rate 0.664 + 0.239 (1 SE) and an ingression rate of 0.259 between July and August.

5.4.7. Summary

In summary then, a number of methods have been used to estimate the size of the small mammal (mouse and vole) population in one hectare of Rowardennan woodland from the same trapping data. This is shown in fig 17 and appendix 4.

The weighted mean, Fisher-Ford and triple catch methods all make a number of assumptions discussed above, most important of which is probably heterogeneity in catchability, both as an effect of capture and as something endogenous within the population. The effect of this will be to underestimate the population size. This assumption has also been made in the calculation of adjusted MNA but may be unimportant as discussed above. The MNA is well recognised to underestimate the population by an unknown amount. The figures are all in broad general agreement apart from the estimate for July using the Fisher-Ford method. Whilst it would be easiest to ignore this figure as an abberancy resulting from small sample statistics, one may note that the Fisher-Ford pattern of seasonal variation is the one that most closely follows that expected for a mouse and vole population, and it is therefore worth looking more closely at the validity of this model in this study.

It was considered that weight was not measured with sufficient reliability in this study and so age at capture cannot be studied. However, the traps had 12mm holes in their sides which meant that young, small mice would escape. This will serve to underestimate the size of the mouse and vole population but increase homogeneity of the population's age-structure. One assumption of the Fisher-Ford model is that the survival rate is uniform and not age-related. This is an assumption usually violated but may be much more valid in this study where only the older population is censused. The other most important way in which Fisher-Ford's assumptions will be violated is in heterogeneity of capture. This can be looked at by studying sub-sets of the population. Analysis of capture rates by X^2 ($X^2 = 12.44$, 3df, $p < 0.01$) shows that *C.glareolus* were caught more often than *A.sylvaticus* but this difference is small and inconsistent. There was no significant difference by X^2 analysis in capture rates of males and females of either species.

Table 13. Capture rates of small mammal subsets

Species	sex	number	mean	SD
Apodemus	males	15	2.67	2.72
sylvaticus	females	13	2.46	2.03
Clethrionomys	males	25	4.28	5.96
glareolus	females	15	3.33	2.77

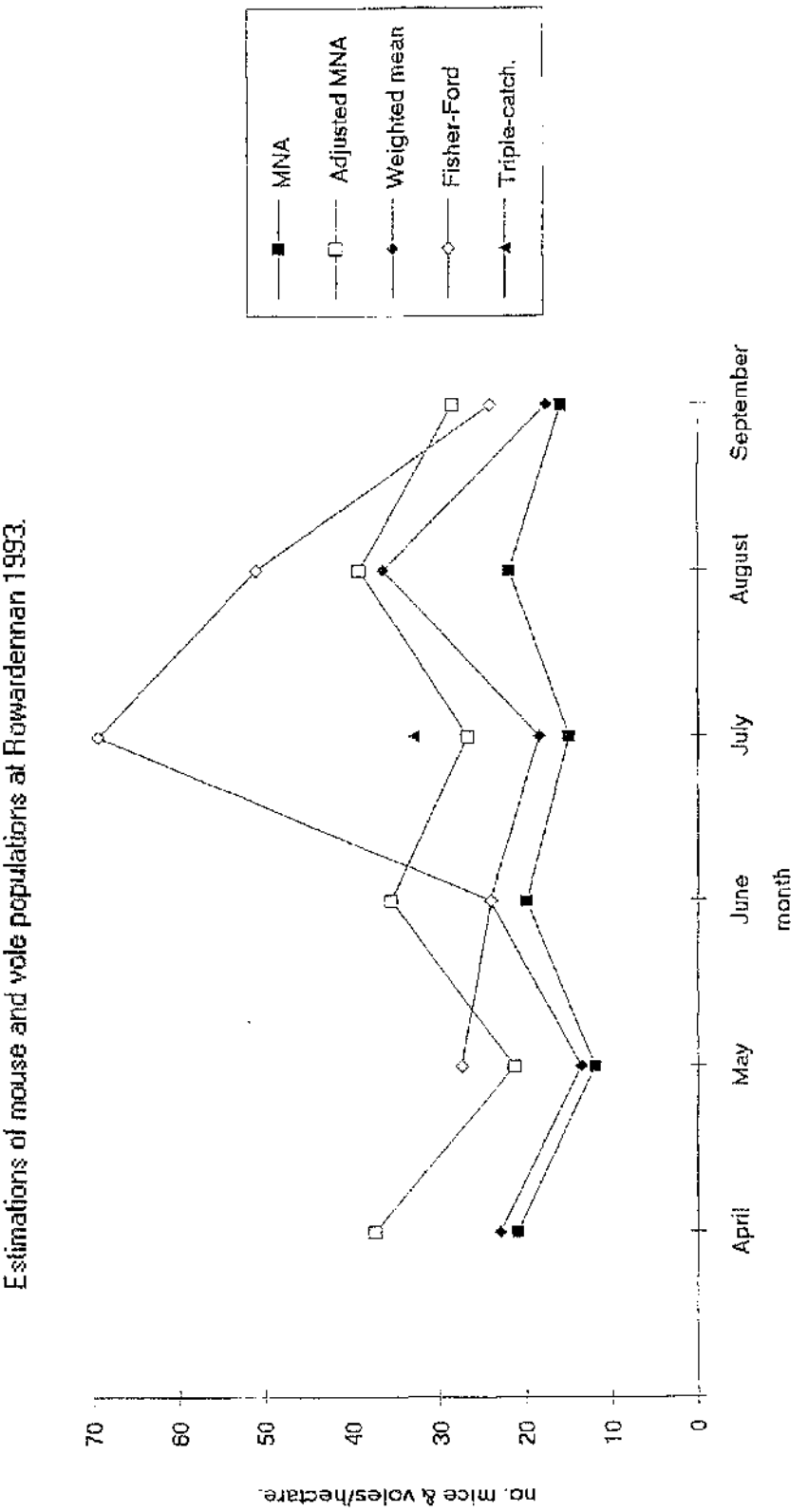


Figure 18. Graph showing seasonal variation in small mammal populations.

It would appear then that sex has little effect upon likelihood of capture and age is also not likely to be important in the validity of the model. However *C.glareolus* are a liitle more prone to capture and so the study is likely to slightly underestimate the mouse population.

In conclusion, the Fisher-Ford method appears to be a reasonable model for analysing the mouse and vole population within the constriants imposed upon the study. If mice and voles are studied as a single group, it will however, underestimate mouse numbers. The combined numbers of mice and voles in the Rowardennan study-site vary between 24 and 70 per hectare.

Tick infestation rates.

The number of ticks infesting each small mammal were counted on 104 small mammals which had been caught for the first time that month. Mammals caught for the second or more time in a session are not included in the analysis since their infestation rates will have been affected by the unknown length of time that they have spent in mammal traps. The mean number of ticks found on small mammals was 3.02 with a standard deviation of 4.99 i.e. Distribution of ticks was highly clustered as in fig 10.

Unfortunately, because ticks were identified at the time of removal and identified at a later date, infestation cannot be analysed by species. However, since only 7% were found to be *I.trianguliceps*, it is unlikely that this species had a significant effect on the results.

Of the 91 small mammals whose sex was determined, males were significantly more infested than females by the Mann-Whitney U test (table 14).

Table 14. A comparison of tick infestation rates of males and female small mammals.

Sex	n	mean rank
male	56	50.33
female	35	39.07
U=737.5 W= 1367.5 Z= - 2.0655		**two-tailed P= 0.0389**

There was no significant difference in infestation rates between *A.sylvaticus* and *C.glareolus* and there was no significant relationship between weight and infestation

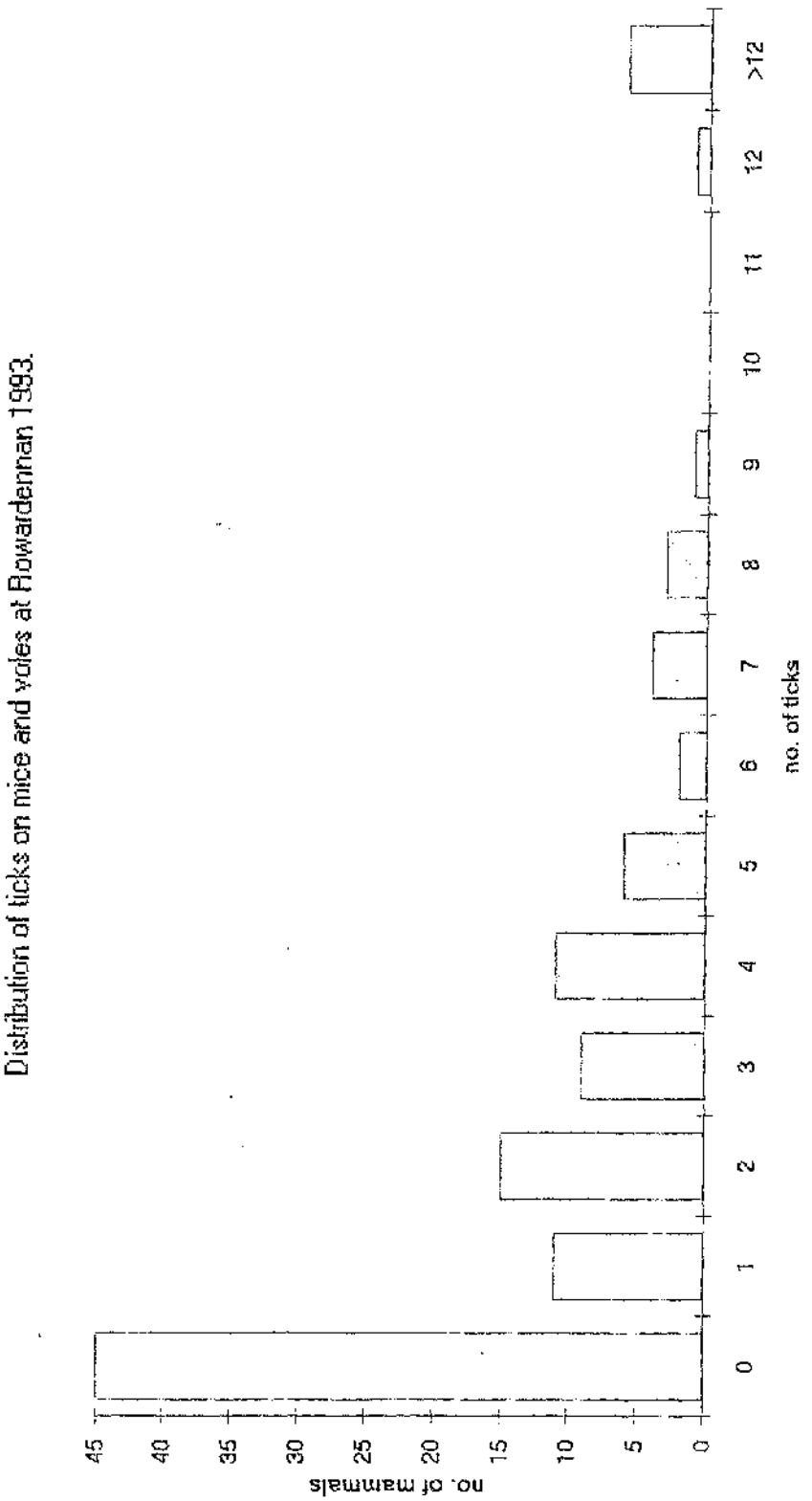


Figure 19. Histogram of mammal infestation rates.

rate by the Mann-Whitney U test. However infestation rate varied significantly between months by the Kruskal-Wallis 1-way ANOVA at a level of significance of P=0.0010 (table 15).

