Contributions to the epidemiology of Louping-ill.

1. The ecology of *Ixodes trianguliceps* Birula, 1895, a potential vector.

2. Prevalence of Louping-ill in northern Scottish cattle.

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Abstract.

Part 1. The ecology of *Ixodes trianguliceps* Birula, 1895.

Ixodes trianguliceps and its small mammal hosts have previously been proposed as serving an occult reservoir role in the cycling of Louping-ill virus. The tick was collected from three host species: *C. glareolus*, *M. agrestis* and *A. sylvaticus*. The main study area was at Balerno, Midlothian where it was the only species of tick recovered. Regular collections were made from July 1974 until August 1976.

C. glareolus had the greatest frequency of parasitised hosts and the heaviest burdens; the males were more heavily burdened than the females. Such sex preference was not found for the other hosts. Larvae infest the hosts throughout the year, burdens being maximal in June and minimal in July, a second, much lower, peak occurs in October. Females are active from early spring and nymphs from late spring until autumn. The ear pinna is the area of the body most heavily infested by larvae and nymphs, particularly the inner surface with voles and the outer surface with the mouse. While larvae are overdispersed on all three species of host, nymphs and females are overdispersed on *C. glareolus* only, and this host alone of the three maintains the instars in appropriate numbers and proportions to qualify it as an independent maintenance host. While *C. glareolus* could maintain the tick population, *M. agrestis* and *A. sylvaticus* could not and may even be deleterious to the tick population.

Larvae have a pre-feeding diapause while attached to the host from December until February. There is no evidence that larvae are active during winter as reported by other workers. Diapause is abandoned when infested hosts are brought into the laboratory and engorgement to repletion is slower on all three hosts than it is during the rest of the year. At any time of the year larvae take longer to feed on *A. sylvaticus* than on the voles.

Only 9% of field captured larvae completed development and it is proposed that the majority of engorged larvae enter a pre-developmental diapause. Similar conclusions are reached for nymphs and females. For larvae and nymphs which develop at constant temperature without manifest diapause delays the relationship between incubation temperature and developmental velocity is linear and direct.

Part 2. Prevalence of Louping-ill in northern Scottish cattle.

In a survey of cattle in the northern counties and inner islands of the west coast of Scotland, 4529 sera were tested for immunoglobulin G to Louping-ill, using antigen derived from Louping-ill strain 31. The incidence of antisera in herds decreases from the west of the country to the east. In a stable herd structure there is a significant, steady increase in antibody with age. It is suggested that cattle are useful indicators of Louping-ill, but their role in maintenance of the disease remains an unknown factor.

Contents

Introduction.

The work reported on here was undertaken as part of a wider project on the epidemiology of the virus disease, Louping-ill. Earlier work on this subject has been reviewed by Gordon, Brownlee, Wilson and Macleod (1962), who were the principal pioneers in the study of the disease, Macleod (1962) and Varma (1964). In summary, Hoogstraal (1966) concludes "The chief cycle of Louping-ill virus appears to be between sheep and I. ricinus". Several considerations leave room a rriori to doubt this conclusion. Its occurrence in cattle and horses among domestic animals is referred to by Hoogstraal himself. Among feral animals, Dunn (1960) has studied the red deer, Cervus elaphus, and Smith, Varma and McMahan (1964) have recorded the recovery of the virus from roderts and shrews. This last finding assumes particular interest in view of Havlík's (1954) observation that fluctuations in rodent populations affect the epidemiology of Central European Tick-borne encephalitis a closely related virus in Czechoslovakia. It thus becomes conceivable that although the sheep - I. ricinus cycle may predominate in the natural maintenance of the virus other cycles may occur which serve an occult reservoir role. The most obvious of such potential cycles is the widespread rodent association with the tick Ixodes trianguliceps.

This work began as a study of the inter-relationships of feral small mammals and their ticks in Scotland with the intention of establishing laboratory cultures of the ticks for experimental work on virus transmission. I. trianguliceps proved a reluctant subject in the laboratory and the intention of laboratory culture was wholly frustrated. Nevertheless, the ecological study of the tick was pursued, and the results are presented in Part 1 of this thesis.

Opportunity occurred to survey the distribution of louping-ill antisera in a series of large samples of cattle-sera which became available in the laboratory from an independent concurrent study of babesiasis in Scottish cattle (Blewett and Adam (1978 A and B) and Adam and Blewett (1978)). The results of this survey are presented in Part 2 which follows.

Contributions to the epidemiology of Louping-ill.

Part 1

The ecology of Ixodes trianguliceps Birula, 1895, a potential vector.

PART 1.

Introduction.

Ixodes trianguliceps Birula, 1895, is the only known European representative of the sub genus Exopalpiger Schulze, 1935. It has been variously described and named, I. tenuirostris Neumann, 1901 I. nivalis Rondelli, 1928 and Endopalpiger heroldi (P. Schulze, 1943) Filipova (1957). Its distribution extends across Europe (Lachmajer (1962), Lichard (1965), Morel (1965), Aeschlimann (1970) and Ulmanen (1972)) and into Asian U.S.S.R. (Korenberg and Lebedeva (1969)) and is closely associated with woodland where the soil remains moist, becoming neither waterlogged nor too dry (Lachmajer, 1962).

I. trianguliceps appears to be restricted in Britain to small burrowing rodents and insectivores. It will feed in all instars on the bank vole (Clethrionomys glareolus), field vole (Microtus agrestis) and woodmouse (Apodemus sylvaticus). The common shrew (Sorex araneus) and pygmy shrew (Sorex minutus) both feed larvae and nymphs, but the common shrew is rarely reported to feed the female tick (Lachmajer, 1962; Randolph, 1975 B). The harvest mouse (Micromys minutus) feeds larvae and females (Randolph 1975 B) but nymphs have not yet been found. Arthur (1963) adds as hosts, 'Arvicola cratensis', Arvicola amphibius var ater, Rattus rattus, Rattus norvegicus, Mus musculus and Talpa europaea.

On continental Eurasia, this tick has a much wider host range, (U.S.S.R: Vysotskaya (1951), Korenberg and Lebedeva (1969), Katelina (1960), Nikitina (1960); Europe : Lachmajer (1962), Lichard (1965), Lutta (1968), Aeschlimann (1970) and Ulmanen (1972)), although not all hosts are known to have had the instars attached, and some are predators of small burrowing mammals and therefore in direct contact with the tick.

With the exception of Randolph (1975 B) there is general agreement that C. glareolus plays an important role in the maintainance of Ixodes trianguliceps, Randolph considers its survival dependent on 4 other

species and concludes that in Sussex, England "C. glareolus would appear to be rather more incidental" as a host.

There is broad agreement on the seasonal distribution of the instars and expecially so for nymphs which have only one record of winter activity (Ulmanen, 1972). Females share the spring to autumn activity period of the nymphs but Randolph (1975 A) found single females engorging in December and January, and Ulmanen (1972) found the females active in winter. The extension of larval activity into winter is generally accepted and has led to the proposal that "I. trianguliceps may take over the role of carrier of infections among mammals from ticks remaining in diapause during this period [autumn and winter]" (Lachmajer, 1962). It will be argued below that during winter larvae are not actively engorging on hosts but are indeed also in diapause. Lachmajer's observation of apparent activity in winter leads her to conclude this is "evidence of their adaption to very low temperatures", and Korenberg and Lebedeva, (1969) state that it is "apperently one of the most 'cold-resistant' species of the genus Ixodes". Certainly the distribution of this tick covers regions which have climates harsh in the extreme; to latitude 67° 43' north in Finland (Ulmanen 1972) and to altitudes of ~~1620~~ 2300 metres in ~~Switzerland~~ the Caucasus (~~Aeschlimann 1970~~ Djaparidze, 1960). In these regions temperatures rise to more equitable levels in summer - albeit for their relatively short summer seasons, but I. trianguliceps has not been shown capable of withstanding extended periods of low temperatures. It has a rival perhaps in I. uriae, which survives the rigours of Baffinland and the sub-antarctic where temperatures may have maxima of less than 10°C, and extended periods of very low temperatures during the long winters.

The exact location of I. trianguliceps when it is off the host is still unknown. Arthur (1963) found males only in nests and burrows, and from his study of its hypostomal dentition concluded that mating must take place off the host. Cotton and Watts (1967) examined "many hundreds of nests" finding there both males and nymphs and have introduced the concept of nidicoly to the literature on this tick. This is not unwarranted but probably premature. This concept is discussed below and nidicoly discounted, the tick being once more relegated to the burrows.

Life-cycles are proposed by Cotton and Watts (1967) and Randolph (1975 A) but they are founded on a profound lack of knowledge about the

time relations of the ~~of the~~ stadia. Little is known of this aspect of the biology in the field and even in laboratory conditions only an exceptional few ticks will complete development. Until this developmental hurdle can be cleared and some data procured on the behaviour of the majority of the population, life-cycles will do little beyond reflecting our present lack of knowledge rather than be a basis for hypotheses.

The trapping sites.

Balerno, Midlothian (Map 1).

The two sites, Goodtrees (Map 3/2) and Cock burn (Map 2/3) lie at an altitude of 650-700 feet (~~594-640~~ 198-213 metres). Underlying rocks are of the Scottish calciferous sandstone series (Lower Carboniferous) covered by glacial drift of boulder till overlayed by clay. Mean monthly rainfall varied between 8.2 mm (December 1975) and 126.1 mm (September 1975). Mean monthly air temperature reached a maximum of 21.7°C, (August 1975) and a minimum of 0.6°C (March 1975).[1]

Both sites are in wooded shelter belts approximately 30 yards (28 metres) wide, which protect the flat cultivated fields of 10 acres, (4 hectares) or more on each side from the prevailing winds. (PLATES 1 & 2) The countryside in this area has a network of these stripwoods more or less interconnected, some of which in addition to their protective role are managed as a timber crop, as were those used in this study.

Hardwoods predominate at the Goodtrees site, sycamore (Acer sp.), beech (Fagus sylvatica), elm (Ulmus glabra) and ash (Fraxinus excelsior) with small stands and individuals of spruce (Abies sp.) and individual larch (Larix europaeus) and Scot's pine (Pinus sylvestris). At a lower level of the canopy, rowan (Pyrus aucuraria) and oak (Quercus sessiliflora) predominate with a few alder (Alnus rotundifolia) and birches (Betula spp.). Brambles (Rubus spp.) and wild rose (Rosa sp) form a lower screen which in wetter parts is replaced by Phragmites communis. The ground cover is of grasses : Agropyron repens, A. caninum, Milium effusum, Agrostis tenuis, A. stolonifera, A. caninum sub species montana, Arrhenatherum elatus, Anthoxanthum odoratum, Helictotrichon sp, Holcus mollis, H. lanatus, Poa sp., Dactylis glomerata and Deschampsia sp., with groups of nettles Urtica spp. and according to season the more transitory dicotyledons.

The Cock burn site (Map 3/2) has a similar flora. Among the trees the proportion of conifers, mainly spruce, is greater. Along the burn-side the variety of dicotyledonous plants is greater but this was not a habitat favoured by the voles and mice.

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1 Meteorological Office Climatological services (Scotland), Edinburgh. Data collected at Turnhouse, approximately 4 km NW from Balerno.

Bush estate site.

Balerno

Stripwood at Goodtrees site.

Balerno
Goodtrees trap site.

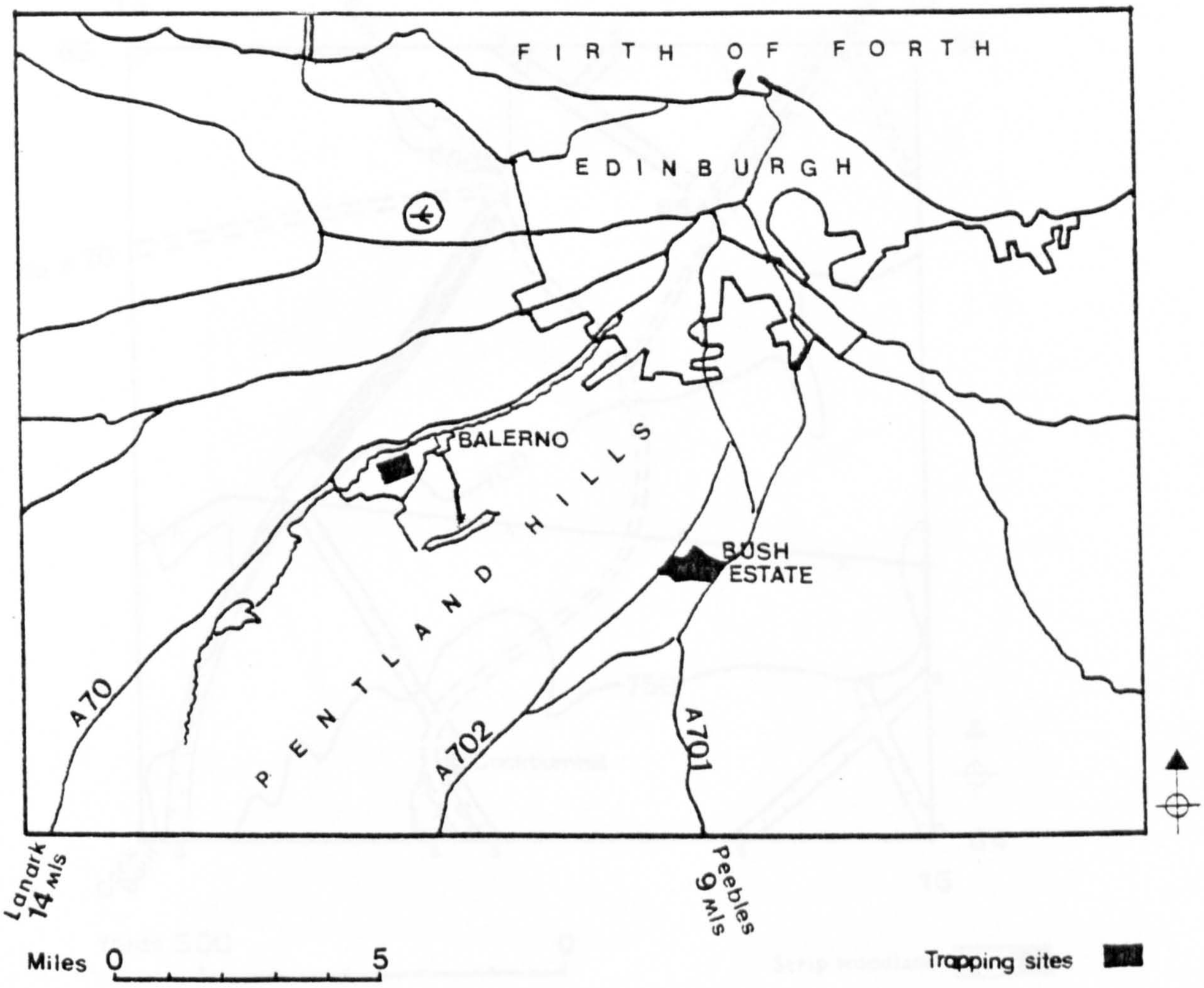
Plate 1. Trapping sites in Midlothian. Bush and Balerno.

Stripwood at Cock burn site.

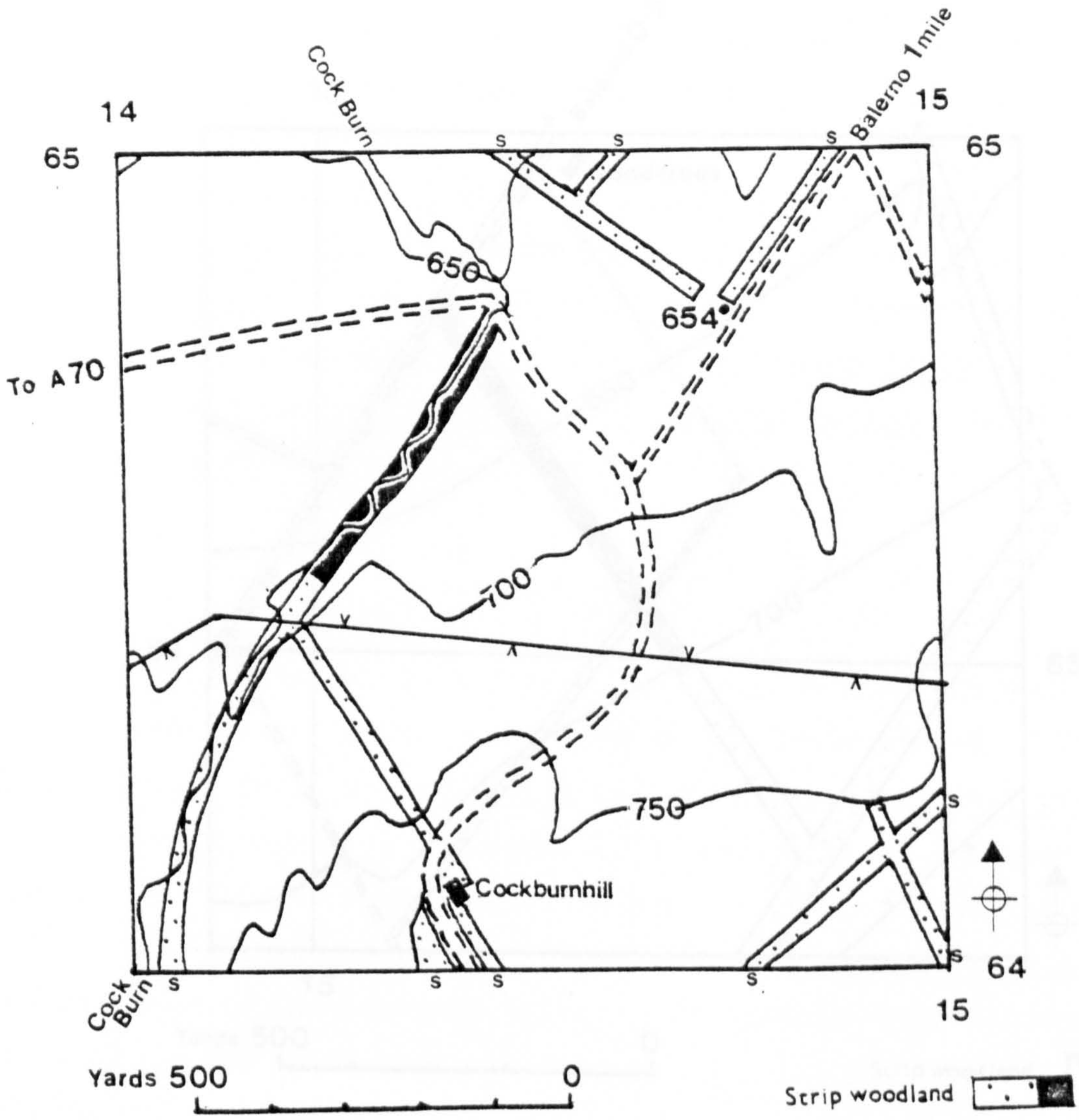
Cock burn trap site in summer.

Cock burn trap site in winter

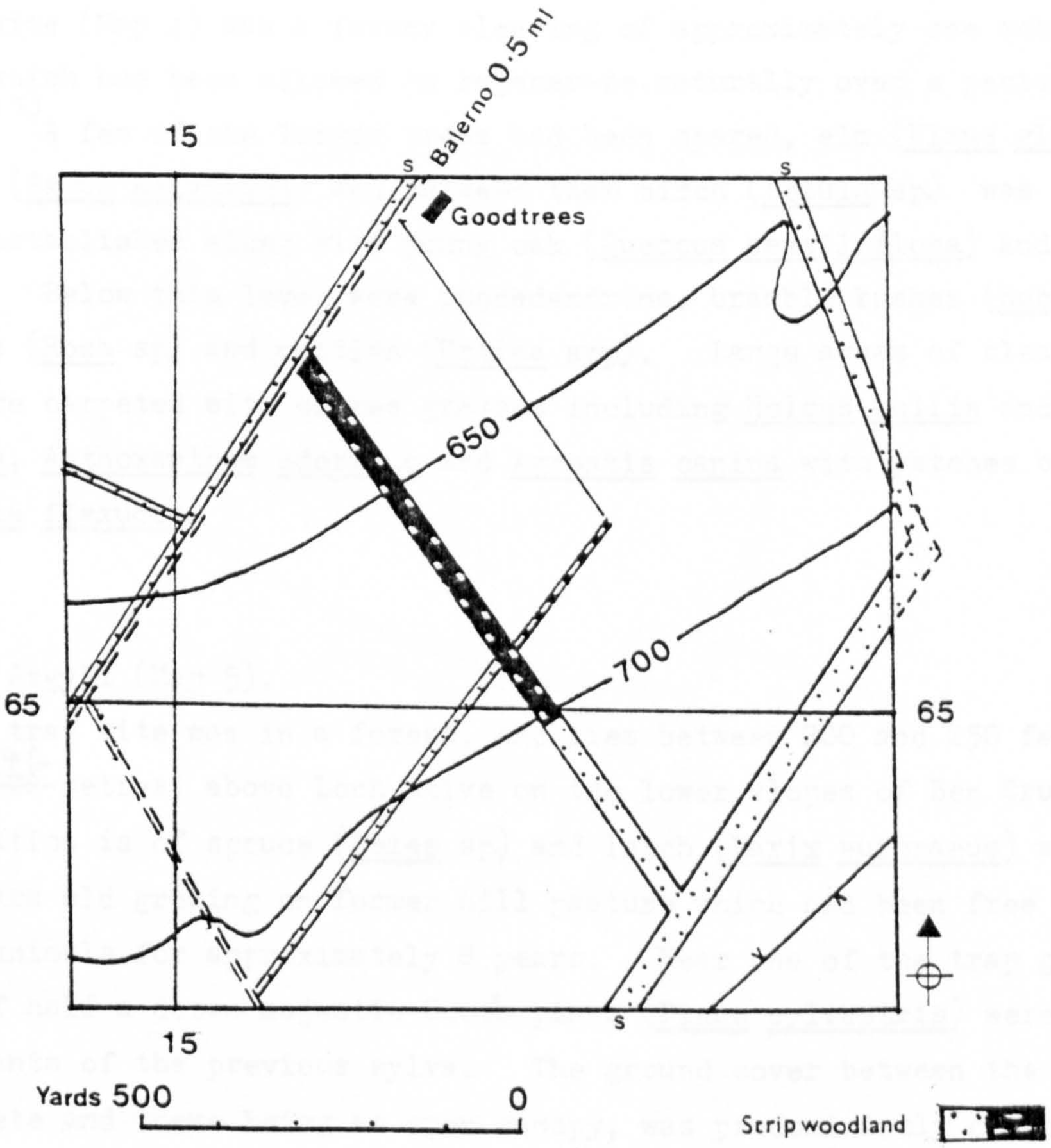
Plate 2. Trapping sites in Midlothian. Balerno



Map 1. Midlothian trapping sites.



Map 2. Balerno, Cock burn trapping site.



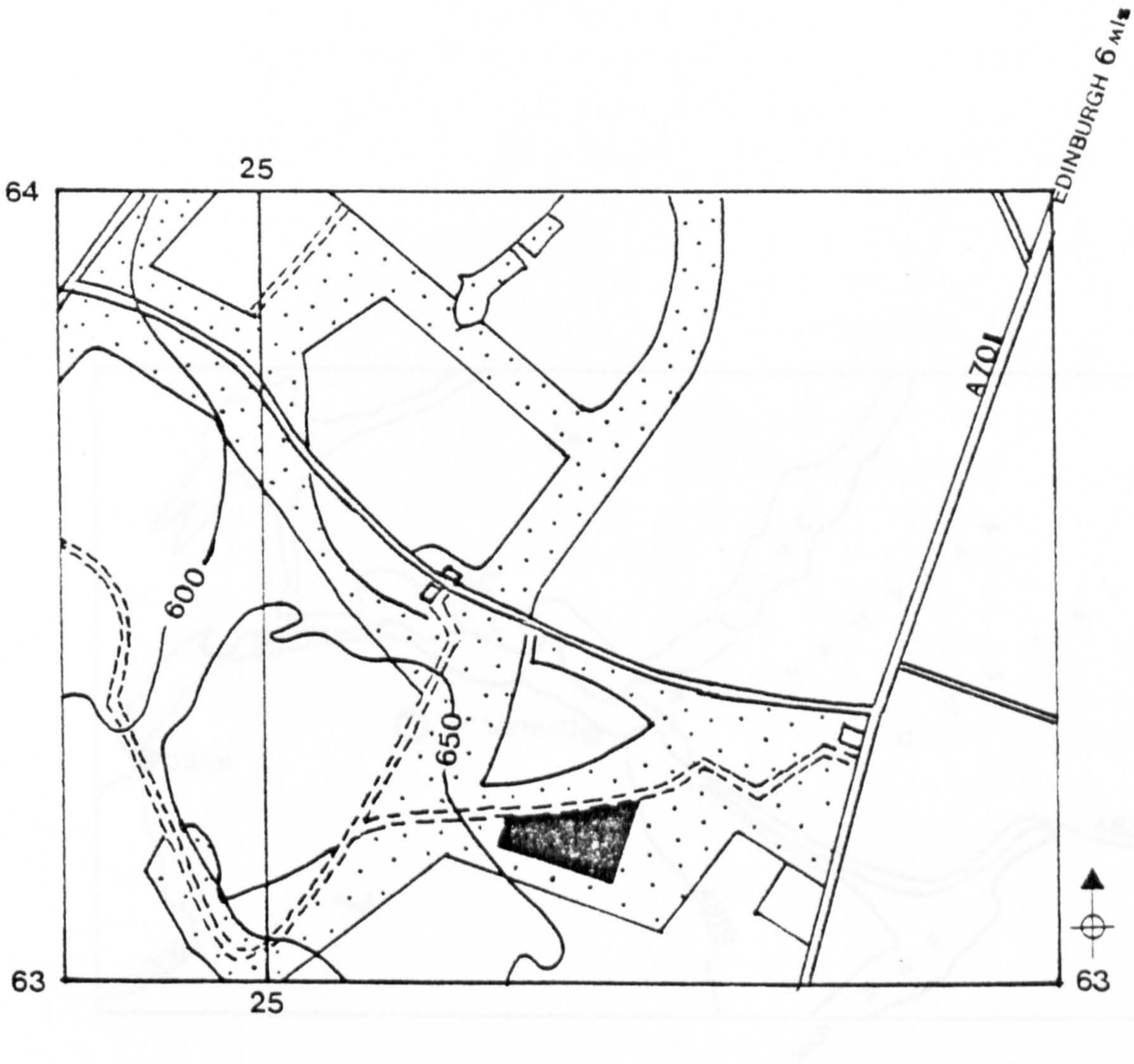
Map 3. Balerno, Goodtrees trapping site.

Bush estate, Midlothian (Map 1).

