Studies on the Biology of Tipula paludosa Meigen (Diptera: Tipulidae) with special reference to Mortality Factors

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Declaration

I hereby declare that this thesis represents, except where a note is made to the contrary, work carried out by myself. It has not been previously submitted for any degree.

N.M. Barbash

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SUMMARY

This thesis describes the results of a general study of the bionomics of the British species of cranefly, Tipula paludosa Meigen in which particular emphasis was given to the identification and evaluation of the various mortality factors affecting the immature stages.

1. General Bionomics

Natural populations were observed throughout the year on a field at Lawmuir School, East Kilbride near Glasgow, Scotland, throughout the years 1984-85 and 1985-86 and estimates of the numbers of eggs, larvae and adult flies were made. Sex ratios were measured by three methods (a) from daily records of males and females obtained in emergence cages, (b) from marking and recapture observations, (c) from the examination of pupal exuviae reared from field-captured larvae. All three methods showed a preponderance of males over females.

Oviposition in the field was studied by means of sunken pots containing a sand layer covering natural turf. The efficiency of this method was tested by controlled experiments on oviposition preference in an insectary where flies were given a choice of sand/no sand and long/short grass. The results were inconclusive because with long grass more eggs were laid in the no-sand treatments whereas with short grass the reverse occurred. Magnesium sulphate, used as a flotation medium for extracting eggs from the sand or soil was found to have no effect on their viability.

Eggs were incubated on moist filter paper at temperatures ranging from 5 to 25^{o}C. and an expression relating rates of development at different temperatures was derived. The percentages hatching were similar at all temperatures within this range. Eggs kept at 30^{o}C. developed partially but died without hatching.

Larvae were sampled in the field using the "Blasdale" extractor and its efficiency was estimated as 96.86%. The growth of larvae in the field was investigated by regular sampling and was compared with that in insectary cultures. Growth rates were largely dependent on temperature and expressions relating these variables within the range 5 to 25°C were derived.

There was a close correlation between the weight of a larva of either sex and that of the pupa it produced and there was a similar close correlation between the weight of a female pupa and the fecundity of the subsequent adult.

An improved method of culturing the species was developed enabling two complete generations a year to be produced.

2. Mortality Factors

(a) Climatic

New-laid eggs kept at 0% relative humidity at 20°C. were markedly susceptible to desiccation but 4 day-old ones seemed resistant. First instar larvae were also susceptible to desiccation and here the time of exposure to a desiccating atmosphere may have been of more importance than the humidity used.

Third instar larvae kept in deoxygenated water showed 50% mortality in 5.6 hours, but a similar percentage survived 204.8 hours in oxygenated water. Third instar larvae subjected to sub-zero temperatures showed increasing mortality with both severity of frost and period of exposure. All survived for 10 hours at -2.5°C. but at -7.5°C. a 10 hour exposure caused 76% mortality and only 1% survived for 30 hrs. Larvae were able to escape from low temperatures by burrowing more deeply into the soil.

(b) **Biotic**

In two types of experiment designed to investigate the effects of population density on mortality of larvae reared from the egg, mortality was found to increase with the degree of crowding. An intraspecific density-dependent regulating factor is inferred.

Data on the rates of infection, within the Glasgow area, of Tipula iridescent Virus, Nuclear Polyhedrosis Virus, of the intestinal gregarines, Gregarina longa, Hirmocystis ventricosa and Actinocephalus tipula and of an unidentified mermithid nematode were obtained.

A single species of parasitoid tachinid, Siphona geniculata was identified and in the laboratory it was shown that the carabid beetles, Nebria brevicollis and Pterostichus madidus would feed on young larvae.

Bird predation appeared to be an important factor in the over-winter mortality of larvae at Lawmuir. In experiments carried out in 1985-86 and 1986-87 grass plots subject to bird predation showed late spring populations which were respectively approximately 62% and 36% of those where birds were excluded. This was reflected in a significantly higher yield of grass on the plots subject to bird predation. Starlings seemed to be the predominant predators.

SECTION 1

INTRODUCTION

The Tipulidae, with some 14,000 described species, is numerically the largest family of the Diptera. In Britain there are 291 species distributed amongst three subfamilies, the Tipulinae, the Cylindrotominae and the Limoniinae.

The adults, or "Craneflies", have mouthparts adapted for imbibing liquids and cause no damage. Some species feed on nectar but there is little other information on feeding habits. Probably most species take no nourishment as adults.

The larvae are hemicephalous and have well developed biting mouthparts. Their feeding habits vary greatly, some are phytophagous, some are fungivorous and a few are predatory. Their habitats are likewise varied; some are fully aquatic or inhabit mud in marshes, some live in decaying wood and others, especially of the Tipulinae, inhabit the soil.

Amongst the latter, out of the 36 species of soil-dwelling species in Britain, some nine, belonging to the genera *Tipula* Linnaeus 1758 and *Nephrotoma* Meigen 1803, occur on agricultural soils and have become pests by virtue of the damage they inflict on plants of economic importance. Their larvae are the familiar "Leatherjackets" of agriculture and horticulture. Of the pest species, *Tipula paludosa* Meigen 1830, rather inappropriately named the "Marsh Cranefly", is of outstanding importance and is the subject of the present work.

1.1. The Life Cycle and Economic Importance of *Tipula paludosa*

Adult flies emerge in the second half of summer and lay numerous eggs in the upper soil. The larvae hatch in about two weeks and start to feed on the plants. Feeding continues throughout the winter and spring, pupation occurs in early summer and the new brood of adults appears shortly after. There is, therefore, one complete generation each year with the larval stage occupying up to 10 months.

The larvae feed amongst the root system and at the base of plants just below or on the surface of the soil. During autumn and winter they feed little and grow slowly and damage is often not apparent but with the warming of the soil in spring, feeding and growth are greatly increased. Infestations may be associated with local patches of extensive plant destruction which may be particularly apparent in a dry spring when there is a retardation of plant growth.

The species is widely distributed throughout the British Isles and much of Northern Europe and is particularly abundant in regions of high rainfall and wet soils. It attacks a wide range of plants including cereals, root crops, vegetables, grassland and many herbaceous garden plants.

It is difficult to put a monetary value on the damage caused by this species. One pre-inflation estimate (1955), £500,000 per annum for damage to crops in the United Kingdom, is probably an understatement as it does not include "unseen", non-acute, damage to grassland.

Broadly speaking, the amount of damage caused is proportional to the size of the population present and decisions on whether or not to initiate control measures are usually based on estimates of larval populations in winter or early spring taken in conjunction with costs and possible benefits. Control invariably consists of blanket treatments with suitable insecticides.

Each female can lay about 300 eggs and for populations to remain stable from one year to another only two of these need survive to produce breeding adults the following year. The species is thus well adapted to withstand an annual mortality of over 99%. Numerous mortality factors have been listed, but have rarely been evaluated. They include diseases, parasites and parasitoids, vertebrate and invertebrate predators, cannibalism, freezing, drowning and dry weather in late summer and autumn.

1.2. The Present Work

The main purposes of the present work are two-fold:

First, an attempt was made to follow a typical infestation of grassland for several successive seasons and to measure the magnitude of the populations of all stages throughout the life cycle.

Second, the various mortality factors were identified, and an attempt was made to assess their importance in the general ecology of the species. (A proper understanding of the roles and modes of operation of these factors might lead to ways of harnessing them to Man's advantage and biological means of reducing the numbers of larvae to levels below the economic threshold before their period of intense feeding in spring could be of immense value to the agriculturalist).

In addition certain relevant aspects of the general physiology of the species were studied.

SECTION 2

GENERAL REVIEW OF LITERATURE

The literature on the Tipulidae and on T.paludosa is very extensive so only those aspects directly relevant to the present work will be considered here. For a summary review of the biology of the family as a whole the work of Pritchard (1983) should be consulted.

2.1. Taxonomy and descriptions

Although the adults of the pest species were clearly distinguished by early economic entomologists such as Curtis (1860), Ormerod (1890) and Theobald (1919), for many years T.oleracea was thought to be the most important pest species. However, Rennie (1916, 1917), working in Scotland, recognized the true importance of T.paludosa by rearing it through the life cycle and he was soon followed by other workers in Britain (Morrison, 1924) and on the Continent (de Jong, 1925).

Several authors provided descriptions or diagnostic tables for identifying pest species (e.g. Oldham, 1928; Audcent, 1932) but it was not until Coe et al. (1950) published their general monograph that it became possible to identify the adults of British species in general with certainty. This book, dealing with 82 of the 84 recorded British Tipulinae, is still the standard work. A valuable adjunct to it is the series of drawings of the male genitalia of Tipulinae which has been privately circulated by the Tipulid Recording Scheme and which enables the rapid identification of any male adult irrespective of its condition provided that the tip of the abdomen is intact. The precise identification of the female of some species can be difficult but, fortunately, the economic species are distinct and T.paludosa can be readily distinguished from its near relatives T.oleracea L. and

T.czizeki de Jong by its having abbreviated wings and 14 rather than 13 antennal segments (Coe et al., 1950).

The works of Chiswell (1956) on the last instar larvae of some British Tipulinae and of Brindle (1958-61) leading to his general account of the larvae and pupae of British Tipulinae have done much to facilitate the identification of the immature stages and it was Brindle (1959) who first satisfactorily distinguished between the larvae of the closely related T.paludosa, T.oleracea and T.czizeki. Blackshaw and Moore (1984) showed how to distinguish the separate instars of T.paludosa by measurement of the spiracular discs.

The eggs of T.paludosa differ from those of T.oleracea and T.czizeki in that they lack an elongate filament (Hemmingsen & Jensen, 1972).

2.2. Bionomics

Cuthbertson (1926 a,b,c,d,e; 1927, 1929 a,b) deals with many aspects of the less important species of crane-flies in the West of Scotland making useful observations on their parasites, swarming and mating behaviour. Rennie (1916, 1917, 1927) discusses the biology and economic importance of T.paludosa in the North of Scotland and Barnes (1937) gives an account of it in the south of England. Sellke (1936) and Maercks (1943a,b) deal with the biology of this, and other pest species in Germany.

Adults of T.paludosa will copulate immediately after eclosion from the pupa and may mate several times with the same or different partners (Rennie, 1916; Barnes, 1937). Egg laying starts immediately and most are laid during the first day. Gravid females are heavily burdened and, having abbreviated wings, cannot fly properly until they have discharged most of their load. There is a tendency, therefore, for most eggs to be laid near the point of emergence (Dobson, 1974).

Individual flies are short-lived (females 4 days; males 7 days) but the whole emergence period is extended over about two months e.g. from early July to early September with a peak in early August in Northern England and Southern and Mid-Scotland (Coulson, 1962; Dobson, 1973, 1974). In Southern England, however, the whole flight period is later (e.g. Barnes, 1937). There may also be differences in timing between less distant sites (Coulson, 1962; Dobson, 1974).

The larvae grow relatively slowly during autumn and winter but Dunnet (1955) noted that there is a period of rapid growth in late spring when they may increase their weight by 250-300% in three weeks.