Table 15. Comparison of tick infestation of small mammals by month (see also fig 17).

Month	n	Mean infestation rate.	Mean Rank
April	18	0.778	29.89
May	18	2.833	55.33
June	9	3.222	55.39
July	17	7.824	73.94
August	24	2.125	52.02
September	18	2.000	51.22

Discussion

Using the data and calculated frequencies above it is possible to estimate the numbers of ticks fed by small mammals in the study area during the tick season. It is assumed that the larval stage feeds for a period of four days such that a host will feed 7.5 times its infestation rate in a calendar month. It is then possible to compile table 16.

Table 16. Estimated numbers of ticks fed by small mammals at Rowardennan in 1993.

Month	Infestation rate	no small mammals /hectare	no. ticks fed / hectare
April	0.778	23	125
May	2.833	27.5	545
June	3.222	24.1	544
July	7.824	69.5	3806
August	2.125	51.1	760
September	2.000	24.2	339
		Total ticks fed	6119

This estimates that about 6000 larvae per hectare were fed by small mammals during the 1993 season at Rowardennan. It is likely to be an underestimate for a number of reasons. Firstly the study did not include other small mammal species such as shrews. Secondly because of the shrew escape holes the smaller mammals escaped and were not counted. It is also likely that a proportion of ticks on the small mammals were not found, leading to an underestimation of infestation rates.

The distribution of ticks among animals warrants further attention. In this study, no difference was found in infestation rates between *A.sylvaticus* and *C.glareolus*. This is at odds with previous work (Randolph 1975 and Nilsson and Lundqvist 1978) that found *A.sylvaticus* to be more infested than *C.glareolus*. Whilst Randolph was studying *I.trianguliceps*, Nilsson and Lundqvist in Sweden found the difference for *I.ricinus*. Randolph attributed it to differences in home ranges for the two species. In this study more *A.sylvaticus* were caught early in the season and *C.glareolus* later. It may be that if the study was large enough for season to be controlled such a difference would be apparent.

In this study more ticks were found upon males than on females. This finding has also been made by the above authors, both of whom were able to sub-analyse their populations and find it confined to the adult *C.glareolus*. Again Randolph postulates that it is a result of differing home ranges and differing levels of activity resulting in the males picking up more ticks.

Lastly no correlation was found here between weight and infestation rate. Weight correlates closely with age and and can be used to predict it (Turner 1986). This may be for two reasons. As stated, the smaller mammals in this study escaped through shrew escape holes so that the study population was biased towards older mammals and was more uniform than that outside of the traps. Secondly, considerable variation was found in the weights of mammals caught a number of times, suggesting that either weight varied considerably or measurements were unreliable.

In summary small mammal numbers fluctuate considerably at Rowardennan but rise steadily as the tick season progresses. They feed a minimum of 6000 larvae per hectare per year with males feeding more than females and *A.sylvaticus* more than *C.glareolus*.

6.0. Birds and the ecology of Lyme disease.

6.1. Introduction

Birds are well known to be hosts of *I.ricinus* and may also act as reservoirs for the *B.burgdorferi* spirochaete. Their relative importance as hosts would be determined by their numbers and their degree of infestation. Their importance as reservoirs would be determined by the number of ticks that they would be able to infect with the spirochaete. The aim of this section of the project therefore was to make an attempt at quantifying some of these parameters to get some idea as to the importance of the role played by birds in the ecology of Lyme disease by Loch Lomond.

6.2. Methods

Birds were caught by a fully trained ringer (RW Furness) by mist-netting on a monthly basis from March to December 1993. The mist-nets were erected in a garden near Tarbet on the west side of Loch Lomond for the period of one day a month when the weather was suitable. This meant that preferably there would be little wind and it would be overcast but not raining. Some birds were also caught in the study area on the east side of the loch in the Rowardennan study area. Mist nets were set up on two occasions in late May and early June and a number of birds were caught whilst sitting on nests in nest boxes on the same occasions. Another investigator (NB Metcalfe) was looking at pied flycatchers in the area at the time of the study and he kindly checked all his study birds for ticks.

After the birds had flown their nests at the end of the season, four nests (including one of a great tit that had been found to be particularly infested) were removed to the laboratory where they were placed on sheets of white paper. The edges of the paper were lined with upturned sticky cellulose tape. A 60W light bulb was then lit over the nest. In preliminary experiments ticks that had been placed under the light in the absence of the nest were found to move quickly to the paper edge where they would become stuck.

6.3. Results

From March to December 1993, 1191 birds were caught and ringed at Tarbet. A total of 453 larvae were removed from 63 birds. The mean infestation rate of birds overall was 0.43 larvae per bird with an SD of 4.19. Distribution was extremely clustered and variable among species of bird.

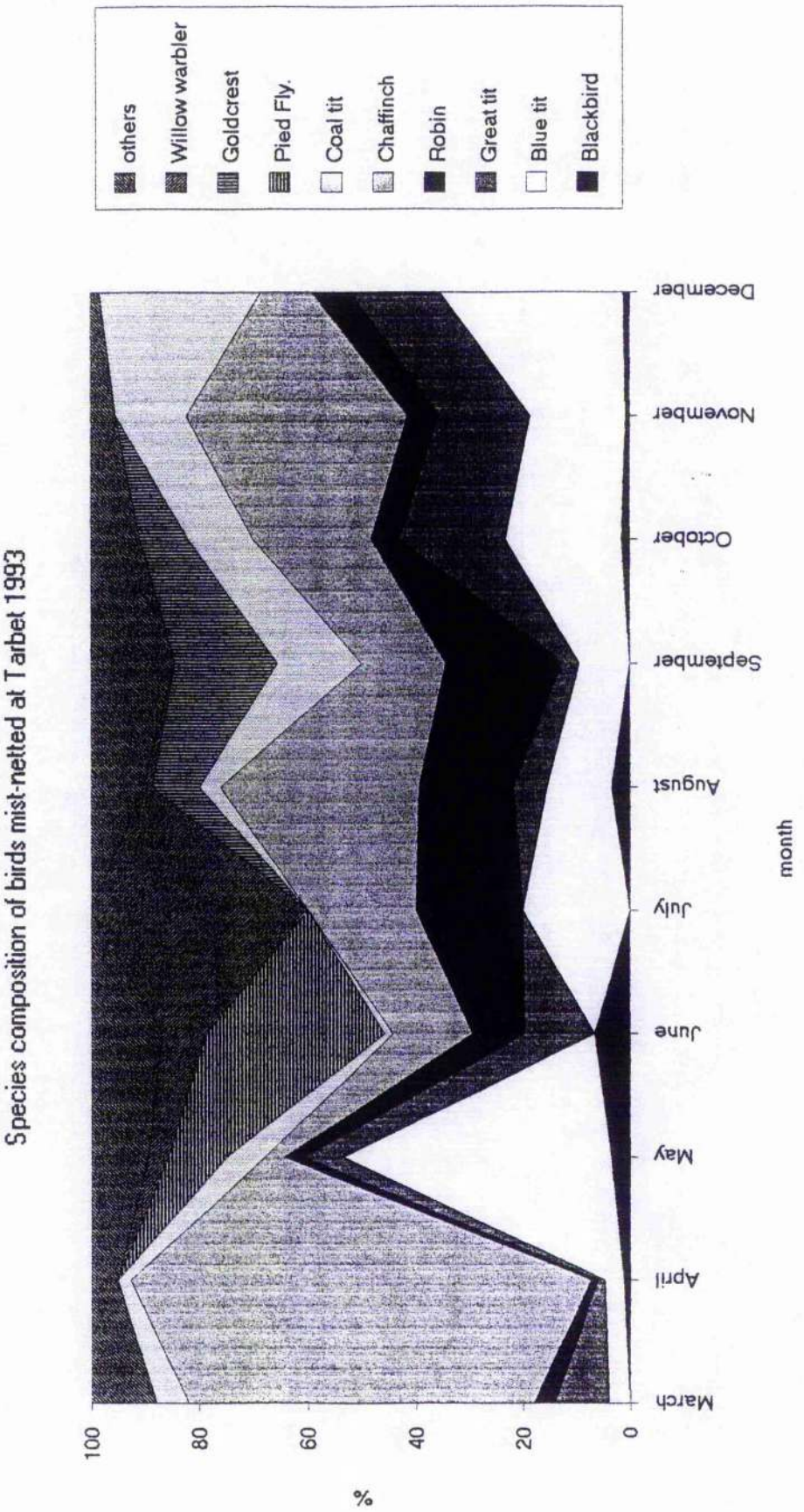


Figure 20. Graph showing seasonal variation in bird species composition.

Infestation rate varied as expected in a seasonal fashion. Unfortunately the seasonal effect on infestation rate is a little masked by the consderable diminution in activity of summer birds which reduced the numbers caught in July quite drastically. However seasonal activity was as is in fig. 21 and appendix 6.

Most activity occurred from May to August and indeed in this experiment only 23 ticks (5%) were caught outside of this time. It appears to peak in July but as only 5 birds were netted in this month there is likely to be a wide margin of error.

23 species of bird were netted at Tarbet, showing considerable variation in infestation rate among species as shown in table 17.

Levels of infestation vary among species but this variation may be due to seasonal variation in rates of capture. This may be because the population composition varies throughout the year as a result of differences in breeding rates or with the arrival and disappearance of summer migrants or simply as a result of sampling error. Most importantly the above infestation rates may play down the importance of some bird species as hosts, if large numbers of that species were caught out of season, thereby biasing the rates downwards.

Table 17. Tick infestation rates of different bird species at Tarbet.

Species	Mean infestation rate	Number of birds caught	SE	SD
Blackbird *Turdus merula*	8.24	21	4.45	20.41
Blue tit *Parus caeruleus*	0.026	230	0.02	0.28
Bullfinch *Pyrrhula pyrrhula*	0.50	8	0.38	1.07
Chaffinch *Fringilla coelebs*	0.34	365	0.07	1.27
Coal tit *Parus ater*	0.006	159	0.006	0.08
Crow *Corvus corone*	0	1		
Dunnock *Prunella modularis*	0.18	17	0.095	0.39

Garden warbler *Sylvia borin*	0	1		
Goldcrest *Regulus regulus*	0	43		
Great tit *Parus major*	0.049	162	0.017	0.22
Greenfinch *Chloris chloris*	0	4		
House sparrow *Passer domesticus*	0	2		
Jay *Garrulus glandarius*	12.88	8	12.45	35.21
Lesser black backed Gull *Larus fuscus*	0	1		
Long tailed tit *Aegitholos caudatus*	0	2		
Pied flycatcher *Ficedula hypoleuca*	0	23		
Redpoll *Carduelis flammea*	0	2		
Robin *Erithacus rubecula.*	0.50	95	0.16	1.59
Siskin *Carduelis spinus*	1.11	18	0.89	3.76
Song thrush *Turdus philomelus*	2.00	3	2.00	3.46
Treecreeper *Certhia familiaris*	0.20	5	0.20	0.45
Willow warbler *Phylloscopus trochilus*	0	11		
Wren *Troglodytes troglodytes*	2.60	10	0.87	2.76

It is reasonable to assume that the pattern of seasonal variation in infestation rates applies equally to the different species of birds between May and August. This assumption may be violated of course, if birds changed their pattern of behaviour during this time such as by moving from ground to tree to feed. However for the purposes of this study it has been assumed that the majority of such movement occurs

before or after this period. It is therefore possible to standardize the infestation rates for May, July and August to those of June by making a seasonal adjustment. This can then provide us with a theoretical mean infestation rate for each species as if all the birds had been netted in June. The following infestation rates were calculated after exclusion of all birds that had no ticks on them between May and August. It was considered that influxes of migrating pied flycatchers, willow warblers or goldcrests that appear to play no part in tick ecology would negatively bias the true seasonal pattern.