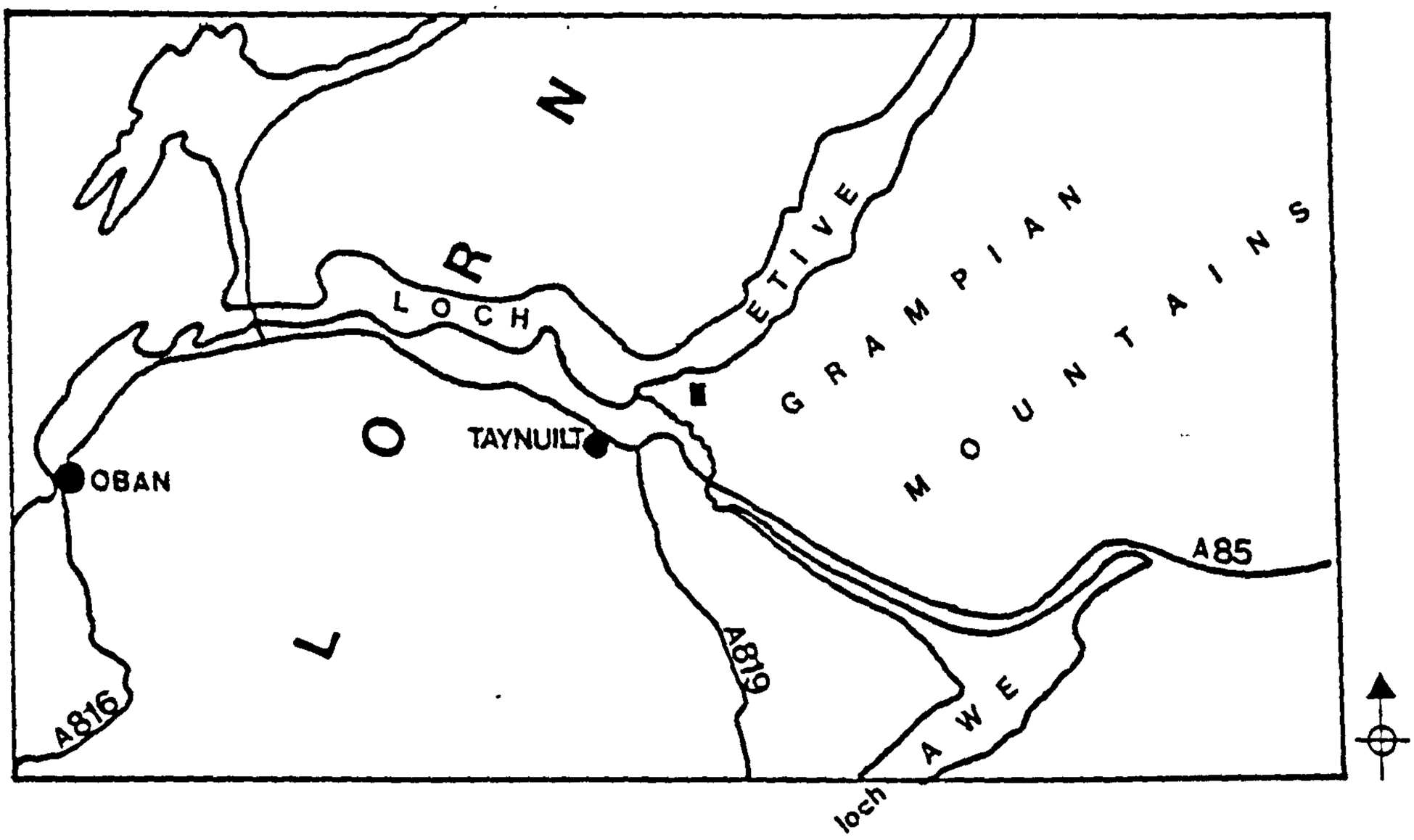
The estate is on the eastern side of the Pentland Hills almost due east from the sites at Balerno and at the same altitude (650feet/~~594~~ 198 metres). The trap site (Map 4) was a former clearing of approximately one acre (0.4 hectare) which had been allowed to regenerate naturally over a period of 10 years (PLATE 1). A few of the larger trees had been spared, elm (Ulmus glabra) and beech (Fagus sylvatica) and between them birch (Betula sp.) was becoming established along with young oak (Quercus sessiliflora) and small conifers. Below this level were rhodedendrons, bramble bushes (Rubus sp.) wild roses (Rosa sp.) and nettles (Urtica spp.). Large areas of cleared ground were carpeted with coarse grasses including Holcus mollis and H. lanatus, Anthoxanthum odoratum and Agrostis canina with patches of Deschamrsia flexuosa.

Taynuilt, Argyll (Map 5).

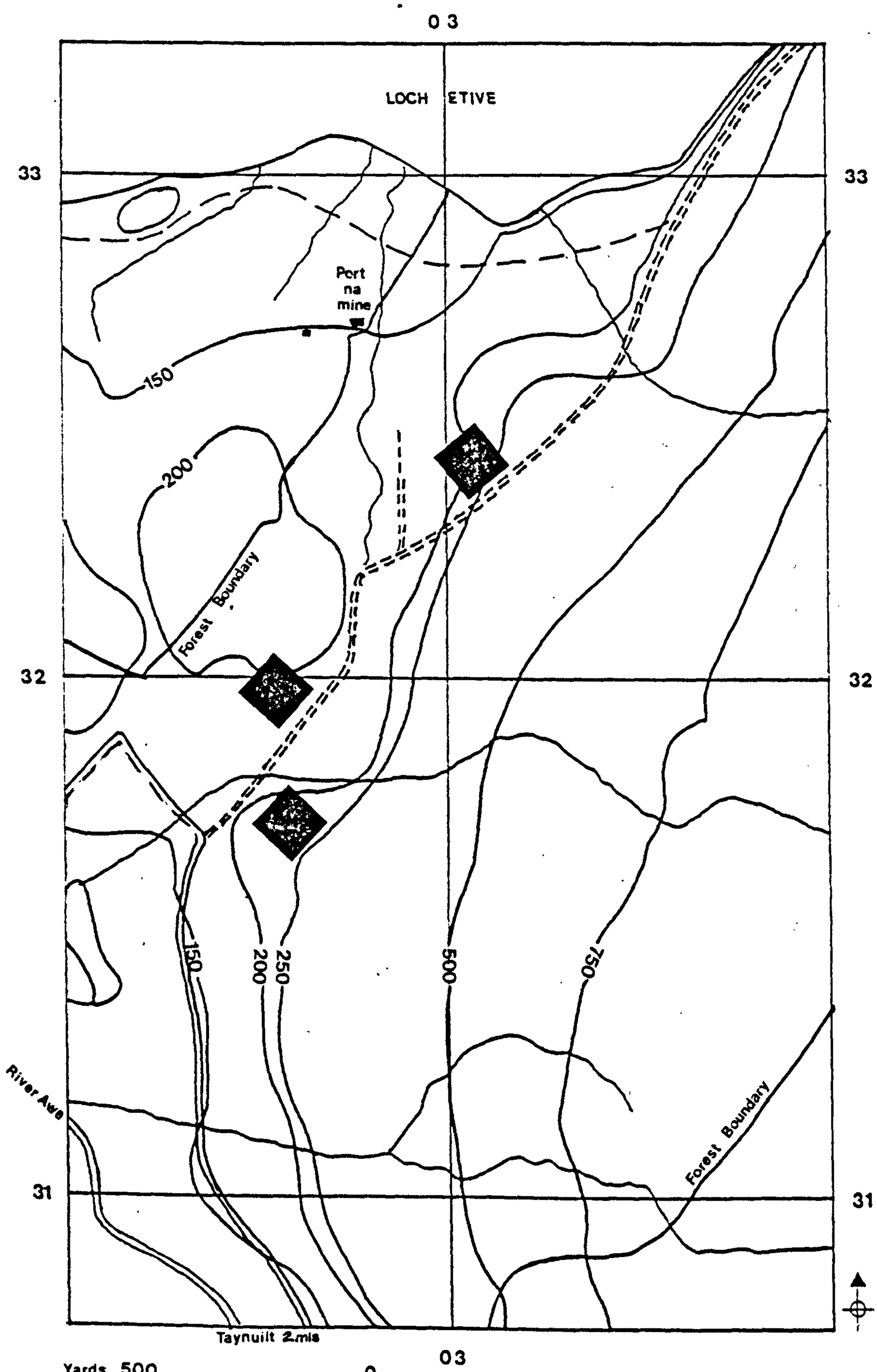
This trap site was in a forest, and lies between 200 and 250 feet (~~163~~ 61 and ~~226~~ 76 metres) above Loch Etive on the lower slopes of Ben Cruachan. The plantation is of spruce (Abies sp.) and larch (Larix europaeus) which were 5 years old growing on former hill pasture which had been free of domestic animals for approximately 8 years. Near one of the trap grids a stand of half a dozen majestic Scot's pines (Pinus sylvestris) were the last remnants of the previous sylva. The ground cover between the trees was complete and there being an open canopy, was predominantly of coarse grasses.



Map 4. Bush Estate trapping site.



Map 5. Argyll trapping site.



Map 6. Taynuilt, forest trapping site.

Hosts.

Ticks were collected from 3 species of rodents: Clethrionomys glareolus (Schreber); Microtus agrestis (Linnaeus) and Apodemus sylvaticus (Linnaeus). Other small mammals were occasionally captured: Sorex araneus Linnaeus and Sorex minutus Linnaeus, but neither was obtained in sufficiently large numbers to be included in the results.

Trapping routine.

To ensure the maximum trapping efficiency on each site, the best trapping points were found and marked, these were then used repeatedly.

Longworth traps (Chitty, D. and Kempson, D.A., 1949) were provided with hay and grain in the nest box and baited with a few grains of cereal spread within the tunnel and around its entrance. Traps were set to operate with a treadle pressure of 10 grams: less than the weight of Sorex minutus, the lightest of the mature hosts expected. Where a trap might be exposed to draughts or direct sunlight, insulation was provided by a covering of grass or bracken.

Traps were set in the forenoon and visited at 24 hour intervals to retrieve captive animals. Occupied or accidentally sprung traps were serviced as required before being reset.

To avoid overtrapping any site, only one trap was set at each trap-point and for a maximum of two consecutive days; the site was then left undisturbed for at least two weeks.

Handling of animals.

Removal from traps.

To cause animals the minimum of disturbance and so avoid loss of engorged ticks during retrieval, traps were first emptied into a transparent plastic bag. The captive animal was then easily immobilised by firmly gripping it by the slack skin along its back.

After identification and sexing of the host, engorged ticks were located and noted in case any should detach during transportation. Animals were then isolated in a numbered, dark compartment of a travelling box, each compartment of which had a thin layer of fine wood shavings which could easily be picked through to recover a detached tick.

Animals which were obviously near parturition were immediately released to help maintain the population.

Isolation of hosts.

To prevent cross-infestation, animals were kept in isolation from the time of capture until they were released. In the laboratory, animals were confined to a quarantine room (9' x 9'), maintained at approximately 21°C in a 12 hour light: 12 hour dark regime, and housed in polypropylene mouse cages[1] modified as shown in Plate which were shaded from an overhead 2x40 watt striplight. Food (Spratts Lab diet 1) was provided in excess of the animals daily requirements, and drinking water was constantly available from water bottles fitted with melamine nozzles and renewed daily.

A record card was attached to each cage giving the date of capture; registration number; species and stage of development; trap point numbers; stage of engorgment and development of ticks during captivity,and any information concerning the captive's welfare.

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1. Type M1, Manufactured by North Kent Plastic Cages Ltd. Dartford. Kent.

TICKS.

Location of ticks on host.

On its removal from a trap, each animal was inspected to locate engorged ticks which might detach before reaching the laboratory. Fully engorged females were easily visible in the fur, while engorged nymphs and larvae were located visually only when on the ear pinnae. Tactile examination located engorged nymphs on the rest of the body. Engorged ticks which detached during transportation were included in the collections of the first 24 hours.

Before being allocated a cage, the ears of one group of voles and mice were inspected in good light, under magnification, to locate attached larvae and nymphs; their point of attachment, life-stage and degree of engorgement were noted on the host's record card. The progress of each attached tick was then followed daily until detachment.

Just prior to release, all caged animals were inspected to ensure that they were tick-free.

Collection of ticks from cages.

Larvae and nymphs were collected daily from the water tank beneath each cage (PLATE 3). Inspection of the water was made in good light, under magnification and continued for a minimum of 30 seconds, or for 30 seconds after a tick had been removed from the tank. Any ticks concealed on the ledge supporting the grid floor of the cage were flushed into the water tank by routine application of a fine jet of water directed onto the side of the cage. The inspection routine was then reapplied to the water tank. Ticks were picked from the water with an '00' size sable paint brush and passed through two petri dishes of tap-water at room temperature before being dried by placing for a few seconds onto a sheet of filter paper; they were then assigned to glass storage tubes.

When a female tick reached the final stage of engorgement (PLATE 4), the host was transferred to a cage which had a double grid floor, the upper grid being large enough to allow all ticks to pass through, the lower allowing the passage of all but engorged females. In this way female ticks were safe from predation by their former host, and

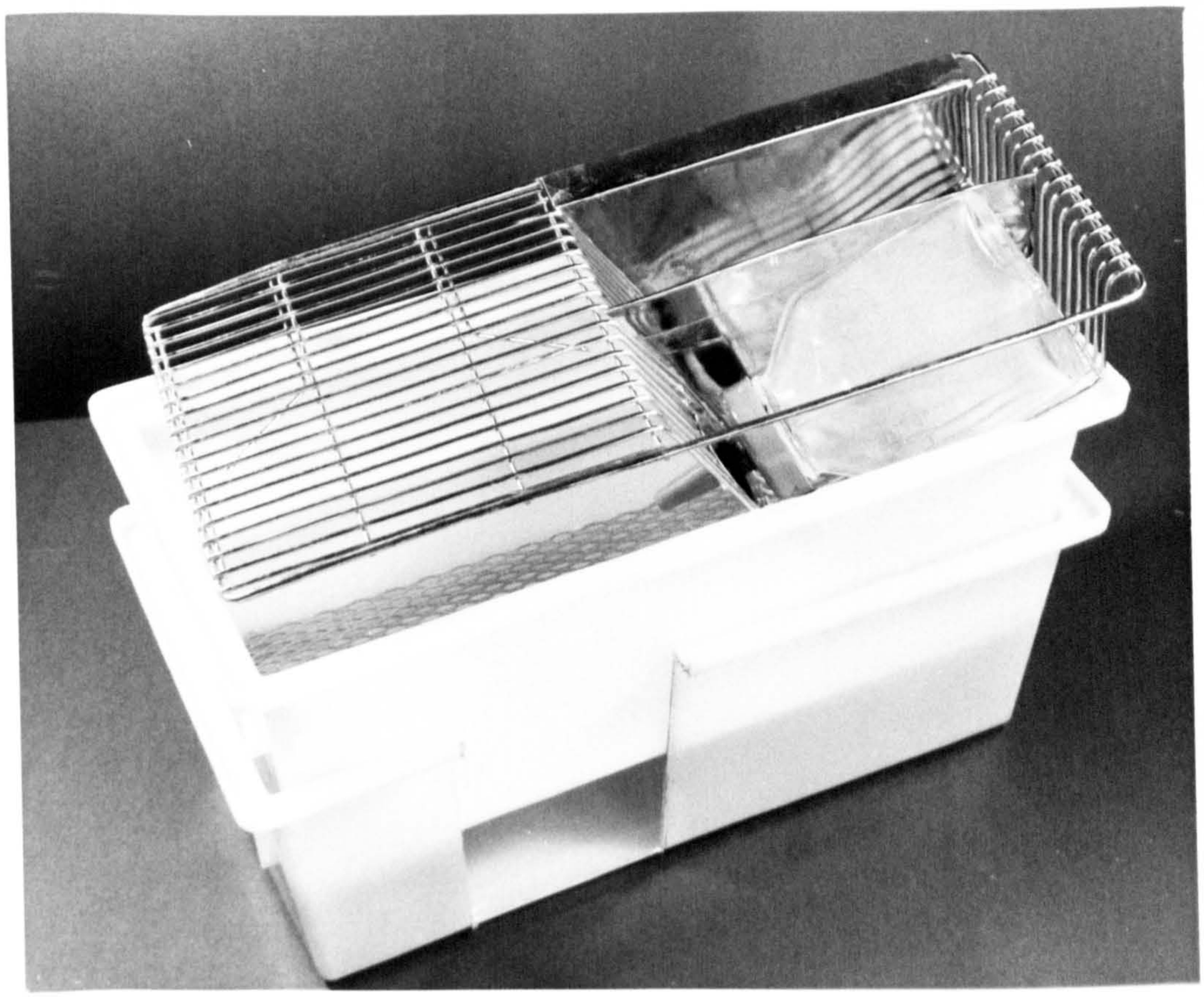


Plate 3. Modified cage stack showing showing base grid and water tank. (Water tank in section).

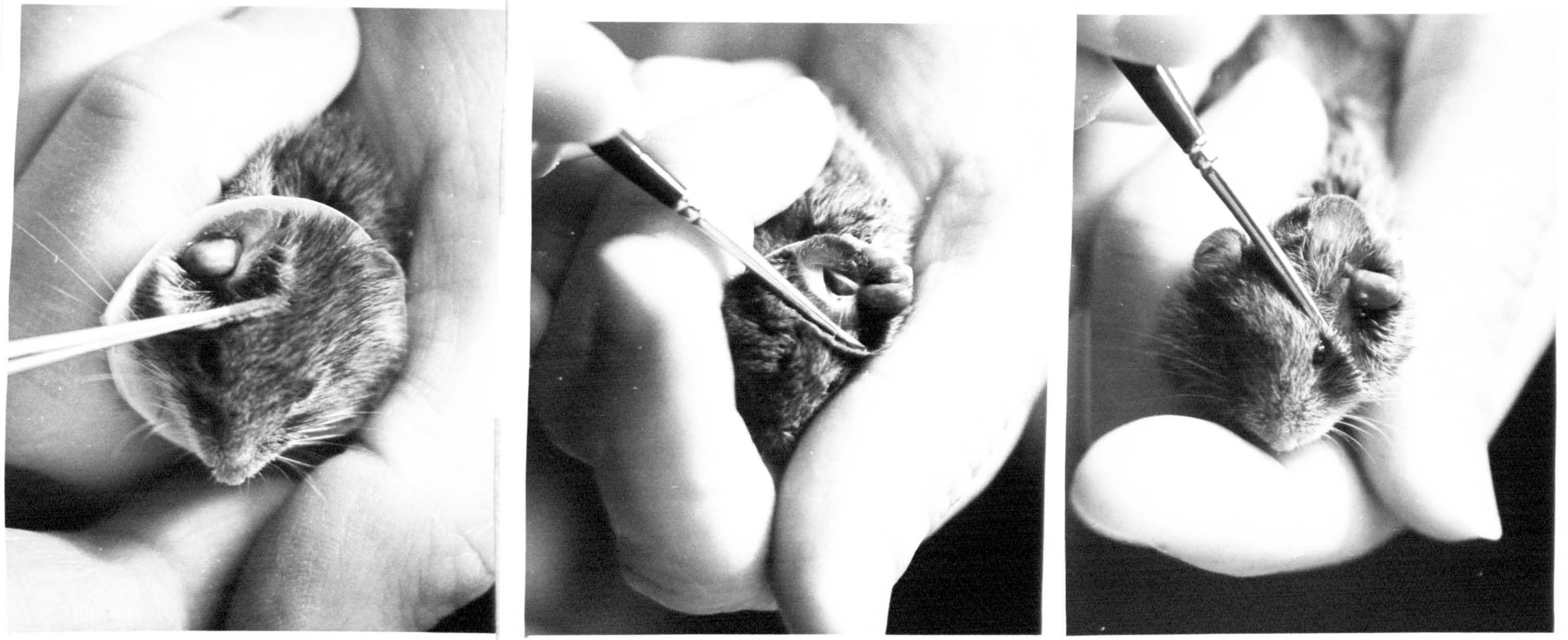


Plate 4. *Ixodes triangulliceps* females engorging on the ears of *C glareolus*.

from immersion. Engorged females were washed and dried in the same manner as larvae and nymphs before being placed in a numbered storage tube.

Storage of engorged ticks.

All ticks except engorged females were stored in glass tubes, 4cm x 1cm, with a maximum of 10 larvae, 3 nymphs or 1 unengorged adult per tube; engorged females were stored individually in polystyrene tubes, 6cm x 1.8cm. Each tube was lined below the plug with filter paper (Whatman) for 2/3 of its circumference; this liner served the dual purpose of preventing the formation of free water from condensation within the tube, and carrying the pencilled tube-number on its outer surface. Tubes were plugged with a loose twist of non-absorbent cotton wool. The larger tubes were stored horizontally while the smaller were kept vertical and held in groups of 11 around a central boss (6cm x 1.8cm polystyrene tube) by a rubber band: a group of tubes could then be handled by the projecting boss, so reducing temperature changes which would result from direct handling. Tubes were stored on the open base of a 9cm diameter Petri dish in a screw-topped, 2lb sweet jar; the air was kept saturated by a moat of water around the Petri dish. Prepared jars, whether or not they contained ticks, were stored in incubators and maintained within a range of $\pm$ 2C^{o} of the designated temperatures; a daily check was made from thermometers, recording minimum and maximum temperatures, kept among the storage jars.

Inspection of engorged ticks.

Routine inspections of all stored ticks were made under magnification, in good light, and results and treatments recorded. Ticks were kept in their plugged tubes during all inspections, being removed only when they required a clean tube; such transfers were made as soon as fungus was seen within the tube. Storage units of 11 tubes were removed from their jar only long enough for the inspection. While away from its incubator each storage jar was kept in a close fitting insulated box made from expanded polystyrene. As soon as their contents had been inspected, storage jars were returned to the incubators.

SECTION II RESULTS.

Field sampling data.

Table 1 presents gross data and Table 2 details the tick population components obtained from routine, weekly trapping at Balerno, Midlothian from July 1974 to August 1976, of three small mammal species: the voles, Clethrionomys glareolus (Schreber) and Microtus agrestis (Linnaeus), and the wood mouse, Apodemus sylvaticus (Linnaeus)(PLATE 5).

Of 875 animals taken alive from the traps and examined for ticks, 397 were C. glareolus, the most frequently trapped of the three species; fewer A. sylvaticus (320) were captured and of the least numerous species, M. agrestis, 158 animals were examined.

Ixodes trianguliceps (Birula,1895) was the only species of tick recovered from these animals, but it fed on each of the small mammal species in all three of the instars.

The host with the greatest frequency of parasitised animals was C. glareolus, 67% of which had tick burdens at capture; this was almost twice as great as the proportion of parasitised animals in the two other host species, M. agrestis (28%) and A. sylvaticus (34%).

TABLE 1 : Trapping record for small mammal hosts of Ixodes trianguliceps.

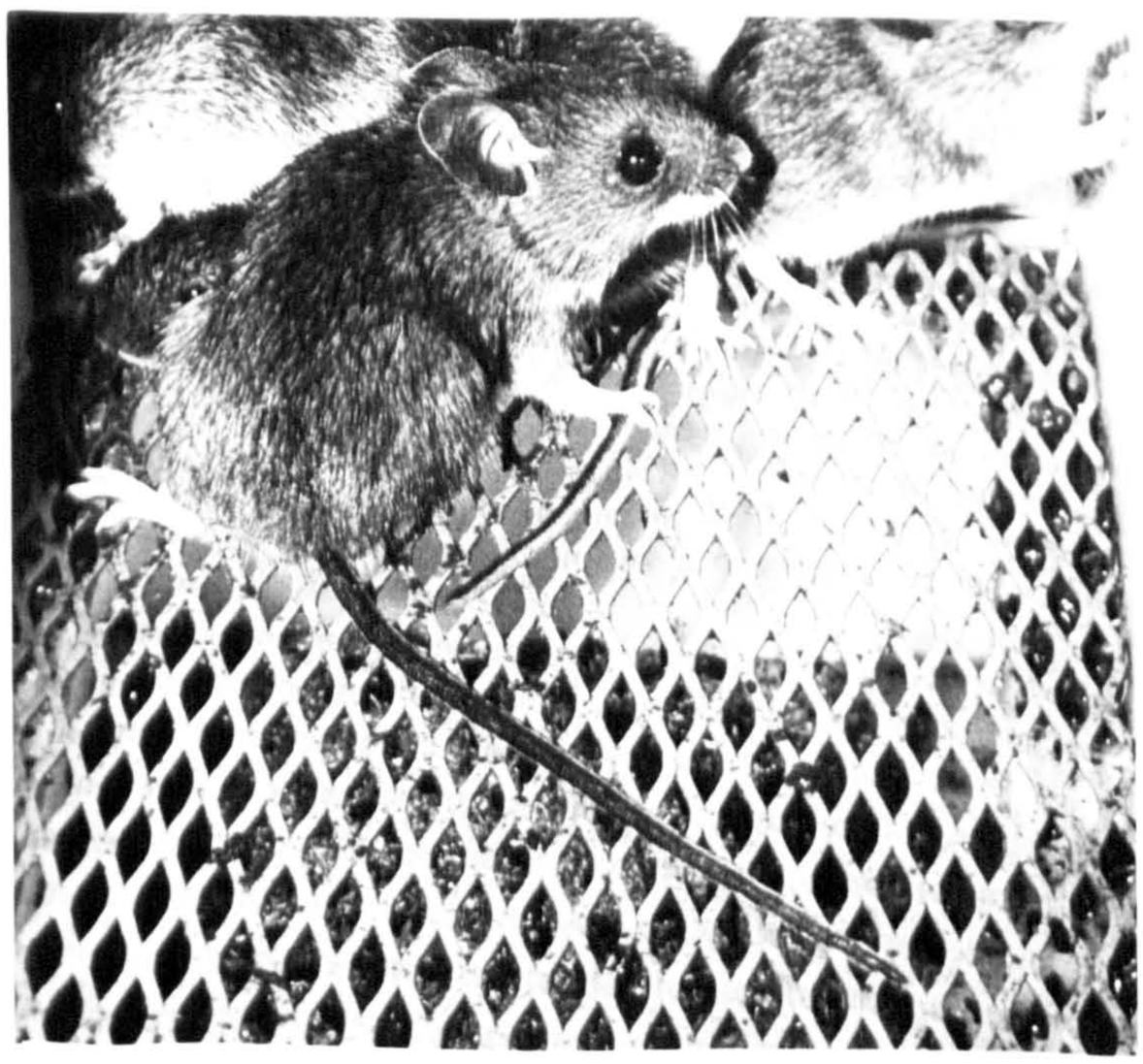
Site : Balerno.

Time period : July 1974 to August 1976.

Host	Number of animals			Percentage of animals infested
	Examined	With tick burden	Tick-free	
Clethrionomys glareolus	397	267	130	67
Microtus agrestis	158	44	114	28
Apodemus sylvaticus	320	110	210	34
Total	875	421	454	48

Clethrionomys glareolus

Microtus agrestis



Apodemus sylvaticus

Plate 5. The host species.

TABLE 2 : Record of Larvae, Nymphs and Females of Ixodes trianguliceps attached to three host species.

Host	Number of ticks at each life-stage			Total number of ticks	Percentage Composition of Tick population		
	L	N	F		L	N	F
C. glareolus	820	208	44	1072	76.5	19.4	4.1
M. agrestis	82	11	14	107	76.6	10.3	13.1
A. sylvaticus	223	17	18	258	86.4	6.6	7.0
Total	1125	236	76	1437	78.3	16.4	5.3

The frequencies of parasitised animals are compared statistically in Table 3.

TABLE 3 : Proportion of infested animals.

Hosts compared	Number of animals: With tick-burden	Tick-free	χ^2	P
C. glareolus	267	130	76.8*	<0.005
A. sylvaticus	110	210		
C. glareolus	267	130	71.2*	<0.005
M. agrestis	44	114		
A. sylvaticus	110	210	2.06	>0.100
M. agrestis	44	114		

* = χ^2 value is significant at the 5% level of probability.

This confirms that C. glareolus has a significantly greater frequency of individuals parasitised by I. trianguliceps than have either M. agrestis ($P < 0.005$) or A. sylvaticus ($P < 0.005$).

However, if this conclusion is to be accepted as a valid one, it has to be shown that the samples from the host populations are indeed comparable and that the composition of the samples is not unacceptably biased.

The ratio of males to females (see Table 4) does not differ from parity ($P > 0.100$) for any of the host population samples.

TABLE 4 : Comparison of the proportions of each sex in the host population samples.