2.3. The effects of climate on the immature stages

2.3.1. Water relations

Moisture plays an important part in the year to year variations of leatherjacket populations. Rennie (1927) recorded that early stage larvae were markedly susceptible to changes in physical conditions, particularly to dryness. When prevented from reaching the moist regions of the soil they died off rapidly. De Jong and Elize (1932) similarly reported that the mortality rate of larvae is greater in dry weather during the first weeks of larval life.

Maercks (1939a,b) showed that soil moisture was important to the larvae and that dry autumns could cause drastic reductions in populations: he concluded that the young larvae were susceptible to desiccation and considered that for development, soils containing three times their dry matter weight of water were optimal. Later (1941, 1943a, 1953) he correlated high densities of T.paludosa larvae in the spring with high rainfall during the autumn when the insects were small. According to him tipulid populations were favoured by average rainfalls of at least 60 cms./annum.

More recently Mayor and Davies (1976) found, in their survey of leatherjacket populations in south-west England, that there was a significant correlation between larval populations and rainfall in September and October.

Cameron (1945) reported heavy infestations of leatherjackets in West Lothian after two successive wet autumns and Milne _et al._ (1965) noting that there had been population "crashes" in 1955 and 1959 in the N.E. of England following unusually dry years devised an experiment in which turves artifically "seeded" with eggs and young larvae were subjected to simulated late summer and autumn rainfalls of previous years. They found that with rainfall regimes simulating the "dry" years mortalities were highest and concluded that, in nature, eggs which had been laid in such dry turf would probably fail to hatch. Their conclusion that prolonged drought during and after the oviposition period would have a catastrophic effect on population levels was supported by Meats (1968).

According to Maercks (1939b, 1941, 1943a) eggs of _T.paludosa_ are very sensitive to dryness and dry weather in autumn kills eggs laid just below the surface of the ground. The mortality of those laid in moorland soil was least when the soil contained twice its dry matter weight of water. Physiological studies on eggs carried out by Maercks (1939b), Coulson (1962) and Meats (1968) help in the understanding of some of these observations.

The larvae may also be able to cope with excessive water in their environment. According to Maercks (1943a) the third and fourth instars survived for twenty days when submerged in water covering the soil and Ormerod (1890) found that larvae immersed for 58 hours recovered after exposing them to air but all were dead by 120 hours. She estimated that death probably occurred at about 96 hours. Meats (1968, 1972), however, found that soil flooding was fatal to both eggs and larvae. Presumably the results of observations on immersion are dependent on the amount of oxygen in the water.

2.3.2. Effects of Temperature

Maercks (1941) considered that tipulid populations are favoured by mild winters and cool summers and observed decreased numbers of larvae of T.paludosa after the hard winter of 1939-40 in Germany. Many larvae were killed when water which had collected on the soil surface subsequently froze. Larsen (1949) investigated the influence of the severe winters of 1939-42 on the soil fauna at Tipperne in Denmark and attributed the reduction in the population of T.paludosa to a combination of the abnormal long periods of frost in winter and the reduced rain in May and June.

Freeman (1967) and Ricou (1967b) showed that T.paludosa larvae had a high mortality when they were exposed to temperatures below 0°C in the laboratory but Ormerod (1890) reported finding larvae "frozen until quite brittle, and yet when they thawed were perfectly active". The same authoress had some larvae artificially frozen to -10° C and, although most died, some survived apparently uninjured. De Jong and Elize (1932) found larvae to be resistant to frost and individual larvae, which had thawed out of ice, to be unharmed.

Milne et al. (1965) and Laughlin (1967) related the abundance and growth rate of T.paludosa in Northern England to climatic conditions such as temperature and rainfall and Coulson (1962) suggested that the earlier emergence of adults in northerly sites may be associated with conditions for larval growth being more favourable. Possibly northern populations have to start their life cycles earlier because low temperatures would otherwise prevent the attainment of suitably sized adults (Meats, 1975).

2.3.3. Natural Mortality Factors

Many workers have recorded the occurrence of fatal and debilitating pathogens in natural populations of Tipulidae, and, in particular, in those of T.paludosa from Scotland (Rennie and Sutherland, 1920; Rennie, 1924; Ahmed, 1968), England (Sherlock, 1973; Carter, 1976; Carter et al., 1983; Beesley, 1977) and France (Ricou & Douyer, 1975). These include viral, bacterial, protozoal and fungal diseases, parasitic Nematoda, parasitoid Diptera and various types of invertebrate predators. Although there is much detailed knowledge of these separate factors few workers (apart from Carter, et al., 1983, who demonstrated total mortalities, due to viruses, bacteria and insect parasitoids, of 10.7% and 7.7% amongst populations of larvae of T.paludosa, in a field near Liverpool for the years 1977-78 and 1978-79 respectively) have attempted to assess the separate and combined effects of them in terms of the mortality they produce and their influence in population regulation.

Birds have long been known as predators of larval and adult Tipulidae and their activities have been well-documented in various parts of the world e.g. U.S.A. - Alexander (1920), Holmes and Pitelka (1968), Hartman and Hynes (1977); Canada - Wilkinson and MacCarthy (1967); Switzerland - Matter (1982); Holland - Tinbergen and Drent (1980); U.K. - Dunnet (1955, 1956), Dunnet and Patterson (1968), Galbraith (1986). The influence of these predators on the populations of Tipulidae is largely unknown but Dunnet (1955) estimated that between 1.86 and 7% of available larvae were consumed by nesting starlings during his observations in Aberdeenshire. He also reported (1956) that leatherjackets were the main source of food of the starling in that area from late autumn until the end of the breeding season in July.

SECTION 3

THE STUDY AREA

The main site used for field observations and experiments was an enclosed area of permanent pasture 105 m. long and 32 m. broad situated at Lawmuir School near East Kilbride, Lanarkshire, Grid Ref. NS5953 (Figure 1). From east to west the area was almost level but there was a gentle slope downwards from north to south and, as drainage was poor, the area adjoining the southern border tended to become somewhat swampy in wet weather.

The field was fenced on all sides and also bordered by a hedge to the west and a hedge with some trees to the south. The adjacent fields were pastures essentially similar in type.

During the course of the experiments grazing animals were excluded as far as possible and the grass was mowed from time to time to facilitate sampling.

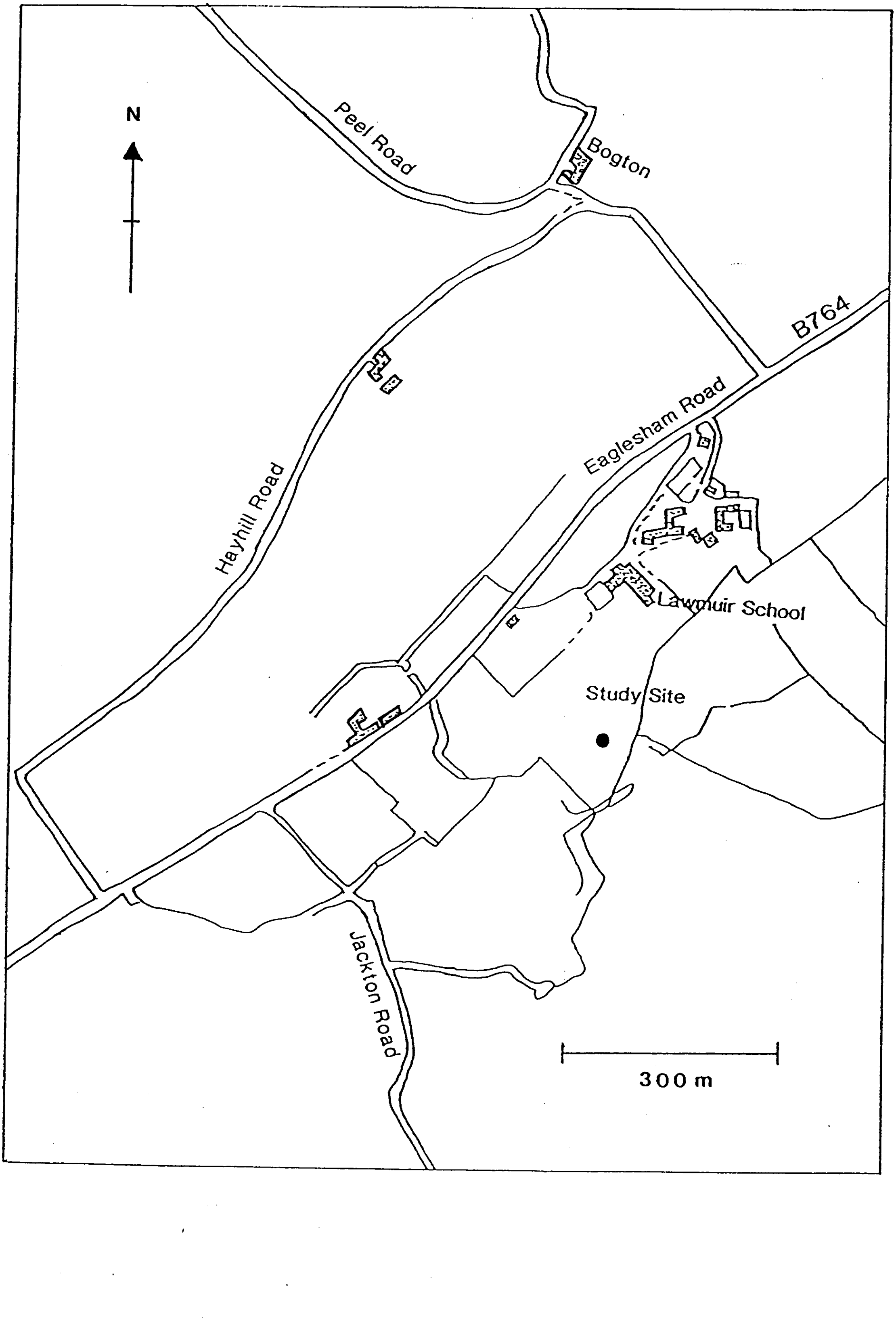
In May 1986 twenty soil cores of diameter 6.5 cm. were taken at random for botanical analysis. Plant species identified were:

(1)	*Poa pratensis*	(Smooth meadow grass)
(2)	*Lolium perenne*	(Perennial Rye grass)
(3)	*Ranunculus repens*	(Creeping butter-cup)
(4)	*Trifolium repens*	(White clover)

Maercks (1939a) showed that the larvae of *T.paludosa* develop best on white clover and grow well on most grasses except oats. The sward at Lawmuir appeared to provide a satisfactory diet.

Less detailed observations were also made at two Glasgow University farms: Garscube, near Bearsden in the N.W. of the city and Cochno, near Duntocher about seven miles west.

Figure 1. Map showing the location of study site


N
Peel Road
Bogton
B764
Eaglesham Road
Hayhill Road
Lawmuir School
Study Site
Jackton Road
300 m

SECTION 4

QUANTITATIVE STUDIES ON FIELD POPULATIONS OF T.PALUDOSA

The populations of T.paludosa were followed throughout the life cycle on the field at Lawmuir during the seasons 1984-1985 and 1985-1986, estimates of the numbers of eggs, larvae and adult flies being made.

4.1. Studies on adult flies

4.1.1. Introduction

The great diversity in the life-cycles, behaviour and habitats of different insect species has led to a corresponding diversity in the techniques used for the quantitative estimation of their populations in the field.