Table 18. Seasonal variation in infestation rate.

Month	Infestation rate	n	Adjustment factor
May	2.54	24	2.60
June	6.60	38	1
July	7.6	3	0.87
August	1.83	152	3.61

The infestation rate was calculated for each species each month and then that infestation rate was adjusted for that month by the adjustment factor. It was then possible to find the mean seasonally adjusted infestation rate (MSAIR) for each species which predicts the mean infestation rate for each species in June 1993. This is shown in Fig 22 and appendix 7.

These results can be compared to those from birds at the study site at Rowardennan on the other side of the loch where birds were caught on the 28th May and 6th June 1993.

5 adult blue tits and 4 adult great tits were caught on the nest. 3 blue tits, a coal tit, 2 wood warblers and a wren were caught in mist nets. Infestation with *I.ricinus* was as in appendix 8 and fig 22.

In general it appears that ticks were more abundant on the Rowardennan side where they were even found on the tree feeding birds. It should be noted that the great tit infestation rate was markedly increased by one great tit infested with 37 larvae. This tit resided in the hectare intensively studied by dragging and which was found to carry very high numbers of ticks.

Nest studies proved less fruitful. Of four nests placed under warm, drying lights only one produced ticks. These were four, larval stage *I.ricinus*. All pied flycatchers (13 pairs) checked by NBM were found to be free of infestation with ticks.

Seasonal Variation in bird infestation by ticks at Tarbet 1993.

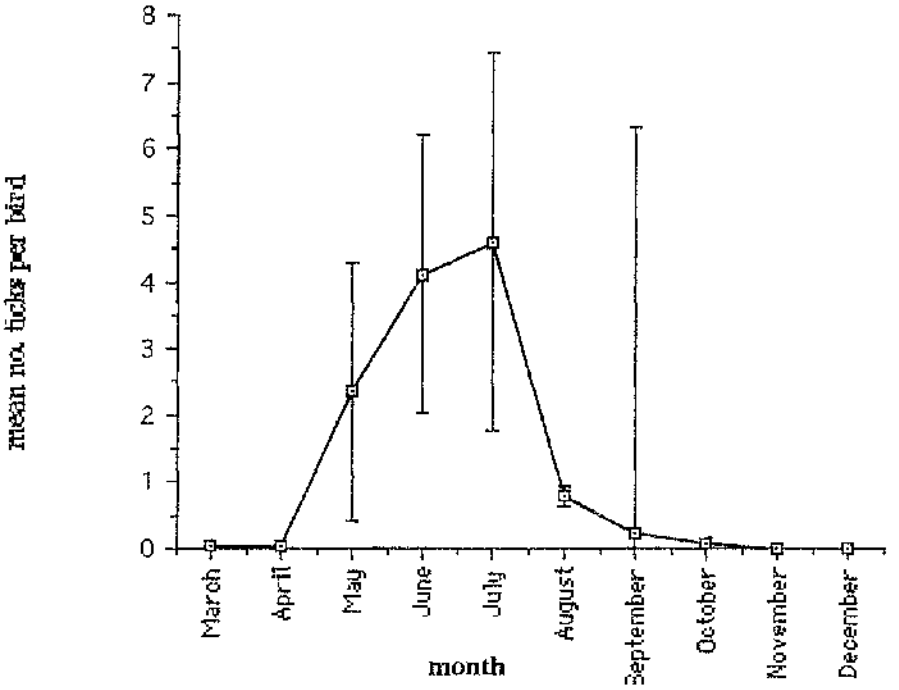


Figure 21. Graph showing seasonal variation in bird infestation rates.

6.4. Discussion

As would be expected, ticks are distributed on birds in a highly clustered fashion. This means that small samples which attempt to look at infestation rates will be highly susceptible to sampling error. Consequently, whilst this study may have presented us with some useful infestation rates for passerines as a group one must be much more wary when interpreting the results for individual species.

On the whole though, this study broadly supports the hypothesis that ticks would be found mostly on ground feeding species of birds and blackbirds and jays were found to be particularly infested. Robins, chaffinches and wrens were also fairly infested but less so. Siskins and blue tits were perhaps surprisingly highly infested considering that they are usually considered tree feeding birds but they do in fact both feed on the ground and perhaps do so more than is generally appreciated. Whilst numbers of dunnocks and song thrushes were low, they were quite surprisingly free of infestation which leads one to speculate that they carry mechanisms protecting them from ticks and that these could be present but less well developed in chaffinches, robins and wrens.
The high infestation rate on the Rowardennan birds is at first very striking. However, the sample of birds is small and they were all caught at the height of the tick season. The birds were also caught in that part of the woodland area that has been shown elsewhere in this study to carry high numbers of ticks. Data are inadequate for a thorough statistical comparison of infestation rates on each side of the loch but there is no reason to believe that they are significantly different. It was not surprising to find an absence of ticks in nests since nestboxes are cleaned out every year. For ticks to have been found they would have had to have been brought in to the nests by hosts that season and therefore would have had to drop off hosts in a state of engorgement. However adult tits appear to spend little time on the nest during the tick season.

This study was unable to measure the size of the passerine population in the Rowardennan study area. However a BTO expedition to Loch Lomond in 1972 (Williamson 1974) addressed this question specifically and it is not felt (Marchant 1994, pers com.) that populations are likely to have changed significantly. Seven sites were studied: two on the mainland and five islands. Density ranged from 780 to 1505 pairs per km^2. The two mainland wood sites (closest to the study site) carried 1090 and 1120 pairs per km^2. As Arrochymore was closest to the site, figures from there will be used. Density of species there was as in table 22.

Estimated importance of bird species as hosts to ticks (percentages refer to % of bird fed ticks fed by that species).

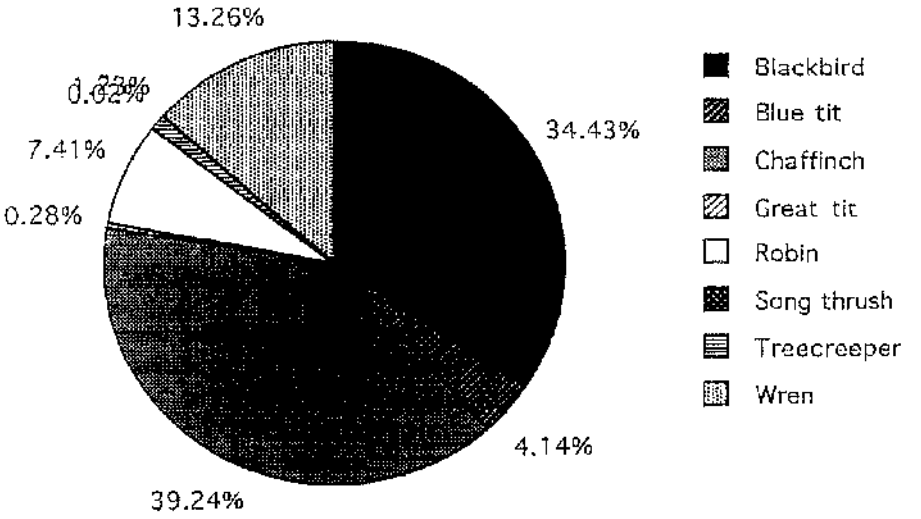


Figure 23. Pie chart showing relative importance of bird species as tick hosts.

Relative levels of infestation of different bird species at Rowardennan and Tarbet

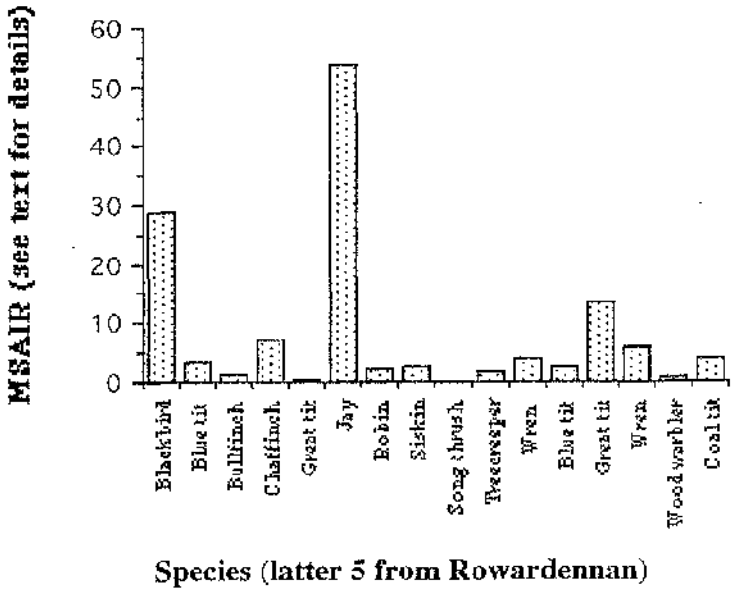


Figure 22. Histogram showing differences in infestation rates between different bird species.

Table 19. Population density of different bird species at Rowardennan in 1972.

	% of population	estimated pairs/km^2
Chaffinch	22	255
Robin	12.75	148
Wren	13.5	160
Willow warbler	11.25	131
Great tit	4	46
Blue tit	4.75	55
Song thrush	2	23
Coal tit	3.25	37
Wood warbler	2	23
Blackbird	4.75	55
Treecreeper	2.75	32
Garden warbler	2.75	32
Others (14 species)	14.25	165

Using these densities and the infestation rates measured above it should be possible to estimate the number of larvae fed by birds and therefore the proportion fed by birds. Larvae normally feed for 3-5 days (Arthur 1963) and so one can calculate that a bird will feed 7x its mean infestation rate of ticks in a month. The results of these calculations are shown in table 20 and fig 23.

These calculations suggest that birds feed in the order of 1800 larvae per hectare per season in the area around Loch Lomond. It is clearly an underestimation as it does not cover the period after August and only includes those species of bird censused in the 1974 study, excluding the jay and the siskin. Jays whilst highly infested hosts are not present in great density. Their density was measured on Inchcailleach east in the 1974 study and they were present at 0.37/hectare. At this density on the mainland they would feed an additional 391 larvae per hectare.

Table 20. Estimated numbers of ticks fed by different species of bird by Loch Lomond.(IR = infestation rate and NF = numbers of ticks fed).

Species	Density no/hct	May		June		July		August		Total ticks fed
		IR	NF	IR	NF	IR	NF	IR	NF	
Black-bird	1.1	11.1	85	28.8	222	33.1	255	8	61	623
Blue tit	1.1	1.34	10	3.5	27	4.02	31	0.97	7	75
Chaffinch	5.1	2.7	97	7.1	253	8.1	290	1.97	70	710
Great tit	0.92	0.12	0.73	0.3	1.9	0.34	2.2	0.83	0.53	5
Robin	2.96	0.88	18	2.3	48	2.6	55	0.64	13	134
Song thrush	0.46	0.038	0.12	0.1	0.32	0.11	0.36	0.028	0.1	0.3
Tree-creeper	0.64	0.69	3	1.8	8	2.1	9	0.5	2.2	22.2
Wren	3.2	1.46	33	3.8	85	4.37	98	1.1	24	240
								Total		**1810**

6.5. Conclusions

Birds act as hosts to *I.ricinus* larvae and their importance as hosts varies among species. This variation is not explained solely by a knowledge of the bird's habits and other factors may be involved. The importance of a bird as a host is not dictated solely by its susceptibility to infestation but also by its population density. It would seem likely therefore that the chaffinch is the most important bird host to *I.ricinus* around Loch Lomond but that the wren, robin blackbird and jay also act as important bird hosts. Overall birds probably feed a minimum of 1800 larvae per hectare per season. The significance of this will be discussed in the final discussion chapter.