Hosts	Sex	Number of Animals (o)	Total number of Animals (n)	Number of animals of each sex expected ($E = \frac{n}{2}$)	$\chi^2 = \frac{(o-E)^2}{E}$	P
C. glareolus	M.	202				
	F.	195	397	198.5	0.06	>0.750
M. agrestis	M.	68				
	F.	90	158	79.0	1.53	>0.100
A. sylvaticus	M.	168				
	F.	152	320	160.0	0.40	>0.500

* M. = male

* F. = female

Grouping of the data for the sexes is, therefore, acceptable since it is unlikely that sex differences will present a source of biased variation in parasite burdens between the species.

The distribution of ticks between the sexes of the hosts must be considered, however, so that the role of each sex in tick-feeding may be determined. Table 5 presents the ratio of parasitised males and females for each host species.

TABLE 5 : Proportions of animals parasitised and tick-free.

Host	Sex	Number of animals: With tick-burden	tick-free	Total	χ^2	P
C. glareolus	M.	148	54	202	6.75*	<0.010
	F.	119	76	195		
M. agrestis	M.	18	50	68	0.11	>0.500
	F.	26	64	90		
A. sylvaticus	M.	56	112	168	0.17	>0.500
	F.	54	98	152		

M. = male
F. = female

The difference in the proportions of animals of each sex parasitised by ticks is within sampling error for M. agrestis ($P > 0.500$) and A. sylvaticus ($P > 0.500$), but for C. glareolus a significantly greater proportion of males than females ($P < 0.010$) carry ticks.

Turning now to the infested animals of each species. (Table 6)

TABLE 6 : Ticks per infested host.

Host	Number of infested animals	Number of ticks	Number of ticks per infested animal
C. glareolus	267	1072	4.0
M. agrestis	44	107	2.4
A. sylvaticus	110	258	2.3

C. glareolus contributed not only more hosts (267) than M. agrestis (44) and A. sylvaticus (110), but in addition the mean infestation of C.glareolus is greater (4.0 ticks per infested host) than either M. agrestis (2.4 ticks per infested host) or A. sylvaticus (2.3 ticks per infested host). This conforms to the pattern of infestations seen when all animals in the sample were considered (Table 1).

Although the seasonal distribution of the ticks and their hosts will be dealt with in detail below it is advantageous to point out here that infestation levels differing with season are not themselves a source of the difference between the species.

Seasonal distributions of the three host species are given in Figure 1 which summates the numbers of captured animals over the trapping period from July 1974 to August 1976.

A greater number of C. glareolus than A. sylvaticus were captured each month from February to August. During the rest of the year A. sylvaticus was numerically greater. In each month except February consistently fewer M. agrestis than C. glareolus were caught, and with the few ticks collected from M. agrestis and the resulting sampling errors, this host will not be treated further in this section.

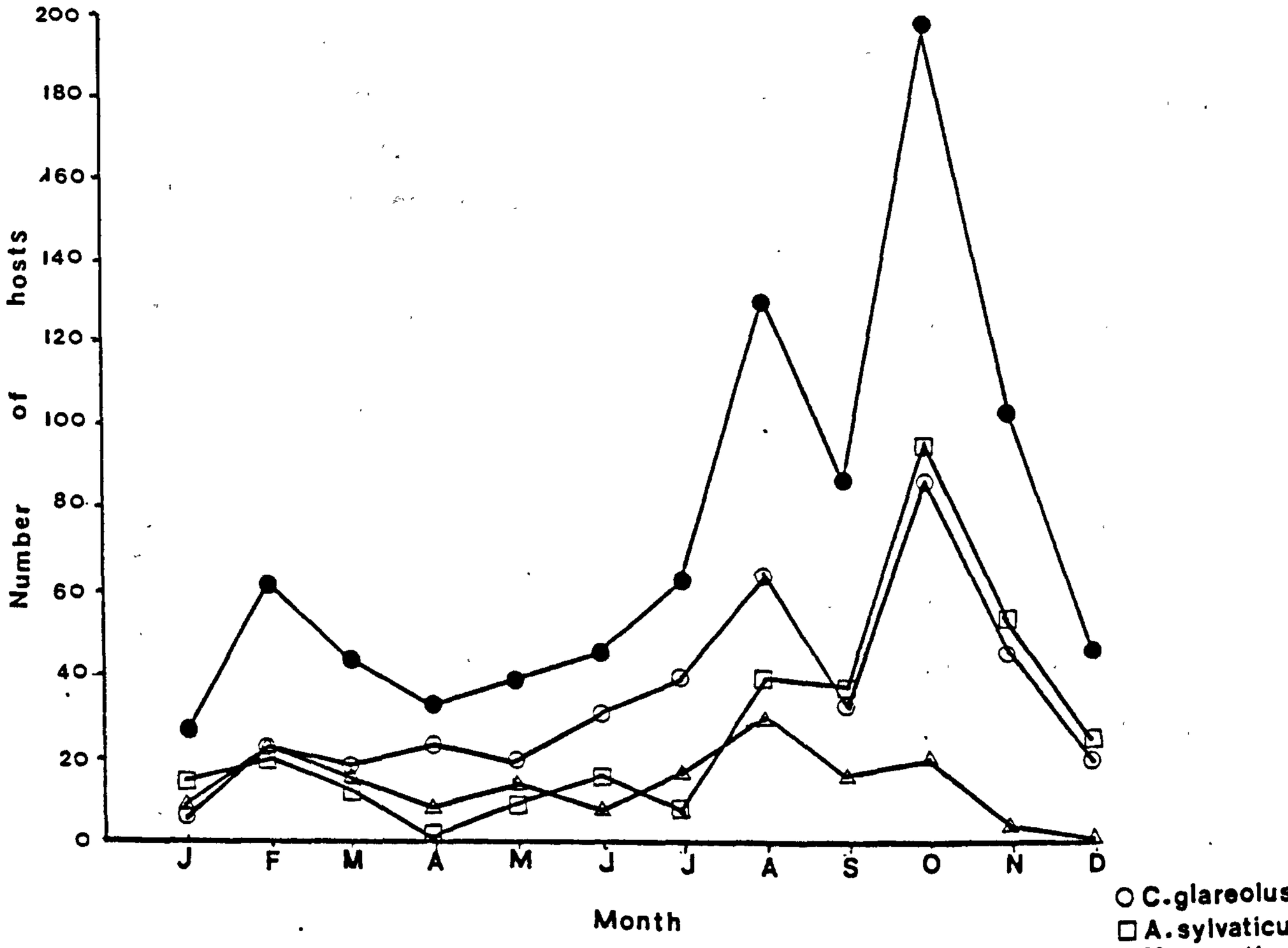


Figure 1. Number of hosts each month.
(Composite graph from data of 1974 - 1976).

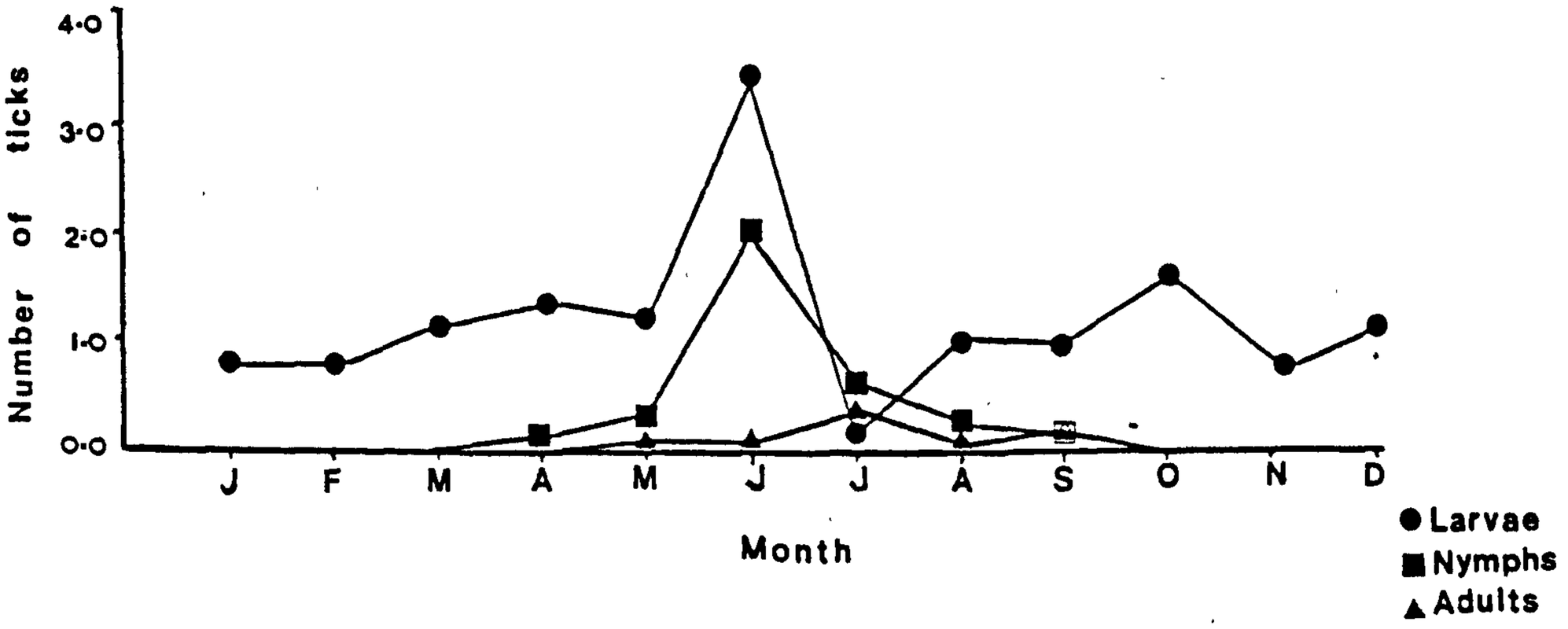


Figure 2. Mean number of ticks per nost..
(Composite graph from data of 1974 - 1976).

The numbers of infested and tick-free animals of C. glareolus and A. sylvaticus are given for three-monthly periods of the composite year in Table 7. Although the sample of C. glareolus is numerically greater than A. sylvaticus during the two seasons from March to August only, it has in each season a significantly greater proportion of infested animals ($P < 0.005$).

TABLE 7 : Seasonal distribution of infested Clethrionomys glareolus and Apodemus sylvaticus.

	C. glareolus			A. sylvaticus					
	No. of animals			No. of animals					
Season	With ticks	Tick-free	total	With ticks	Tick-free	total	Totals compared	χ^2	P
Dec - Feb	35	11	46	22	37	59	C < A	15.7*	<0.005
Mar - May	49	12	61	5	19	24	C > A	26.3*	<0.005
Jun - Aug	88	43	131	14	44	58	C > A	30.0*	<0.005
Sep - Nov	95	64	159	69	110	179	C < A	15.2*	<0.005

Only in autumn does the proportion of infested C. glareolus differ significantly from the winter level ($P < 0.050$) (Table 8).

TABLE 8 : Comparison by season of the number of hosts infested.

	C. glareolus				A. sylvaticus			
	Number of animals				Number of animals			
Season	With ticks	Tick-free	χ^2 +	P	With ticks	Tick-free	χ^2 +	P
Dec - Feb	35	11	-	-	22	37	-	-
Mar - May	49	12	0.28	>0.500	5	19	2.10	> 0.100
Jun - Aug	88	43	1.28	>0.250	14	44	2.37	> 0.100
Sep - Nov	95	64	4.10*	<0.050	69	110	0.03	> 0.750

+ each season is compared with the results for winter.

The proportion of A. sylvaticus infested does not differ significantly in any season from that of winter ($P > 0.100$).

Larvae infest the hosts throughout the year (Fig. 2), and, most strikingly, after increasing to the higher peak of infestation (3.5 larvae per host) in June drop to a minimum for the year in July, (χ^2 larvae per host) even in the presence of increasing numbers of hosts. From August the larvae again become increasingly numerous on the hosts until the lower peak is reached in October (1.7 larvae per host). The peak of larval parasitism in June and minimum in July is common to C. glareolus and A. sylvaticus (Fig. 3).

Nymphal infestation also reaches a peak in June on both hosts (Fig. 4), but while this is minimal by July on A. sylvaticus, burdens on C. glareolus decline progressively until November. From November until March the hosts are wholly free of attached nymphs.

The pattern of infestation by female ticks resembles that of the nymphs, but they reach a peak later in the year: in July on C. glareolus and in September on A. sylvaticus, (Fig. 5). No females were seen on the hosts after October, and although there were a few individuals from March onwards on M. agrestis, infestation of C. glareolus was delayed until June, and until August on A. sylvaticus.

A priori there is no reason to suspect that under the same environmental conditions, if these host species each maintain a seperate population of ticks, there will be a very different seasonal pattern of parasitisation.

The distribution of infestation levels for each instar (Table 9) are compared with Poisson distributions[1] having the same means (Tables 10a - c). Larvae are overdistributed on all three species of host (Table 11). On C. glareolus, nymphs and females are also over-distributed. The infestation levels for nymphs on M. agrestis and A. sylvaticus are too low to allow proper application of the test (the third and later classes are too small to allow the use of χ^2). This feature alone distinguishes the distributions on these two species from that on C. glareolus. Although the distribution of nymphs on M. agrestis and A. sylvaticus cannot be properly tested, inspection of the constituent classes of infestation reveals that there is no significant deviation from the expected numbers in the first two classes for M. agrestis (χ^2 for class zero = 0.02, and for

1. Snedecor and Cochran, 1974.

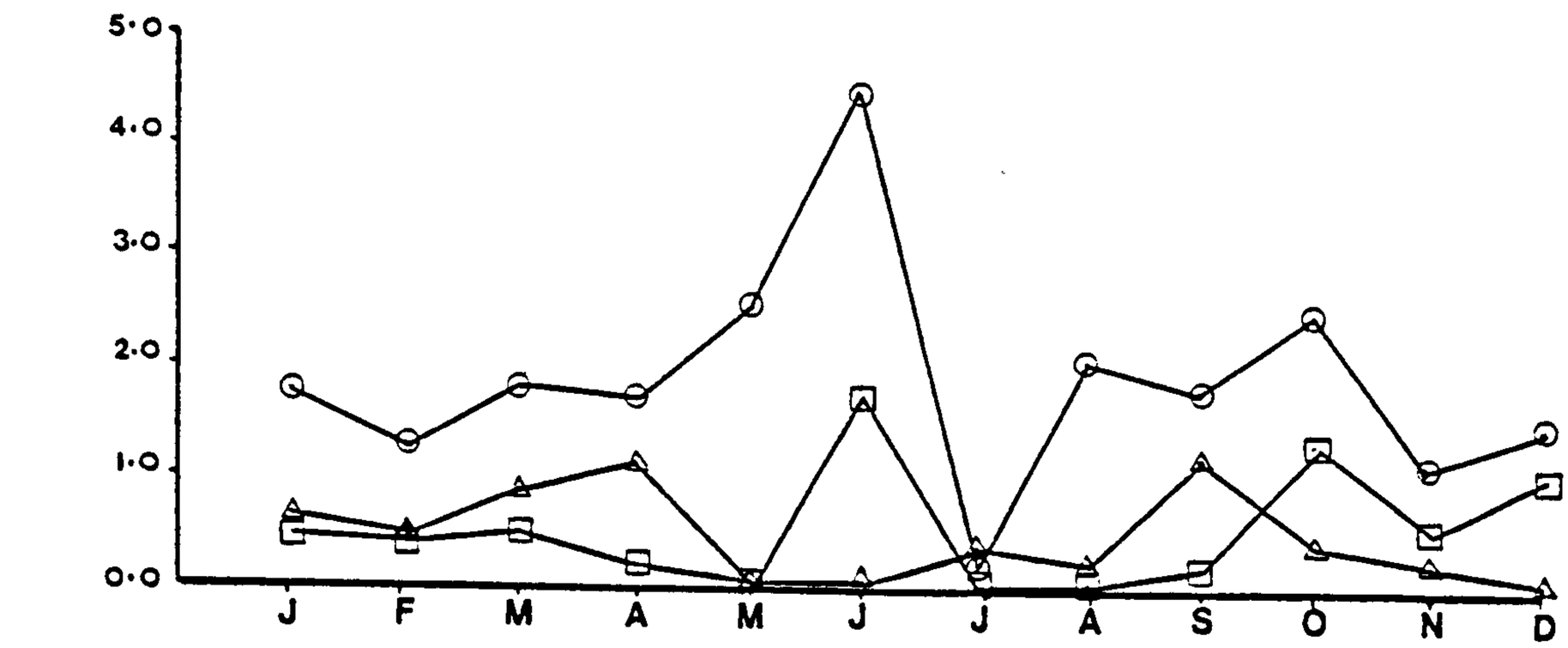


Figure 3. Composite monthly mean larvae per host, 1974 - 76.

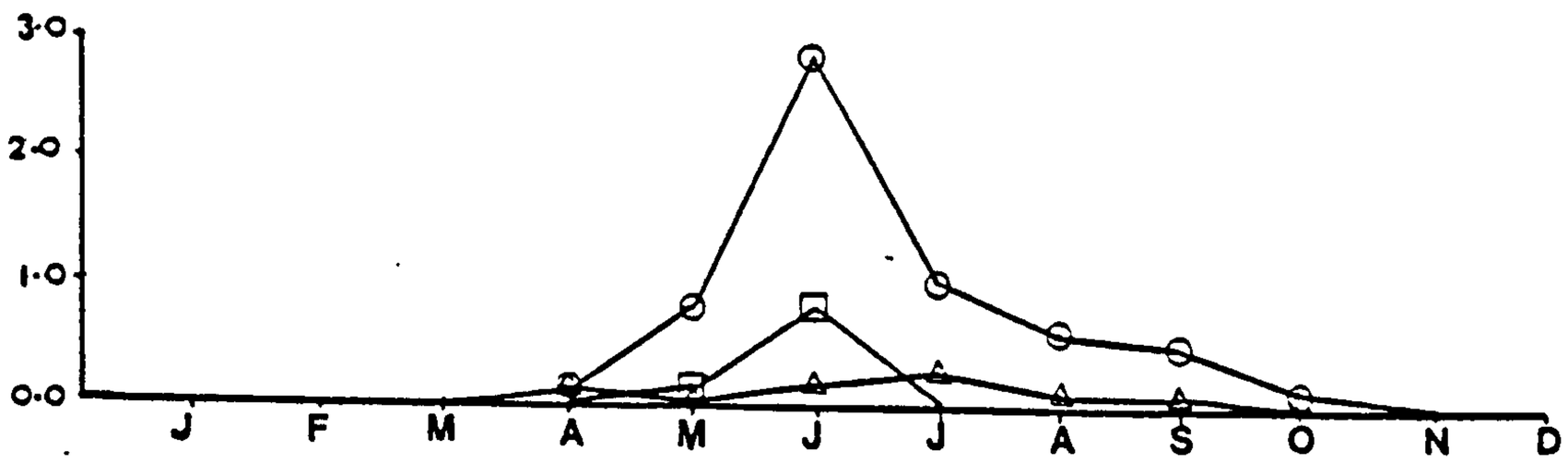


Figure 4. Composite monthly mean nymphs per host, 1974 - 76.

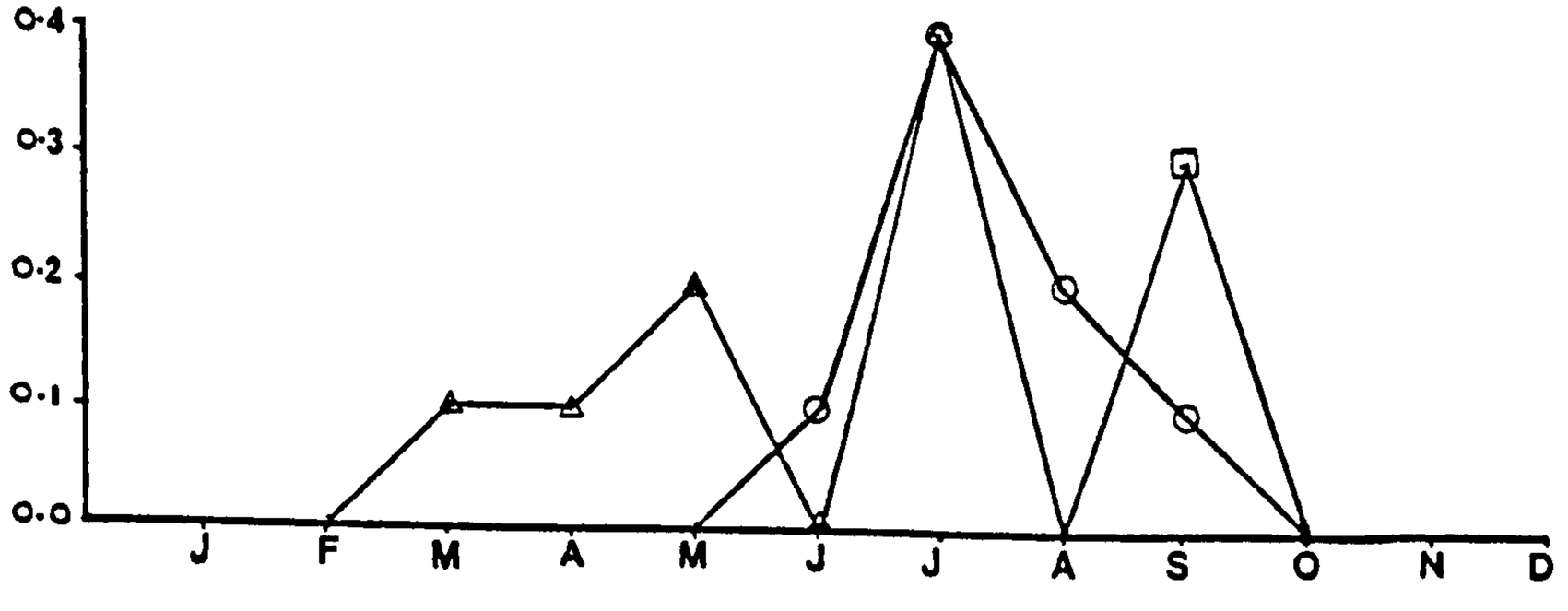


Figure 5. Composite monthly mean females per host. 1974 - 76.

○ C.glareolus
□ A.sylvaticus
△ M.agrestis

TABLE 9 : Frequency distribution of ticks on hosts.

Number of ticks	LARVAE			NYMPHS			FEMALES		
	glareolus	agrestis	sylvaticus	glareolus	agrestis	sylvaticus	glareolus	agrestis	sylvaticus
	Ol	Ml	Al	Ol	Ml	Al	Ol	Ml	Al
0	181	119	230	224	99	220	264	124	191
1	82	23	44	50	7	7	18	4	8
2	46	9	17	22	2	3	8	2	3
3	23	3	12	9			2	2	
4	28		7	4		1	1		1
5	7		2	2					
6	4		5	2					
7	9	3		1					
8	3			1					
9	1								
10	1		1	1					
11	1	1							
12				2					
13									
14	2		1						
15	1								
16									
17	1		1						
18									
19	2								
20	1								
29	1								
34	1								
46	1								
62	1								
≥63									
No. of hosts	397	158	320	318	108	231	293	132	203
No. of ticks	820	82	223	208	11	17	44	14	18

TABLE 10a : Comparison of distribution of infestation levels with Poisson distributions.

LARVAE

No. of larvae	Clethrionomys glareolus fobs.	f. Poisson	χ^2	Microtus agrestis fobs.	f. Poisson	χ^2	Apodemus sylvaticus fobs.	f. Poisson	χ^2
0	181	50.322	339.35	119	94.028	6.63	230	159.401	31.27
1	82	103.940	4.63	23	48.801	13.64	44	111.086	40.51
2	46	107.344	35.06	9	12.664	1.06	17	38.708	12.17
3	23	73.906	35.06	3	2.191		12	8.992	1.01
4	28	38.163	2.71				7	1.567	
5	7	15.765	4.87				2	0.218	
6	4	5.427	0.38				5	0.025	
7	9	1.601		3	0.000	10.56			
8	3	0.413							
9	1	0.095							
10	1	0.020					1	0.000	127.48
11	1	0.004		1	0.000				
12				⩾ 12					
13									
14	2	0.000					1	0.000	
15	1	0.000	267.06						
16									
17	1	0.000					1	0.000	
18							⩾18		
19	2	0.000							
20	1	0.000							
29	1	0.000							
34	1	0.000							
46	1	0.000							
62	1	0.000							
⩾ 63									

No.of hosts (N)=397	χ^2total = 689	No.of hosts (N)=158	χ^2total = 31.89	No.of hosts (N)=320	χ^2total = 212.4
No.of larvae =820	d.f. = 6	No.of larvae = 82	d.f. = 2	No.of larvae =223	d.f. = 3
μ = 2.0655	P <0.005	μ = 0.5190	P <0.005	μ = 0.6969	P <0.005
$Ne^{-\mu}$ = 50.3218		$Ne^{-\mu}$ = 94.0283		$Ne^{-\mu}$ = 159.4007	

TABLE 10b : Comparison of distribution of infestation levels with Poisson distributions.

NYMPHS

Clethrionomys glareolus					Microtus agrestis and Apodemus sylvaticus Combined data.				
No. of Nymphs	fobs.		f Poisson	χ^2	No. of Nymphs	fobs.		f Poisson	χ^2
0	224		165.333	20.8	0	319		312.12	0.15
1	50		108.142	31.2	1	14		25.78	5.38
2	22		35.367	5.0	2	5	6	1.06	-
3	9		7.711	0.2	3				
4	4		1.261		4	1			
5	2		0.165		⩾5				
6	2		0.018						
7	1		0.002						
8	1	13	0.000	1.446					
9									
10	1		0.000						
11									
12	2		0.000						
⩾13									
No. of hosts (N)=318				χ^2 total = 149.5	No. of hosts (N)=339				
No. of nymphs =208				d.f.= 3	No. of nymphs = 28			d.f. = 0	
μ = 0.6541				P< 0.005	μ = 0.0826				
$Ne^{-\mu}$ = 165.333					$Ne^{-\mu}$ = 312.12				

TABLE 10c : Comparison of distribution of infestation levels with Poisson distributions.

FEMALES

Clethrionomys glareolus					Microtus agrestis and Apodemus sylvaticus Combined data.			
No. of females	fobs.	f Poisson		χ^2	No. of females	fobs.	f Poisson	χ^2
0	264	252.144		0.6	0	315	304.64	0.35
1	18	37.865		10.4	1	12	20.94	3.82
2	8	2.843			2			
3	2 } 11	0.142 }	2.990	21.4	3 }	8	9.42	0.21
4	1	0.005			4			
⩾5					⩾5			
No. of hosts (N) = 293			χ^2total = 32.4		No. of hosts (N) = 335			χ^2total = 4.38
No. of females = 44			d.f. = 1		No. of females = 32			
μ = 0.150					μ = 0.095			d.f. = 1
$Ne^{-\mu}$ = 252.144			P<0.005		$Ne^{-\mu}$ = 304.64			P<0.05

TABLE 11 : Summary; comparison of observed distributions of infestation levels with Poisson distributions. Results of χ^2 tests.

Host		Larvae	Nymphs	Females
Clethrionomys glareolus	χ^2	689	149	32
	d.f.	6	3	1
	P	<0.005	<0.005	<0.005
Microtus agrestis	χ^2	32	-	-
	d.f.	2	0	0
	P	<0.005	- d.f=0	- =4
Apodemus sylvaticus	χ^2	212	-	-d.f=1 P<0.005
	d.f.	3	0	0
	P	<0.005	-	-

class 1 = 0.85) and it is considered probable that nymphs on this species do not deviate significantly from random. On A. sylvaticus, however, the values are for class zero, χ^2 = 0.13 and for class 1, χ^2 = 4.9, thus being strongly suggestive of a non-random distribution. Nevertheless, the bulk of the weight of the deviation is due to the occurrence of 4 individuals which between them carried 10 ticks. The removal of two of these reduces the χ^2 values to 0.014 for class zero and to 0.024 for class 1*. We have here a situation where a tail of two individuals out of 231 (< 1%) produces the main weight of the deviation from random in contrast to the 44 individuals out of 318 (13.8%) which produce the deviation from random in C. glareolus. This is considered to be strongly suggestive of a random dispersal in A. sylvaticus as well as in M. agrestis.