Morris (1960) has stressed the importance of defining the objectives of sampling clearly as there is an important distinction between sampling to estimate population density and sampling to investigate other aspects such as the degree of parasitism, or the developmental stages present.

Most species of adult Tipulidae are highly mobile and, as with many ecological studies of non-sessile animals, there is difficulty in obtaining reliable absolute measures of their population density. Hitherto, sampling techniques have included the use of sweep nets, capture-recapture, emergence, light, suction and sticky traps and the counting of vacated pupal exuviae (Southwood, 1978).

In the present observations the populations of adults were studied during the summers of 1984, 1985 and 1986 at the Lawmuir site.

4.1.2. Methods and materials

Two methods of estimating populations were used. Each year emergence cages were set out during the whole of the expected flight season of the adults and in 1984 and 1985 this was supplemented by capture-recapture observations. Both methods have their limitations: emergence cages may affect the microclimate of the area enclosed and hence influence the phenology of the organisms within them (Southwood & Siddorn, 1965) and insects may remain deep in the vegetation and escape detection. Capture-recapture techniques depend on the marked specimens mingling freely with the unmarked ones and they are much influenced by emergences, deaths, immigration and emigration of the species concerned.

4.1.3. Emergence cages

In 1984 a rectangular area of 100m. x 30m. was marked out and, after mowing the whole area and removing the cut grass, ten emergence cages were set out at randomly selected points within it just before the emergence of the first flies was expected. The cages were made by sinking four wooden stakes into the ground in the form of a square 50cm. x 50cm. and enclosing the staked area with a cover made from nylon curtain netting of hole size 2mm. This was sufficiently fine to contain the flies but was open enough to allow observations to be made through it. No meteorological observations were made in these cages which were 15cm high but it is thought that the effects on temperature, humidity, light intensity and wind speed would be less than those recorded in the field cage experiment of Dobson _et al._ (1958) where a smaller mesh was used.

In 1985 and 1986, to ensure a more even coverage of the area under observation a rectangle of 50m. x 20m. was marked out and then subdivided into a 2 x 5 array of 10m. x 10m. plots each of which was identified by a letter ranging from A - J. In each plot two emergence cages were set out at random (Figure 2) and, to avoid some of the difficulties experienced in managing and maintaining those used in

1984 new cages were devised. The frames of these were made from heavy duty wire sheep fencing which, when cut off in suitable lengths, could be transformed into very robust cages by simply turning down the edges to form bottom-less boxes 50 x 50 x 15cm. The cut-off ends were left projecting to form prongs which could be pushed into the ground (Figure 3). As before, the framework of each cage was covered with fine netting secured by string.

Each year the traps remained in position throughout the emergence period and were inspected daily between 10.00 and 12.00 hours, the numbers of males and females being noted separately.

4.1.4. Results from observations with emergence cages

The numbers of males and females caught in the daily observations in the emergence cages and their accumulated numbers are given in Appendix I Tables A1, A2 and A3 for 1984, 1985 and 1986 respectively. These are shown also in Figures 4, 5, 6, 7, 8, 9 and summarised in Table 1.

TABLE 1

Data from emergence cages summarised for 1984, 1985 & 1986

Sex	Year	Totals caught	Date of first catch	Date of 50% of catch	Date of last catch
Male	1984	84	25/7	19/8	7/9
	1985	21	5/8	18/8	30/8
	1986	178	20/7	20/8	4/9
Female	1984	47	29/7	21/8	14/9
	1985	9	10/8	26/8	10/9
	1986	65	29/7	19/8	29/8

Figure 2. Distribution of emergence cages in the field

Figure 3. Emergence cage construction in the field

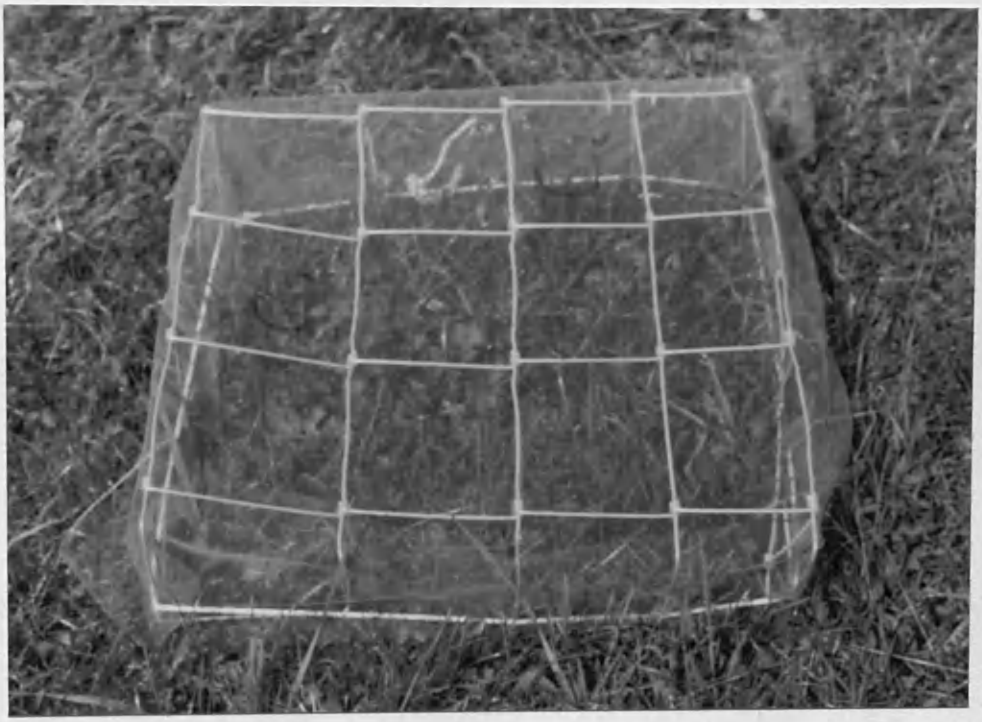


Figure 4. The number of adult males and females emerged daily from 10 emergence traps in 1984.

Solid line with triangles = Males

Broken line with circles = Females

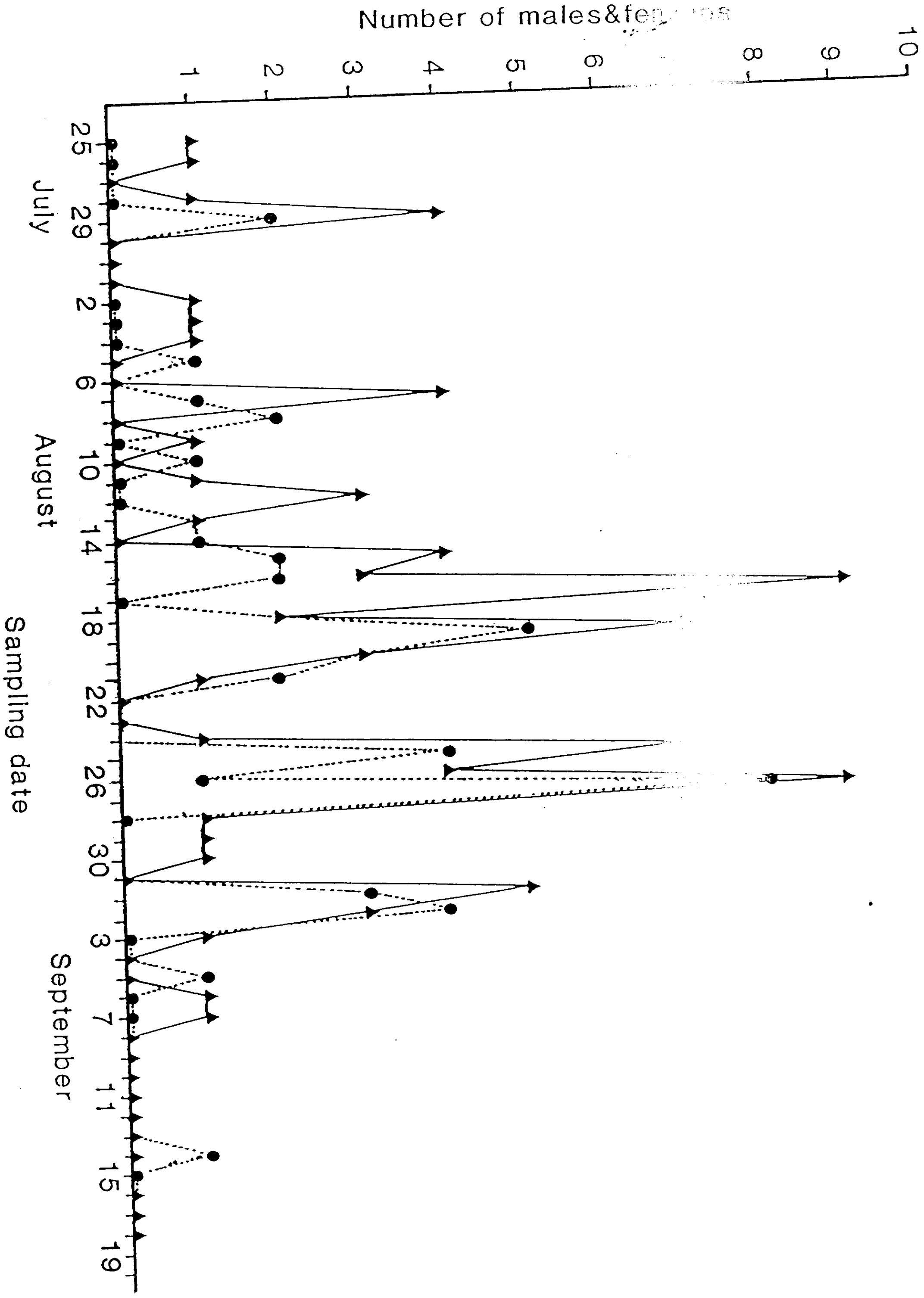

Number of males&females
1
2
3
4
5
6
8
9
10
25
29
July
2
6
10
August
14
18
22
26
30
Sampling date
3
7
September
11
15
19

Figure 5. The accumulative percentage emergence of male and female adults from emergence cages in 1984.