7.0. *Borrelia burgdorferi* in *Ixodes ricinus.*

7.1. Introduction.

The level of risk of Lyme disease to users of the rural environment will be determined both by the numbers and species of ticks present and by the proportion which serve to transmit the spirochaete. An important aspect of this project was therefore to attempt to measure the proportion of ticks that carried the spirochaete. Furthermore, as has been discussed earlier, the nature of the animal reservoirs of Lyme disease is still poorly understood. Since it has been shown that only a very small proportion of larvae, in the order of 1%, inherit the spirochaete, the removal of spirochaete-positive larvae from a host would provide strong evidence that that host was acting as a reservoir. In this section a method was developed for examining ticks for the presence and abundance of *B.burgdorferi* in ticks by Loch Lomond.

7.2. Method.

General

Once ticks had been identified to life cycle stage and species (according to Arthur 1963) they were transferred to a slide in a small drop of 0.9% saline solution. They were then smeared by first tearing open the ventral surface with two 19g needles and then by washing the tick contents out onto the slide in the saline. The slide was then air dried rapidly. In some experiments *Borrelia burgdorferi* were added to the slide at this point. These were commercially prepared (Kirkgaard and Perry Labs., Inc.), heat-killed wet cells in dextran solution, stored in 50% glycerol and 50% water. They were the American B41 sub-culture. The *Borrelia* were added in varying amounts to the tick and the slide was again rapidly air dried. The slides were fixed for 20 minutes in acetone and if not ready for immunofluorescence, they were stored with silica gel and frozen at -20°C. For immunofluorescence the primary antiserum used was goat anti-*B.burgdorferi* antiserum, affinity purified (Kirkgaard and Perry Ltd.). After application the slides were then incubated in a humidity chamber for 1 hour before being washed twice for 5 minutes each time in PBS. Secondary antibodies were flourescein labelled anti-goat/sheep antibodies. These were incubated for twenty minutes with the slides before two further brief washings in PBS and mounting of the slides in mowiol.

Experiment 1.

B.burgdorferi were dried on slides in the absence of tick extract, diluted in PBS in concentrations of 1:25, 1:50 and 1:100. They were counterstained with DAPI which stains DNA. The 1:25 dilution resulted in conglomorations of borrelia, 1:50 dilution

resulted in countable numbers of cells present and a 1:100 dilution resulted in cells too low in number to be found easily.

Experiment 2.

B.burgdorferi were dried on slides at a dilution of 1:50. Primary antibodies were added at dilutions of 1/20, 1/40, 1/80, 1/160 and 1/180 and saline control. DAPI was added to the primary antibody solution. This would serve to confirm the presence of bacteria in the absence of staining by the antibody. Secondary antibody was added in the arbitrarily chosen dilution of 1:50.

Results:

Dilution: 1/20: Spirochaetes glowed very brightly.

1/40: Fewer spirochaetes seen but glowing brightly.

1/80: Well stained but no bright glow.

1/160: Spirochaetes visible but dull.

1/320: Few visible spirochaetes seen and staining was patchy.

Saline control: no staining visible.

DAPI stained spirochaetes were equally visible at all dilutions.

It was concluded that primary antibody worked best at a 1:50 dilution.

Experiment 3.

B.burgdorferi were dried on slides at a dilution of 1:50 and primary antibody was added at a similar dilution. Secondary antibody was added at dilutions of 1:12.5, 1/25, 1/50, 1/100, 1/200 and normal saline was added as a control. In opposition to the experiment where the primary antibody concentration was varied there was no clear trend in the degree to which the spirochaetes glowed and at present this is unexplained. One major problem was the tendency of the bacteria to clump, resulting in few very brightly glowing patches. However since the use of 1:50 dilution of secondary antibody gave satisfactory results in the previous experiment, this level was chosen for use in further experiments.

Experiment 4.

B.burgdorferi solution was added to tick extract and the slides were then air dried. Again primary and secondary antibodies were added in varying concentrations. Primary antibody was added in concentrations of 1:10, 1:20, 1:40, 1:80 and saline control with the secondary antibody at a concentration of 1:50. In a replicate experiment the secondary antibody was added at a dilution of 1:100. Once again there

was no clear trend to indicate the most suitable levels of dilution though there was a brighter picture with secondary antibodies at the 1:50 dilution. The main problem that became apparent here was the affinity of the primary antibody for the tick extract resulting in a high level of background staining. Smeared tick connective tissue often stained in strands similar in appearance to spirochaetes making counting and identification of spirochaetes very difficult. Secondary antibody in the absence of primary antibody did not lead to any fluorescence which suggests that direct binding of the conjugate to tick material was not the cause of this cross reactivity.

Experiment 5.

It was considered that the use of blocking reagants may decrease the level of background staining and thereby make spirochaetes more visible. To test this possibility, 15 field collected *I.ricinus* nymphs were smeared and fixed. A solution of PBS was prepared with 0.5% tween-20 and 0.5% bovine serum albumin. A second solution of 5% skimmed milk (marvel) in PBS was also prepared. Primary antibody was diluted in these blocking solutions and in PBS. 5 ticks were stained with each solution as described above. Neither blocking agent made any visible difference to the degree of background staining.

Experiment 6.

In order to estimate the sensitivity of the immunofluorescence method in detecting spirochaetes in wild caught ticks, it was necessary to determine the limit of detection. To do this tick extracts were spiked with defined numbers of *Borrelia.* A prerequisite of this method therefore was to estimate the *B.burgdorferi* density in the supplied suspension from Kirkgaard and Perry. Unfortunately the company were unable to supply this information and so this had to be determined.

Slides were used with pre-marked wells and borrelia were stained as follows:

well no.	ul of borrelia	ul of PBS + DAPI.
1	8.0	08
2	4.0	12
3	2.0	14
4	1.0	15
5	0.5	15.5

Only in wells 4 and 5 were borrelia sufficiently well diluted that clumping did not prevent their being counted. In each well numbers of borrelia in 8 different fields at a magnification of 630x were counted with results as follows:

Well 4: mean number per field = 26.8
Well 5: mean number per field = 12.4

The number of fields of view in one well is calculated by:

$$\text{Fields per well} = \frac{\text{area of well}}{\text{area of field}} = \frac{(W/2)_2}{(F/2)^2} = \frac{W^2}{F^2}$$

(where W = the diameter of the well and F= the diameter of the field.)

Using the microscope stage, well diameter was measured in four places to give a mean well diameter of 7.675mm. The number of fields of view in a 1-well-diameter was counted 6 times to give a mean of 21.7 fields per diameter and therefore a field diameter of 0.354mm and the number of fields per well of 471.

Borrelia counts demonstrate the presence of 26.8 spirochaetes per field at 1ul of undiluted borrelia solution per well. Therefore in one well, a total of 12,622 spirochaetes were calculated to be present and it can therefore be estimated that the borrelia solution used in these experiments carries approximately 12,000 borrelia per ul.

Experiment 7.

28 ticks were smeared onto 4 slides. On each slide, ticks were spiked with 5ul *B.burgdorferi* solution in increasing dilutions of 1/50, 1/100, 1/200, 1/400, 1/800 and 1/1600. The final tick on each slide was spiked with saline only. A fifth slide was fixed with borrelia only. Each tick was then examined at 630x magification through 30 fields of view.

Results:

slide number	dilution						
	1/50	1/100	1/200	1/400	1/800	1/1600	saline
1	50	59	13	6	5	14	0
2	37+	10+	4	6	4	2	0
3	23	50	30	30	4	10+	0
4	55	33	12	7	4	2	0
(mean)	(41)	(38)	(15)	(12)	(4)	(7)	(0)
5	15+	12+	4	0	0	1	0

(+ indicates that clumps were present)

The larger number of spirochaetes present at high dilutions in slides 1 and 3 may be explained if wild spirochaetes had been present and were included in the count. If however it is assumed that this was not the case, the proportion of borrelia that were used to spike the tick and were consequently seen by immunofluorescence can be estimated.

Dilution	mean number borrelia seen /30 fields	mean number expected	mean number borrelia added per tick
1/50	41+	76	1200
1/100	38+	38	600
1/200	15	19	300
1/400	12	9.6	150
1/800	4	4.8	75
1/1600	7	2.4	38

Therefore, for a tick to appear positive on immunofluorescence, it would need to contain greater than 15.8 organisms per tick.

Experiment 8.

50 field collected nymphs, 25 from leaf litter and 25 from blaeberry bush were smeared and acetone fixed as previously described. They were stained by immunofluorescence using primary and secondary antibodies both at dilutions of 1:50. Each tick was examined for evidence of spirochaetes through 30 fields. A tick was deemed positive if one or more *borrelia* were identified. For a strand to be deemed a borrelia it had to meet three criteria, namely: a) regular width over the whole of its length; b) appropriate length and width and c) bright fluorescent staining.

Of those ticks collected in leaf litter 4/25 ticks were positive for *Borrelia burgdorferi*. In each positive tick, only the one borrelia was found. Of those ticks collected in blaeberry 6/25 were positive. Numbers of borrelia seen in each tick were : 8,5,5,1,1 and 1. There is therefore a hint that blaeberry collected nymphs are more likely to be infectious though data are insufficient to apply statistical analyses with confidence. Overall it appears that approximately 20% of field collected nymphs from Rowardennan by Loch Lomond were *Borrelia burgdorferi* positive.

Experiment 9.

35 larvae were removed from small mammals in differing states of engorgement. They were smeared and acetone fixed within 3 hours of removal and then frozen at -20°C. Slides were then stained using the immunofluorescence methods described above.

Only 2/35 larvae were considered positive for the spirochaete and in both cases only one spirochaete was seen and their identification was considered equivocal. One tick flouresced with a number of rods that were short and blunt, unlike any artefact previously seen and dissimilar in shape to *B.burgdorferi*. This raises the possibility that the antibodies cross-reacted with another tick vectored organism.