In the above estimates for nymphs and adults the figures are for animals captured only in the months when these stadia were active, whereas for larvae winter samples are also included.

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* When the same two individuals are discarded from the analysis of the joint data for A. sylvaticus and M. agrestis as in Table 10b, the figures become μ = 0.065, N = 337 and the observed and Poisson expected classes are

	Observed	Expected	χ^2
Class 0	319	315.7	0.03
1	14	20.61	2.12
2	4	0.69	-

again strongly implying random distribution.

TABLE 12 : State of engorgement of larvae at capture.

Month	Monthly totals		Season's totals			
	Number of Larvae which were engorged at capture	Number of Larvae which engorged during hosts' captivity	Number of Larvae which were engorged at capture	Number of Larvae which engorged during hosts' captivity	χ^2 +	P
December	0	49				
January	0	21	1	95	-	-
February	1	25				
March	1	44				
April	6	39	12	125	7.1*	< 0.01
May	5	42				
June	8	85				
July	2	10	>35	203	>16.6*	< 0.005
August	>25	115				

+ Spring and summer are each tested against winter

TABLE 13 : Number of engorged larvae collected from hosts during the period July 1974 to August 1976.

Month	Engorged larvae detached in first two days of captivity	Number of Larvae which engorged during hosts' captivity	Season	Engorged larvae detached in first two days of captivity	Number of larvae which engorged during hosts' captivity	χ^2 +	P
December	2	60					
Jan/Feb.	4	69	Winter	6	129	-	-
Mar/Apr.	25	90					
May	17	49	Spring	42	139	29.7*	<0.005
June	35	154					
July	8	13	Summer	94	285	38.9*	<0.005
August	51	118					
September	17	88					
October	59	343	Autumn	88	512	12.9*	<0.005
November	12	81					

+ Spring, summer and autumn are each tested against winter.

Engorgement of larvae.

During the period December 1975 to August 1976 the state of engorgement of larvae was ascertained on the day of capture: the results are shown in Table 12.

During December and January, all 70 of the larvae which had their mouthparts embedded in the ear pinnae were in a flat, unengorged condition. For the rest of this period, until August, larvae at various stages of engorgement were also present on the hosts.

From winter when, in December and January, none of the attached larvae were engorging at capture, the proportions of engorging larvae increased significantly through spring to summer, (Table 12).

These data were collected over a limited period only, for the rest of the time the state of engorgement at capture can be deduced to some extent from the number of larvae which completed their feed during the first two days, (Table 13).

A significantly lower proportion ($P < 0.005$) of larvae completed engorgement in the first two days of captivity during the winter months.

The median detachment time for engorged larvae is longer in December than any other month, and there is progressive change from winter to summer (Table 14).

TABLE 14 : Median detachment times of engorged larvae.

Month	Dec.	Jan/ Feb.	Mar/ Apr.	May.	June	July	Aug.	Sep.	Oct.	Nov.
Number of days	5.5	4.8	3.4	3.2	3.2	1.8	2.2	3.0	3.8	4.1
Season	Winter		Spring		Summer		Autumn			
Number of days	5.0		3.3		2.7		3.3			

The time taken for the last engorged larvae to drop from the host (Table 15) was, like the median detachment time, longer in December than any other month, and there is progressive change from winter to summer.

TABLE 15 : Longest engorgement times for larvae in the laboratory.

Month	Dec.	Jan/ Feb.	Mar/ Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.
Number of days	>16	12	11	6	8	5	7	7	13	13
Season	Winter		Spring		Summer			Autumn		
Number of days	>16		11		8			13		

If the change in engorgement time were a simple response to the constant temperature of the laboratory (21°C) it should be the same throughout the year.[1] Since there is a progressive change in both the median and longest engorgement times so must there be a physiological change which is slower in winter to respond to the laboratory conditions but faster in summer.

The most numerous of the infested hosts was C. glareolus (267) whose mean burden over the trapping period was 3.1 larvae, (Table 16); the lowest mean burden was in July (0.2 larvae), immediately after the highest level in June (5.1 larvae); from November until February mean burdens remained almost constant (2.1 - 2.2 larvae).

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1 It might be argued that the larvae are showing a seasonal climatisation response. This, however, would be expected to operate in the reverse direction and it will be argued later that the difference observed reflect diapause phenomena.

TABLE 16 : Larval burdens of C.glareolus

Combined results for 1974 to 1976.

Month	Number of Engorged Larvae	Number of tick-infested hosts	Larvae per tick-infested host
Jan.	11	5	2.2
Feb.	29	14	2.1
Mar.	33	12	2.8
Apr.	41	19	2.2
May.	52	18	2.9
Jun.	148	29	5.1
Jul.	7	28	0.2
Aug.	135	31	4.4
Sep.	61	21	2.9
Oct.	218	51	4.3
Nov.	51	23	2.2
Dec.	34	16	2.1
Total	820	267	-
Larvae per tick-infested host			3.1

A. sylvaticus (110 animals) had a mean infestation rate of 2.0 larvae. Low numbers of infested A. sylvaticus were captured in all months except October when 41 animals were trapped, (Table 17).

TABLE 17 : Larval burdens of A. sylvaticus

Combined results for 1974 to 1976.

Month	Number of Engorged Larvae	Number of tick-infested hosts	Larvae per tick-infested host
Jan.	6	4	-
Feb.	9	6	1.5
Mar.	6	3	-
Apr.	1	1	-
May.		1	
Jun.	17	8	2.1
Jul.			
Aug.	2	6	-
Sep.	6	13	0.5
Oct.	121	41	2.9
Nov.	29	15	1.9
Dec.	26	12	2.2
Total	223	110	-
Larvae per tick-infested host			2.0

Least numerous of the infested hosts was M. agrestis (44), the monthly capture never exceeded 9 infested animals, (Table 18).

TABLE 18 : Larval burdens of M. agrestis

Combined results for 1974 to 1976.

Month	Number of Engorged Larvae	Number of tick-infested hosts	Larvae per tick-infested host
Jan.	5	3	-
Feb.	10	9	1.1
Mar.	14	3	-
Apr.	9	3	-
May.		1	
Jun.		1	
Jul.	7	6	1.2
Aug.	6	6	1.0
Sep.	21	6	3.5
Oct.	9	5	1.8
Nov.	1	1	-
Dec.			
Total	82	44	-
Larvae per tick-infested host			1.9

TABLE 19 : Summary of larval infestations.

		Class of Host					
		All Animals Examined		Infested with any instar		Infested with Larvae	
Month	Number of Engorged Larvae	Number of hosts	Larvae per host	Number of hosts	Larvae per host	Number of hosts	Larvae per host
C. flareolus							
Jan	11	6	1.8	5	2.2	5	2.2
Feb.	29	21	1.4	14	2.1	14	2.1
Mar.	33	17	1.9	12	2.8	12	2.8
Apr.	41	24	1.7	19	2.2	18	2.3
May.	52	20	2.6	18	2.9	16	3.2
Jun.	148	33	4.5	29	5.1	25	5.9
Jul.	7	37	0.2	28	0.2	5	1.4
Aug.	135	61	2.2	31	4.4	17	7.9
Sep.	61	32	1.9	21	2.9	15	4.1
Oct.	218	85	2.6	51	4.3	49	4.4
Nov.	51	42	1.2	23	2.2	23	2.2
Dec.	34	19	1.8	16	2.1	16	2.1
Total	820	397		267		215	
Larvae per host			2.1		3.1		3.8
M. agrestis							
Jan	5	9	0.6	3	-	3	-
Feb.	10	20	0.5	9	1.1	9	1.1
Mar.	14	15	0.9	3	-	3	-
Apr.	9	8	1.1	3	-	3	-
May.		13		1			
Jun.		4		1			
Jul.	7	16	0.4	6	1.2	2	-
Aug.	6	30	0.2	6	1.0	5	1.2
Sep.	21	17	1.2	6	3.5	6	3.5
Oct.	9	20	0.4	5	1.8	5	1.8
Nov.	1	5	-	1	-	1	-
Dec.		1					
Total	82	158		44		37	
Larvae per host			0.5		1.9		2.2
A. sylvaticus							
Jan	6	14	0.4	4	-	4	-
Feb.	9	20	0.4	6	1.5	6	1.5
Mar.	6	12	0.5	3	-	3	-
Apr.	1	4	-	1	-	1	-
May.		8		1			
Jun.	17	10	1.7	8	2.1	7	2.4
Jul.		8					
Aug.	2	40	-	6	-	1	-
Sep.	6	37	0.2	13	0.5	6	1.0
Oct.	121	91	1.3	41	2.9	35	3.4
Nov.	29	51	0.6	15	1.9	15	1.9
Dec.	26	25	1.0	12	2.2	12	2.2
Total	223	320		110		90	
Larvae per host			0.7		2.0		2.5

TABLE 20

Number of engorged larvae detached from each host species and number of engorged larvae recovered from cages on each day after capture.

Days after Capture \ Engorged Larvae	*Clethrionomys glareolus*		*Microtus agrestis*		*Apodemus sylvaticus*		Total number of engorged larvae	
	Detached	Recovered	Detached	Recovered	Detached	Recovered	Detached	Recovered
1	18	16	3	2	1	1	22	19
2	32	29	5	4	1		38	33
3	48	41	5	5	1	1	54	47
4	46	41	9	8	3	3	58	52
5	40	34	9	8	6	4	55	46
6	29	27	3	2	8	8	40	37
7	11	11			7	6	18	17
8	4	3			6	3	10	6
9					9	9	9	9
10	1				6	6	7	6
11					4	4	4	4
12	1				1	1	2	1
13					1	1	1	1
14								
Total	230	202	34	29	54	47	318	278
Number of infested hosts	82		16		29		127	

It was apparent during the routine examinations of trapped animals that the ear pinna was the most heavily infested area of the body, therefore, the progress of engorgement of 318 larvae on the ear pinnae of 127 hosts (82 C. glareolus; 16 M. agrestis and 29 A. sylvaticus) was followed daily, (Table 20).

There is no significance between the number of larvae seen to have detached and larvae recovered from the cages on the same day ($\chi^2 = 6.7$, $P > 0.75$): 263 larvae were recovered from the cages on the day they were found to be detached, (Table 21); 3 were recovered within the next 24 hours, and 52 were never recovered. In addition to these, 12 larvae were collected from the cages before any were seen to have detached from the ears; these must have engorged elsewhere on the body.

TABLE 21 : Record of Engorged larvae detached from ear pinnae of hosts*and recovered from cages.

Number of engorged larvae detaching from ear pinnae (Day 0)	Number of engorged larvae recovered from cages on Day 0	Number of engorged larvae not recovered after detaching	Number of engorged larvae recovered after concealed engorgement
318	263	52	12
Total	315		

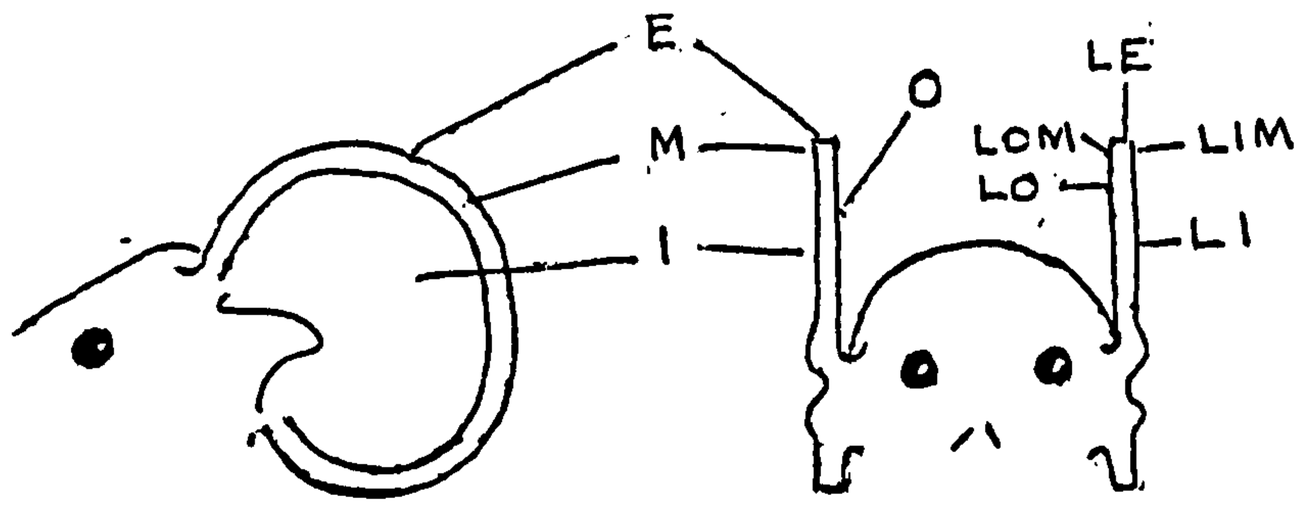
* 82 C. glareolus
16 M. agrestis
29 A. sylvaticus

Together with the above 127 animals, 142 others (42 C. glareolus ; 68 M. agrestis and 32 A. sylvaticus) which had no larvae attached to the ear pinnae, were examined daily. No larvae were recovered from their cages, although during this period, 27 of the animals (21 C. glareolus; 5 M. agrestis and 1 A. sylvaticus) fed a total of 24 nymphs and 7 female ticks, and were, therefore, from a habitat infested by I. trianguliceps.

The majority of larvae engorged on the ear pinnae (318 : 12), and after detaching from the site of engorgement leave the host within 24 hours.

In summary, almost without fail it can be considered that only hosts with observable larvae on the ear pinna are likely to be infested with that instar.

As would be expected there is no significant difference in the numbers or larvae infesting the two ears ($\chi^2 = 0.23$, $P > 0.5$) (Table 22). Nevertheless they are not randomly distributed over the pinna, (Fig. 11).



L - left
R - right
I - inside
O - outside
M - margin
E - edge

Figure 11: Areas of the ear to which larvae and nymphs attached.

On voles the inner surface carries the greater infestation ($\chi^2 = 15.7$, $P < 0.005$), while for A. sylvaticus the converse is true ($\chi^2 = 5.9$, $P < 0,005$). On voles the greatest proportion (0.84) was attached within 1 mm (0.2 of the surface area) of the edge of the ear ($\chi^2 = 74$, $P < 0.005$). In marked contrast, this marginal area of the mouse ear (0.2 of the surface area) carried a lower proportion of larvae ($\chi^2 = 10$, $P < 0.005$) than the rest of the pinna.

TABLE 22 Summary : Sites of attachment and numbers of larvae on each host species.

Host		C. glareolus										M. agrestis									A. sylvaticus									
Month		Dec	Jan	Feb	Mar	Apr	May	Jun	Jly	Aug	Total	Dec	Jan	Feb	Mar	Apr	May	Jun	Jly	Aug	Total	Dec	Jan	Feb	Mar	Apr	May	Jun	Jly	Aug	Total
No. of hosts infested with larvae		11	4	6	11	15	12	19	4	13	95	0	3	6	3	2	0	0	4	2	20	8	4	5	3	1	0	7	0	1	29
Larvae attached to ears	* L E	7	3	1	6	7	4	6	1	9	44		2	2	7	1			1		13	2						1			3
	L I M	4		3	4	5	10	12	1	11	50			3	1	3			1		8	1		1		1		1			4
	L I	1	2					3	1	6	13									1	1	2						1			3
	L O M	1		2		3	1	5	1	10	23								2		2							2		1	3
	L O						1	6	1	1	9											7	2		2			5			16
	Total for left ear	13	5	6	10	15	16	32	5	37	139		2	5	8	4			4	1	24	12	2	1	2	1		10		1	29
	R E	5	1	3	8	4	6	9		6	42		2	2	5	1					10			1	1						2
	R I M	1	2	3	6	9	16	12		7	56		1			1			1		3										
	R I	2	3		2	3	1	5		6	22					1					1	3		3				1			7
	R O M	2		1	6	1	4	9		3	26					1				1	2										
	R O							7		1	8											6	3	1	4			6			20
	Total for right ear	10	6	7	22	17	27	42		23	154	3	2	5	4				1	1	16	9	4	5	4			7			29
	Total No. on ears	23	11	13	32	32	43	74	5	60	293	5	7	13	8				5	2	40	21	6	6	6	1		17		1	58
No. of Larvae on rest of body		3				5		2	1	5	16			1					1	1	3		1								1
No. of Larvae on Host		26	11	13	32	37	43	76	5	65	309	5	7	14	8				6	3	43	21	7	6	6	1		17		1	59

* Abbreviations as Fig. 11

In terms of density of infestation, however, it is the edge of the vole ear which carries the greatest concentration of larvae. The proportions 0.4 of the burden of C. glareolus and 0.6 on M. agrestis were concentrated on 2% of the surface area.

A major proportion of nymphs (0.90) on C. glareolus occurred on the ears, (Table 23), but this was significantly less ($\chi^2 = 4.2$, $P < 0.005$) than the proportion of larvae (0.95) (Table 22). Infestation levels on the two ears were at parity ($\chi^2 = 0.12$, $P > 0.5$), but a significantly greater number fed on the inner surface ($\chi^2 = 4.8$, $P < 0.05$). Unlike larvae, nymphs did not engorge within 1 mm of the margin of the pinna. Data for M. agrestis and A. sylvaticus are too few for analysis but they appear to conform to the findings for C. glareolus.

From the data presented in Table 20, the median engorgement time of larvae is seen to be greater on A. sylvaticus (7 days) than on either of the voles: C. glareolus (4 days), M. agrestis (4 days). Admittedly A. sylvaticus included a higher proportion (0.59) of winter captures than did the voles (0.28) ($\chi^2 = 9.5$, $P < 0.005$), and winter larvae are known to remain attached for longer periods (this is discussed below). This, however, does not account wholly for the difference between mice and voles and reference to Table 24 which contains the data for the whole trapping period 1974 -76 confirms that engorgement on A. sylvaticus was consistently more prolonged than on the voles.

Engorgement has two overt stages: the tick retains its 'flat' condition for some time after attaching then distension of the idiosoma follows. The slightest change of the dorsum from flat to convex is readily seen in the reflective quality of the cuticle: when flat, the whole surface reflects light from an overhead source and has a relatively matt appearance; immediately this flatness

TABLE 23 Summary : Sites of attachment and numbers of nymphs on each host species.

Host		C. glareolus										M. agrestis										A. sylvaticus									
Month		Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total
No. of hosts infested with nymphs		0	0	0	0	2	7	17	14	12	52	0	0	0	0	0	0	1	5	1	7	0	0	0	0	0	1	2	0	0	3
Nymphs attached to ears.	* L E																														
	L I M																														
	L I					1	5	12	9	5	32							1	2	·	3						1				1
	L O M																														
	L O							9	4	3	16																1				1
	Total for left ear					1	5	21	13	8	48							1	2	·	3						2				2
	R E																														
	R I	1	3	18	5	7	34											2	1	3						1				1
	R O M																														
	R O							10	3	6	19	1						1			2					1				1
	Total for right ear	1	3	28	8	13	53					1						3	1	5						2				2
	Total No. on ears	2	8	49	21	21	101					1	1	5	1	8										4				4
No. of nymphs on rest of body				2	6	1	2	11																							
No. of nymphs on host		2	10	55	22	23	112					1	1	5	1	8										4				4	

* Abbreviations as Fig. 11

TABLE 24 : Number of engorged larvae dropped each day after capture.

Time period	Days after capture of host 1	2	3	4	5	6	7	8	9	10	11	12	13	14	No. of engorged larvae	No. of hosts examined	median time to detach
Host : C. glareolus																	
Dec. - Feb.	2	1	10	14	19	13	9	3				1			72	46	5
Mar. - May	13	21	16	24	23	9	3			1					110	61	4
Jun. - Aug.	29	59	65	58	27	15	3	2							258	131	3
Sep. - Nov.	34	46	82	80	55	29	2					1			329	159	4
Total	78	127	173	176	124	66	17	5	-	1	-	2	-	-	769	397	
Host : M. agrestis																	
Dec. - Feb.	2	1	2	5	3			1	1						15	30	4
Mar. - May	2	5	4	4	4	3									22	36	3
Jun. - Aug.	2	3	2	1	2										10	50	2
Sep. - Nov.	3		5	8	7	4	4								31	42	4
Total	9	9	13	18	16	7	4	1							78	158	
Host : A. sylvaticus																	
Dec. - Feb.				3	2	4	3	9	8	6	4	1	1		41	59	8
Mar. - May		1		1	1	1	1		1		1				7	24	6
Jun. - Aug.	1		2	2	3	4	4	1							17	58	6
Sep. - Nov.	1	4	9	28	27	28	20	10	8	10	3	2	2		152	179	6
Total	2	5	11	34	33	37	28	20	17	16	8	3	3		217	320	

is lost the reflection becomes limited to a circular area which progressively decreases in size and increases in brilliance as engorgement continues. These criteria were used in compiling Table 25.

On A. sylvaticus the median time for both the 'flat' and the 'round' stages is greater (3 and 4 days respectively) than on C. glareolus (2 days and 1 day respectively) and M. agrestis (2 days for each stage). The prolongation of larval engorgement on A. sylvaticus is not, therefore, attributable to any particular stage in feeding.

Development of larvae after engorgement.

The original purpose of this work was to establish a laboratory colony of Ixodes trianguliceps hence all individuals recovered were routinely placed at 19°C with the intention of bringing them through to the next instar; it became apparent, however, that development was comparatively rarely completed in the laboratory.

The ticks appear to fall into two groups : those which completed development within a period of 33 to 118 days; and the majority which, though they survived for some months, did not go into the moult phase.

An incubation temperature of 19°C was chosen to approximate summer conditions, but the poor results forced a series of empirical trials with other conditions to establish a method of inducing the ticks to moult in useable numbers; this has still not achieved a moulting rate exceeding 50% in any constant conditions of incubation.

Between 10°C and 25°C there was a qualitatively different effect on the developing ticks; it is now seen that 23° - 25° is the best of the temperatures to use for constant conditions, but this had been considered a priori a very high temperature of incubation for a tick living in a cold temperate region. Diapause was suspected where ticks moulted after prolonged developmental periods, hence extreme temperatures were tried to induce its abandonment.

From the data for these trials information has become available on the biology of ticks, and for that reason is analysed here.

TABLE 25 : Number of larvae and the time spent in 'flat' and 'round' stages during engorgement.

C. glareolus

Month	Number of days at 'flat' stage										Number of days at 'round' stage										Number of Larvae	Number of Hosts
	<1	1	2	3	4	5	6	7	8	9	<1	1	2	3	4	5	6	7	8	9		
Dec		3											3								3	2
Jan			1	2		1	1	1				2	2		1	1					6	3
Feb		1	3		1	3	2				1	7	1	1							10	5
Mar		3	4		2	3	3				4	9	2								15	7
Arr	5	4	2	1	4						5	6	4		1						16	11
May	6	8	4	8	6	1					7	16	9			1					33	10
Jun	4	7	9	10	6	2					6	21	6	3	1		1				38	13
July	1	1	2									3		1							4	4
Aug	4	5	10	8	1						4	11	11		1	1					28	9
Total	20	32	35	29	20	10	6	1			27	75	38	5	4	3	1				153	64

M. agrestis

Month	Number of days at 'flat' stage										Number of days at 'round' stage										Number of Larvae	Number of Hosts
	<1	1	2	3	4	5	6	7	8	9	<1	1	2	3	4	5	6	7	8	9		
Dec																						
Jan				1		1					1	1									2	1
Feb	1	3	1		1							1	2	1	2						6	6
Mar	1		2	5	1	1					2	1	5		2						10	1
Apr	1	5		1							1	2	3	1							7	1
May																						
Jun																						
July	1	2	1								2	1			1						4	3
Aug			1		1						1			1							2	2
Total	4	10	5	7	3	2					7	6	10	3	5						31	14

A. sylvaticus

Month	Number of days at 'flat' stage										Number of days at 'round' stage										Number of Larvae	Number of Hosts
	<1	1	2	3	4	5	6	7	8	9	<1	1	2	3	4	5	6	7	8	9		
Dec			1	1												1	1				2	1
Jan		2		1		1	1		1				1		1	3		1			6	4
Feb		1				3	2						.	3	1		2				6	5
Mar		2	1	1	2						2			1	1			1	1		6	3
Arr	1				1									1		1					2	1
May																						
Jun	3		3	2	2						1	1	2	5	1						10	4
Jul																						
Aug				1								1									1	1
Total	4	5	5	6	5	4	3		1		3	3	7	8	6	3	2	1			33	19

The relationship of larval development to the month of engorgement throughout the year can be discussed for those incubated at 19°C only, (Table 26).

TABLE 26 : Development of larvae incubated at 19° C.

Number	Month												
of larvae	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jly	Aug.	Sep.	Oct.	Nov.	Dec.	Total
incubated	21	48	32	13	28	108		37	88	343	81	60	859
moulted	7	9			4		3	1			8		32

A significantly greater proportion of the larvae developed to nymphs when they had engorged during the winter months than when they engorged between March and September ($P < 0.005$) (Table 27) of 424 larvae which engorged in October and November, none completed development.

TABLE 27 : Proportions of engorged larvae completing development.

Month of Engorgment	Number of larvae completing Development	Number of larvae failing to complete development	Total	χ^2	P
Dec. to Feb.	24	105	129	34.1*	<0.005
Mar. to Sep.	8	298	306		
Oct. & Nov.	0	424	424	-	-
Total	32	827	859		

The development period of larvae decreased progressively over the range of incubation temperatures, (Table 28).

TABLE 28 : Development of Larvae.

Developmental Period (days)	Incubation Temperature 10°	15°	19°	21°	23°	25°
Minimum	132	46	33	44	33	23
Median	-	128	65	58	36.5	35

The time range of development periods was greater at 19° C (33 - 118 days) and 15° C (46 - 279 days) than at the other temperatures, (Appendix 2); excessively long times are probably associated with diapause and are discussed in this context below.

This developmental period has two distinct, successive, components, (Table 29): during the first phase ticks are active for a variable period, but eventually become quiescent and remain so until moulting is complete; the second phase is overt moulting accompanied by a lightening of cuticular colour and immobility.

TABEL 29 : Developmental phases of Larvae.

Developmental Phase	Developmental Period (days)	Incubation Temperature (°C) 10°	15°	19°	23°	25°
Pre-moulting	Minimum	78	30	16	15	9
	Median	78	55	29	20	20
	Maximum	100	233	74	36	29
Moulting	Minimum	32	16	14	12	13
	Median	56.5	31	19.5	16	15
	Maximum	59	46	24	21	19

Both the pre-moulting and moulting phases are temperature controlled. The relationship between incubation temperature and developmental velocity (the reciprocal of developmental period) is linear and direct for both the pre-moult and moulting component of development, (Figures 6 and 7).

For any given temperature, moulting time shows a tendency to regress on pre-moulting time, i.e. the longer the time spent in the pre-moult the slower is the development which follows. Figure 8 displays the relationships obtained and although none is significant statistically the sample at 23^{o} C has a regression whose t value of 1.95 ($t = r\sqrt{\frac{n-2}{1-r^2}}$) is very close to an 0.05 probability.