Solid line with triangles = Males

Broken line with circles = Females

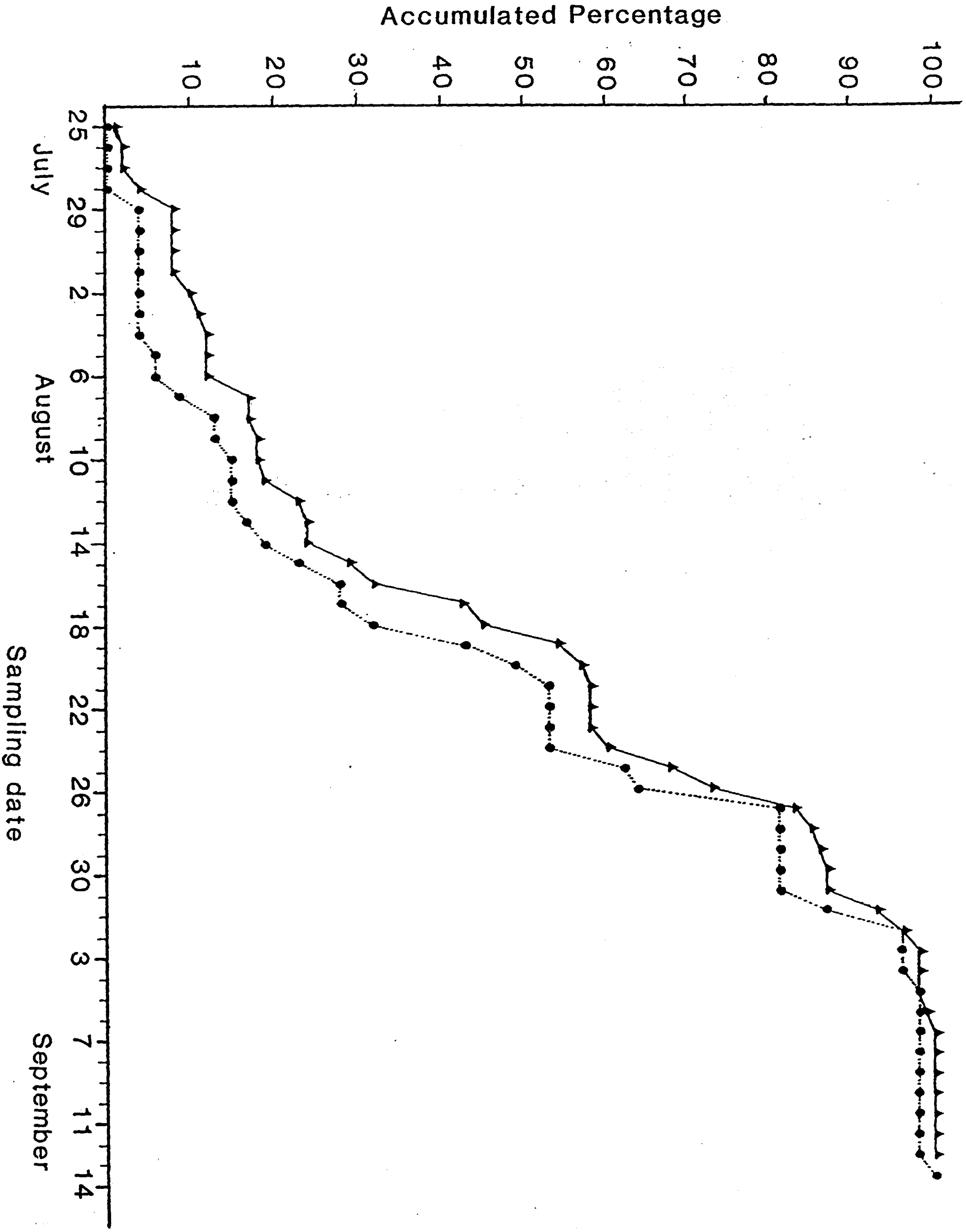

Accumulated Percentage
10
20
30
40
50
60
70
80
90
100
25
29
2
6
10
14
18
22
26
30
3
7
11
14
July
August
September
Sampling date

Figure 6. The number of adult males and females emerged daily from 20 emergence traps in 1985.

Solid line with triangles = Males

Broken line with circles = Females

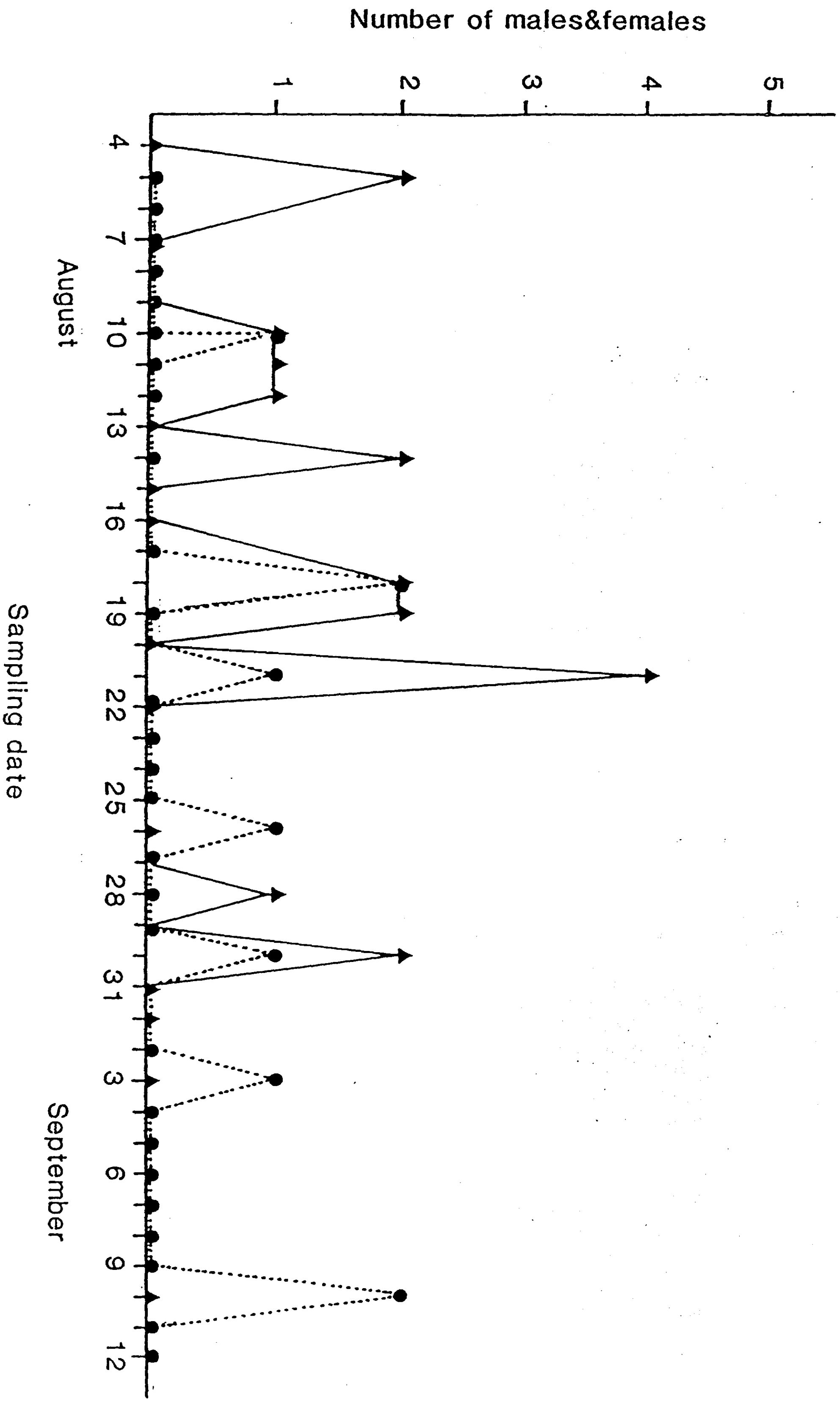

Number of males&females
1
2
3
4
5
4
7
10
13
16
19
22
25
28
31
3
6
9
12
August
September
Sampling date

Figure 7. The accumulative percentage emergence of male and female adults from emergence cages in 1985.

Solid line with triangles = Males

Broken line with circles = Females

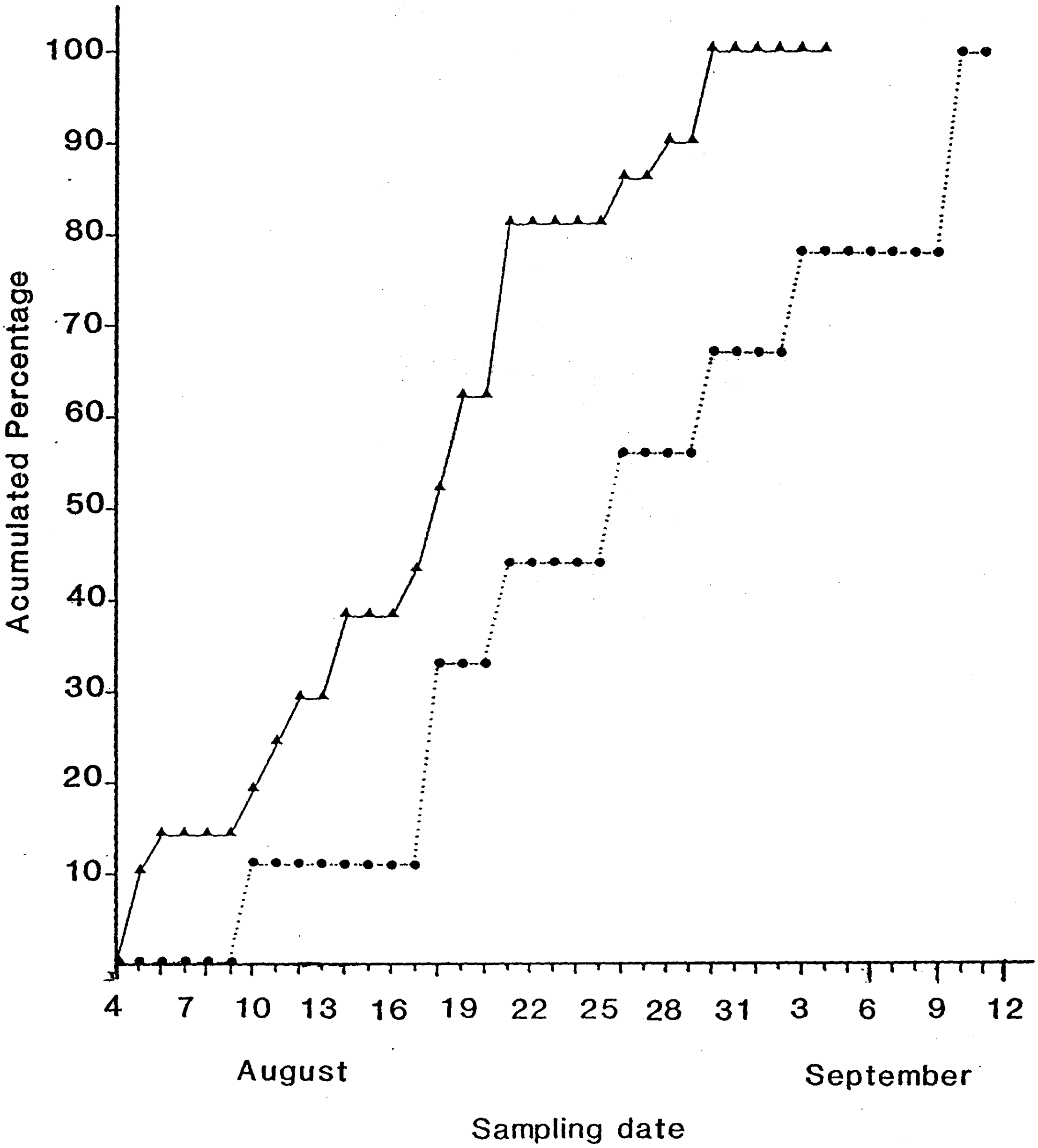

Acumulated Percentage
100
90
80
70
60
50
40
30
20
10
4
7
10
13
16
19
22
25
28
31
3
6
9
12
August
September
Sampling date

Figure 8. The number of adult males and females emerged daily from 20 emergence traps in 1986.