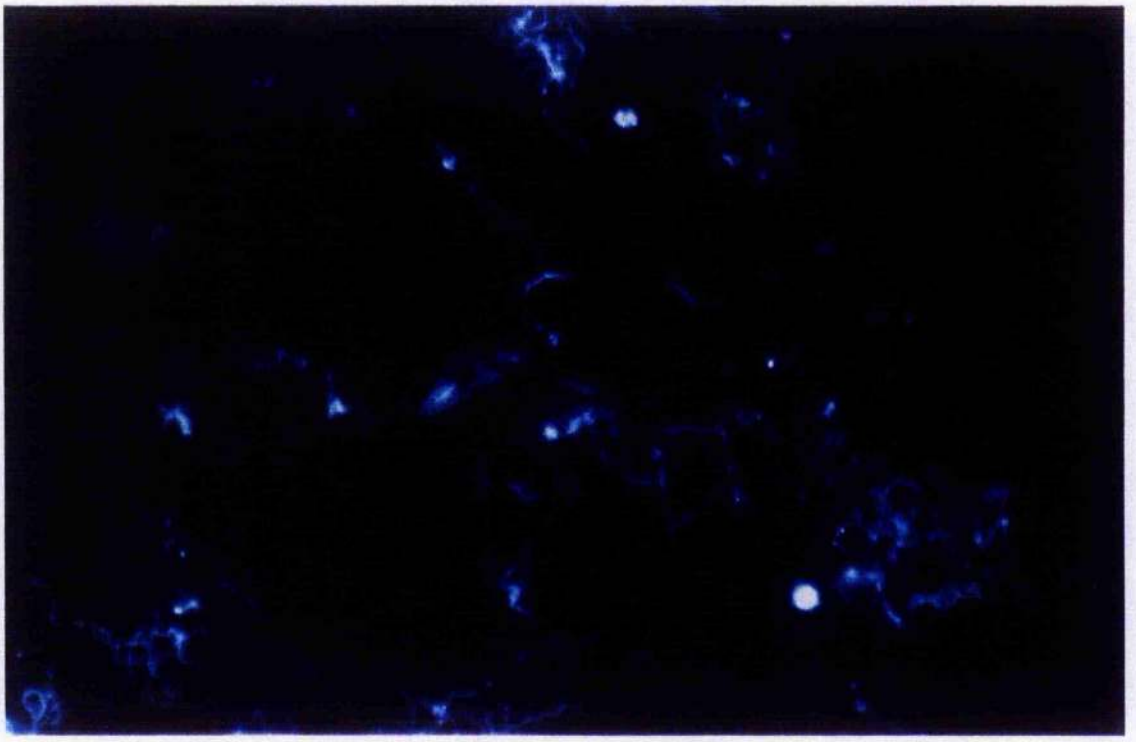
7.3. Discussion.

The greatest compounding problem in this study was the high level of background immunofluorescence, which combined with the very fibrous nature of the tick tissue, led to the common presence of artefacts. Since this background activity was not prevented by blockers such as 'Marvel', tween-20 or BSA and binding of secondary antibody to tick tissue did not occur, it seems likely that it was the direct result of cross-reactivity between borrelial and tick antigens. It would perhaps not be surprising that *B.burgdorferi* should present an antigenic phenotype similar to that of its host. This would lead to it being less likely recognised as foreign and to its more likely being accepted by its tick host.

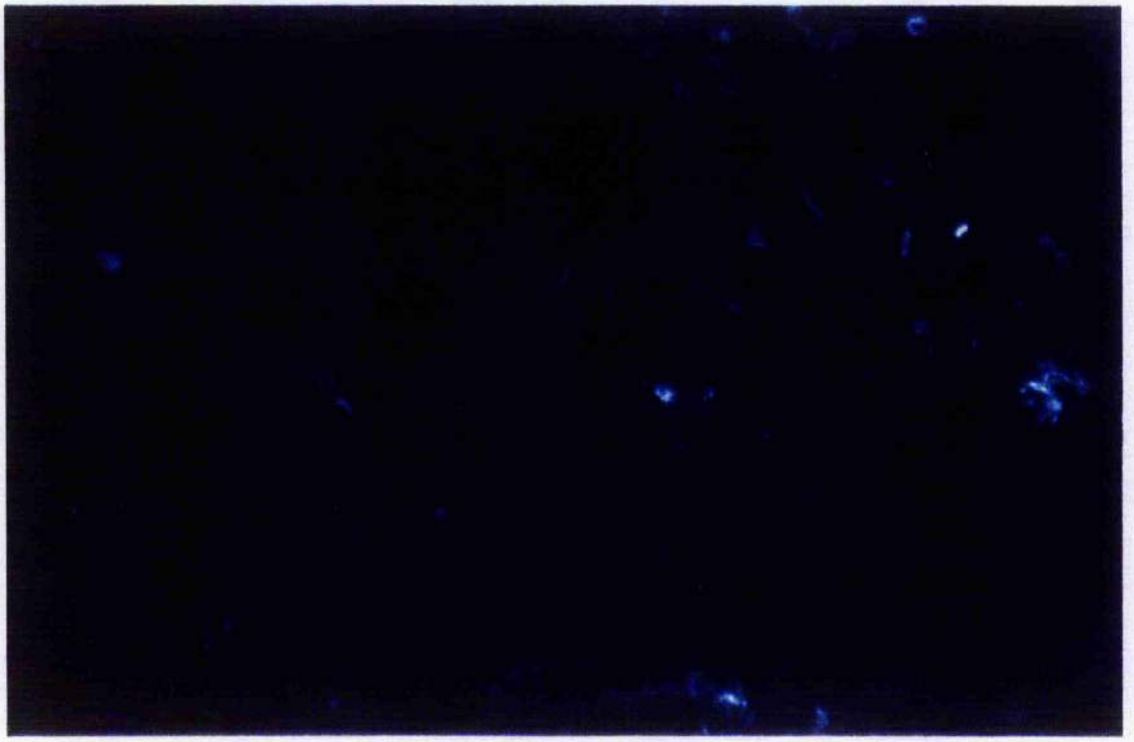
However, this means that whilst the immunofluorescence methods described are apparently sufficiently sensitive in picking up any substantially infected tick, caution must be expressed as to whether or not those wild spirochaetes recorded as being present were accurately recognised. Certainly, very few spirochaetes were seen whose appearance exactly matched that of the American culture. Whilst British spirochaetes have not been cultured, their behaviour in the disease state has been shown to be significantly different to both American and continental European types. It does not seem unreasonable therefore to suppose that their antigenic presentation and even their appearance may also differ from the American organisms. The experiments above showed that immunofluorescence was sensitive when used to detect that strain of bacteria that had been used to generate the antibodies. Unfortunately there is no evidence that it is adequate when using American antibodies to detect British bacteria, possibly because the antibody binds to tick tissue and bacteria with similar tenacity.

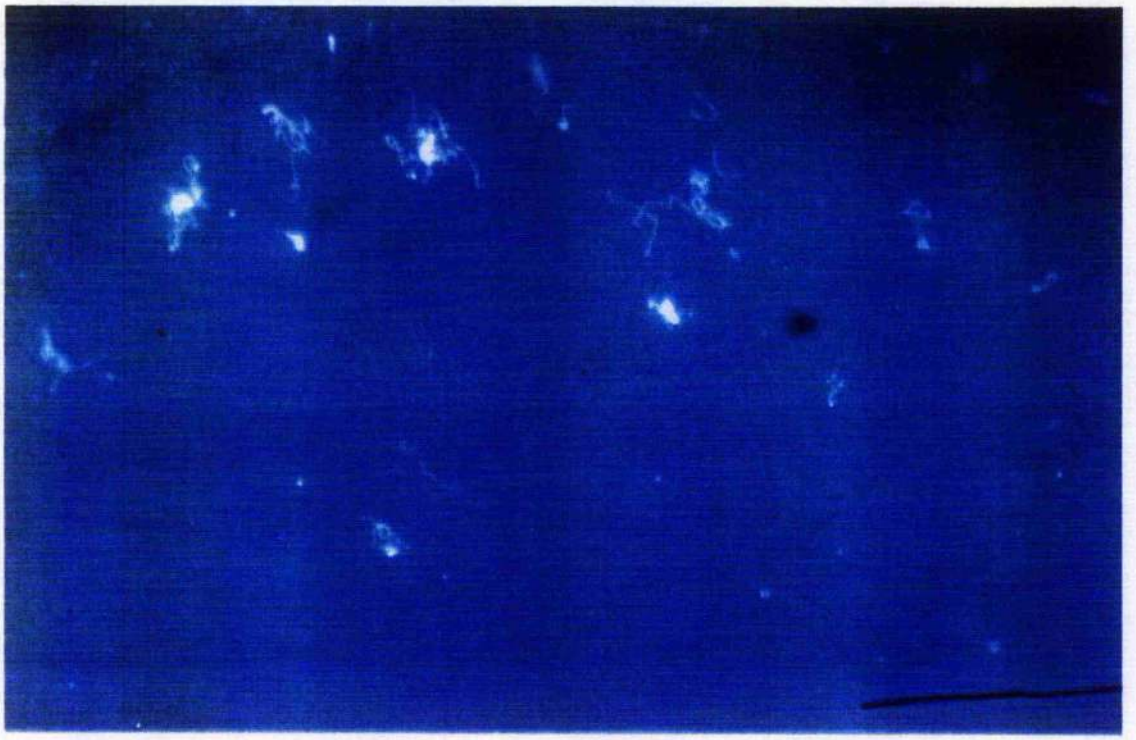
Larvae from small mammals were only equivocally positive twice. However most were not fully engorged and some were only a little so. As discussed above, immunofluorescence may not have picked up bacteria that were present. Therefore whilst there is very little evidence that small mammals are infecting ticks with *B.burgdorferi* and thereby are acting as the animal reservoir, this study must at present remain inconclusive on this issue.

There was a suggestion in one mammal derived tick of the presence of an organism that bound the primary antibody but which was not *B.burgdorferi.* Since it now appears that the antibodies used were not highly specific for *B.burgdorferi,* it may be interesting to speculate on the nature of the organism. Whilst *Leptospira* are the most likely organisms known to be present to cross react, the protozoan organisms, *Grahamella,* often present in small mammals (though usually intracellularly), which are also blunt rod-like in shape would also be suitable candidates. One may expect for similar reasons to the bacteria, that they could also antigenically mimic their tick host.