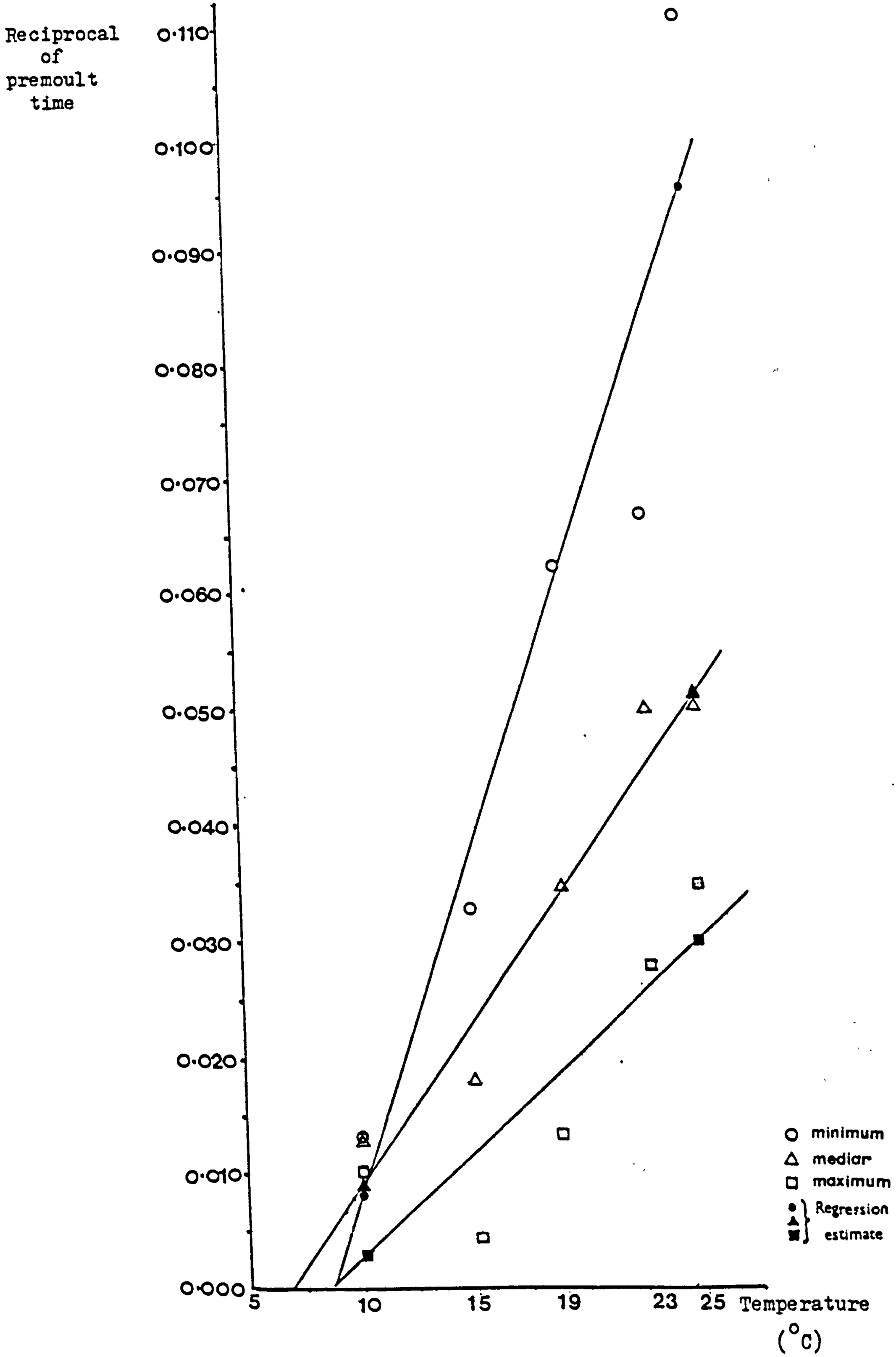


Figure 6. Larvae, relationship between temperature and duration of premoulting phase.

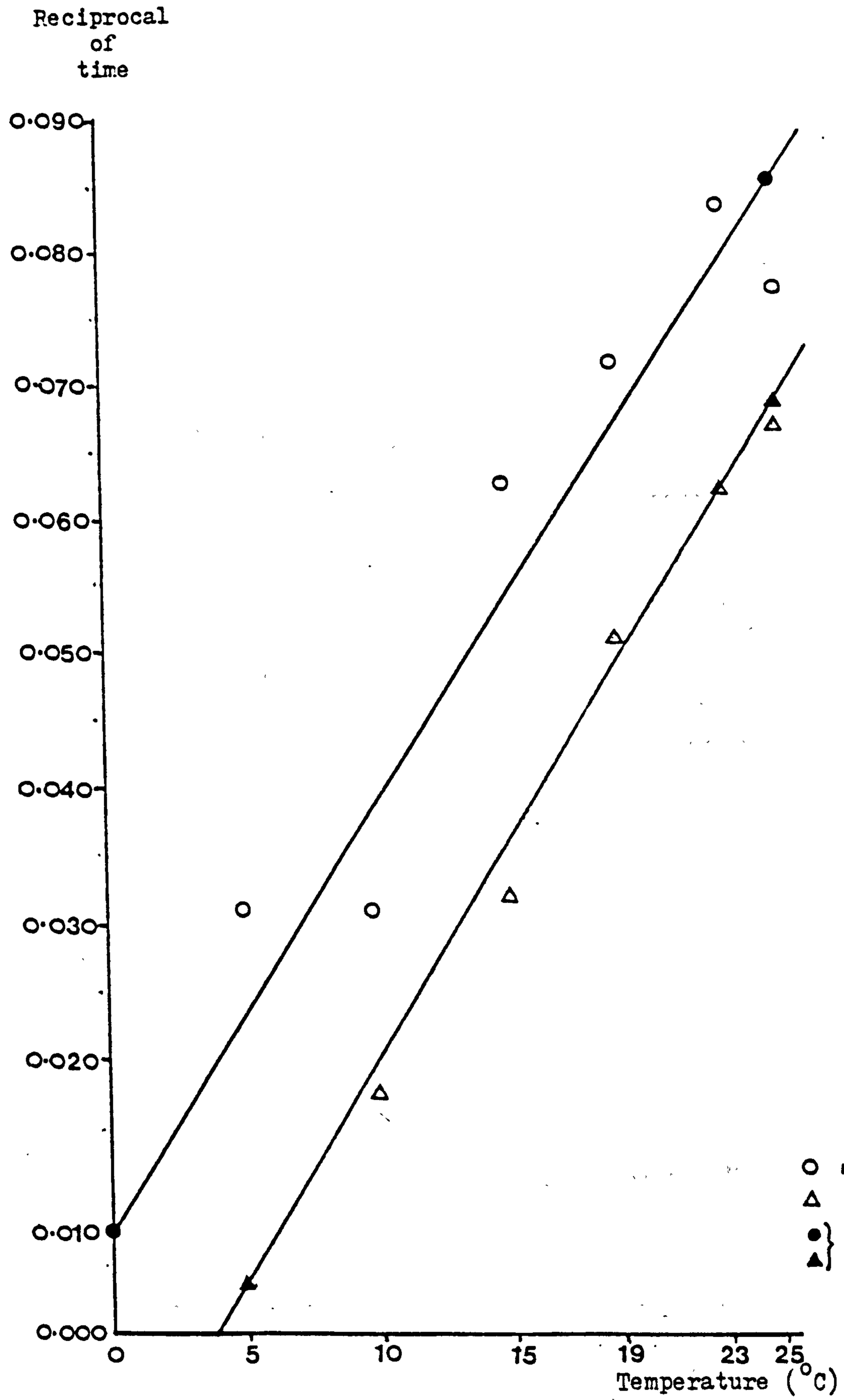


Figure 7. Larvae. Relationship between temperature and moulting velocity.

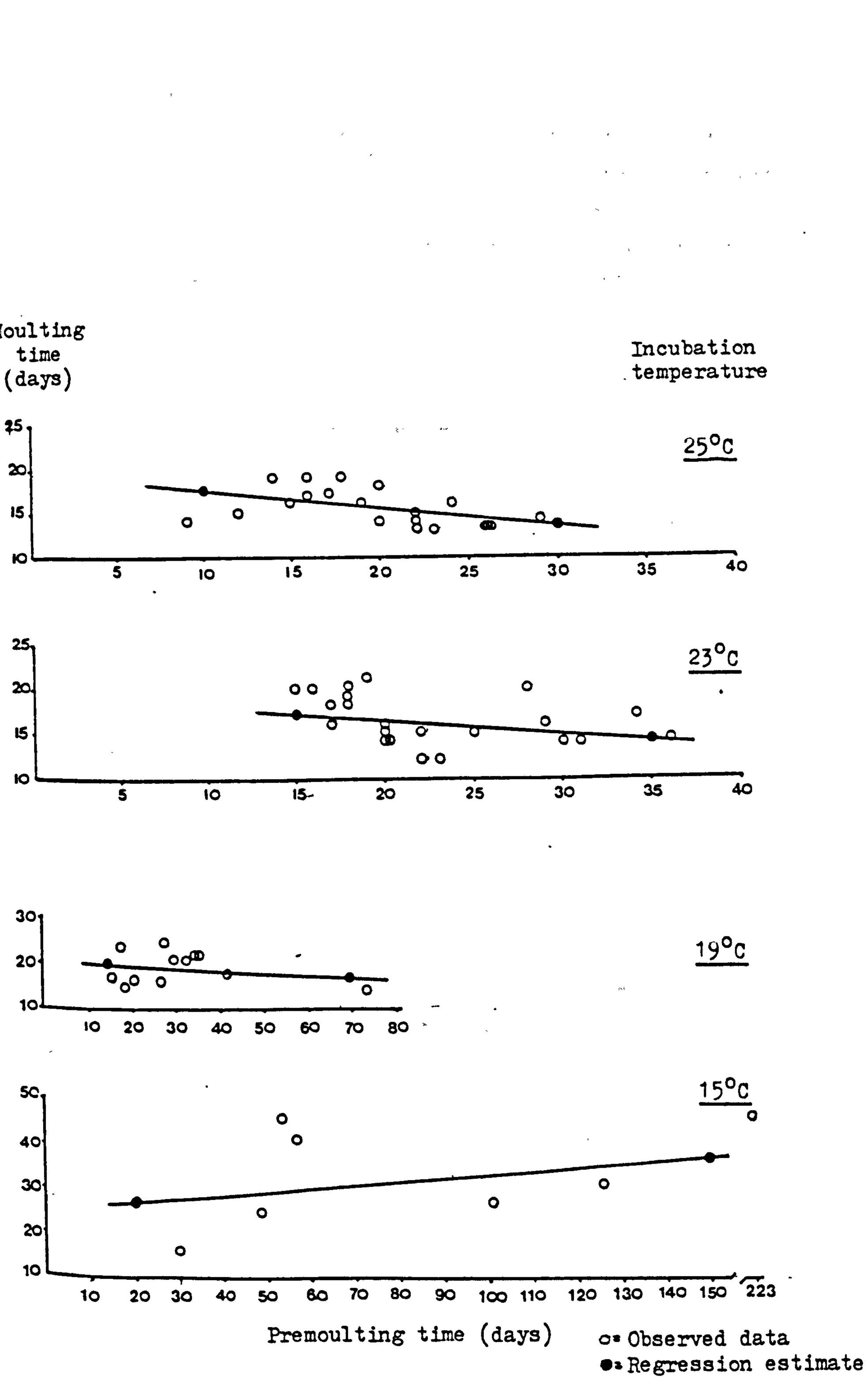


Figure 8. Larvae. Regression of moulting time on premoulting time.

Engorgement of nymphs.

Nymphs occurred on the three host species during the period April to November only (Fig. 4). A significantly greater proportion of C. glareolus was infested than either of the other two host species ($\chi^2 = 62$, $P < 0.005$), and a significantly greater proportion of males than females was infested ($\chi^2 = 7.9$, $P < 0.005$). Sex preference is not significant for M. agrestis and A. sylvaticus, ($\chi^2 = 0.15$, $P > 0.5$) (Table 30).

TABLE 30 : Distribution of engorged nymphs on the host species.

Host Species	Sex	Number of hosts Inspected	Number of hosts Infested	Number of ticks in burden
C. glareolus	M	161	59	158
	F	157	35	50
M. agrestis	M	42	4	4
	F	66	5	5
A. sylvaticus	M	125	5	6
	F	106	6	11

M = male
F = female

Between April and August 1976, 112 nymphs had their mouthparts embedded at capture, of these, 90 were in a flat condition and 22 partly engorged (Table 31).

TABLE 31 : State of engorgement of nymphs at capture.

State of engorgement	Month April	May	June	July	August	Total
'flat' *	2	8	40	20	20	90
'round'	-	2	11	6	3	22
Total	2	10	51	26	23	112

* definitions as given on page 25

C. glareolus was the most numerous of the infested hosts and most heavily burdened, (Table 32); over the trapping period its mean burden per infested host was 2.3 nymphs. Nymphs first appeared on C. glareolus in April and peak burden was reached in June (4.2 nymphs per infested host), thereafter, mean burdens declined progressively until November when the last nymph attached.

TABLE 32 : Nymphal burdens of C. glareolus
Combined results for 1974 to 1976.

Month	Number of engorged nymphs	Number of infested hosts	Number per infested host
January			
February			
March			
April	2	2	
May	16	11	1.4
June	92	24	3.83
July	40	23	1.73
August	35	17	2.05
September	16	12	1.3
October	6	4	-
November	1	1	-
December			
Total	208	89	
Nymphs per infested host			2.2

Only a few M. agrestis (Table 33) and A. sylvaticus (Table 34) were infested with nymphs, these occurring within the infestation period for C. glareolus.

TABLE 33 : Nymphal burdens of *M. agrestis*

Combined results for 1974 to 1976.

Month	Number of engorged nymphs	Number of infested hosts	Nymphs per infested host
January			
February			
March			
April	1	1	-
May			
June	1	1	-
July	5	3	-
August	2	1	-
September	2	1	-
October			
November			
December			
Total	11	7	
Nymphs per infested host			1.6

TABLE 34 : Nymphal burdens of *A. sylvaticus*

Combined results for 1974 to 1976.

Month	Number of engorged nymphs	Number of infested hosts	Nymphs per infested host
January			
February			
March			
April			
May	1	1	-
June	8	3	-
July	1	1	-
August	2	2	-
September	2	2	-
October	3	2	-
November			
December			
Total	17	11	
Nymphs per infested host			1.5

TABLE 35 : Summary of nymphal infestations.

	Class of host					
	All animals examined		Infested with any instar		Infested with nymphs	
Month	Number of engorged nymphs	Number of hosts	Nymphs per host	Number of hosts	Nymphs per host	Number of hosts	Nymphs per host
C. glareolus							
January		6		5			
February		21		14			
March		17		12			
April	2	24		19		2	
May	16	20	C.8	18	C.9	11	1.4
Jure	92	33	2.8	29	3.2	24	3.83
July	40	37	1.1	28	1.4	23	1.73
August	35	61	C.6	31	1.1	17	2.C5
September	16	32	0.5	21	0.8	12	1.3
October	6	65	C.1	51	0.1	4	-
November	1	42	-	23	-	1	-
December		19		16			
Total	208	397		267		89	
Nymphs per host			0.52		0.78		2.2
M. agrestis							
January		9		3			
February		20		9			
March		15		3			
April	1	8	-	3	-	1	-
May		13		1			
June	1	4	-	1	-	1	-
July	5	16	0.3	6	0.8	3	-
August	2	30	-	6	-	1	-
September	2	17	-	6	-	1	-
October		20		5			
November		5		1			
December		1					
Total	11	158		44		7	
Nymphs per host			0.07		0.25		1.6
A. sylvaticus							
January		14		4			
February		20		6			
March		12		3			
April		4		1			
May	1	8	-	1	-	1	-
June	8	10	0.8	8	1.0	3	-
July	1	8	-	1	-	1	-
August	2	40	-	6	-	2	-
September	2	37	-	13	-	2	-
October	3	91	-	42	-	2	-
November		51	-	15			
December		25		12			
Total	17	320		111		11	
Nymphs per host			0.05		0.15		1.5

Development of nymphs after engorgement.

Although the number of nymphs is very much lower, the relationship of development with temperature (Table 36) is comparable and consistent with the minimum time taken by larvae (Table 22).

TABLE 36 : Development of nymphs.

Developmental Period (days)	Incubation temperature (°C) 10°	15°	19°	23°	25°
Minimum	103	46	28	26	28
Median	-	52	93	32.5	-
Maximum	-	71	240	34	-
Number of Nymphs	1	7	26	4	1

There is no obvious difference between the two instars in their time relations at any given temperature, (Fig. 9).

Developmental periods at 19°C fall into two distinct groups: those completing development in less than 125 days (19 nymphs) and more than 195 days (7 nymphs) (Appendix 4).

The long time periods involved have affected the median value which is greater than at 15°C and 23°C presumable because at 15°C a higher proportion of the long tail (i.e. the diapause members) fails, while at 23°C the proportion in diapause is reduced. The relationship between pre-moulting and moulting phases of development can be given at 15°C only (Fig. 10).

For individual ticks at this temperature the regression of moulting on pre-moulting periods is not significant ($t = 0.58$). This again is wholly consistent with the patterns observed in the laboratory.

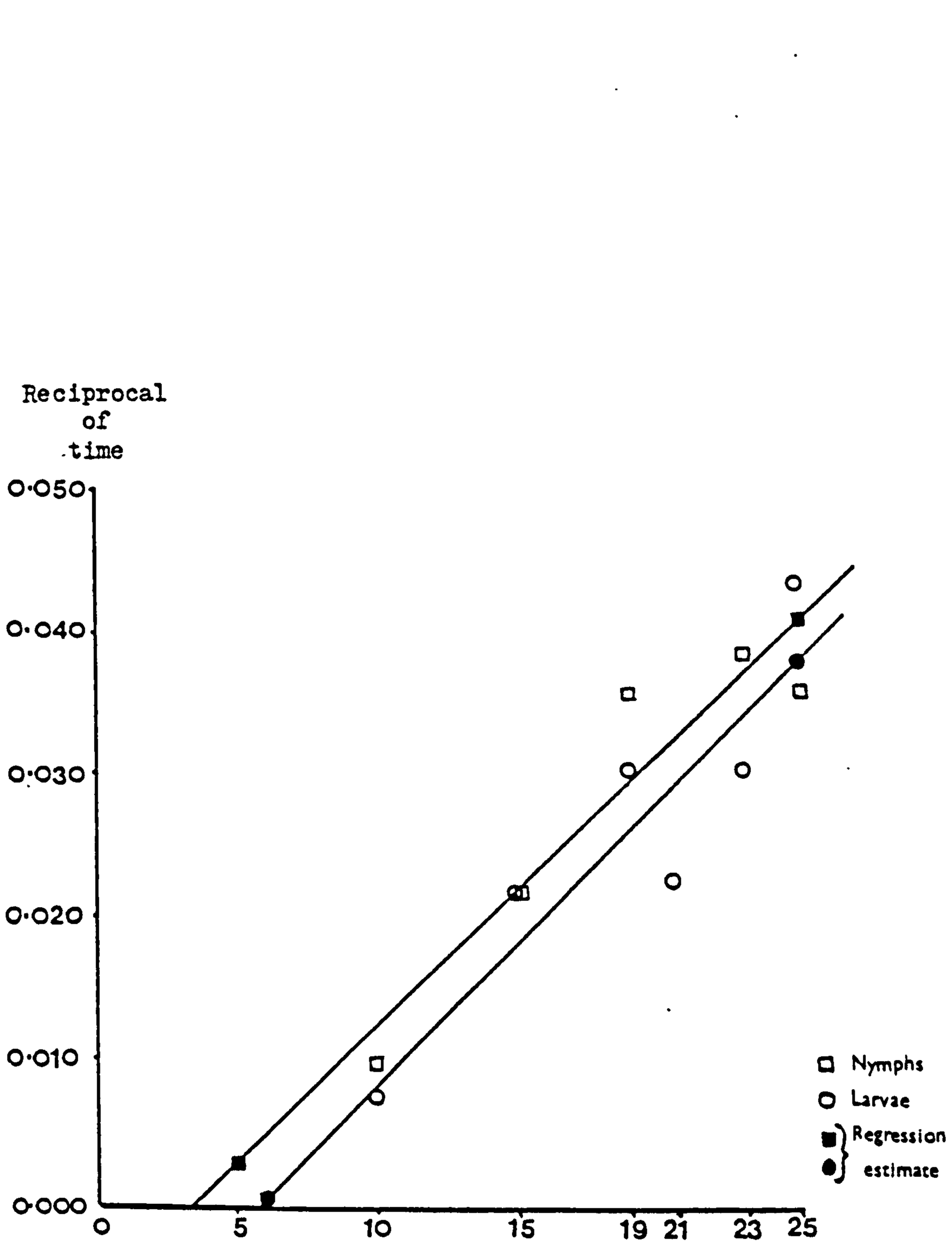


Figure 9. Relationship between temperature and moulting velocity. Mimimum developmental periods of larvae and nymphs.

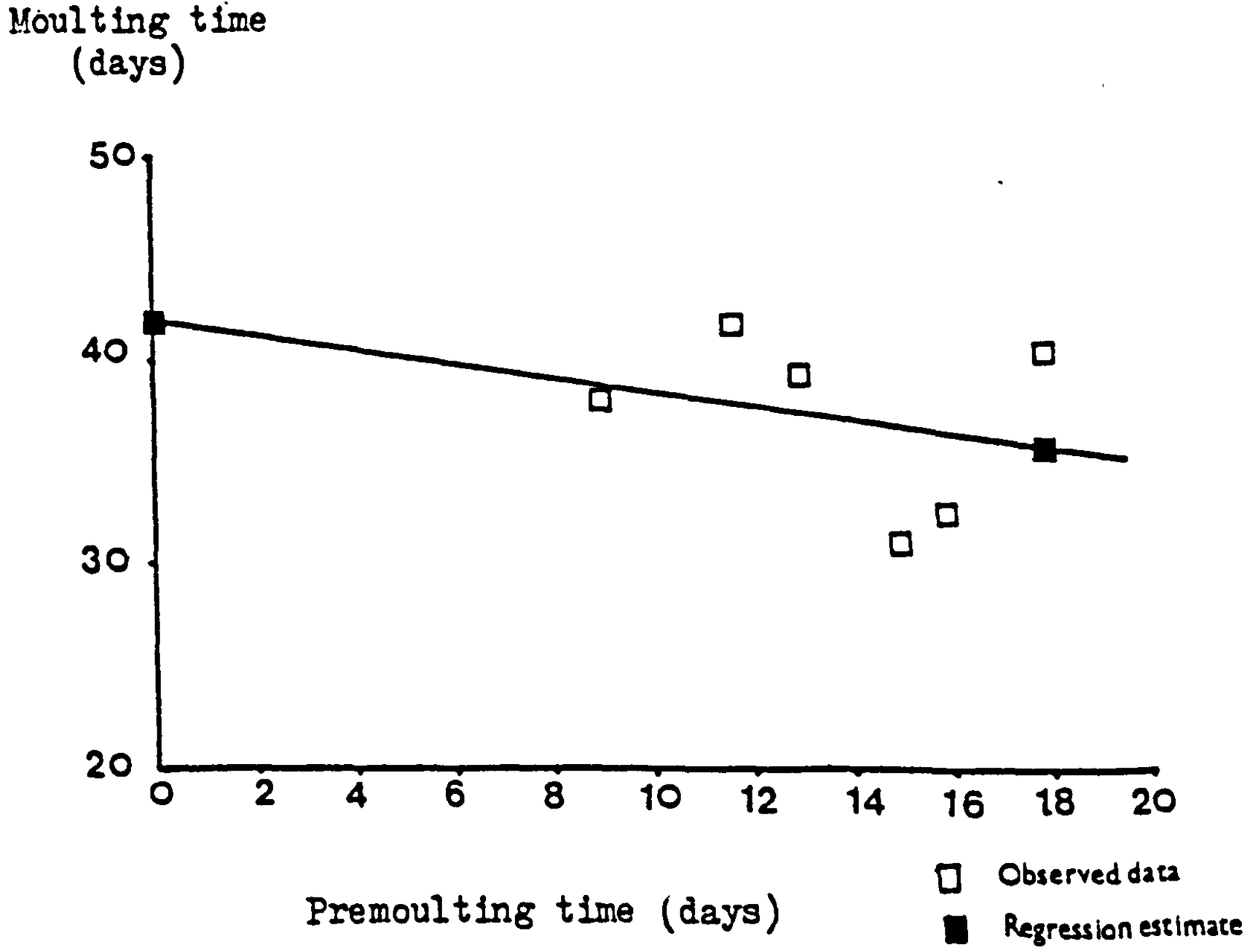


Figure 10. Nymphs. Regression of moulting time on premoulting time.

Incubation temperature: 15°C.

Capture of adult ticks.

Adult ticks occurred on the three species of host during the period February to November only (Fig. 5), with the level of infestation greatest in July (0.43 females per C. glareolus, 0.44 females per M. agrestis). There is no significant difference between the proportions infested of C. glareolus and M. agrestis, (χ^2 = 1.7, P>0·1) or of C. glareolus and A. sylvaticus (χ^2 = 2.5, P>0·1); nor do the female ticks show a sex preference in infestation of hosts: C. glareolus (χ^2 = 0.004, P=0·95); M. agrestis plus A. sylvaticus (χ^2 = 2.2, P>0·1) (Table 37).

TABLE 37 : Distribution of adult I. trianguliceps on the three species of host.

Host Species	Sex	Number of hosts Examined	Number of hosts Infested	Number of Adult ticks Female	Number of Adult ticks Male	Number of Female ticks Per Host	Number of Female ticks Per Infested Host
C. glareolus	M	150	15	25	-		
	F	143	14	19	2	0.15	1.52
M. agrestis	M	56	6	12	-		
	F	76	2	2	-	0.12	1.75
A. sylvaticus	M	108	7	12	-		
	F	95	5	6	1	0.09	1.50
Total		628	49	76	3	0.12	1.55

M = Male
F = Female

Multiple infestation by female ticks was seen, with an upper limit of four: this occurred on only 2 of the 47 hosts carrying female ticks. (Table 38).

TABLE 38 : Frequency distribution of I. triangulicеps females.

Host Species	Sex	Infestation Level 0	1	2	3	4	≥5	Number of Infested Hosts	Number of ticks
C. glareolus	M	186	8	5	1	1	-	15	25
	F	182	10	3	1	-	-	14	19
M. agrestis	M	63	2	2	2	-	-	6	12
	F	87	2	-	-	-	-	2	2
A. sylvaticus	M	161	4	2	-	1	-	7	12
	F	147	4	1	-	-	-	5	6
Total		826	30	13	4	2	-	49	76

Males were much rarer than female ticks (3 males, 76 females), (Table 37). On each of the three occasions when a male tick was found it was in copula with an engorging female, and left the host before the second day of captivity. This relatively short time males spent on the host was also observed when engorging females were mated in the laboratory, (Table 39).

TABLE 39 : Numbers of applications of I. triangulicеps males to engorging females and the time they spent on the host.

Days spent by male on host	Source of Engorged females Laboratory Field	Laboratory Stock	Total
<1	9	22	31
1 - 2	2	2	4
2 - 3	-	2	2
3 - 4	-	1	1
>4	-	-	-
		Total	38

On only two occasions did male ticks remain hidden after they were seen to have detached from the female: in one case the male was taken from the cage next day, the other on the second day.