Solid line with triangles = Males

Broken line with circles = Females

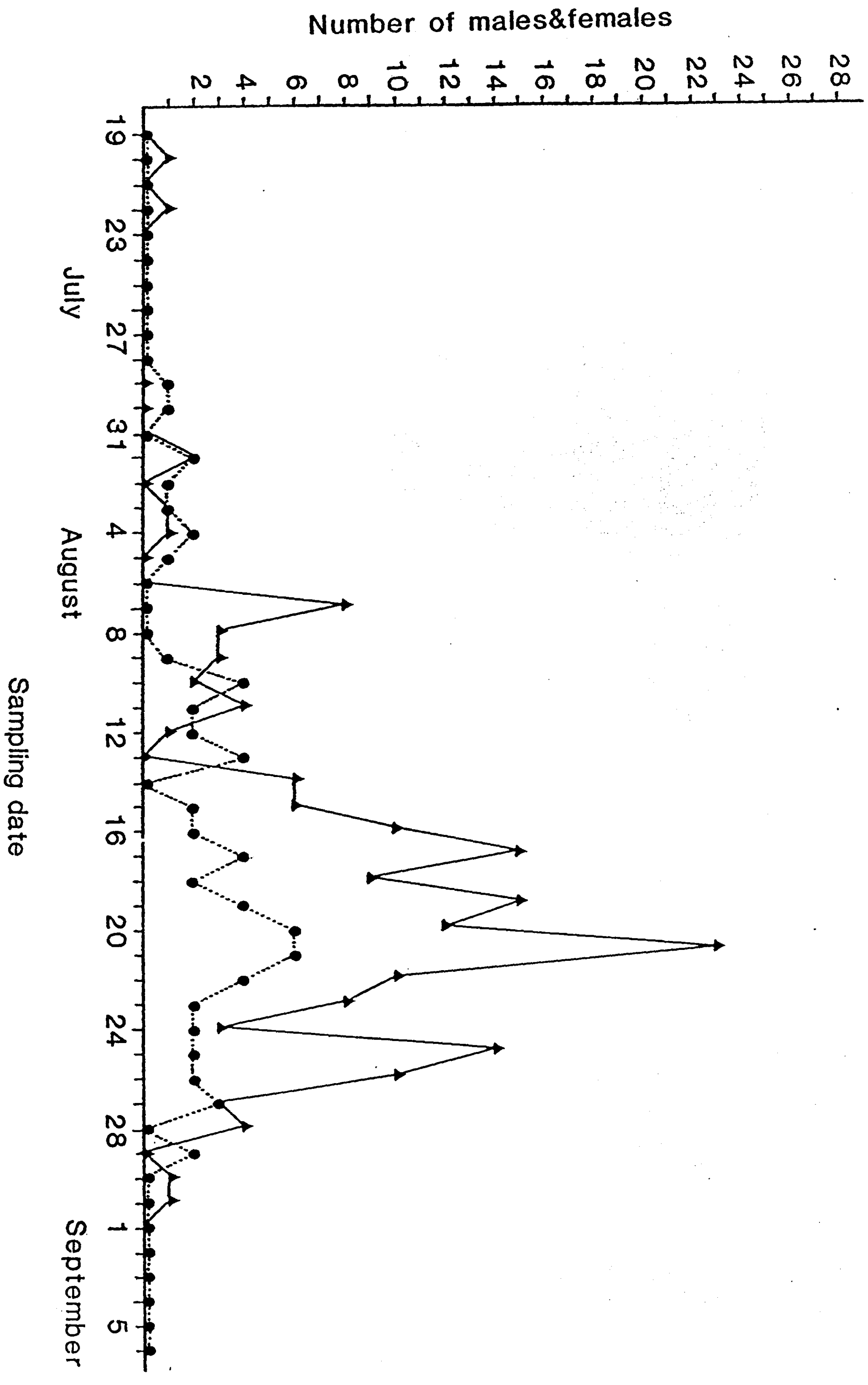
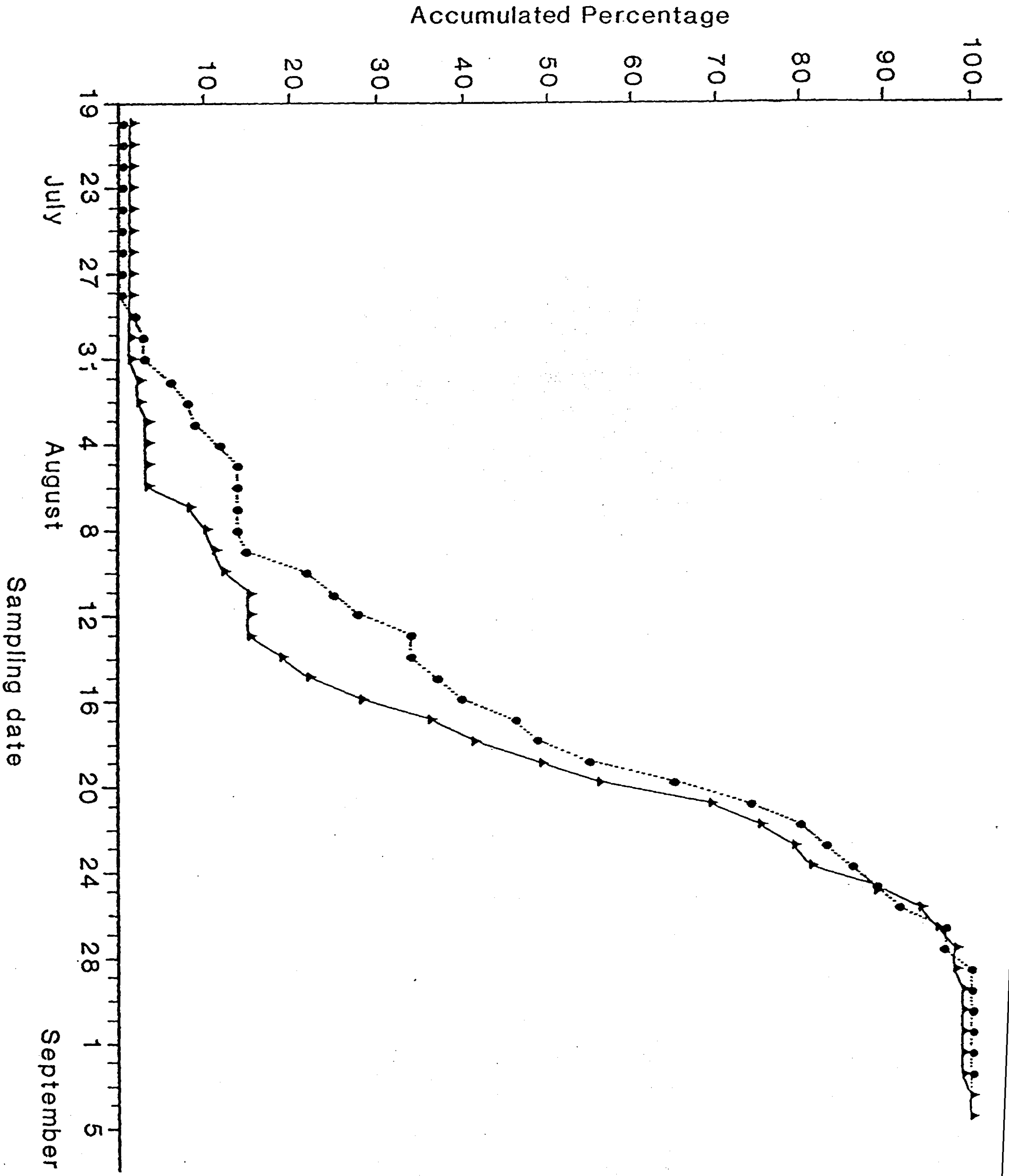

Number of males&females
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Sampling date
July
August
September
19
23
27
31
4
8
12
16
20
24
28
1
5

Figure 9. The accumulative percentage emergence of male and female adults from emergence cages in 1986.

Solid line with triangles = Males

Broken line with circles = Females


Accumulated Percentage
10
20
30
40
50
60
70
80
90
100
19
23
27
31
4
8
12
16
20
24
28
1
5
July
August
September
Sampling date

In 1984 the catches for all traps were grouped together to give daily totals for each sex but in 1985 and 1986 the catches on the individual plots were recorded separately so that sampling errors could be estimated. Table 2 shows estimates of the mean total numbers of flies emerging from the study area/square metre for each year and, for 1985 and 1986, the means minus and plus one standard error. The original counts showed asymmetry in distribution, this being particularly evident in the 1985 data where counts were very low, so the analyses were made using square root ($\sqrt{x + 0.5}$) transformation and the figures quoted are the detransformed values.

TABLE 2

Estimates of mean population density of adults on Lawmuir field in 1984, 1985 & 1986

Year	Sex	Mean pop. density (flies/sq.m.)	Mean - 1 S.E.	Mean + 1 S.E.
1984	Male	33.60	N.A.	N.A.
	Female	18.80	N.A.	N.A.
1985	Male	3.47	2.35	4.76
	Female	1.50	0.96	2.11
1986	Male	34.43	30.28	38.85
	Female	12.49	11.15	13.89

4.2. Sex Ratios in T.paludosa

4.2.1. Introduction

The sex ratios of adult insects in natural populations are often difficult to determine because of differences in behaviour, time of emergence, life span and distribution of the two sexes and this is most evident in the published studies of Tipulidae where sex ratios have been measured by various means. For instance, Dobson (1974), in a study of the spatial distribution of flying Tipulinae in Scotland, determined the sex ratios of adults taken in suction traps and found that, taking all observations into consideration, in 20 out of the 21 species of Tipula and Nephrotoma where both sexes were taken, the males outnumbered the females, sometimes by a factor as high as 26:1. Similar preponderances of males were found by Lovibond (1937) and Robertson (1939) using light traps.

The most accurate estimates of the sex ratio of insects in a given area can probably be made by sampling over the entire area throughout the whole of the emergence season and by using methods which are, as far as possible, independent of bias caused by differences in distribution and activity. Barnes (1937) solving this problem by rearing samples of larvae of T.paludosa to maturity in the laboratory, found an unbiased sex ratio (M:F) of 1.64:1, and Coulson (1962), working on the same species, found a ratio of 1.72:1 based on the collection of pupal exuviae from the field throughout the whole of the emergence period. Freeman (1964) measured the head-capsules of 4th instar T.luna Westoff larvae and found a sex ratio of 2:1 (M:F).

4.2.2. Materials and Methods

In the present studies sex ratios were derived from three sources:

1) The daily records of males and females obtained throughout the emergence periods in emergence cages.
2) The data obtained from the capture-recapture observations.
3) The examination of pupal exuviae bred out from larvae taken from the field.

4.2.3. Results and Discussion

The numbers of insects observed in these observations and their sex ratios are shown in Table 3. Clearly the data from the capture-recapture observations varied widely between the two years (chi square: 16.4 1 d.f. $P<0.001$) and they are presumably much influenced by the differences in behaviour, activity and life span of the sexes. Females are partially brachypterous and when gravid can barely fly and spend most of their time amongst the grass whereas the males are fully active from the outset. Females are also less long-lived than males (Barnes, 1937). Figure 10 shows adult male and gravid female T.paludosa in copula in the field.