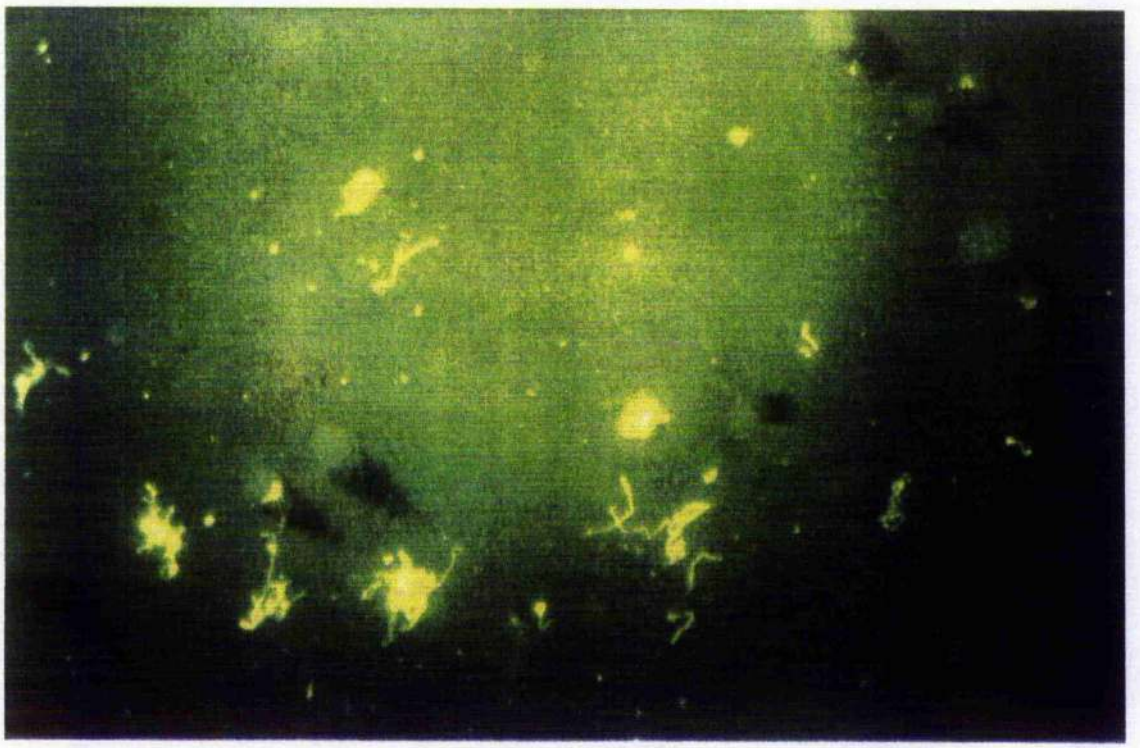


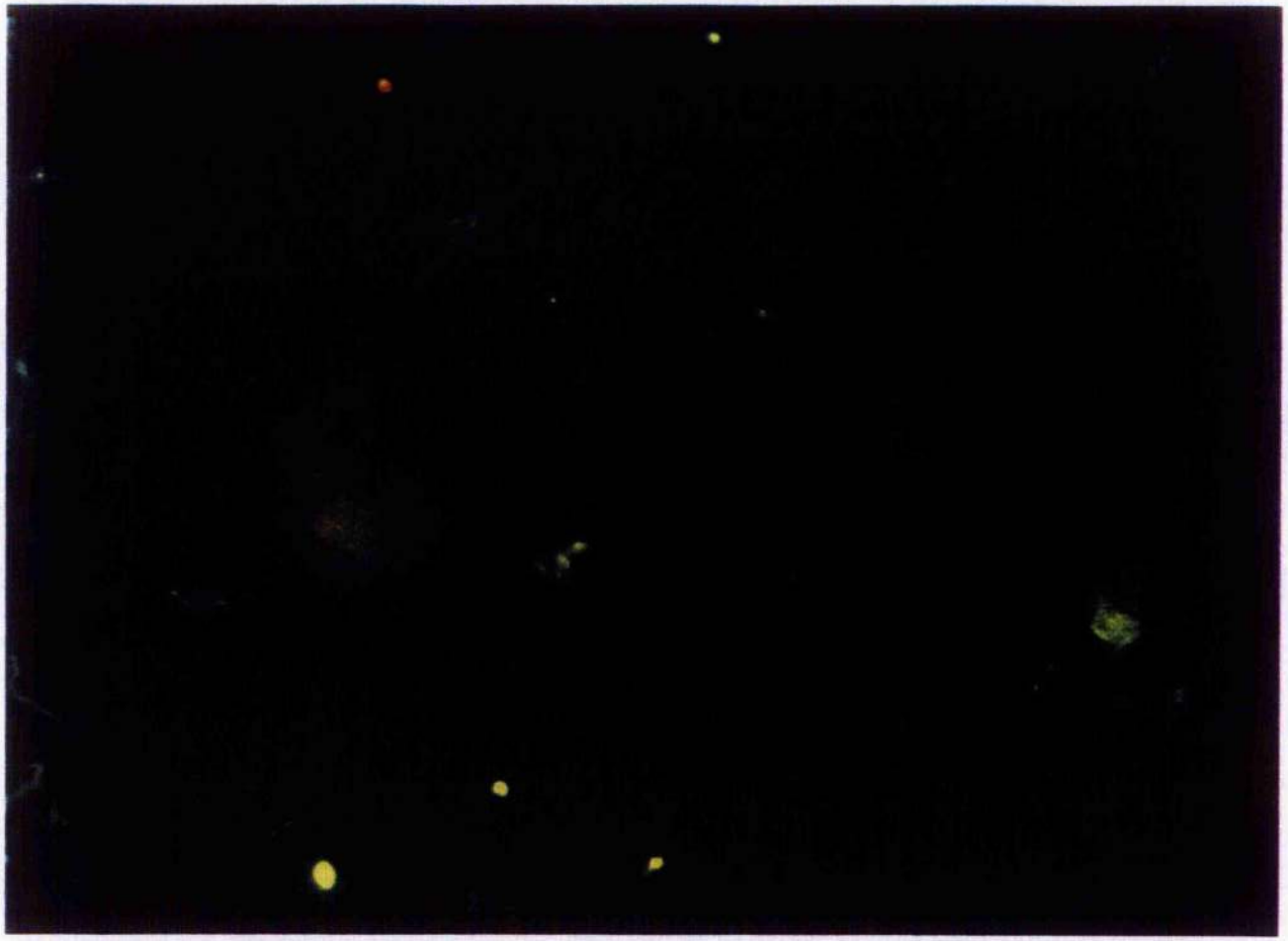
Borrelia burgdorferi, DAPI stained at high and low concentrations.



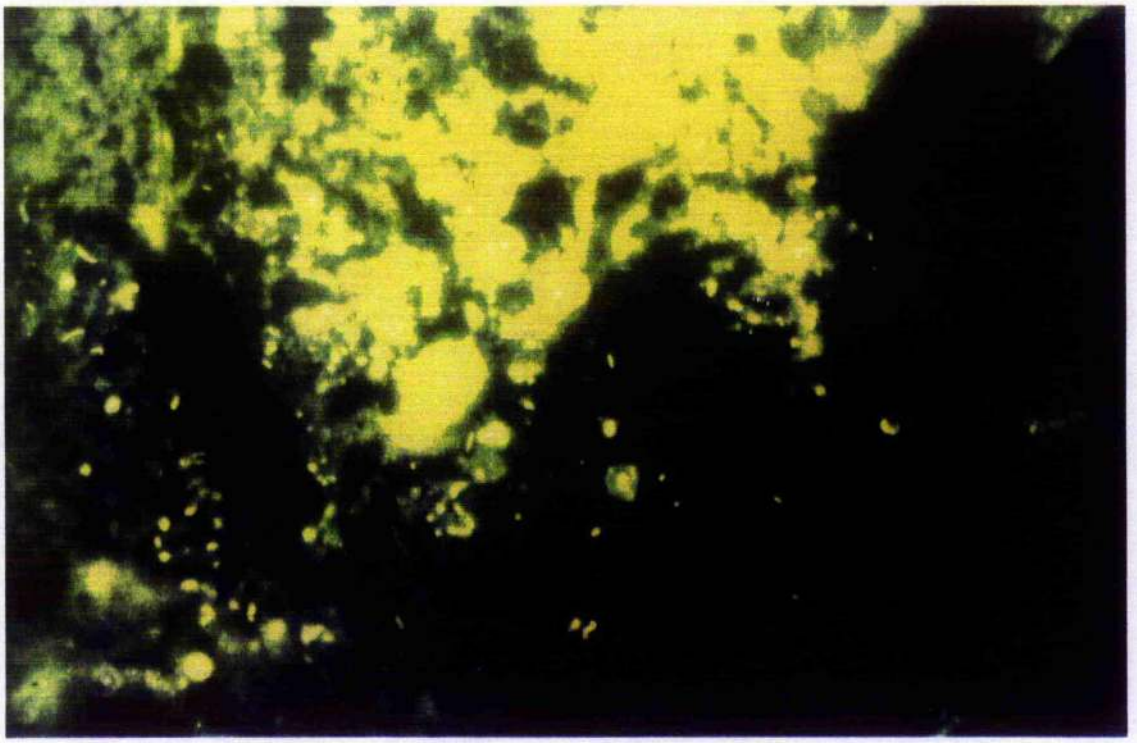


Borrelia burgdorferi. The same bacteria, stained above with DAPI and below by immunoflourescence.

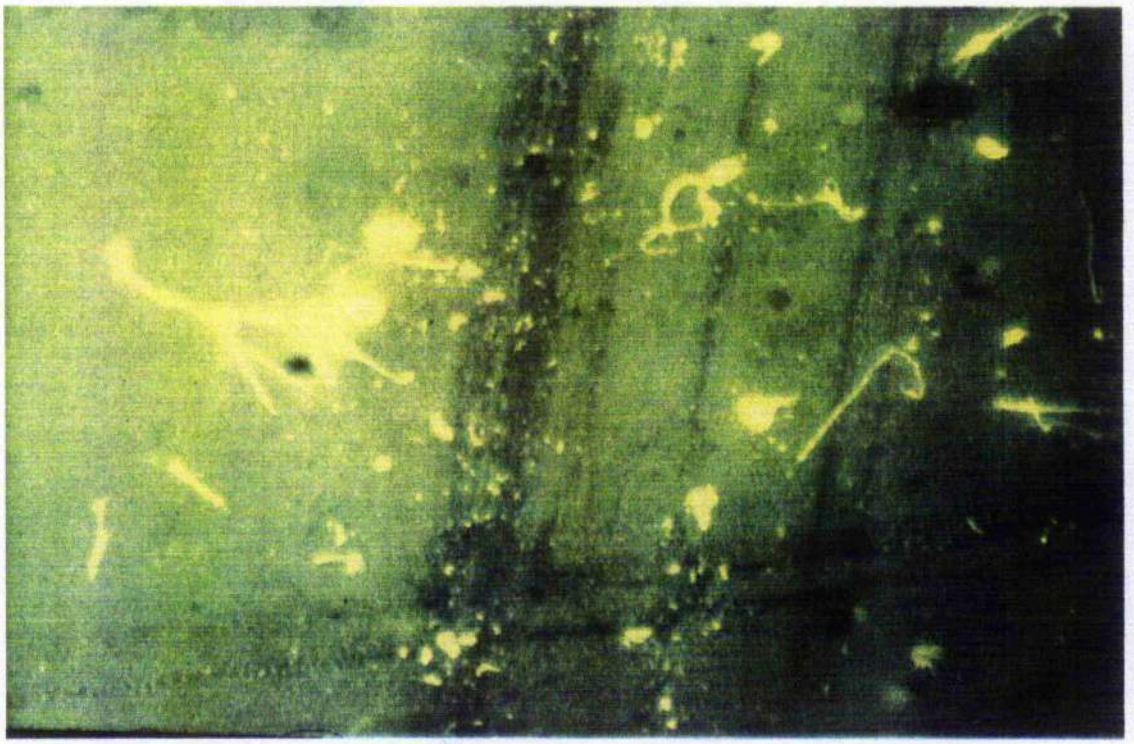




A tick smear spiked with cultured *B.burgdorferi*. Spirochaetes can be distinguished from artefeact.



Artefact. Above: non-specific flourescence disrupts any views of spirochaetes. Below: Strands of connective tissue immitate *B.burgdorferi.*



8.0. Final Discussion

8.1Tick ecology

The study site at Rowardennan was found to have a high abundance of ticks. It also appears that distibution was not evenly spread throughout the woodland and that the site most intensively studied was particularly well populated. Here, it was estimated that the ground carried a minimum of 8 nymphs per m^2 that quested in a season. In paired dragsets this site picked up a mean of 36 nymphs per dragset compared to 12 nymphs per dragset on the north side of the hill. Therefore it would seem that other areas of the woodland carry in the order of 3 nymphs per m^2.

The ratio of adults: nymphs: larvae found in this study was 1: 30: 142. Therefore we would estimate that the population of ticks per hectare in the highly populated site would be a minimum of 2700 adults, 80,000 nymphs and 380,000 larvae. Elsewhere numbers would be a third of these.

If one assumes that this population stays roughly constant from year to year then one can see that 21% of larvae become nymphs. The nymphal population must have fed the previous season as larvae and there will have also been a mortality of fed larvae. However, at least 27,000 larvae were fed per hectare at Rowardennan in 1992.

This study looked at the possible contribution of small mammals and birds as hosts of these ticks. It was estimated that birds at Rowardennan fed a minimum of 1800 larvae in 1993 and if this was the same as in 1992 then this would amount to approximately 7% of the 27,000 larvae known to have fed. This figure is subject to considerable error with the study being conducted over a single season and as the birds were examined on the opposite side of the loch to the blanket dragging. It was estimated that small mammals fed a minimum of 6,000 larvae per hectare in 1993 which means that small mammals feed a significant proportion of larvae, in the order of 22%. Because small mammal populations fluctuate so much from year to year and measuring them is fraught with so many difficulties this figure is also subject to considerable error. However small mammals are clearly an important host.