Males were never found in the absence of females; the three males collected in copula give the only indication of how they may get onto hosts; possibly carried there by the female. Neither this postulate nor other possible mechanisms can be discussed from evidence in the data. That males remain on the host for a much shorter time than females indicates that the proportions of the sexes in collections from the hosts do not accurately reflect the proportions in the adult tick population. Proportions of the sexes in ticks which moulted in the laboratory support this theory: 31 engorged nymphs moulted to produce 15 males and 16 females, proportions which are at parity.

On initial inspection, 56 of the female ticks were seen to be already attached, the remaining 20 being first seen on the hosts up to 6 days after capture. But on no occasion was a female tick seen to be wandering freely on a host.

During attempts to feed laboratory-moulted females, individuals which were not seen to have attached were either found within one day in the water-baths beneath the cages, or were never recovered; since the chance of escape was minimal, they were probably groomed off the fur and eaten by the host. The wild-caught ticks may, therefore, have all been attached at the time the hosts were first inspected.

Engorgement of females.

All 76 of the female ticks on the trapped hosts detached within 23 days of host capture, (Table 40).

TABLE 40 : Number of engorged females dropped from hosts each day after capture.
Combined results for 1974 to 1976.

	Days on which females dropped from the host																				
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20≥21	Total
Jan.																					
Feb.													1								1
Mar.										1											1
Apr.							1				1										2
May.	3																			1*	4
Jun.							1		2												3
Jly	4		2	2	1		4	2		3				2	1				1	1 1*	24
Aug.	2	2		2	1	5	3	1									1	1		1*	19
Sep.	1	1		3	4	2					2	1							1		15
Oct.	1		1	2				1									1		1		7
Nov.																					
Dec.																					
Total	11	3	3	9	6	7	9	4	2	4	3	1		3	1		2	2	3	3	76

*23 days

This was within the maximum engorgement time of 31 days recorded for laboratory-moulted females which fed on C. glareolus and laboratory mice, (Table 41).

Not all female ticks engorged fully before detachment; from July 1974 to October 1975, 7 of 33 females detached prior to complete engorgement. After one attached female which had apparently stopped feeding on the host for a period of 13 days was provided with a male it completed engorgement 2 days after mating, and it became a routine practice to apply a stock male to such female ticks.

Because of the scarcity of female ticks no controlled experiments could be made on the effects of fertilisation on feeding behaviour, but it has been shown for Rhipicephalus simpsoni, a parasite of the African grasscutter (Thryonomys swinderianus) that females will not complete engorgement without mating, Ntiamoa-Baidu, (pers. comm.)

TABLE 41 : Engorgement times of laboratory moulted females.

Days+ on which females dropped from the host																													Total
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
		3	1	2	1						1		2		1						1								1		13

+ Days after application to hosts

Development of females after engorgement.

Of the 76 engorged females, 64 were taken alive from the cages, and 12 were lost. The engorged females varied greatly in size, and to determine the extent of this all 14 which completed engorgement during the period July to September 1976 were weighed on recovery, (Table 42)

TABLE 42 : Weights of engorged females after detachment.

Host		Female tick				
Species	Sex	Days on host after capture	offered male	date collected	weight mg	Incubation temperature (°C)
C. glareolus	M	10		July 1976	120.3	10
"	M	10		"	127.8	10
"	F	7	yes, mated	"	78.3	15
M. agrestis	M	5		"	110.9	10
C. glareolus	M	8	yes, mated	"	89.2	15
"	M	0		"	30.0	15
"	F	11		"	120.3	15
"	M	8		Aug. 1976	79.4	15
M. agrestis	M	21	yes, mated	"	16.5	15
C. glareolus	M	6		"	44.8	15
M. agrestis	F	8		"	84.1	15
"	F	24	yes, mated	"	44.0	15
C. glareolus	M	17	yes, mated	"	28.4	15
"	F	11		Sep. 1976	135.1	15

M = Male

F = Female

None of these females laid eggs although conditions of incubation were suitable for long-term survival: up to 335 days at 15°C (Table 43).

The incubation progress of 24 engorged females was followed to determine the patterns of activity and survival, (Table 43). Inspections were made daily for the first week, weekly until week 13, and thereafter at approximately 2 week intervals until death. A tick was regarded as 'active' if any movement of the legs could be seen during a 15 second inspection under magnification in good light, and was considered 'dead' when any part of it was black or attacked by fungus. It will be seen that the times given for 'activity' are minima while those for 'survival' are maxima.

TABLE 43 : Number of days engorged females were active and survived at each of three incubation temperatures.

Incubation Temperature								
10°C			15°C			21°C		
Active (days)	Non-Active (days)	Survived (days)	Active (days)	Non-Active (days)	Survived (days)	Active (days)	Non-Active (days)	Survived (days)
4	160	164	0	3	3	0	3	3
7	21	28	0	3	3	1	8	9
22	20	42	2	17	19	2	6	8
45	31	76	6	12	18	8	9	17
74	24	98	9	156	165	10	3	13
194	38	232	16	21	37	16	14	30
206	42	248	21	25	46	16	16	32
			37	31	68			
			81	162	243			
			185	150	335			
Median Time (days)	45	31	98	12.5	23	41.5	8	8	13

Periods of activity (0 - 206 days) and survival (3 -335 days) varied considerably, but the median times for both classes are inversely proportional to the incubation temperature.

The extended periods for which the females were active, especially at 10°C (maximum time 206 days) and 15°C (maximum time 185 days) imply a state of diapause which regrettably, none were induced to abandon.

One female which laid eggs took 66 days pre-ovipositional development before eggs were produced, nevertheless, this was at a very low temperature (10°C), and by analogy with other species, (Ixodes ricinus, 34 - 55 days at 10°C, Campbell 1948), is the sort of period that would be expected to reach successful oviposition at this temperature. Regarding the others, it would appear that the conditions under which they were being maintained were adequate for their survival but lacked the stimulus to induce the abandonment of diapause and entry into oviposition; this is a problem that still remains.

Discussion.

Of the three species of burrowing rodent trapped at Balerno, Midlothian, Clethrionomys glareolus, the bank vole, was the most frequently and heavily infested by the tick, Ixodes trianguliceps. From a total sample of 397 bank voles 2.70 ticks per host were recovered. Infestation on Microtus agrestis, the field vole and Apodemus sylvaticus, the field mouse were markedly lighter at 0.68 and 0.81 ticks per host in total samples of 158 and 324 animals respectively. In varying degrees, the bank vole appears to assume the dominant role in supporting this tick elsewhere. In Southern Finland, Ulmanen (1972) states, "The bank vole (C. glareolus) was the most heavily infested [small mammal]"; Cotton and Watts (1967) found in Berkshire, England that "Bank voles were more heavily infested than field voles...."; and Lachmajer (1962) asserts "The principal host for [adult ticks] in Biálowieza National Park [Poland], as was shown by the number of individuals collected, is Clethrionomys glareolus", and this species was one of three on which nymphal ticks occurred most frequently.

Two other localities in the present study contained bank voles in the trapped sample. In both these areas the bank voles appeared in lower proportions than at Balerno, and the tick infestations were appreciably lower. The three areas form a series in which tick numbers increase as the proportion of bank voles increases. Thus:

Locality	Total rodent sample	Proportion of bank voles	Mean infestation
Taynuilt, Argyll	324	18%	Trace
Bush, Midlothian	135	39%	0.22
Balerno, Midlothian	878	45%	1.64

Ulmanen (1972) comments on the size of tick populations in different areas. Those he observed in Finland were comparable with populations from Soviet Karelia described by Vysotskaya (1951) and Lutta (1968). These in turn were comparable to, but somewhat smaller than, those studied by Lachmajer (1962) in Poland and Lichard (1965) in Czechoslovakia, and much smaller than the English population

of Cotton and Watts (1967). In two only of these reports do the published data allow a comparison between tick numbers and the host-population structure, namely, those of Lachmajer (1962) and Ulmanen (1972). In Finland 0.38 ticks per host were found on a sample of 626 rodents containing 15% of bank voles, while in Poland 0.32 ticks per host occurred on 918 rodents containing 60% of bank voles. These two similar infestation levels occurring in rodent samples containing such very different proportions of bank voles denies the simple conclusion implied by the Scottish data that there should be a regression of tick population size on the proportion of bank voles in the host populations. Thus, even where workers recognise the predominant role of the bank vole in supporting I. trianguliceps the interactions with other host species must vary considerably.

The study of Randolph (1975 A and B) in Sussex, England records data which are greatly at variance with the findings elsewhere, and lead her to substantially different conclusions. Randolph (1975 B) states ".... all these four species [Sorex minutus, S. araneus, Apodemus sylvaticus and Micromys minutus] should be considered as fundamental to the continuing survival of the Ixodes trianguliceps population in Common Wood, but Clethrionomys glareolus and Microtus agrestis would appear to be rather more incidental as hosts". Several features in Randolph's data allow room for doubting her conclusions. The two shrews included in the group of "fundamental" hosts carried one female tick only on some 186 individuals captured during the two years of study. Indeed, Sorex minutus has nowhere been recorded as a host of adult ticks. Whatever might be their role it is incontrovertible that neither of these species can play an independent role in the maintenance of this tick. Equally, although Randolph's evidence is of quite large numbers of larvae and adults parasitising the mice, Micromys minutus, no nymphs were observed on 81 captures, and this host has to be discounted as an independent maintenance host. The harvest mouse, M. minutus, is exceptional among the rodents studied by Randolph, in that it was unrepresented in the traps from May to September, the period of nymphal activity. Randolph (1975 B) draws attention to the findings of Kikkawa (1964), Tanton (1965, 1969) and Brown (1969) that the trappability of different

rodent species varies throughout the year but bases reliance on Kikkawa's (1964) conclusion that provided enough traps are available individual differences in trap response will not significantly affect random catches. The continued absence of M. minutus from traps for half the year cannot be interpreted on the basis of trappable and untrappable components in the population but must indicate the absence of the species from the trapping environment. Its intermittent contact with the tick population demonstrably negates the possibility of an independent role in tick maintenance. Of the four species nominated by Randolph (1975 B), one only, Apodemus sylvaticus, carries ticks of the requisite stadial proportions to qualify it as, "fundamental to the continuing survival" of the tick population in Common Wood and the three others, on the evidence presented, appear more appropriately to be regarded as relegated to subordinate roles.

Ulmanen (1972) refers to the clumped distribution of larvae on hosts in Finland but asserts that clumping was not observed for the other tick instars. This presumably implies that he considers nymphs and adults to be randomly distributed on their hosts. Randolph (1975 B) on the other hand, considers that clumped distributions occur for all stadia and she denies random association.

These conclusions derive from a statistical analysis of the difference between observed ratios and random (Poisson) ratios of varience : mean. The samples tested are the total captures of all species for each of the two study years; and the same samples are employed for each of the three instars. Not surprisingly, the difference between the observed and random (Poissonian) ratios are significant for all three instars. The procedure ignores several significant features contained in the gross data:

(1) Both nymphs and adults are markedly seasonal in their activity and in their occurrence on hosts. The author recognises elsewhere in her paper a distinction between low and high seasons. Hence zero records in the inactive season are wholly irrelevant to a test for random or non-random occurrence of ticks on hosts. The clumping effect which is recognised must be a resultant of the proportions of low and high season captures fortuitously included in the test samples.

(2) The sample for nymphal analysis contains an inflated number of zeros through the inclusion of Micromys minutus captures none of which occurred during the season of nymphal activity.
(3) The sample for adult analysis has its zero component grossly inflated by the inclusion of shrews which are recognised by all previous authors to play a negligible role in the feeding of the adult instar.

The analysis, since it ignores the gross biological evidence, is meaningless, its results deriving from the random weights of its individual components, and the conclusion, that all tick instars are overdispersed on the hosts, remains unsupported.

The findings, concerning the host parasite relations of this tick, upon which all authors are in agreement relate to the larval instar. First, the larvae are much more widely distributed on the available host species than are the later instars; all rodents and insectivores carry appreciable larval infestations. Secondly, larval infestations occur throughout the year; although there is clear evidence of seasonal variation in numbers there is no time when hosts are wholly free of larvae. Finally, larval burdens on the various host species are grossly overdispersed. This last feature is the inevitable consequence of the oviposition habits of females and behaviour of flat larvae. Eggs are deposited in single adherent batches of up to 2000 and the larvae after emergence remain aggragated, displaying little or no tendency to disperse. Hence, random contact of host with larval batches invariably produces clumping or overdispersal on host samples. Larval burdens are subject to a further modification in that larval residence on the host may vary according to the season. The finding that all of the 70 larvae captured during December and January were in a flat condition at capture whereas in spring and summer 47 of 328 individuals were already at least partially engorged (Table 12) indicates strongly that the time relations of the parasitic phase of larvae differ with the season It is concluded that winter-attached larvae probably remain attached continuously until the end of winter. A diapause mechanism is implied. The Table (Appendix 1) showing the requisite times for

the completion of engorgement after capture at different times of the year supports this view. Lachmajer (1962) suggests a continuous winter activity on the part of larvae and goes so far as to suggest a winter role for larvae of this species vis à vis viral circulation. This appears an unlikely explanation of the data in Table 12 and Appendix 1.

The seasonal patterns of larvae present a further striking feature of a sharp mid-year infestation minimum on all hosts in all areas. It occurs in July in Scotland (Fig. 3). This corresponds to minima in July in the Tula region of Soviet Russia, (Katelina, 1960), in July and August in Finland (Ulmanen, 1972), Sussex, England (Randolph 1975 A) and in August in Berkshire, England (Cotton and Watts, 1967). In Poland, (Lachrajer, 1962) the minimum occurs as early as May. In Soviet Karelia (Vysotskaya, 1951) the pattern is unique with peaks in March, June and October, and minima in May and September.

A disappointing feature of work with this tick is the low success rate in moulting of engorged ticks under laboratory conditions, and this appears to have been the experience of other workers (Katelina, 1960 and Randolph, 1957 A). In the present studies only 9% of over 1000 field captured engorged larvae succeeded in moulting to the nymphal instar. The success rate, however, depends upon the time of year when captures are made. Of the 382 larvae collected before the July minimum, 13% moulted successfully in laboratory conditions, while only 5.8% of 690 collected after July were successful ($\chi^2 = 17$, $P<0.005$). Some of the successful individuals required inordinately long times to complete the moult (up to 279 days, and the extended development period was most marked at temperatures of 19°C and below. The long delays were due to extension of the premoult phase and the performance of these larvae was wholly comparable to that of Ixodes ricinus larvae from the dispausing autumn-feeding population (Campbell, 1949 ; Kemp 1968). The successful completion of the moult after a prolonged diapause has not been recorded elsewhere although Randolph (1975 A) refers to long developmental periods for larvae fed in late season and maintained at 12.5°C; but the general failure to achieve more than a 1 in 10 success rate in the laboratory strongly implies an almost universal developmental diapause in the larval instar of this tick. The failures must be due to failure to provide proper circumstances for completion of diapause

and resumption of development. We have evidence, therefore, of diapause mechanisms operating at two points in the life of the larval instar. The first is when larvae attached to hosts in the winter months, December - February, present no evidence that they are actively feeding at this time in the field. They can be stimulated, however, to feed and detach when the hosts are brought indoors although the time required is significantly greater than it is at other times of the year. It is probable that such larvae do not become free to develop in nature until March at the earliest.

The second diapause point is manifest in premoulting engorged larvae and although the evidence is far from complete it suggests that diapause is most firmly established in the individuals fed in the second half of the year. Unlike I. ricinus, however, this species also displays a high incidence of a perhaps less firmly established diapause in individuals whose engorgement occurs in the first half of the year.

In contrast to larvae, nymphs display a much more uniform pattern of activity throughout the distribution range. Infestation of hosts is seen everywhere during the period May to September and this may be extended from as early as March in Finland and Soviet Karelia to as late as November in Finland and England. Varma and Smith (1971) postulate a spring maximum of nymphal activity in Ayrshire, Scotland and they contrast this situation with that described for Berkshire, Oxfordshire (sic), England by Cotton and Watts (1967). Their sampling, however, was too erratic to allow difinitive conclusions and their opinion, which is unique, is discounted. Elsewhere, the peak of nymphal infestation occurs between June and August and the most common peak month is July. At Balerno, infestation maxima were observed in June in both years 1975 and 1976, with activity extending from April to October.

Although at very different infestation levels, the three hosts C. glareolus (0.65/host), A. sylvaticus (0.07/host) and M. agrestis (0.10/host) showed the same seasonal patterns. The frequency distributions of nymphs within each host sample were, however, markedly different. A strongly clumped distribution characterises nymphal infestation on C. glareolus and the deviation from random is highly

significant, $\chi^2 = 150$, $P < 0.005$ (Table 10b).

A different pattern occurred in both A. sylvaticus and M. agrestis. On these there was no suggestion of clumping but the data were insufficient for the application of a χ^2 test. Randolph (1975 B) claimed that this tick is overdispersed on its hosts in all its instars. This conclusion, based upon frequency distributions on composite host samples, ignores differences between host species. Randolph's data, when seperately analysed according to the host species show that nymphal infestations on C. glareolus were indeed overdispersed, (Appendix 5) although the divergence from a random dispersal ($\chi^2 = 4.5$, $P<0.005$) is much less pronounced than in the Midlothian data. The Sussex data for nymphs on A. sylvaticus are sufficiently close to a Poisson distribution to deny rejection of the null hypothesis ($\chi^2 = 1.68$, $P>0.1$).

Overdispersal is, thus, a clearly established feature of nymphal infestation on C. glareolus in both Midlothian and Sussex. Random dispersal equally clearly characterises infestations on A. sylvaticus in Sussex and almost certainly in Midlothian as well. The evidence for M. agrestis is limited in both areas but this species resembles A. sylvaticus more closely than C. glareolus.

The development of field-engorged nymphs in the laboratory, (Appendix 4) was significantly more successful ($\chi^2 = 21$, $P<0.005$) than that of larvae. Nevertheless, the failure rate of 4 out of 5 was still extremely high. Randolph (1975 A) also asserts a higher success rate for nymphs but, regrettably, she presents no figures for mortality in either instar. Nymphs in Midlothian showed no seasonal influence on moulting rates such as was noted for larvae. The difference between moulting in pre-July samples and post-July samples was non-significant ($\chi^2 = 1.3$, $P>0.25$). According to Randolph, Sussex nymphs did not present the variations in moulting times comparable to those observed for larvae. Midlothian nymphs maintained at 10°, 15°, 23° and 25° moulted, allowing for direct temperature effects, at comparatively uniform rates within from 1 to 3 months. The mortality in this group of ticks (80.2%) was the same ($\chi^2 = 0.01$, $P = 0.1$) as that of a group maintained at 19°C (79.6%). In this second series, however, moulting times ranged from 28 to 240 days with 9 of 26 individuals completing

their development in the 1 - 2 month period and 7 of the 26 showing an extension of their development to 6 - 8 months. The remaining 10 individuals developed in periods between 3 and 6 months. Again, the major part of the time in these extended developmental periods was occupied by the pre-moult phase. There can be little doubt the phenomenon observed here is directly comparable to that recorded for larvae and that diapause is an important feature in nymphal development. The data provide positive evidence of diapause in a proportion of 0.27 of the successful moults at 19°C but none at other temperatures of incubation. Nevertheless, a proportion of 0.80 of all nymphs subjected to laboratory conditions failed to moult and it is proposed that this, as with the moulting failure in laboratory-maintained larvae, is indirect evidence for an almost universal tendency within this population to diapause in the development process in the engorged nymphal instar.

During the whole period of study 78 female ticks in all were recovered. C. glareolus was the principal host with 46 of them, but A. sylvaticus (20) and M. agrestis (14) carried appreciable numbers. This is strikingly different from Randolph's Sussex recoveries where these species yielded: C. glareolus - 1, M. agrestis - 7 and A. sylvaticus - 42 out of a total catch of 64. The seasonal appearance of adults in Midlothian was somewhat different in the years 1975 and 1976 in that none were recovered before July in 1975 while in the following year a female was taken in February, 2 each in March and April and 3 in May. The activity patterns in other areas, insofar as the small numbers allow comparisons are broadly similar, and we find the following records:

	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	
Soviet Russia (Tula)	-	-	9	7	4	2	8	3	-	Katelina (1960)
Poland	-	-	2	8	7	1	3	2	-	Lachmajer (1962)
England (Sussex)			18					46		Randolph (1975B)
Midlothian	1	2	2	3	4	23	19	15	9	

The milder climate of Britain is the possible cause of the occasional early season activity here (February and March above and Randolph (1975A) records single individuals in December and January). Nevertheless, in both Sussex and Midlothian the greatest activity appears to be in the latter half of the year (Sussex 18 - 46, Midlothian 12 - 66) whereas in the two continental sites above the early part of the year dominates (Russia 20 - 13, Poland 17 - 6). Randolph's claim that adult activity is trimodal with spring, summer and autumn peaks cannot be taken very seriously since the spring and summer peaks depend upon the tenuous basis of partition of 9 individuals in each of two years.

Only British samples provide evidence of the frequency distributions on different hosts (Table 10c and Appendix 5). In Midlothian adults are overdispersed on C. glareolus ($\chi^2 = 32$, $P<0.005$) but are not far different from random on the other two hosts, whereas in Sussex the infestation on A. sylvaticus is demonstrably a random one.

The paucity of detailed information on adults is doubtless the consequence of their relative scarcity in field samples. Males are particularly scarce in host collections and this has led to some quite unsupported claims by earlier workers. Arthur (1963), for example, considered the male to be morphologically ill-adapted for mating while on the host and goes so far as to state that "copulation undoubtedly takes place off the host". Katelina (1960) claimed priority for her observation of males on host animals but overlooked the fact that Neumann's (1902) description of an Ixodes tenuirostris male was based on an individual taken from a bank vole in S. Wales, and that Nuttall et al. (1911) also recorded Jordan's finding of 2 males on hosts in Switzerland. Cotton and Watts (1967) describe males in corula on host animals. In this present study 3 males were taken in copula from host animals. There appears to be no record of males attached to the skin of their hosts, all have been taken either in corula or free in the fur. The paucity of host records is not an a priori reason for assuming that mating off the host must predominate. Ignoring qualitative statements and occasional records the literature contains evidence of recoveries of adult ticks in the proportion 26 males to 208 females, i.e. 1 : 8 (including Midlothian 3 males 78 females; Russia, Tula 3 males 33

females (Katelina, 1960); England, Sussex 7 males 64 females (Randolph, 1975 A); Poland 11 males 23 females (Lachmajer, 1962) and Firland 2 males 10 females (Ulmanen, 1972)). If we compare these proportions to those of records of other Ixodes species we find several species in which male : female captures are comparable to or even much scarcer than 1 : 10. The data of Aeschlirann (1967) in the Ivory Coast include:

I. aulacodi	8	males	56	females	ratio	1 : 7
I. muniensis	7	"	211	"	"	1 : 30
I. oldi	2	"	71	"	"	1 : 35
I. cumulatimpunctatus	4	"	141	"	"	1 : 35
I. rasus	1	"	9	"	"	1 : 9

These proportions are confirmed by Campbell (pers. comm.) ~~(1979)~~ in samples from Ghana :

I. aulacodi	361	males	1850	females	ratio	1 : 5
I. muniensis	20	"	478	"	"	1 : 24
I. oldi	9	"	45	"	"	1 : 5
I. cumulatimpunctatus	6	"	123	"	"	1 : 20
I. rasus	0	"	11	"	"	-
I. moreli	32	"	193	"	"	1 : 6

Only two of these ticks are associated with nidicolous hosts, viz. I. oldi and I. cumulatimpunctatus which occur on small carnivores and giant rats respectively. Indeed, three of them are almost exclusively parasites of antelopes where mating off the host is most improbable. In none of these species has the male been found attached to the host, they are always in copula or free in the fur. We know that the nymphal moult of I. aulacodi produces the sexes in a ratio of 0.5 : 0.5 (Ntiamoa-Baidu, pers. comm. ~~1979~~). It is concluded, therefore, that these species normally copulate on the host but that the male residence on the host is very brief. Direct evidence for this view is provided by I. rasus for which the combined collections from

animals in Ghana and Ivory Coast contained 1 male to 20 females; collections from humans in the Ghana forest, where ticks were removed as soon as their presence was felt, contained 25 males to 60 females. This observation recalls the patterns observed when male I. triangulicers are introduced to hosts carrying females: copula follows almost immediately upon contact and males always quit the host within 24 hours. It has also to be mentioned again that of the successful nymphal moults in this species, 31 nymphs produced 15 males and 16 females.

The most disappointing feature of the present study was the almost complete failure to induce oviposition in engorged females. Of field-captured individuals three only out of 76 laid eggs. This was in spite of subjecting them to a wide range of temperatures, high and low, and variations and alterations thereof. The three successful individuals together with a single laboratory fed specimen were all maintained at constant conditions. One at 10°C captured in April produced over 1000 eggs after a 66 day preoviposition period and 98% of these eggs hatched after about 200 days of embryonic development. Of the others, one, a June capture, laid eggs after 10 days preoviposition, and the other an August capture had a 56 day preoviposition period, both at 19°C. The laboratory raised individual required 12 days before oviposition began at the same temperature. All these ticks at 19°C laid small egg batches of 272, 292 and 310 eggs. Nevertheless hatching ratios of 21%, 44% and 71% indicated their viability. Randolph (1975 A) reports a relative failure, "only 2 females laid eggs at constant temperature" but she omits to record its magnitude. Nevertheless, it is clear that by the use of simulated natural conditions, i.e. long term lowering and raising of temperatures Randolph achieved greater success. Two features stand out in her data: (1) Great variations occurred in the preoviposition periods and led her to suspect the possibility of diapause and (2) Although eggs were obtained very few hatched successfully and this was particularly evident for those derived from autumn-fed females whose hatching rates were distinctly inferior to the few results obtained from the Midlothian ticks.

It is concluded from these considerations that attempts to obtain eggs from Midlothian ticks were frustrated by the failure to cope with diapause. Randolph had a comparable experience but when the female diapause was partially overcome the resultant eggs failed. It is proposed that again the mortality in eggs is most probably a diapause effect.

The objection might be raised that at each point of difficulty in the foregoing discussion a facile resort is made to the proposition of diapause intervention whereas earlier authors have only occasionally and tentatively suggested such a possibility. The distribution of the tick, on the other hand, strongly discounts the alternative that its existence could be maintained by direct responses to environmental conditions. Korenberg and Lebedeva (1969) have drawn attention to the establishment of this tick in particularly inhospitable regions of the U.S.S.R, from as far north as 65° 20' North on the river Tsilma, (Dunayeva et al, 1961), and from altitudes as great as 2050 meters in the Carpathians (Emchuk, 1960) and 2300 metres in the Caucasus, (Djaparidze, 1960). Aeschlimann et al (1970) have recorded it at altitudes up to 1820 metres in Switzerland.

It is remarkable that so much attention should have been devoted to this tick by so many workers in the field yet Randolph (1975 A) stands alone in reporting on work on the developmental stages. Arzamazov (1966) admittedly provides some information but it is entirely qualitative. It is difficult to understand why with so much material available nobody reports on its development, if only to confirm the identification of immature instars, unless it be that the experience of failure recorded here (c. 90% of larvae, 80% of nymphs and 96% of females failed to develop further) has been general. If this be true it is regrettable that non-success should have been withheld from publication, since this is probably important evidence of widespread diapause problems.

More direct evidence for the view that the ticks which fail in the laboratory do so for lack of appropriate stimuli is the individuals which fail to develop survive for very long periods. For example, engorged females have remained healthy and active for as long as 335 days, nevertheless they have remained unfruitful. In short, they are in diapause.