TABLE 3

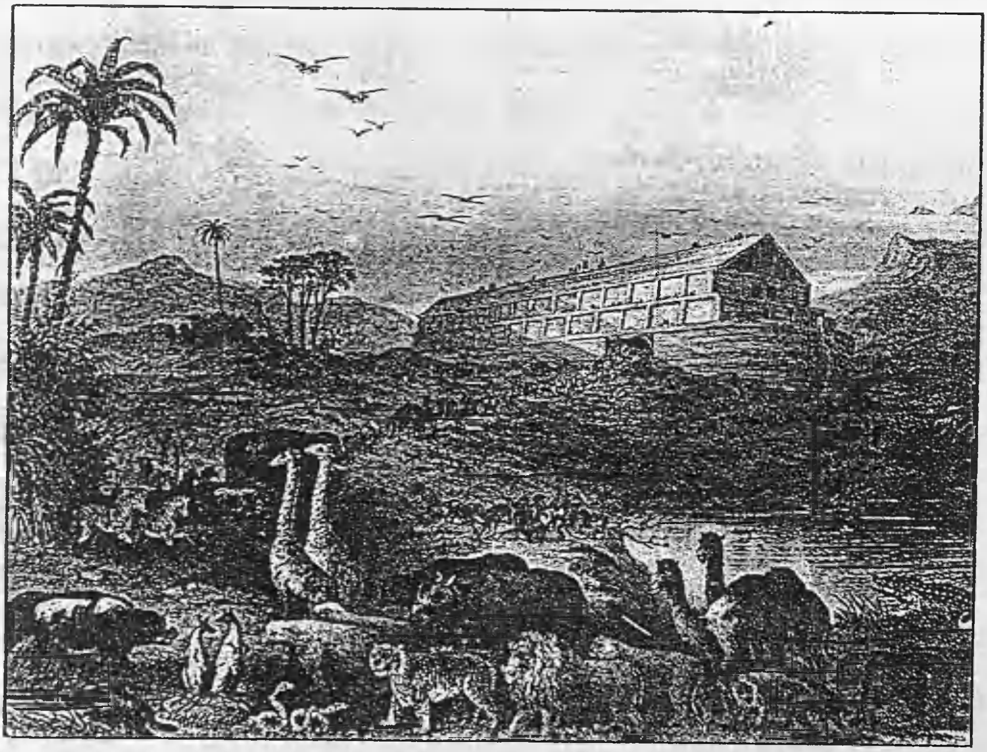
Numbers of adults of T.paludosa observed and their sex ratios

	Year					
	1984		1985		1986	
Type of Observation	Male	Female	Male	Female	Male	Female
Emergence cages	84	47	21	9	178	65
	(1.79 : 1)		(2.33 : 1)		(2.74 : 1)	
Capture-recapture	3270	703	455	53	N.A.	
	(4.65 : 1)		(8.58 : 1)			
Pupal exuviae	N.A.		65	36	271	168
			(1.81 : 1)		(1.61 : 1)	

The data from the emergence cages are probably much more representative of the true sex-ratio. They were consistent from year to year (chi square: 3.38, 2 d.f. $0.10<P<0.20$) but are still likely to

Figure 10. Adult male and gravid female T.paludosa in copula in the field

Figure 11. Noah knew a thing or two: The ideal sex-ratio is one to one (New Scientist 1985)



be influenced by behavioural differences because females are more difficult to find than males. The figures may be combined to give an overall ratio 2.34:1.

The most reliable estimate is probably that obtained from the pupal exuviae. Here the data were wholly consistent from year to year (chi square: 0.14, 1 d.f. $P>0.70$) and are best combined to give an overall sex ratio of 1.65:1 which is almost identical to the 1.64:1 ratio observed by Barnes (1937), based on bred-out material and closely similar to the 1.72:1 ratio obtained for this species by Coulson (1962) who collected pupal exuviae in the field.

The reduction in the numbers of adult females at eclosion has been attributed to there being higher mortality amongst female late-instar larvae than amongst male ones particularly during the final stage (Coulson, 1962; Butterfield, 1973; Pritchard, 1976). The biological significance of this has not yet been explained (Fig. 11).

4.3. Studies on Eggs

4.3.1. Materials and Methods

An accurate direct estimate of the numbers of eggs laid in grassland is probably impossible due to the difficulties of separating them from the soil and vegetation in which they are laid. To overcome this problem special oviposition sites were prepared in the following way.

On each of the ten plots of the experimental area five plastic plant pots, of diameter 11cm., were scattered at random, each being inserted into a hole dug in the soil so that its rim was flush with the surface. The turf removed when digging the hole was replaced in the pot but a space of 2.5cm. was left at the top so that a thin layer of white sand could be added (Figures 12 and 13). The site of each pot was then made to look as "natural" as possible. Females of <u>T.paludosa</u>

Figure 12. A diagram showing the position of plant pot in the ground

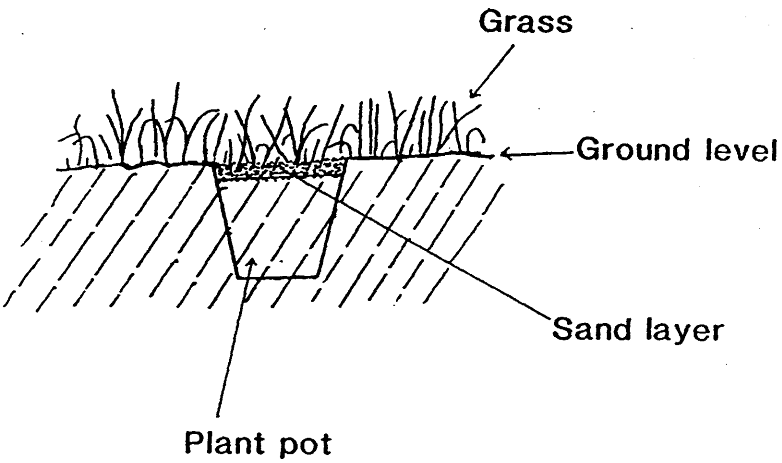

Grass
Ground level
Sand layer
Plant pot

Figure 13 showing the plastic pot and its hole in the field.

place only the tips of their ovipositors in the soil when ovipositing (Cuthbertson, 1929a; Coulson, 1962; personal observation) and it was hoped that they would accept the sand in place of the natural soil surface. The sand layer could easily be removed from each pot by tipping it into a plastic container and eggs could be removed from the sand by flotation in magnesium sulphate solution of specific gravity 1.250.

The pots were placed in position in early July in 1984, 1985 and 1986 just before emergence of the flies was expected, and they were examined and the eggs were extracted at weekly intervals until October. For reasons concerned with the availability of transport sampling had always to be carried out on Sundays so that in each year it started one calendar date earlier. However, to avoid undue complication, the results are presented with the weeks dated according to the intermediate year, 1985.

In 1984 the sand from all fifty pots was mixed to give one composite sample for each date but in subsequent years, that from each plot (i.e. amalgamated for the five separate pots) was extracted separately to enable estimates of sampling errors to be obtained.

4.3.2. Results

The numbers of eggs recovered each week from the samples during the three years are shown in Table 4 and the detailed plot counts for 1985 and 1986 are given in Appendix II Tables B3, B4, B6 and B7.

As explained previously the data for all plots were bulked together in 1984 and no errors can be attached to the estimates for that year. However, the data for 1985 and 1986 (Appendix II Tables B3, B4, B6 and B7) can be examined further.

TABLE 4

Weekly totals of eggs recorded on Lawmuir plots

Week ending	Numbers of eggs found		
	1984	1985	1986
28 July	0	0	10
4 August	12	0	19
11 "	47	33	27
18 "	646	50	222
25 "	698	81	379
1 September	250	129	268
8 "	64	109	148
15 "	29	55	159
22 "	7	32	22
29 "	6	14	11
6 October	0	0	0
Totals	1759	503	1265

The original counts showed marked assymmetry in their distribution so were examined after square root transformation $\sqrt{(x + 0.5)}$ of the original counts for each date. Table 5 shows the detransformed means for each date and the detransformed means ± one standard error expressed as the numbers of eggs/square metre. Similar values are given for the totals for each season. These values are expressed graphically in Figures 14 and 15 the latter showing the accumulated totals during each season.

TABLE 5

Numbers of eggs/square metre

(Calculated after square root transformation)

Week ending	1985			1986		
	Mean	Mean ± S.E.		Mean	Mean ± S.E.	
28 July	0	-	-	14	24	7
4 August	0	-	-	34	45	24
11 "	67	76	58	44	63	28
18 "	99	117	83	442	515	374
25 "	152	190	118	777	861	698
1 September	213	295	144	532	622	449
8 "	140	227	73	298	341	257
15 "	101	129	75	331	352	310
22 "	62	76	49	40	52	28
29 "	20	32	9	19	27	12
6 October	0	-	-	0	-	-
Totals	854	1142	609	2531	2902	2187

Figure 14. Number of eggs/square metre in 1985.