This means that the hosts for about 70% of the larvae have not yet been accounted for. Furthermore this study failed to identify the hosts for the majority of nymphs and adults. The most likely hosts are the deer which have been shown elsewhere to be very important as tick hosts. Wilson *et al* (1988) showed *I.dammini* numbers dropping considerably after elimination of deer from an island. Gray *et al* (1992) showed large

differences in *I.ricinus* populations either side of a deer fence. Milne (1947) reports infestations of up to 1000 female adults per deer with usually 150-300 adults per deer at peak activity and this observation was supported by discussions with gamekeepers at Rowardennan who had noted large numbers of ticks on deer.

The Handbook of British Mammals (Corbet and Harris, 1991) lists densities of deer in Scottish woodlands as follows:
Red deer *Cervus elaphus* 5-15 /km^2 (with a mean of 9/km^2)
Roe deer *Capreolus capreolus* 8-25/km^2
Sika deer *Cervus nippon* 8-40/km^2.

This totals a density of 21-80 deer/km^2 feeding the previously unaccounted for 1,917,000 larvae/km^2 per year that are fed and which become nymphs. If this was spread over a four month season or 28x 4 day periods, the mean infestation of a deer during this time would be 214-815 larvae per deer. This would not be a surprising number of larvae to find upon a deer.

It is likely that other wild species act as tick hosts. Milne (1947) discussed the importance of squirrels, pine martens, and capercaillie, all of which inhabit the Rowardennan woodland. However they exist in small numbers and it seems unlikely that they would have a significant effect upon tick populations.

On open grazing land, sheep are often infested with ticks. A farmer in the Rowardennan area reported to me a significant mortality of his lambs due to pyaemia resulting from tick bites. He noted that his sheep became particularly infested if they escaped from their relatively tick free enclosure into the woods. However since the sheep are most likely to spend the majority of their time on the grazing land it seems unlikely that ticks that had fed upon them would drop off when they were in the woods.

It is worth noting here that most hosts to the ticks will have fairly large ranges and that their habitats are not confined to the woods. There is therefore likely to be a considerable flux of tick populations from the woods to grazing land and vice versa.

In conclusion, deer are most likely the principal tick hosts in Scottish oak woodland. Small mammals also feed a significant number of larvae and birds are probably only minor hosts feeding just a few larvae. The tick population in the woodland is unlikely

to be an isolated one and will mix with that in other habitats. Further work is required to quantify the tick loads of sheep and deer in Loch Lomond

It would seem likely then, that if deer feed nearly all nymphs and adults and the majority of larvae in the woods, that they will dictate tick distribution within this habitat. This has been shown to be the case in two instances and it is also apparent that areas in the UK with high tick numbers such as the New Forest, Thetford Forest and the Isle of Rum are also known for their dense deer populations. This may explain the clustered tick distribution at Rowardennan. The area of highest tick density was a sheltered area on the south side of a hill that perhaps provides a habitat very suitable for deer.

Ticks were found to be more abundant in blaeberry bush than amongst leaf litter. This may be accounted for by deer having a preference for blaeberry as grazing material rather than grass and leaf litter and presumingly this would depend partly on how grassy the latter was. However a significant proportion of ticks were fed by small mammals which were most definitely inclined towards the blaeberry habitat since traps laid on open ground were rarely found occupied (appendix 11). Blaeberry bush is also likely to maintain a higher humidity than grass and leaf litter both at ground level and at the questing point so that tick dessication and consequent mortality is likely to lower in this habitat.

8.2. Implications for the ecology of *Borrelia burgdorferi.*

With these high numbers of ticks at Rowardennan it is unlikely that any vertebrate that spends a significant amount of time on the ground will pass through the season without being infested by ticks. In this study no mammal ever escaped infestation and even mainly tree dwelling birds such as the wood warbler *Phylloscopus sibalatrix* or the treecreeper *Certhia familiaris* were found to get infested. Conditions would therefore be expected to be ideal for the transmission of the parasites to which many ticks act as vectors.

However, in the case of Lyme disease this would depend upon the suitability of hosts as reservoirs for the *B.burgdorferi* spirochaete. It has been proposed that in North America, deer are unable to act as reservoirs and that the white footed mouse *P.leucopus* acts as the principal reservoir. If such a similar situation existed in Scotland, up to 40% of larvae could pick up infection if all small mammals acted as infective reservoirs. Small mammals are only rarely fed upon by nymphs and adults and

the mortality of small mammals from one tick season to the next is very high. For bank voles *C.glareolus* less than 50% survive for more than 4 months and by late summer the population is made up almost totally of animals born that year. Few wood mice *A.sylvaticus* survive for more than one year and mean life expectancy at one month old is 2.9-3.6 months (Corbet and Harris 1991). Therefore, as the tick season progresses, most mammals coming into the population will be free of infection. They will become more often positive as it progresses and as such will be able to infect other larvae that feed upon them. If mice are the only reservoirs, therefore, the ticks would then have to remain positive for the spirochaete whilst feeding as nymphs and as adults and then transovarially transmit the spirochaete to the eggs and the new generation of larvae. It is thought that only 2-3% of the eggs of an infected *I.dammini* female will be infected and figures are not available for *I.ricinus.* It was therefore thought that this was an unimportant stage of transmission. However, it was pointed out by Randolph (1991), that because the female lays 1500 eggs, a 1% rate of transmission means that one infected female gives rise to 15 infected larvae. In this study, 1 female gave rise to 142 larvae and so with these rates one would expect to find about 3 questing infected larvae for two infected females. It is therefore a point at which the disease can expand within the population.

If small mammals are the only reservoirs, with the figures above, one infected female could give rise to 1.5 infected larvae of which up to half (0.75) would feed on a suitable host. If every host became infected and fed a further 0.25 larvae which became infected and always passed the infection on to the next stage, then one infected female would have given rise to an infected female of the next generation and the reproductive rate of the disease in the population would be 1.0. The disease would then be viable endemically. Of course this is a gross simplification and whilst infectivity rates are probably well below 100%, each infested host probably feeds a much larger number of larvae. However these figures show it may be possible to build some sort of model which would predict how these various factors affected the reproductive rate of a tick born disease. It is reasonable to conclude though that whilst data are insufficient at present, it does not exclude the possibility that small mammals are the only Lyme disease reservoirs. The number of larvae that a host would have to feed to maintain the reproductive rate of the disease at >1.0 would depend upon infectivity rates between vector and host. One would also need to know to what extent hosts could become immune to the parasite and to what extent the parasite and immunity to it could be transplacentally transmitted. It may be that newborn small mammals inherit an immunity to the parasite by inheriting maternal IgG and only become susceptible after some weeks. On the other hand the parasite may get transmitted between members of

the population via the placenta rather than by ticks. If so, an infected mother could give rise to 6 infected young and as such the disease could spread rapidly in the population. Congenital syphillis, is well recognised and congenital Lyme disease has been reported (O'Neill and Wright 1988).

Unfortunately tick studies in this project failed to demonstrate the unequivocal presence of Lyme disease spirochaetes in the woods around Loch Lomond. However other workers using polymerase chain reaction technology (Curtin 1993, Nuttall *et al* 1993) have shown infection to be ubiquitous in association with ticks in the UK. Curtin found about 30% of nymphs to be infected in Scotland with significantly more on the west than the east coast. She also found kidney and liver samples from deer to be positive. If most larvae feed on deer and deer are not acting as reservoirs it would be surprising to find 30% of questing nymphs positive. The west coast is much more populated with deer than on the east and if deer act as a reservoir and make larvae positive this would explain Curtin's results. Other evidence supporting the notion that ungulates may act as a reservoir comes from work demonstrating Lyme disease in horses (Carter *et al* 1993).

The evidence that birds may act as disease reservoirs has been discussed and from this work it appears that birds feed far too small a number of ticks to become an important component of the reservoir. However they are clearly subject to considerable levels of infestation and to tick born viruses, bacteria, rickettsia and protozoan infections. These are likely to have a considerable effect upon bird population size and structure. In the Rowardennan area in particular Capercaillie populations are of notable conservation interest. As mentioned earlier Milne (1948) indicated that they are most susceptible to infestation and so it seems highly likely that their populations will be significantly affected by ticks.

In summary, this project has indirectly supported the idea that deer are the most important tick hosts in Scottish oak woodland. However small mammals and birds also play a significant role. It may be possible for Lyme disease to be maintained in the population with only small mammals as reservoirs but there is a little evidence supporting the notion that ungulates and birds could also act as reservoirs. Ticks may also have a significant impact on wild animal populations. This may be of conservation interest in birds such as the Capercailie or economical in the case of deer. It should be possible to develop models of Lyme disease epidemiology and the possible effects of transplacental transmission should be taken into account.

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Appendices.

Appendix 1. Numbers of ticks collected in dragsets at Rowardennan.

		Drag number within dragset.									
		1	2	3	4	5	6	7	8	9	10
All	total	387	222	123	120	77	71	65	60	34	30
(n = 54)	mean	7.17	4.11	2.28	2.22	1.43	1.32	1.20	1.11	0.63	0.56
	S.E.	1.49	0.94	0.55	0.61	0.37	0.37	0.27	0.28	0.17	0.27
cumulative mean		7.17	11.3	13.6	15.8	17.2	18.5	19.7	20.8	22.1	24.1
Blaeberry	total	201	116	61	66	43	44	32	30	13	19
(n = 20)	mean	10.1	5.80	3.05	3.30	2.15	2.20	1.60	1.50	0.65	0.95
	S.E.	3.73	2.42	1.38	1.58	0.97	0.93	0.65	0.70	0.41	0.69
cumulative mean		10.1	15.9	18.8	22.2	24.4	26.6	28.2	29.7	30.3	31.3
Leaf litter	total	186	106	62	54	34	27	33	30	21	11
(n = 34)	mean	5.48	3.12	1.82	1.59	1.00	0.79	0.97	0.88	0.62	0.32
	S.E.	0.35	0.42	0.32	0.28	0.16	0.18	0.19	0.17	0.15	0.11
cumulative mean		5.47	8.59	10.4	12.0	13.0	13.8	16.2	17.1	17.7	18.0

Appendix 2. Cumulative numbers of ticks collected in dragsets in blaeberry and leaf litter

drag number in dragset.

	1	2	3	4	5	6	7	8	9	10	
blae-berry	30	48	57	67	74	80	85	89	91	94	mean % of total
leaf litter	29	46	56	64	70	74	87	92	95	97	ticks collected

Appendix 3. Seasonal variation in tick abundance as measured by blanket dragging.

	April	May	June	July	August	Sept.
Dragsite A.	21	31	46	37	22	14
Dragsite B.	19	26	24	20	7	2
All drags.	170	580	238	126	60	23
nymphs per drag.(n)	15.45 (11)	34.12 (17)	29.75 (8)	21.00 (6)	10.00 (6)	5.75 (4)
S.E.	3.75	16.64	8.09	5.35	3.34	1.39
larvae per drag. (n)	Nil seen	122.57 (7)	345.38 (8)	112.5 (4)	71.33 (6)	79.25 (4)
S.E.		89.97	142.5	56.25	23.78	15.93
adults per drag. (n)	0.923 (13)	0.941 (17)	0.778 (7)	0.667 (6)	0.333 (6)	0.250 (4)

Appendix 4. Estimated numbers of small mammals (mice and voles) at Rowardennan in 1993.