Arzamazov (1966), Cotton and Watts (1967) and Randolph (1975 A) are the only authors who have essayed an account of the life-cycle timing and pattern. The Russian author considers that in White Russia a complete generation requires 3 years and is possibly extended at times to 4 years. His account, however, is confined to verbal statements and provides no quantitative information. Cotton and Watts base their account on field evidence of seasonal incidence of the parasitic stages but interpret their evidence somewhat naïvely on the basis of the findings of Arthur (1951) on Ixodes hexagonus. Randolph alone has made a serious attempt to model the life-history, and within the premises available to her has produced an acceptable interpretation.

From the evidence in Midlothian it is clear that our knowledge is based wholly on a minority, and that probably aberrant, of the ticks examined. We know nothing of the time relations of the diapause phenomenon which it is claimed occurs at so many points in the life-cycle and which apparently affects all but the recorded exceptions. It is the time-relations of this proportion which will determine the course and duration of the life-cycle in nature and until this section of the population has been studied closely attempts to model the natural life-cycle are manifestly premature. There seems no reason to dissent from Arzamazov's 3 year plus life-cycle except to suggest that his estimate could be minimal.

The site at Balerno, Midlothian is exceptional among study areas reported by earlier workers in that it is situated in comparatively open country interrupted by narrow tree (shelter) belts, whereas nearly all other workers report on sites in woodland or forest. The Russian authors, in particular, regard I. trianguliceps as a forest tick. Secondly, the rodent fauna in the Midlothian site is restricted to three species. Of these C. glareolus is the dominant host while M. agrestis is the least important. The preference of M. agrestis for wetter situations could account for its reduced role since Lachmajer (1962) emphasises the effect of waterlogging in reducing tick-populations. The general concensus is that C. glareolus is of major importance in most areas. Data on frequency distributions are sparse and Randolph (1975 B) alone provides information of value for comparative purposes, yet her information on the relative roles of the

available host species is the most at variance with the rest. Her environment is obviously a more complex one than the Midlothian one described here, nevertheless a reconciliation between her data and ours remains to be achieved. The complex interdependence which she describes appears not to apply to the Midlothian conditions. C. glareolus alone of the three species here maintains the instars in appropriate numbers and proportions to qualify it as an independent maintenance host. Moreover, it alone presents clumped or over-dispersed distributions of all instars. On the other two species randomness is strongly suggested for nymphs and adults although our data are not so unequivocal as those of Randolph appear to be for A. sylvaticus. These two species, furthermore, present infestations where the numbers and proportions of the instars are grossly unbalanced. These two lines of evidence imply strongly a qualitatively different role as between C. glareolus on one side and the other two species on the other. It is difficult to envisage either M. agrestis or A. sylvaticus in this area as capable of playing independent roles in tick maintenance, and the infestations on these two species must be derived at least in part from C. glareolus maintained populations. If this deduction be correct then it is a strong possibility that the two subsidiary hosts are deleterious in their effects on the general success of the tick population.

The location of the developing stages, as Randolph (1975 A) states, is uncertain. She quotes the occasional recovery of ticks from rodent burrows and nests by Vysotskaya (1951), Arthur (1963) and Cotton and Watts (1967) and assumes that survival and development are possible only below ground in rodent burrows and/or nests. There is no evidence to encourage dissent from this view. Cotton and Watts (1967) enter into an inconclusive discussion on whether or not I. trianguliceps is a truly nidicolous species. They adduce mating behaviour as an argument but this has been dealt with above. The important feature of nidicoly is that it involves detachment from the resting host. If this were to occur we should expect quite strict isolation of the various population components and infestation of each host species to

present regular numerical proportions of the sequence of instars. The complex interplay between species such as Randolph describes would appear to be most unlikely. The weight of evidence favours the opinion that the species is anidicolous and detachment is from the active host. This is much more likely to provide a basis for Randolph's findings. Rodents and shrews are potential invaders of underground runways and burrows of other species but are less likely to trespass into nests. Survival and development above ground are unlikely in Britain, and in the inhospitable high latitude and high altitude regions of mainland Europe are impossible.

On these grounds a basis is provided for the difference between the infestations of the three rodent species in Midlothian. C. glareolus has a fairly restricted home range and its movements are predominantly hypogeal. It consequently provides optimal opportunity for dropping ticks in favourable developmental sites in its homerange burrows and in due course the tick has optimal opportunity for host contact. M. agrestis favours different and usually wetter terrain which in most parts of the distribution range is a less suitable environment for the tick. A. sylvaticus has the widest home range of all these species and it is the most likely of the three to undertake epigeal movements. This implies the loss of ticks in wholly unfavourable environments, hence, the suggestion that the subsidiary hosts may play a not wholly favourable role in this locality. Overdispersal on C. glareolus samples results from their being drawn from a population whose components each within their burrow system have a closed relationship with their own local tick population. The other two species which become infested at least in part in passage through C. glareolus burrows sample the tick population across its local sections hence their infestations approach random frequencies.

In view of the difficulty encountered in promoting development of captured ticks it is inevitable that the original purpose of setting up laboratory cultures for virus transmission studies should have been frustrated. The experience has highlighted the need for study of diapause or delayed development mechanisms in this tick and the factors involved in their induction and regulation.

Contributions to the epidemiology of Louping-ill.

Part 2.

Prevalence of Louping-ill in northern Scottish cattle.

PART 2.

Introduction.

This survey of 4529 cattle is the first to be undertaken in the 9 northern counties and on 9 of the inner islands on the west coast of Scotland to determine the incidence of antiserum to Louping-ill virus, (Moredun strain 31, (Reid and Doherty, 1971)) in this animal. To the south in lowland Stirlingshire, Walton (1967) tested 314 cattle and 204 sheep but found no antisera to this virus. Dunn's (1960) survey of the red deer (Cervus elaphus) covering herds in the greater part of Scotland embraced the whole of the area in this present study. In the work described below only the immunoglobin G fraction was determined in the haemagglutination inhibition test. The results, therefore, reflect the individual's experience of challenge in the long-term.

Part of the data relating to the isle of Eigg has already been published in another context (Blewett et al, 1978) where the same sera had been used as part of an independent concurrent study of babesiasis in Scottish cattle (Blewett and Adam, 1975 A and B, and Adam and Blewett, 1978).

Materials and Methods.

The test sera.

Sera were obtained through the officers of the Scottish Veterinary Investigation Centres from sera collected for the Brucellosis eradication scheme. Information relating to the specimens was restricted to the donor animal's age, sex, eartag number, the date of sampling and the Parish and herd reference numbers. A condition of supply was that communication with the herd owners was not permissable.

Specimens received as serum were stored at - 20°C from arrival; those received as serum on the clot were stored at 5°C if they were to be used within a week, otherwise the serum was withdrawn and deep frozen. Sera were allowed to thaw-out completely at room temperature immediately before use.

Testing of sera for antibody to louping-ill virus.

For the haemagglutination inhibition (HI) test the techniques of Clarke and Casals (1958) were followed; where changes or additions have been made these are indicated in the text.

In preparation for this test, sera underwent a series of extraction processes to remove factors which would inhibit haemagglutination of goose (Anser cinereus) erythrocytes by Louping-ill antigen, or would cause agglutination of these cells. In addition to Clarke and Casal's (1958) methods it was necessary to deactivate the immunoglobin (Ig) M fraction. Along with each group of test sera, 2 control sera were prepared: one of known positive titre (the positive control), the other known to have zero titre (the zero titre control).

Preparation of sera.

A bijou bottle for each serum specimen was charged with 0.8ml of borate saline (BS) (Appendix 6) and its cap labelled before 0.2ml of serum was added.

Removal of non-specific inhibitors of arbovirus haemagglutination.

Following dilution of the serum, 0.1ml of a 25% weight per volume suspension of acid-washed kaolin in BS was added and the capped bottle kept at room temperature for 20 minutes with occasional shaking. The kaolin with bound inhibitors was sedimented by centrifugation at 2500 rpm and 4°C for 15 minutes,[1] and supernatant decanted into a clean bijou bottle and sealed with the labelled cap.

Removal of naturally occurring agglutinins for goose erythrocytes.

Supernatant from kaolin extracted serum was chilled before adding 0.1ml of packed, washed goose erythrocytes. After capping the contents were allowed to stand for 20 minutes at 4°C with the occasional shaking after which the erythrocytes with adsorbed agglutinins were sedimented by centrifugation at 1500 rpm and 4°C for 10 minutes.

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1. Clarke and Casals (1958) recommend 30 minutes at room temperature

Deactivation of Ig M fraction.[1]

Agglutinin-free supernatant was loosely capped and the bijou bottle placed to half its depth in a large capacity pre-heated water-bath and held at 64.5°C (± 0.5°C) for 30 minutes. After tightening the caps the extracted serum was cooled to 4°C, and was then ready for the HI test. This extracted serum was considered to be equivalent to a 1:10 dilution of the serum.

Haemagglutination inhibition test.

HI tests were made in lucite plates, 5 11/16" x 7 1/16", containing 80 hemispherical wells of ¾" diameter arranged in 8 ranks of 10 wells. Each plate accommodated 2-fold serial dilutions from 1:10 to 1:2560 and serum control for each of 8 sera.

For each titration, 0.2ml of borate saline was added to wells 2 to 9, to be used for serial dilution of the extracted serum, and to well 10 for the serum control. 0.2ml of extracted serum was then added to wells 1 (1:10 dilution) and 2 (giving a 1:20 dilution) and the serum control well. Serial 2-fold dilutions were made starting from well 2 until 1:2560 was reached at well 9, 0.2ml of this last dilution was discarded. At this stage the serum control well contains 0.4ml of a 1:20 dilution, and each serial dilution well, 0.2ml.

To each of the serial dilution wells (1-9) was then added 0.2ml of louping-ill virus antigen diluted with bovine albumin (Armour fraction V) in borate saline (BABS) (Appendix 6) at pH9 to give 4-8 units of haemagglutinin per well.

Prepared plates were covered with sheets of thin polythene to minimise evaporation during overnight incubation at 4°C. On the following morning the plates were allowed to return to room temperature before adding to each well, 0.4ml of washed, standardised goose erythrocytes in adjusting diluent (Appendix 6) at pH6. The covered plates were then incubated at room temperature for 1 hour. Inspection of the test controls (for antigen, erythrocytes, positive and zero titre sera, (vide infra)) was then made before the test was accepted; and each serum control consulted before individual

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1. IgM is not deactivated in Clarke and Casals (1958) preparation of sera.

titres read. Plates were read from above, against a white background. With complete agglutination of the erythrocytes (absence of antibody) the erythrocytes formed a uniform, thin transluscent shield of cells over the submerged surface of the well. In the absence of haemagglutination (presence of antibody), erythrocytes formed a dense ring on a button of cells in the bottom of the well.

Controls for HI test.

Serum control.

Used to detect unwanted haemagglutinins in the test serum if the goose erythrocytes settled in a dense, well-defined ring or button at the bottom of the well all naturally occurring haemagglutinins had been removed in preparing the serum and the titre could be read. Failure of this control involved rejection of that particular serum extract.

Positive serum control.

Serum of known titre (1:640) was prepared and tested along with the test sera. For the titres of the test sera to be acceptable the control had to be free from agglutination in its own serum control and have the correct titration. The particular form taken by the sedimented goose cells in the 1:640 well determined the titration and point for the test sera. Failure of this control involves rejection of all sera extracted along with it.

Zero titre serum control.

Serum of known zero titration was prepared and tested along with the test sera. This should show no haemagglutination in its serum control well, while all serial dilution wells have complete agglutination of the goose cells. If these criteria are met, inhibitors of agglutination have been reduced to undetectable levels during preparation of the test sera.

Antigen control.

To titrate the antigen dilution used in the HI test, 0.4ml of BABS was added to wells 1-6 of a rank on an agglutination plate. To well one was added 0.4ml of the diluted antigen, this was mixed thoroughly with the contents of the well and left to incubate overnight with the test sera. Next day a serial 2-fold dilution was made from well 1 to 6, and 0.4ml discarded from the last.0.4ml of the goose erythrocyte dilution was added to each well, and the control left to incubate along with the HI test. The first 4 wells should have complete agglutination of the goose cells when 4-8 units of haemagglutinin per 0.2ml of the antigen dilution are present. Failure of the antigen control through incorrect dilution requires that the HI test be repeated.

Goose erythrocyte control.

0.4ml of BABS was incubated overnight with the HI test. Next morning, 0.4ml of the goose cell dilution was added. If after the 1 hour incubation period they formed a clearly defined button of cells at the bottom of the well, the reagents were known to be agglutinin-free. When more sera were to be tested than could be allocated the goose cell dilution in 10 minutes, this control was duplicated. Goose cells were added to the first well immediately the dilution had been prepared, and to the other when the last test well had been treated. This ensured that while being dispensed, the erythrocytes had not suffered damage from the relatively low pH of the adjusting diluent.

Antigen and goose erythrocytes.

Both the antigen, strain LI 31 (Reid and Docherty, 1971), and goose erythrocytes (in the form of packed cells for use in the extraction of sera, and as cells of stated concentration for use in the HI test) were supplied by Dr. H. Reid, Moredun Research Institute, Edinburgh.

Cleaning methods.

Agglutination plates.

Immediately after use, agglutination plates were rinsed with warm,running water. Following overnight immersion in a dilute NaOH solution (Appendix 6) the plates were rinsed in warm running water and immersed for 1 hour in a dilute solution of HCl, (Appendix 6). After rinsing, the plates were washed for 1 hour in warm, running water, then immersed for 1 hour in each of 2 changes ofdistilled water. Drying was in a warm-air cabinet.

Pipette tips.

The polypropylene pipette tips[1] whether used to dispense serum or the test reagents were subjected to the same cleaning routine as used for agglutination plates.

Glassware.

Bijou bottles were rinsed out with water jets from a bottle washer before being thoroughly brushed. After rinsing with warm running water they were steeped overnight in a detergent solution ('Pyroneg')[2]. The bottles were then rinsed at least six times in hot water before immersion for 1 hour in hot, running water; final rinsing was in distilled water. The bottles were inverted in wire baskets and dried in a hot-air cabinet.

Other glassware was treated in the same way as the bijoux except that immersion in the detergent was limited to 1 hour.

Bijou bottle caps.

Before washing, the peelable, self-adhesive labels, ('Presson') were removed taking care to remove all traces of adhesive from the metal caps. The plastic seals were then removed before they and the caps were brushed. After immersion in warm detergent solution

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1. Jencon (Scientific) Ltd, Hemel-Hempstead, Herts, England.
2. Diversey Ltd Northampton, England.

('Pyroneg') for 1 hour they were rinsed and immersed in hot, running water for a further 1 hour. A final rinse in two changes of distilled water was given to both caps and seals before drying in a hot-air cabinet. The seals were re-inserted using forceps and the assemblies stored in sealed plastic containers.

RESULTS.

The most complete set of data is from the Isle of Eigg where 417 serum samples were taken between 1974 and 1976; 333 (80%) of these were positive for antibody to Louping-ill virus (Table 44).

Positive sera are not uniformly distributed throughout the age range (1 to 13 years), but increase significantly in their frequency from the 1 - 4 year age group to the 5 - 8 years old ($\chi^2 = 20$. $P<0.005$) and to those older than 8 years ($\chi^2 = 27$, $P<0.005$). There is no significant difference between the two groups of older animals ($\chi^2 = 0.43$, $P>0.05$).

The incidence of positive sera is not uniform over the island. Two herds, A (324 sera) and B (93 sera) (Table 45) were sampled; herd A has a significantly greater proportion of its sera positive ($\chi^2 = 107$, $P<0.005$).

Sera with zero titre have distributions which differ between the herds : only one of 30 in herd A was outside the 1 - 3 years age group whereas in herd B the 54 animals with zero titre cover the whole range of ages. Even so, a significantly greater proportion of these sera occur in animals less than 5 years old ($\chi^2 = 14$, $P<0.005$). In both herds, therefore, although proportions vary, the zero titres are associated predominantly with the younger animals.

Positive sera were titrated (and will be quoted as reciprocals of titre, i.e. at their end-point of dilution). In herd A, higher titration levels were reached (>2560) than in herd B (maximum 640), and the geometric mean titre (GMT) of sera increased more quickly in herd A than herd B (Table 45). The increase of titre with age was remarkably consistent in both herds over the 3 years they were sampled (Table 46).

During the 3 years, 110 individuals were sampled more than once (Table 47). The increase in titre with age which was seen in the herds applies to individual animals also. The 3 categories, namely no change, rise and fall of titre, were not randomly distributed ($\chi^2 = 8.3$, $P<0.005$) but almost the whole of the deviation from random lies in the unexpectedly low number of declensions ($\chi^2 = 5.5$)

The changes over a 2 year period were qualitatively similar in both herds (Table 47) but were too few to test statistically. In

TABLE 44 : Incidence of antibody to Louping-ill virus in sera.

County	Number of sera. Tested	Positive	G.M.T.*
Aberdeenshire	474	3	0.02
Banffshire	520	27	0.2
Mainland			
Inverness-shire	722	263	4.8
Kincardineshire	275	3	0.05
Morayshire	478	116	2.2
Nairn	433	58	0.7
Northern Perthshire	281	151	6.8
Ross-shire	150	73	9.8
Sutherland	104	4	0.2
Islands:			
Skye	335	220	26
Canna	54	4	0.3
Rhum	38	9	1.4
Eigg	417	333	47.
Muck	69	6	0.3
Colonsay	10	8	7.8
Oronsay	15	5	7.8
Jura	66	57	57
Gigha	88	0	-
Total	4529		

* Geometric mean titre

TABLE 45 : Island of Eigg. Age and titre distributions.

Herd A.

Titre	Age 1	2	3	4	5	6	7	8	9	10	11	12	13	Total
Zero	20	2	7								1			30
10	5	1	4	4	1		1							16
20	5	2	4	3	3	1	1	1						20
40	3	4	2	2	3	3	1	1	3	2	2		1	27
80	3	4	5	9	5	5	2	5	2	3	5	5	3	56
160	3	4	8	10	8		6	5	5	5	3	1	6	64
320	4	5	6	5	3	5	5	8	9	2	3	4	6	65
640		1	2	3	1	2	2	1	2	4	2	2	8	30
1280	1		2	1	2	2		1		1	1	2		13
2560			1											1
>2560									1				1	2
Total	44	23	41	37	26	18	18	22	22	17	17	14	25	324
GMT*	8.1	65.6	50.4	103.	114.	167.	149.	176.	220.	205.	124.	250.	295.	89.0

Herd B.

Titre	Age 1	2	3	4	5	6	7	8	9	10	11	12	13	Total
Zero	10	5	10	10	2	2	5	2	4	2	1		1	54
10		1							2	2	1	1		7
20						2	3		1		1			7
40			1	2	1	1	1	1		1				8
80					1		1		2			1		5
160	1		2		1		2		1					7
320	1		2											3
640										1		1		2
1280														
2560														
>2560														
Total	12	6	15	12	5	5	12	3	10	6	3	3	1	93
GMT*		1.1	4.4	0.8	9.0	8.8	6.1				12.3			4.3

*GMT = Geometric mean titre

TABLE 46 : Geometric Mean titre (GMT) of serum samples from Eigg.

Serum reference number	AGE GROUP ≤ 5 GMT	No. of sera tested	6-8 GMT	No. of sera tested	≥ 9 GMT	No. of sera tested	GMT of sample	Number tested
Herd A. 1	39.4	44	126	25	173	42	89.8	111
Herd A. 2	45.2	89	167	18	217	39	81.0	146
Herd A. 3	46.2	38	255	15	410	14	107.4	67
Herd B. 1	0.3	19	4.6	13	11	10	2.5	42
Herd B. 2	4.7	31	-	7	9.5	13	6.6	51
Total		221		78		118		417

TABLE 47 : Eigg sera. Changes in titration levels in animals sampled over intervals of one and two years.

Time between samplings		One year period		Two year period	
Date serum samples taken		1974 1975	1975 1976	1974 1976	1974 1976
Change in titration levels	increase 7				
	increase 6	1			1
	increase 5	2		2	1
	increase 4	2	1	1	1
	increase 3	2		2	5
	increase 2	4	2	7	3
	increase 1	24	10	10	0
	No change	31	18	12	12
	decrease 1	15	5	3	2
	decrease 2	4		1	
	decrease 3		2		
	decrease 4				
Number of sera		85	38	38	25
Herd		A	A	A	B

herd A only one animal decreased its titre (by 2 dilutions) whereas 12 increased and 25 remained unchanged. In herd B, the only change of more than 1 dilution was in an increase of titre by 11 sera.

Over the one year period maximum decrease in titre was 3 dilutions and maximum increase 6 dilutions. For the two year period decrease was limited to 2 dilutions but increase was by a maximum of 6 dilutions.

The greatest changes in titre occurred in the younger animals (Table 48), over the year changes exceeding 2 dilutions were confined to the 1-5 year age group.

The proportion of positive animals increased with age in both herds. In herd A, 160 of 198 animals of 6 years or less were positive, whereas for the older animals the proportion had risen to 134 out of 135. Similarly in herd B the proportion positive in the same two age groups is 16/55 and 23/38.

The island of Skye provided 335 sera from 11 herds all of which had been exposed to challenge. The proportion of positive sera was significantly greater in animals of 7 years and older (0.8) than in the younger group (0.6) ($\chi^2 = 18$, $P<0.005$). Zero titre distribution on the island (Table 49) resembles that of herd B on Eigg in its spread over the whole age range.

The GMT increases from 17.1 for the group of animals up to 6 years old, to 54 for animals of 7 or more years. In 3 herds (51, 52 and 58)(Table 50) all sera were positive. Herd 51 had the highest GMT (436) with herd 58 next in rank (GMT = 209). Unlike the herd samples from Eigg, GMT does not invariably increase with age; certainly it does so in herd 54 where for three age-classes GMT is 1.0, 7.6, 24.3; but remains stable over the extreme age classes in herd 52 (80.6 and 84.2), and decreases in the largest herd 51 (483, 481 and 360). As on Eigg, challenge appears not to be uniform over the island.

TABLE 48 : Change in titration level of Ab to L.I. virus in 123 animals over a period of one year.

Isle of Eigg. (1974-75 : 85 Sera) (1975-76 : 38 Sera)

Animals ages when sampled	Change in titration level: Reduction ≥4	3	2	1	No Change	Increase 1	2	3	4	5	6	≥7	Number of pairs tested
1.2							1			1			2
2.3				2	1	2			1		1		7
3.4		1	3	3	6	5	1	2	1	1			23
4.5		1		3	12	5			1				22
5.6				1	5	2							8
6.7					2	1							3
7.8				3	2	5							10
8.9				1	5	2	1						9
9.10				2	5	2	1						10
10.11				1	3	1	1						6
11.12				2	5	1							8
12.13				1	2	2	1						6
13.14			1	1	1	6							9
Total	0	2	4	20	49	34	6	2	3	2	1	0	123

TABLE 49 : Skye. Summary of age and titre distributions.

	Age													
Titre	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
Zero	28	19	17	11	12	4	6	6	5		3		4	115
10		1	3	1	1	1	3	3			1	1	1	16
20		2	2	1	2				1		1	3		12
40	2	1	3	2	3		1	2		1	2	1	2	20
80	2	2	5	6	9	2	6	2	4		1	1	1	41
160	2	4	5	6	6	2	4	4		1	2	3	5	44
320	2	1	3	8	3	4	1	4	4	1	1	3	3	38
640	1	1	3	5	2	5	3	1	4	1		5	2	33
1280			1	1	1	1	1	2		1	1	1		10
2560		1		3									1	5
>2560				1										1
Total	37	32	42	45	39	19	25	24	18	5	12	18	19	335
GMT				17						54				26.0

TABLE 50 : Geometric mean titre (GMT) of serum samples from Skye.

	AGE GROUP							
	5		6-8		9			
Serum reference number	No. of sera GMT	tested	No. of sera GMT	tested	sera GMT	tested	GMT of sample	Number tested
51	482.6	22	418.2	13	368.0	10	436.0	45
52	80.6	21	-	5	84.2	16	83.4	42
53	6.7	58	23.0	16	-	-	8.8	74
54	1.0	17	7.6	10	24.3	10	4.9	37
55	3.0	38	6.1	10	-	6	4.0	54
56	-	8	-	7	-	7	77.8	22
57	22.0	11	-	1	-	4	20.5	16
58	-	5	-	2	-	6	209.3	13
59	-	6	-	1	-	6	8.6	13
510	-	5	-	1	-	4	36.7	10
511	-	4	-	2	-	3	114.8	9
Total		195		68		72		335

The two southernmost islands in the survey were Gigha and Jura. From Gigha, the farthest south, 88 sera from 6 separate herds covering the period 1973 - 1975 had zero titres for each year. The age range of the animals was 2 to 9 years.

Gigha was the only island of 9 in the Inner Hebrides which, although it has ticks (Ixodes ricinus) present, was wholly free of positive sera.

Jura provided a total of 66 sera from 4 herds, 9 sera with zero titre were spread over the whole 2-13 year age range, (Table 51). A greater proportion of the sera from older animals (7 years or more) was positive (33/35) than from the younger age group (24/31 sera), and in this older group the GMT was higher (125) than it was for the younger animals (23).

The other islands provided sera from single herds only and these fall into two groups. Rhum, Colonsay and Oronsay, (38, 10 and 15 sera respectively) had positive sera (9, 8 and 5 respectively) from a wide range of ages, with maximum titres in the middle range (160-640). In the second group are Canna and Muck.

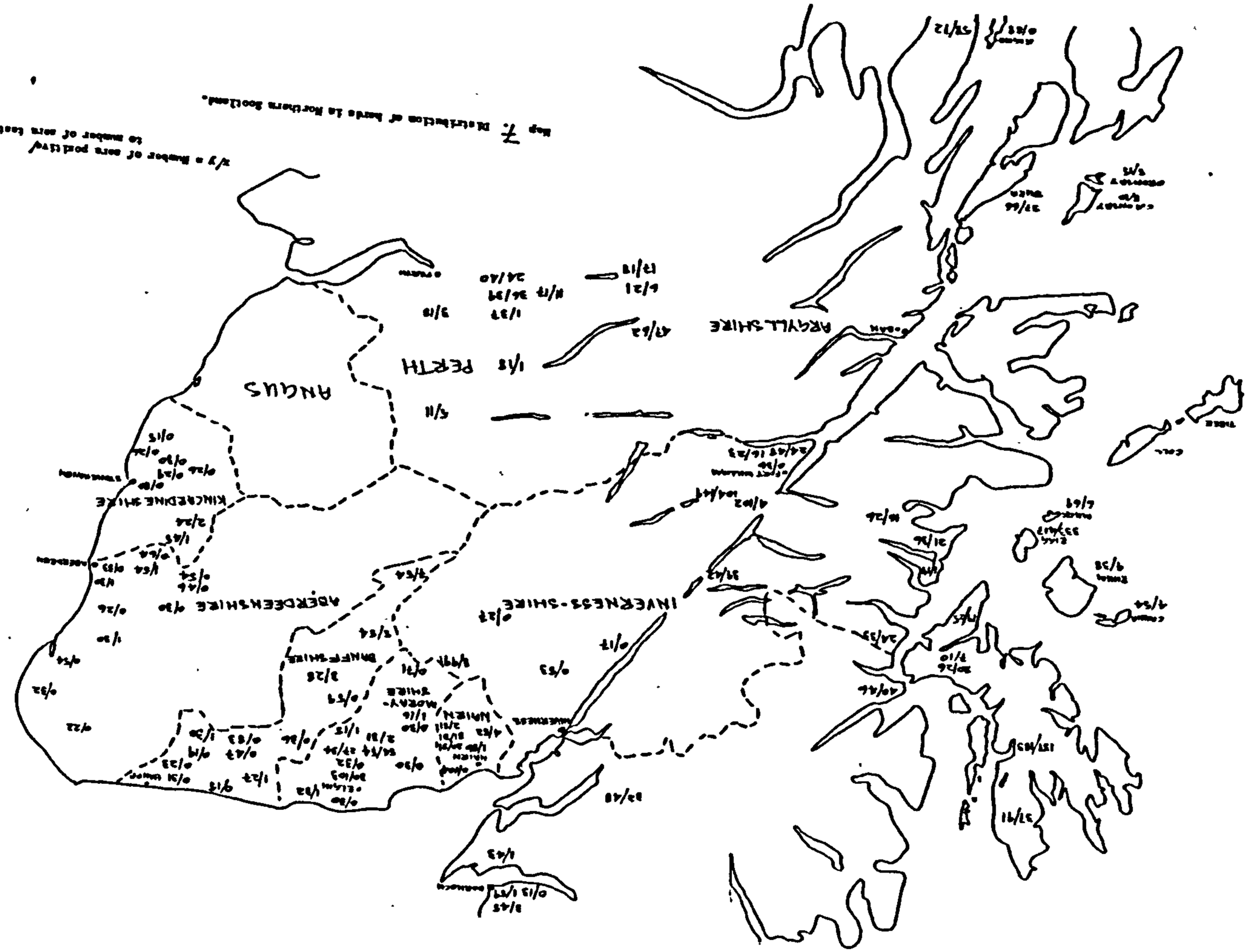
On Canna, a herd of 54 animals from 2-13 years old had only 4 individuals positive, 1 each aged 4 and 8, and 2 aged 6 years with a maximum titre of 40.