Closed circles = Mean number

Open circles = Mean number - st. error

Triangles = Mean number + st. error

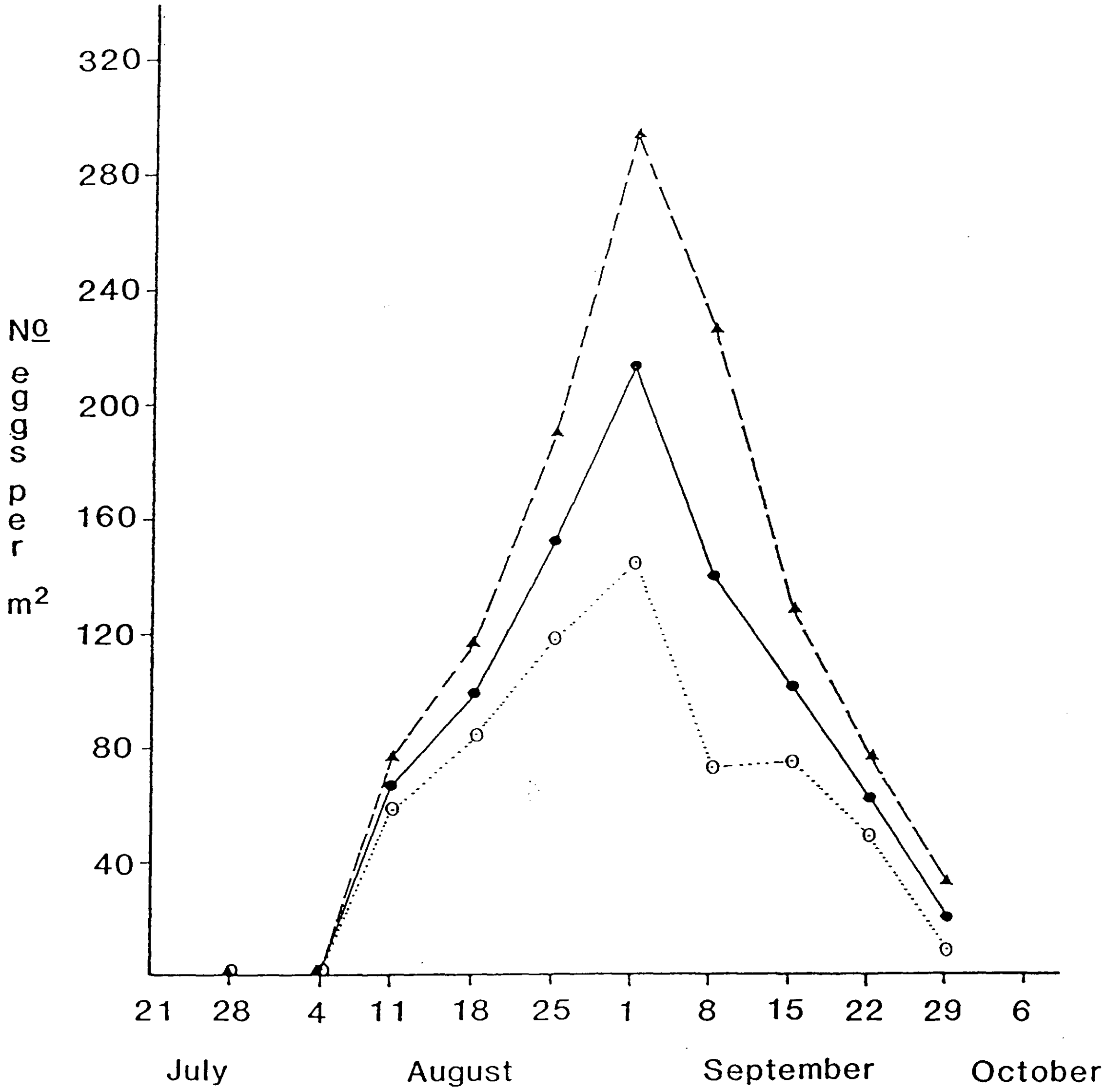
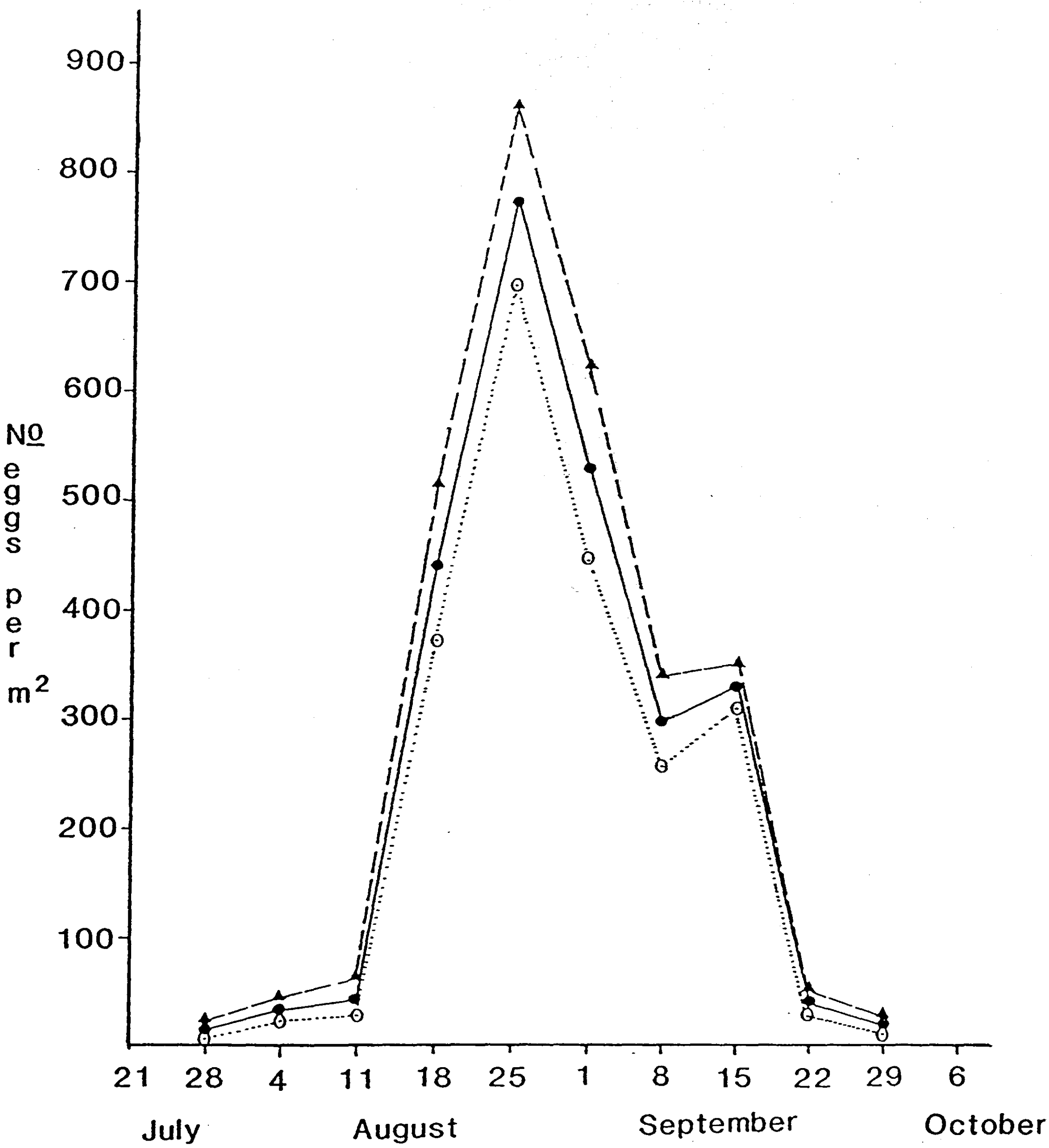

No eggs per m²
320
280
240
200
160
120
80
40
21
28
4
11
18
25
1
8
15
22
29
6
July
August
September
October

Figure 15. Number of eggs/square metre in 1986

Closed circles = Mean number

Open circles = Mean number - st. error

Triangles = Mean number + st. error



900
800
700
600
500
400
300
200
100
Nº eggs per m²
21
28
4
11
18
25
1
8
15
22
29
6
July
August
September
October

SECTION 5

TEST OF TECHNIQUE OF STUDYING EGG OVIPOSITION

5.1. Oviposition preference of **T.paludosa**

5.1.1. Introduction

In the field observations on oviposition it is possible that the technique of allowing the flies to lay in sand might have introduced errors into the estimates as it might have influenced the ovipositional behaviour of the flies. It is also possible that the behaviour might have been affected by the length of the grass. (Jones & Jones, 1984, advocate the close grazing of leatherjacket areas in September as craneflies accumulate on the long growth and oviposit there).

5.1.2. Materials and Methods

To test the effects of sand/no sand and long/short grass on oviposition a 2 x 2 factorial experiment was set up in the insectary. The treatments were:

1. No sand + 20cm. grass
2. Sand + 20cm. grass
3. No sand + 5cm. grass
4. Sand + 5cm. grass

Each treatment "plot" consisted of a perforated plastic tray 21cm. long, 17cm. wide and 5cm. deep containing a closely fitting piece of turf taken from the field at Lawmuir. Each turf was thoroughly washed with a jet of water before being placed in its tray to ensure that there were no eggs present at the outset and then the treatments were applied, care being taken to ensure that the upper surface of the soil was level with the top of each tray. The treated trays were arranged at random within a large tray containing water, to keep the soil moist, in 2 x 2 blocks, each containing all four treatments. In all, four blocks were set up arranged into a 2 x 8 rectangle with no spaces between continuous treatments (Fig. 16).

The whole experiment was enclosed in a cage of fine nylon netting (Fig. 17) and 25 gravid female T.paludosa and an equal number of males, caught in the field, were released into and allowed to remain within the cage for four days. Mating was seen amongst these shortly after their release. At the end of the four day period, the trays were examined for eggs using the following technique.

The grass was cut short and all dead flies were removed and then each tray was immersed separately in a container of saturated Magnesium Sulphate solution and the soil surface was gently, but thoroughly, agitated. Finally, the soil was broken down by hand and the vegetation was separated out. Eggs floating to the surface were removed by means of a Pasteur pipette and were counted.

The entire experiment was repeated three times using the same design on each occasion.

5.1.3. Results

The numbers of eggs found are recorded in Table 6. A general Analysis of Variance (Table 7) showed that the effects of the treatments, Sand/No sand (S) and 20cm./5cm. grass (H) were slight but that there was a marked interaction (S x H) between the two types of treatment indicating that their effects were different in different combinations. There was also a marked difference between the separate experiments but this was probably explained by the greatly differing total numbers of eggs laid in them and does not affect the assessment of treatments.

To investigate this further it was necessary to consider the treatments in isolation. The full analyses are given in Appendix III Tables C1, C2, C3 and C4 and in summary form in Table 8 below.

Figure 16. Randomization of trays in the cage

Figure 17. Experimental cage in the insectary

3	2	3	1	1	2	4	2
1	4	4	2	4	3	1	3
Block A		Block B		Block C		Block D	

1 - Grass 20 cm Turf only
2 - Grass 20 cm Turf + layer of sand
3 - Grass 5 cm Turf only
4 - Grass 5 cm Turf + layer of sand

On the plots not treated with sand almost twice as many eggs were laid in 20cm. grass as in 5cm. grass and this led to the high F value observed (22.06, P < 0.01). There was also an appreciable Grass Height x Experiment interaction but as this was caused by variations in the magnitude of the differences observed in the three experiments (Table 9) there was no cause to dispute the hypothesis that more eggs are laid on longer grass.

TABLE 6

The Numbers of Eggs Found in the Three Oviposition Preference Experiments

Treatment (S)		Sand		No Sand	
Treatment (H)		20cm. grass	5cm. grass	20cm. grass	5cm. grass
Block	Exp.				
A	I	149	98	397	168
	II	58	39	151	89
	III	72	94	83	109
B	I	141	214	299	115
	II	154	315	69	40
	III	95	43	208	40
C	I	85	180	175	80
	II	99	103	37	19
	III	67	140	73	45
D	I	153	267	328	170
	II	67	30	58	52
	III	70	91	123	108

TABLE 7

General Analysis of Variance of Oviposition Preference Experiment Data

Source	df.	Sum of square	Mean square	Var. rat.	P.
Treat. (S)	1	936.33	936.33	0.27	
Treat. (H)	1	6580.08	6580.08	1.88	
Blocks (B)	3	17278.00	5759.33	1.64	
Expt. (E)	2	106671.79	53335.89	15.22	<0.001
Int.S x H	1	39102.08	39102.08	11.16	<0.01
Int.H x B	3	5510.92	1836.97	0.52	
Int.B x E	6	24977.88	4162.98	1.19	
Int.S x B	3	29030.01	9676.67	2.76	
Int.S x E	2	19952.05	9976.03	2.85	
Int.H x E	2	6163.80	3081.90	0.88	
Residue	23	80612.73	3504.90		
Total		336815.67			

TABLE 8

Summary of Separate Analyses of Oviposition Preference Experiment

Comparison		Between Grass Heights		Between Sand & No Sand	
		Sand	No Sand	20 cm grass	5 cm grass
Source	df.	Variance Ratios			
Sand + (S)	1	-	-	14.39**	3.96
Grass Ht. (H)	1	2.15	22.06**	-	-
Blocks (B)	3	1.86	5.42*	3.35	0.43
Expts. (E)	2	3.91	28.91***	22.48**	4.45
H x B Int.	3	0.59	1.07	-	-
B x E Int.	6	2.09	1.17	1.49	1.58
S x B Int.	3	-	-	1.57	2.49
S x E Int.	2	-	-	9.63*	0.46
H x E Int.	2	0.29	6.38*	-	-
Residue	6				

* = $P<0.05$ ** = $P<0.01$ *** = $P<0.001$

TABLE 9

Eggs found on plots without sand according to grass heights

		Grass Height	
		20 cm	5 cm
	I	1199	533
Expt.	II	315	200
	III	487	302

Also, in the observations on 20cm. grass, there were apparently more eggs laid on the plots without sand than on those with sand. However, in this instance, the high Sand/no sand x Experiment interaction suggests that this difference should be disregarded as the direction of the difference was reversed in one of the three experiments carried out (Table 10).