Method	April	May	June	July	August	September
MNA	21	12	20	15	22	16
Adjusted MNA*	37.5	21.4	35.7	26.8	39.3	28.6
Weighted mean	23	13.6	24.2	18.5	36.58	17.7
Fisher-Ford	-	27.5	24.1	69.5	51.1	24.2
Triple-catch	-	-	-	-	33.1	-

*assumes that 56% of small mammals were captured.

Appendix 5. Numbers of times that small mammals were trapped.

Number of times mammal trapped	1	2	3	4	5	6	7	8	9	>9
Number of mammals.	30	17	7	6	1	3	3	2	3	3

Appendix 6. Numbers of birds inspected for ticks and levels of tick infestation at Tarbet in 1993.

Month	No. birds ringed	mean larvae per bird	SE	SD
March	50	0.04	0.03	0.20
April	83	0.04	0.02	0.19
May	28	2.36	1.93	10.2
June	61	4.11	2.09	16.32
July	5	4.60	2.82	6.31
August	175	0.79	0.15	2.04
September	32	0.22	6.10	0.61
October	244	0.08	0.02	0.30
November	258	0	0	0
December	254	0	0	0

Appendix 7. Adjusted infestation rates estimating differing importance of different bird species as tick hosts.

Species	No. of birds ringed	No. infested	MSAIR
Blackbird	11	8	28.8
Blue tit	36	1	3.5
Bullfinch	3	1	1.2
Chaffinch	75	18	7.1
Great tit	21	3	0.3
Jay	2	2	53.6
Robin	38	22	2.3
Siskin	8	4	2.7
Song thrush	3	1	0.1
Treecreeper	2	1	1.8
Wren	7	5	3.8

Appendix 8. Tick infestation of birds caught at Rowardennan in June 1993.

Species	number caught	number infested	mean infestation rate
Blue tit	8	6	2.38
Great tit	4	3	13.5
Wren	1	1	6
Wood warblers	2	1	1
Coal tit	1	1	4

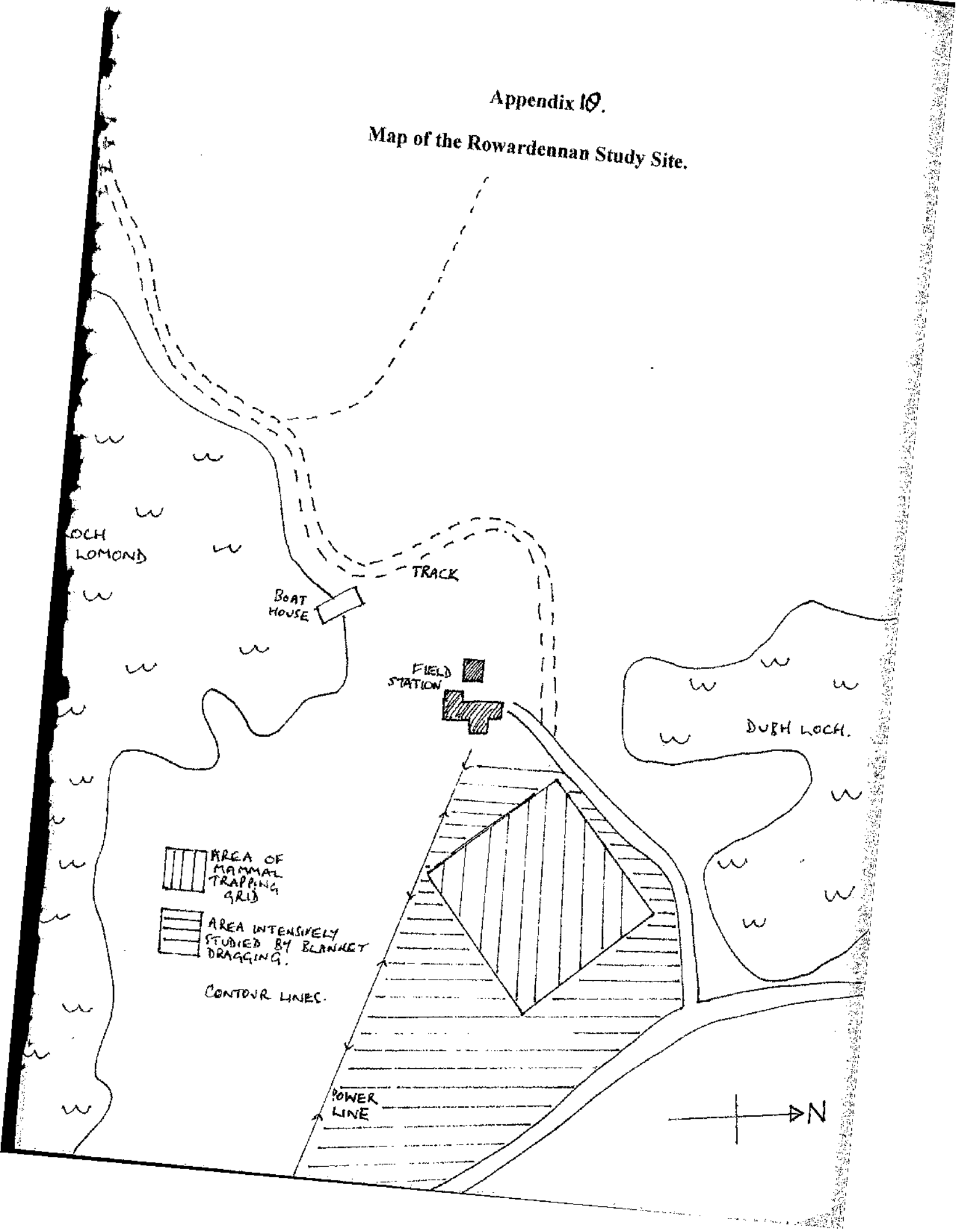
Appendix 9. The history of Captures of small mammals caught at Rowardennan in 1993.

Mammal number	April								May									June						
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7
1	I							I																
2	I																							
3	I		I			I																		
4	I		I					I																
5	I		I					I																
6	I																							
7		I	I		I	I																		
8		I																						
9			I																					
10						I																		
11			I																					
12			I					I																
13			I																					
14				I	I			I	I			I	I	I	I	I		I			I	I		I
15					I			I					I		I									
16					I	I																		
17								I																
18								I																
19								I																
20									I		I		I			I	I		I	I			I	
21									I															
22									I		I	I	I	I	I	I	I							
23											I	I	I			I	I	I	I	I	I			
24													I		I			I		I		I		I
25												I	I	I	I	I					I	I	I	I
26													I	I						I	I	I	I	I
27													I		I									
28																		I			I			
29																		I						
30																		I	I	I			I	I
31																		I		I				I
32																			I			I		
33																				I				I
34																					I			I
35																					I			
36																					I			
37																					I	I		
38																						I		
39																							I	

Mammal number.	July							August						September					
	1	2	3	4	5	6	7	1	2	3	4	5	6	1	2	3	4	5	6
40	I		I		I	I	I					I							
41	I																		
42	I																		
43			I							I									
44	I			I	I		I												
45	I																		
46	I		I																
47	I																		
48	I																		
49		I		I															
50		I																	
51			I		I		I			I									
52			I		I									I	I		I	I	I
53								I						I	I	I	I		I
54								I		I									
55								I		I				I		I			
56								I											
57								I	I			I							
58								I	I	I	I	I	I	I		I	I	I	I
59								I											
60								I				I				I		I	
61									I			I	I		I	I	I	I	I
62									I			I							
63									I				I						
64									I	I	I		I			I	I		I
65										I			I	I					
66											I								
67											I		I				I		
68												I		I					
69														I					
70																I			
71																I			
72																			I
73																		I	
14	I		I	I	I			I	I	I		I	I	I	I	I	I	I	I
25	I		I		I	I			I		I								

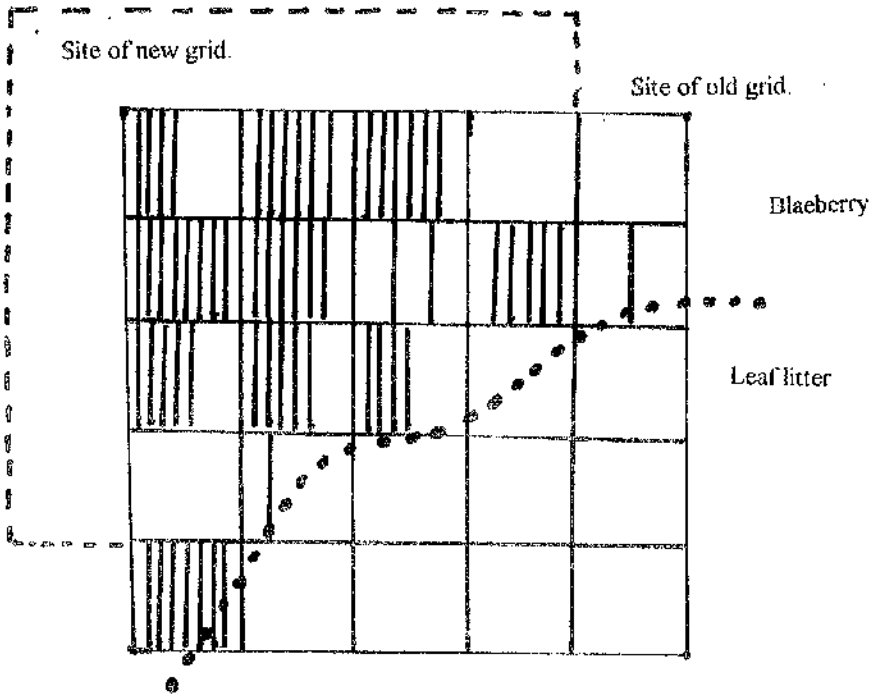
Appendix 19.

Map of the Rowardennan Study Site.



Distribution of captures of small mammals on the trapping grid.

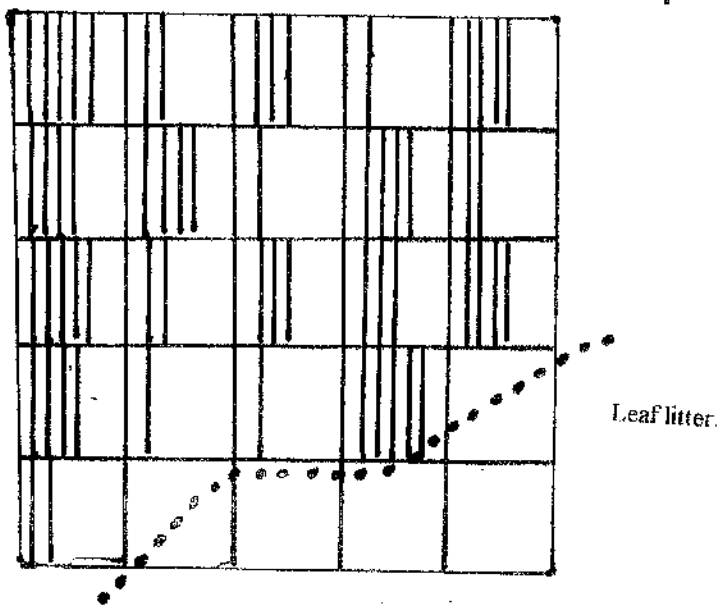
April



Approximate boundary between blaeberry and leaf litter habitats.

August

= 1 capture



NAME	ADDRESS AND AFFILIATION	DATE