69 sera from Muck were from 2-13 year olds, but all 6 positive sera (maximum titre, 80) came from a group of 18 animals, 9 and 10 years old. This isolated group of sera-positive individuals is considered to reflect their importation rather than indicate the presence of infected ticks on Muck.

The greater movement of livestock about the mainland will also involve the risk of ascribing the disease to areas which have no such infection in the tick population or which may even be tick-free.

TABLE 51 : Jura. Summary of age and titre distributions.

	Age													
Titre	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
Zero		6		1						1			1	9
10		5		1			1		1					8
20			2					1						3
40				1	2	2			1	3	1			10
80						1			1	1			2	5
160			2		2	3	2	1	1	2			4	17
320						2			1				2	5
640								2		1	1			4
1280													1	1
2560													2	2
>2560						1				1				2
Total	11	4	3	4	9	3	4	5	9	2		12		66
GMT		23							125					



x/y = Number of acre holding
to number of acre tested

Map 7: Distribution of Barley in Northern Scotland.

Discussion.

Every county and island, bar one, (Gigha) harboured seropositive animals. The incidence of these animals decreased from the Western Isles to Aberdeenshire and Kincardineshire. However there was great variation in the incidence of positive sera within counties, e.g. a complete seronegative herd of 30 animals was found as far west as Fort William, Inverness-shire and even within this parish, 16 of 23 animals in 2 other herds were seropositive. Similarly in Glenalmond, Perthshire of 3 herds one was completely negative (0/22), the others having 1/18 and 36/39 seropositive individuals. (Map 7)

Because of the mountainous nature of the western half of the survey area, grazing is restricted to isolated areas which often have more in common with the neighbouring county than with their own, e.g. in western Ross-shire, Kyle of Lochalsh has an incidence of antibody (40/46) which is similar to Glenelg, 10 miles away in Inverness-shire and to neighbouring Broadford Parish on Skye (20/26); Whereas a herd in Tain parish in eastern Ross-shire had only 1 of 43 sera positive. In these areas it is more meaningful to use natural groupings rather than county boundaries.

The data available for individual herds was limited by the conditions pertaining to the supply of the sera and is a severe constraint on interpreting the results. However, certain assumptions can be made. Movement of cattle is considerably easier and of greater volume from region to region on the mainland than to and between the islands. The few seropositive individuals (4, 6, 8 and 10 years old) in Aberdeenshire and Kincardineshire (Table 44) cannot be presumed indicative of transmission in the herds and are assumed to have been bred and challenged elsewhere.

In the apparently transitional county of Nairn, herds fall into two classes, the majority (12) below 6% incidence of positive sera and two only with greater incidence (25% and 100%) both of which are in Glenferniss.

In both herds on Eigg the incidence of infected animals increases with age but this is not linear. There is a significant increase in incidence from the 1-4 year age group to the 5-8 and 9-13 year groups;

the rise between the two older groups is not significant. This is the form of increase expected with a constant rate of infection and this can be calculated for each age group according to the relationship $x_t = 1-e^{-ht}$ (Muench, 1959) where x is the proportion positive at time t and h is the rate of infection.

Only herd A is large enough to allow h to be calculated for each age (Table 52) but to delay the appearance of the 100% incidence level a higher threshold (titre = 80) for sera to be considered positive has been used.

Rearranging the equation gives the infection rate $h = \frac{-\ln(1-x_t)}{t}$ The mean $h = \frac{\Sigma h}{n} = 0.12$ where n = the number of age classes. There is no significant difference between the observed distribution of x_t and that calculated when $h = 0.12$ ($\chi^2 = 2.27$, $P>0.995$). For comparison with herd A, the infection rate for Skye is 0.1 ($\chi^2 = 2.14$, $P>0.995$) and for the mainland parish of Roybridge in south west Inverness-shire is 0.06 ($\chi^2 = 2.26$, $P>0.995$).

GMT increases with age in both the herds on Eigg but to a greater degree in herd A. The difference is probably related to differences in challenge levels. Although the infection rate could not be calculated for herd B by the same process used for A, a lower rate is implicit in its lower proportion of seropositive individuals. On Skye and Jura GMT also increases with age but Skye lacks the consistent increases in individual herd samples such as those in herd A on Eigg. This apparent inconsistency is considered to reflect the movement of cattle about the country which results in mixed herds where age is no indication of previous challenge. Samples from Jura were too small for calculation to be meaningful, but in mainland Inverness-shire the GMT of a herd at Arisaig rises from 3.4 (1-5 year old group) to 588 (9years and older) and demonstrates that the increase of GMT with age is not peculiar to Eigg.

TABLE 52 : Eigg, herd A. Comparison of observed proportions of positive sera with calculated distribution when $h = 0.12$

Threshold titre for positive sera = 80.

Age (=t)	1	2	3	4	5	6	7	8	9	10	11	12	≥ 13
Number of sera tested	44	23	41	37	26	18	38	22	22	17	17	14	25
Observed x_t	0.250	0.609	0.585	0.757	0.731	0.778	0.833	0.909	0.864	0.882	0.824	1.00	0.960
$h = -\frac{\ln(1-x_t)}{t}$	0.12	0.20	0.13	0.15	0.11	0.11	0.11	0.13	0.10	0.09	0.07	-	0.11
$\bar{h}$	0.12 (s = 0.033)												
$x_t = 1-e^{-\bar{h}t}$	0.113	0.213	0.302	0.381	0.451	0.513	0.568	0.617	0.660	0.699	0.733	0.763	0.790
χ^2	2.27 df = 12 P>0·995												

The incidence of infected individuals in herd A on Eigg can be explained on the basis of a constant challenge rate of 0.12 over a period of 13 years. Antibody levels continue to rise with no apparent limiting value being reached. This is in marked contrast to the levels of babesial antibody measured in these same individuals (Blewett and Adam 1978 B) which after an initial rise dropped to a lower level and remained thus from the 4th year onwards in face of continued challenge.

These data add further to the value already stressed in Blewett and Adam of age and incidence patterns in serological surveys. The feature relating to Louping-ill antibody which is in marked contrast to babesial antibody is that decline in titre with age appears not to characterise Louping-ill and this is particularly significant since the sera described here are the same sera on which Blewett and Adam (1978 A and B) reached their conclusions vis á vis Babesia.

This survey is the outcome of a unique opportunity to handle large quantities of cattle sera. While it throws considerable light on the epidemiological patterns of Louping-ill in Northern Scotland, it has to be emphasised that serological surveys provide no information on the role of the animals studied in the natural maintenance and cycling of the virus since this depends upon the levels of viraemia production in the animals and the susceptibility to infection (infection threshold) of the tick vectors.

Summary

1. The tick Ixodes trianguliceps Birula, 1895, was collected from three species of small mammal: Clethrionomys glareolus, Microtus agrestis and Apodemus sylvaticus from three localities in Scotland. I. trianguliceps was the only tick recovered in Midlothian and was most abundant at Balerno. In Argyll, Ixodes ricinus was the predominant species; I. trianguliceps was only rarely found.

2. The main trapping area was Balerno where regular collections were made from July 1974 to August 1976. Of the three hosts C. glareolus has the greatest frequency of parasitised hosts and the heaviest burden, and a greater proportion of male than female C. glareolus were parasitised with larvae and nymphs. The proportions were equal in M. agrestis and A. sylvaticus.

3. Larvae infest the hosts throughout the year. Burdens are maximal in June and minimal in July, and a second, much lower, peak of activity is reached in October. Nymphal infestation is restricted to spring to autumn with a single peak in June, while female infestation lasts from early spring until autumn, with infestation greatest in autumn.

4. The ear pinna is the area of the body most heavily infested by larvae and nymphs, particularly the inner surface with voles and the outer surface with the mouse.

5. Larvae are overdistributed on all three host species. Nymphs, and females also, are overdistributed on C. glareolus.

6. Larvae have a pre-feeding diapause while attached to hosts in winter and may remain thus from December until February.

7. Larvae engorge more quickly on the voles than on the mouse.

8. Only 9% of field captured, engorged larvae develop in the laboratory but a greater proportion of engorged larvae develop at 23° and 25°C at saturated vapour pressure than at lower temperatures. A greater proportion of larvae which fed in January to June developed than when feeding was between July and December. A pre-developmental diapause which laboratory conditions cannot overcome is proposed for all three ~~studies~~ stadia. Nevertheless, in the minority with direct development in laboratory conditions (i.e. without manifest diapause delays), the relationship between incubation temperature and developmental velocity is linear and direct.

9. In a survey of cattle in the northern counties and ~~minor~~ inner islands of the west coast of Scotland, 4529 sera were tested for immunoglobin (IgG) to Louping-ill using antigen derived from Louping-ill strain 31 (supplied by the Moredun Research Institute, Edinburgh). The broad findings are:

   (a) The incidence of antisera in the herds ~~in~~de creases from west to east.

   (b) In a stable herd structure there is a significant, steady increase in antibody with increasing age.

   (c) It is suggested that cattle are useful indicators of Louping-ill, but their role in Louping-ill maintenance remains an unknown factor.

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Appendix 1

Summary : Number of engorged larvae dropped each day after capture.

	Days after capture																
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	≥16	Total
Jan/Feb.	3	1	6	13	15	8	6	8	3	1	3	2					69
Mar/Apr.	8	17	14	18	16	10	4		1	1	1						90
May	7	10	6	11	12	3											49
Jun.	13	22	33	39	23	16	5	3									154
July	4	4	2	1	2												13
Aug.	15	36	34	21	7	3	2										118
Sep.	5	12	28	24	7	8	4										88
Oct.	26	33	56	76	66	40	20	10	7	4	3	1	1				343
Nov.	7	5	12	16	16	13	2		1	6		2	1				81
Dec.	1	1	6	9	9	9	6	5	6	5	1		1			1	60

APPENDIX 2 : Combined results for 1974 - 1976

Number of engorged larvae incubated each month and number moulting.

Incubation temperature	Class	Month Jan	Feb	Mar	Apr	May	Jun	Jly	Aug	Sep	Oct	Nov	Dec	Total	Number of days taken to develop
°C	incubated					5	11	5						21	132 132 137 137
10°	moulted						4							4	
	incubated						2		28					30	46 74 239 245 279
15°	moulted						1		4					5	
	incubated	21	48	32	13	28	108		37	88	343	81	60	859	33 34 37 42 43 51 52 52 54 55 57 58 60 60 64 65 65 73 81 81 81 82 82 82 83 83 83 104 112 112 118
19°	moulted	7	9				4		3	1			8	32	
	incubated			14	31	15								60	44 47 54 56 58 58 58 58 58 59
21°	moulted			9	1									10	
	incubated					1	15	4	26					46	33 34 34 34 35 35 35 35 36 36 36 37 37 38 40 40 44 45 45 48 50 51
23°	moulted						10	1	11					22	
	incubated						18	4	27					49	23 27 31 33 34 34 35 35 35 36 36 37 37 38 39 39 40 43
25°	moulted						5	1	13					19	
Total No.	incubated	21	48	46	44	49	154	13	118	88	343	81	60	1065	
Total No.	moulted	7	9	9	1		24	2	31	1			8	96	
percentage moulted															

Number of days taken to develop

19°c	19°c	21°c	21°c
55	64	47	44
60	81	54	
65	81	56	
65	81	58	
73	82	58	
83	82	58	
112	82	58	
	82	58	
	83	59	

23°c
48
25°c
43
June
10° 23°
132 34
132 34
137 34
137 35
15° 40
74 44
19° 45
52 45
104 50
112 51
118 25°
27
34
35
36
36

19°c
33
August
15°c 25°c
46 23
239 31
245 33
279 33
34
19° 35
34 35
37 37
43 37
23° 38
33 39
35 39
35 40
35
36
36
37
37
38
40

19°c
42
51
52
54
57
58
60
88

APPENDIX 3 : Summary : Number of engorged nymphs dropped each day after capture.

	Days after capture														Total number
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	⩾14	of nymphs
April				1	2										3
May	2	3	3	2	4	2	1								17
June	9	18	24	18	13	8	3	2							95
July	5	5	6	10	7	6	2								41
August	2	8	5	3	10	4	3	1							36
September	2	1	5	5	4	2				1					20
October	3	1	1					1		1	1		1		9
November			1						1						2
														Total	223

APPENDIX 4 : Combined results for 1974 - 1976

Number of engorged nymphs incubated each month and number moulting

Incubation temperature	Class	Month Jan	Feb	Mar	Apr	May	Jun	Jly	Aug	Sep	Oct	Nov	Dec	Total	Number of days taken to develop
°C	incubated					2	8	4						14	103
10°	moulted					1								1	
	incubated						1	11	12					24	46 47 48 52 53 58 71
15°	moulted							5	2					7	
	incubated				3	7	51	21	16	20	8	2		128	28 33 38 42 42 44 46 53 69 75 83 84 93 93 94 95 102 125 125 195 195 204 226 230 235 240
19°	moulted						8	6	2	5	3	2		26	
	incubated					2	6	1	3					14	26 32 33 34
23°	moulted					2	1	1						4	
	incubated						7	3	4					14	28
25°	moulted						1							1	
Total No.	incubated				3	11	75	40	35	20	8	2		194	
Total No.	moulted					3	10	12	4	5	3	2		39	

Number of days taken to develop

10°	19°	15°	15°	19°	19°	19°
103	93	46	47	28	102	94
	93	48	53	44	230	95
23°	125	52		46	240	
33	125	58	19°	42		
34	195	71	69	53		
	204		75			
	226	19°				
	235	33				
	23°	38				
	32	42				
	25°	83				
	28	84				
		195				
		23°				
		26				

APPENDIX 5 : Comparison of distribution of infestation levels published by Randolph (1975 B) with Poisson distributions.

LARVAE

C. glareolus

N = 314
μ = 1.0382
$Ne^{-\mu}$ = 111.1848

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	199	111.185	69.36
1	55	115.432	31.64
2	24	59.921	21.53
3	13	20.737	2.89
4	13	5.382	10.78
5	4	1.118	
9	1	0.000	
12	1	0.000	
15	1	0.000	70.56
16	1	0.000	
24	1	0.000	
30	1	0.000	

χ^2_{Total} 206.8

df= 4
P<0.005

M. agrestis

N = 126
μ = 0.8333
$Ne^{-\mu}$ = 54.7612

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	80	54.761	11.63
1	25	45.632	9.33
2	10	19.013	4.27
3	5	5.281	0.02
4	1	1.100	
6	1	0.026	
7	2	0.003	21.02
8	1	0.000	
13	1	0.000	

χ^2_{Total} 46.3

df= 3
P<0.005

A. sylvaticus

N = 653
μ = 1.8668
$Ne^{-\mu}$ = 100.9654

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	291	100.965	357.68
1	143	188.482	10.98
2	70	175.929	63.78
3	33	109.475	53.42
4	27	51.092	11.36
5	26	19.076	2.51
6	17	5.935	20.63
7	6	1.583	
8	9	0.369	
9	9	0.077	
10	3	0.014	
11	1	0.002	
12	9	0.000	944.36
13	1	0.000	
14	1	0.000	
15	5	0.000	
17	1	0.000	
34	1	0.000	

χ^2_{Total} 1464.7
df= 6
P<0.005

NYMPHS

C. glareolus

N = 39
μ = 0.3846
$Ne^{-\mu}$ = 33.8102

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	30	26.55	0.45
1	5	10.21	2.66
2	2	2.24	
3	2	0.32	0.81

χ^2_{Total} = 3.92
df= 1
P<0.05

M. agrestis

N = 18
μ = 0.0556
$Ne^{-\mu}$ = 17.0265

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	17	17.02	0.000
1	1	0.95	

df= 0

A. sylvaticus

N = 125
μ = 0.3120
$Ne^{-\mu}$ = 91.4977

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	94	91.50	0.07
1	24	28.55	0.72
2	6	4.45	
3	1	0.46	0.84

χ^2_{Total} = 1.63
df = 1
P>0.1

FEMALES

N = 134
μ = 0.0075
$Ne^{-\mu}$ = 132.9988

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	133	132.999	0.000
1	1	0.998	0.000

df = 0

N = 84
μ = 0.0833
$Ne^{-\mu}$ = 77.2863

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	81	77.286	0.18
1	2	6.438	
2			
3			1.84
4			
5	1	0.000	

df = 0

N = 330
μ = 0.1273
$Ne^{-\mu}$ = 290.5550

Burden	f_{obs}	$f_{Poisson}$	χ^2
0	293	290.555	0.21
1	32	36.987	0.67
2	5	2.354	2.97

χ^2_{Total} = 3.85
df= 1
P<0.05

Appendix 6.

Reagents.

Borate-saline, pH 9·0 (BS):

80 ml 1·5M NaCl
100 ml 0·5M H_3BO_3
24 ml 1·0M NaOH
To one litre with water.

Bovalbumin borate saline pH 9·0 (BABS):

0·4% solution of bovine albumin (Armour fraction 5) in BS.

Adjusting diluent:
8.77g NaCl
6.25g Na_2HPO_4
24.34g $NaH_2PO_4 2H_2O$
To 1 litre with water (distilled)

Plate cleaning solutions.

Acid wash:
90 ml conc. HCl in 4·5 litres of water.(distilled).

Alkali wash:
90 ml M NaOH in 4·5 litres of water (distilled).

References

Adam, K.M.G.,and Blewett, D.A. (1978)
A serological survey for Babesia in cattle in Scotland.
2. The occurrance of antibody in the population and the distribution of infected herds.
Ann. Trop. Med. Parasit. 72(5): 417 - 428.

Aeschlimann, A. (1967). Biologie et écologie des tiques (Ixodoidea) de Côte d'Ivoire.
Acta Tropica, 24: 281 - 405.

_____, Büttiker, W.,Diehl,P.A., Eichenberger, G., Immler, R. and Weiss,N.
Présence d'Ixodes triangulíceps (Birula, 1895) et d'Ixodes apronophorus (Schulze,1824) en Suisse(Ixodoidae; Ixodidae).
Rev. Suisse Zool. 77(3): 527 - 536.

Arthur, D.R. (1951).
The bionomics of Ixodes hexagonus Leach in Britain.
Parasitology, 41: 82 - 90.

_____ (1963). British ticks.
Butterworths, London.

Arzamazov, I.T. (1966).
K izucheniyu biologii Ixodes triangulíceps na Belorussia.
[The study of the biology of Ixodes triangulíceps in Belorussia]
Dokl Akad Nauk Belorusk SSR. 10(4): 290 - 292.

Brown, L.E. (1969).
Field experiments on the movements of Apodemus sylvaticus L using trapping and tracking techniques.
Oelogica, 2: 198 - 222.

Blewett, D.A. and Adam,K.M.G. (1978A).
A serological survey for Babesia in cattle in Scotland.
1. Assessment of the method by results from the outlying islands.
Ann. trop. Med. Parasit. 72(5): 405 - 415.

_____ and ____ (1978B).
A serological survey for Babesia in cattle in Scotland.
3. The rates of aquisition and loss of antibody to Babesia and their effects on observed levels of antibody incidence.
Ann. Trop. Med. Parasit. 72(6): 513 - 522.

_____ , Beasley, S.J., Campbell, J.A., Turnbull, D. McD. (1978).
A simple model for the interpretation of serological data for tick-borne diseases.
In, Tick-borne diseases and their vectors. Ed. J.K.H. Wilde.
Edinburgh University Press.

Campbell,J.A. (1948).
The life history and development of the sheep tick Ixodes ricinus L. in Scotland under natural and controlled conditions.
PhD Thesis. University of Edinburgh.

Chitty, D. and Kempson, D.A. (1949).
Prebaiting small mammals and a new design of live trap.
Ecology, 30: 536 - 542.

Clarke, D.H. and Casals, J. (1958).
Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses.
Am. J. Trop. Med. and Hyg., 7: 561 - 573.

Cotton, M.J. and Watts, C.H.S. (1967).
The ecology of the tick Ixodes triangluliceps Birula (Arachnida; Acarina; Ixodoidea).
Parasitology, 57: 525 - 531.

Djaparidze, N.L. (1960)
(Ixodid ticks of Georgia) (In Russian)
Thilisi: 1 - 295. [In Korenberg and Lebedeva, 1969].

Dunayeva, T.N., Kulik, I.L., Petrov, V.G., Nikitina, N.A. and Uglovoy, G.P. (1961).
Tezisy dokl. mezhoblastnoy nauchnopraktich. konf. po. prirod-noochagovym infektsiyam Tyumen. (No. 48), 161 - 164. (In Russian)
[In Korenberg and Lebedeva, 1969].

Dunn, A.M. (1960).
Louping-ill: The red deer (Cervus elaphus) as an alternative host of the virus in Scotland.
Brit. Vet. J., 116:-284 - 287.

Emchuk,E.M. (1960).
(Fauna of Ukraine, 25. Ixodid ticks)
1 Kiev : 1 - 162. No.s 82, 159. (In Russian)
[In Korenberg and Lebedeva, 1969].

Filipova, N.A. (1957).
Systematic groups of palaearctic ticks of the sub family ixodinae.
Biul. Mosk. Obsh. Ispytal. Prir., 62:(6): 31 - 34.

Gordon, W.S., Brownlee, E.A., Wilson, D.R. and Macleod, J. (1962).
The epizootiology of louping-ill and tick-borne fever with observations on control of these sheep diseases.
In Aspects of disease transmission ~~of disease~~ by ticks.
Symp. Zool. Soc. London, 6 : 1 - 27.

Havlik, O. (1954).
Sygnificance of increase in rodent populations in the epidemiology of Czechlovakian tick encephalitis.
J. Hyg. Epidem. (Praha), 3: 300 - 303. (In czeck).

Hoogstraal, H. (1966).
Ticks in relation to human diseases caused by viruses.
Ann. Rev. Ent., 11: 261 - 308.

Katelina, A.F. (1960).
On the distribution and biology of the tick Ixodes triangluliceps Bir. in Tula region.
Zool. Zh., 39: 1612 - 1617. (In Russian. English summary)

*Kemp.

Kikkawa, J. (1964).
Movement, activity and distribution of the small rodents Clethrionomys glareolus and Apodemus sylvaticus in woodland.
J. Anim. Ecol., 33: 259 - 299.

Korenberg, E.I. and Lebedeva, N.N. (1969).
Distribution and some general features of the ecology of Ixodes triangulíceps Bir. in the Soviet Union.
Folia Parasitologica (Praha)., 16: 143 - 152.

Lachmajer, J.(1962).
The ecology of the tick Ixodes triangulíceps Bir.,1895.
Bull. Inst. mar. Med. Gdansk. 13: 149 - 160.

Lichard, M. (1965).
Notes on the occurrence and ecology of the tick Ixodes triangulíceps Birula, 1895.
Biólogia Bratisl. 20: 348 - 358.

Lutta, A.S. (1968).
Distribution of Ixodes triangulíceps Bir in Karelia.
Parazitologiya, 2: 142 - 147.

Macleod, J. (1962).
Ticks and disease in domestic stock in Great Britain.
(In Aspects of disease transmission by ticks.).
Symp. Zool. Soc. London, 6: 29 - 50.

Morel, P.C. (1965).
Présence en France de Exopalpiger triangulíceps (Birula, 1895) (Acariens, Ixodoidea).
Ann. Parasit. hum. comp., 40: 240 - 242.

Neumann, L.g. (1901).
Révision de la famille des Ixododes. 4e Mémoire.
Mém. Soc. Zool. de France. 14: 249 - 272.

_____ (1902).
Notes sur les ixodidés.
Arch. Parasitol., Paris. 6(1): 109 - 128.

Nuttall, G.H.F., Warburton, C. Cooper, W.F. and Robinson, L.E. (1911).
Ticks: A monograph of the Ixodoidea. Part 2: The genus Ixodes.
105 - 348. Cambridge University Press.

Randolph, S.E. (1975A).
Seasonal dynamics of a host-parasite system: Ixodes triangulíceps (Acarina: Ixodidae) and its small mammal hosts.
J.Anim Ecol., 44: 425 - 449.

_____ (1975B).
Patterns of the tick Ixodes triangulíceps Birula on its hosts.
J. Anim.Ecol. 44: 451 - 474.

Reid, H.W., and Doherty, P.C. (1971).
Experimental louping-ill in sheep and lambs. 1. Viraemia and the antibody response.
J. Comp. Pathol. 81(2): 291 - 298.

Schulze, P. (1935).
Zeit. Morph. Ökol. Tier. 30: 135 - 40[illegible].

_____ (1943).
Zool. Anz. 143: 253 - 259.

Smith, C.E.G., Varma, M.G.R. and McMahon, D. (1964).
Isolation of louping-ill virus from small mammals in Ayrshire, Scotland.
Nature. London. 203: 992 - 993.

Snedecor, G.W. and Cochran, W.G. (1974)
Statistical methods. 6th Edn.
Iowa State University Press, U.S.A.

Tanton, M.T. (1965).
Problems of live trapping and population estimation for the wood mouse, Apodemus sylvaticus (L).
J. Anim. Ecol. 34: 1 - 22.

_____ (1969).
The estimation and biology of populations of the bank vole (Clethrionomys glareolus (Schr.)) and the wood mouse (Apodemus sylvaticus (L.))
J.Anim. Ecol. 38: 511 - 529

Ulmanen, I. (1972).
Distribution and ecology of Ixodes trianguliceps Birula (Acarina, Ixodidae) in Finland.
Ann. Zool. Fennici. 9: 111 - 115.

Varma, M.G.R. (1964).
The acarology of louping-ill.
Acarologica fasc. h.s. 1964: 241 - 254.

_____ and Smith, C.E.G. (1971).
The epidemiology of louping-ill in Ayrshire, Scotland.
2. Ectoparasites of small mammals (Ixodidae).
Folia Parasitologica (Praha). 18: 63 - 72.

Vysotskaya, S.O. (1951).
[On the biology of the ixodod tick Ixodes trianguliceps Bir.].
(In Russian).
Parazit. sborn. Zool. Inst. Akad. Nauk SSSR. 13: 105 - 110.

Walton, G.A. (1967).
Relative status in Britain and Ireland of Louping-ill virus.
J. Med Ent. 4: 161 - 167.

* Kemp, D.H. (1968).
Physiological studies on hard ticks (Ixodidae).
PhD. Thesis, University of Edinburgh.

I hereby declare that this thesis has been composed by myself and that the work reported in it is my own.

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