TABLE 10

Eggs found on 20 cm grass according to sand or no sand

		Sand	No sand
	I	528	1199
Expt.	II	378	315
	III	304	487

These experiments failed to produce the information that they were designed to provide and an unexpected aspect of them was the low numbers of eggs recovered. With 25 fully gravid females used each time, numbers in excess of 7,500 were expected in each experiment. In reality only Experiment I, with 3,019 eggs found approached expectation, and many of the plot totals could have been the production of single individuals. To obtain convincing results, the experiment should, perhaps, be repeated with much larger numbers of females.

I am grateful to Mr J. Bruce for help with the execution of these experiments.

5.2. The Effects of Saturated Solutions of Magnesium Sulphate on Newly-laid Eggs of T.paludosa

5.2.1. Introduction

One of the routine methods of extracting the eggs of T.paludosa from soil and similar substrates involves floating them from the medium in a saturated solution of Magnesium Sulphate. Although this process usually only involves immersing the eggs for from 2 to 5 minutes it is possible it has a deleterious effect which would be of importance if the eggs were needed alive for experimental purposes.

An experiment was designed therefore to investigate possible effects on the hatching rates of eggs immersed for different periods in the solution.

5.2.2. Materials and Methods

Batches of 150 newly-laid eggs were immersed in a saturated solution of Magnesium Sulphate for periods of 5, 10, 20, 40 and 80 minutes at laboratory temperatures. They were then washed several times in tap water, placed on damp filter paper in petri dishes, and incubated at laboratory temperature. A control batch was immersed in tap water for one minute and then incubated similarly.

Observations were made at frequent intervals until all the eggs had hatched or died.

5.2.3. Results

Table 11 shows the numbers of eggs which hatched and failed to hatch in the various treatments. The results for immersions of from 0-40 minutes showed no obvious differences but by 80 minutes there seemed to be a marked decrease in viability. Chi^2 contingency tests on the entire data and on the data with the 80-minute observations excluded upheld these hypotheses.

It may be suggested therefore, that short periods of immersion, such as occur during routine extractions, are unlikely to affect the viability of eggs but unnecessarily prolonged immersions could be harmful and should be avoided.

TABLE 11

Numbers of eggs hatching and failing to hatch after immersion in saturated Magnesium sulphate solution

	Time immersed in solution (mins)					
	0 (water)	5	10	20	40	80
No. hatching	136	143	131	136	130	120
No. dying	14	7	19	14	20	30

Chi^2 : All data 19.5; 5 d.f., $P < 0.01$

Chi^2 : 0 - 40 mins. 8.0; 4 d.f., $P > 0.05$

SECTION 6

EFFECTS OF TEMPERATURE ON THE DEVELOPMENT AND HATCHING OF EGGS

6.1. Introduction

Temperature is one of the most important physical components of an insect's environment and influences all phases and processes of its life. The rates of metabolic processes, like those of chemical reactions, are temperature dependent and to some extent follow the laws of physical chemistry such as laid down by Arrhenius and van 't Hoff. Usually for every metabolic process there is a lower temperature threshold, below which no reactions take place. This is followed by a range where increase in temperature causes increased activity until an upper limit is reached. Further increases in temperature usually cause a decrease in activity and ultimately lead to the thermal death of the individual.

Various attempts have been made to express these relationships mathematically (see Wigglesworth, 1972) but as the processes concerned depend on many different chemical and physical reactions which may show different responses to temperature changes none appear completely satisfactory. Also instances are known where exposure to fluctuating temperatures has caused an acceleration in response e.g. eggs of Melanoplus kept in a regime alternating between 12 and 32°C. developed more quickly than when kept at a constant 32°C. (Parker, 1930). With these limitations in mind, however, laboratory investigations such as those following on the effects of temperature on the development and hatching of eggs of T.paludosa are justifiable as they help to interpret events in the field.

6.2. Materials and Methods

Adult males and females of T.paludosa were collected from the field and placed in 2 lb. Kilner jars where they were allowed to mate.

A few vertical stems of grass were provided in each jar for the flies to stand on and to keep the humidity high.

After mating was complete the legs and wing of the females were cut off and their bodies were floated on water in petri-dishes (cf. Laughlin, 1958b). Under these conditions eggs were laid in quick succession and could be collected on filter paper at the bottom of the dish from where they could be removed to separate petri dishes lined with moist double-thickness filter paper for incubation in batches of 50 at a time.

Initially all eggs were placed in an incubator at 15°C. and thereafter batches were removed at intervals of 1, 3, 6 and 10 days and placed at constant temperatures 5, 10, 15, 20 and 25°C. and observed daily until they hatched or died. With a single exception (3 days/5°C.) when only 160 were used, 200 eggs (four batches) were placed in each temperature/time combination. In a separate experiment batches of eggs were incubated at 30°C.

This design enabled investigations of the rates of development at different temperatures and also of the effects on development of changing the temperature regimes for differing periods. If the rate of development is directly proportional to temperature then the results from all regimes should be similar irrespective of when the temperature change was made. If, however, changing the temperature during development results in disproportionate acceleration or retardation of development this will be revealed. Such effects were noted by Lin _et al._ (1954) but were criticised by Howe (1967).

The detailed results of egg hatching at different temperatures are given in Appendix IV. Tables D1-D6.

6.3. Results

All the eggs kept at 30°C. died without hatching although they developed to the point where the head capsule, mouth-parts, spiracular discs and hair tufts could be distinguished. Evidently this temperature is too high for normal development.

The remaining results are shown in Table 12. At 15°C. the mean period for complete development (based on 800 eggs) was 15.5 days, therefore each day 1/15.5 of complete development takes place at this temperature. To obtain the rates of development at the other temperatures corrections were applied to the observed incubation periods to allow for the amount of development which had already taken place before the switch to the new temperature.

For example, the mean time required for complete development of eggs transferred to 10°C. after 6 days at 15°C. was an additional 19.33 days. During the time they had spent at 15°C. they had completed 6/15.5 (= 0.3871) of their development; therefore the amount of development still to be completed at 10°C. was 1.000 - 0.3870 = 0.6130. the time required for complete development (d) at 10°C. can thus be calculated as:

$$d = 19.33 / 0.6130 = 31.53 \text{ days}$$

The reciprocal of this, $1/d = 0.0317$, is a measure of the rate of development at 10°C.

Values for 1/d were calculated separately for each regime and are entered in Table 13, the separate columns of which give independent estimates of the rate of development at each temperature and can be summarised as regression lines thus:

1 day Transfer: $y = -0.0170 + 0.0054x$ (S.D. 0.0077)
3 day " : $y = -0.0081 + 0.0044x$ (S.D. 0.0083)
6 day " : $y = -0.0113 + 0.0051x$ (S.D. 0.0054)
10 day " : $y = -0.0114 + 0.0046x$ (S.D. 0.0050)

($y = 1/d$, x = temp.)

TABLE 12

Effects of temperature on hatching of eggs

Temp. °C.	Age of eggs (days)	No. of eggs used	No. of eggs hatched	% hatched	Mean days to hatch ± S.E.
5	1	200	177	88.5	59.25±0.2095
	3	160	108	67.5	46.10±0.1785
	6	200	170	85.0	35.29±0.6304
	10	200	176	88.0	22.75±0.4523
10	1	200	182	91.0	33.7 ±0.076
	3	200	184	92.0	31.45±0.0918
	6	200	181	90.0	19.33±0.2202
	10	200	173	86.0	13.04±0.2968
15	1	200	186	93.0	14.18±0.044
	3	200	184	92.0	12.79±0.072
	6	200	178	89.0	9.08±0.1418
	10	200	179	89.5	5.95±0.1083
20	1	200	185	92.5	9.47±0.066
	3	200	187	93.5	9.36±0.0584
	6	200	174	87.0	6.62±0.1488
	10	200	178	89.0	4.73±0.1161
25	1	200	173	86.5	8.028±0.042
	3	200	168	84.0	8.29±0.0736
	6	200	171	85.5	5.41±0.0681
	10	200	165	82.5	3.44±0.0596

TABLE 13

Rates of Development of Eggs at Five Different Temperatures Calculated from Eggs Transferred From 15°C. after 1, 3, 6 and 10 days

Temp.°C.	Transferred from 15°C. after:			
	1 day	3 days	6 days	10 days
5	0.0158	0.0175	0.0174	0.0156
10	0.0278	0.0256	0.0317	0.0272
15	0.0645	0.0631	0.0675	0.0597
20	0.0988	0.0862	0.0926	0.0812
25	0.1165	0.0973	0.1133	0.1032

Analysis of Co-Variance on these results showed that there were no differences in the slopes ($F = 1.21$, 3/12 d.f.; $P > 0.20$), or elevations ($F = 1.68$, 3/15 d.f., $P > 0.20$) of the lines so all the data could be combined to give a single line (Figure 18). Thus:

$$Y = -0.01196 + 0.004873X \quad (\text{S.D. } 0.007293)$$

The Coef. of Det. (r^2) showed that 96% of the total variation was accounted for by this regression. There were no indications that changes of temperature during incubation caused disproportion in the rates of development. Figure 19 shows the relationship between time and development of eggs and temperature based on this regression line Figure 18).

6.4. Hatching Rates of Eggs

The % hatching rates of eggs kept in the various regimes are shown in Table 12. Examination of these by 2-way Analysis of Variance after angular transformation (Table 14) showed that hatching rates were similar throughout.

TABLE 14

Results of Analysis of Variance on Angularly Transformed Hatching Rates of Eggs in Different Regimes

Source	d.f.	Sum of Sq.	Mean Sq.	F	P
Temperatures	3	34.2695	11.4232	0.78	>0.20
Age at transfer	4	167.1150	41.7788	2.86	>0.05
Residual	12	175.2929	14.6077		

Figure 18. Rates of development of eggs transferred from 15°C to second temperature after 1, 3, 6 and 10 days.

Open circles = 1 day

Closed circles = 3 days

Open star = 6 days

Closed star = 10 days

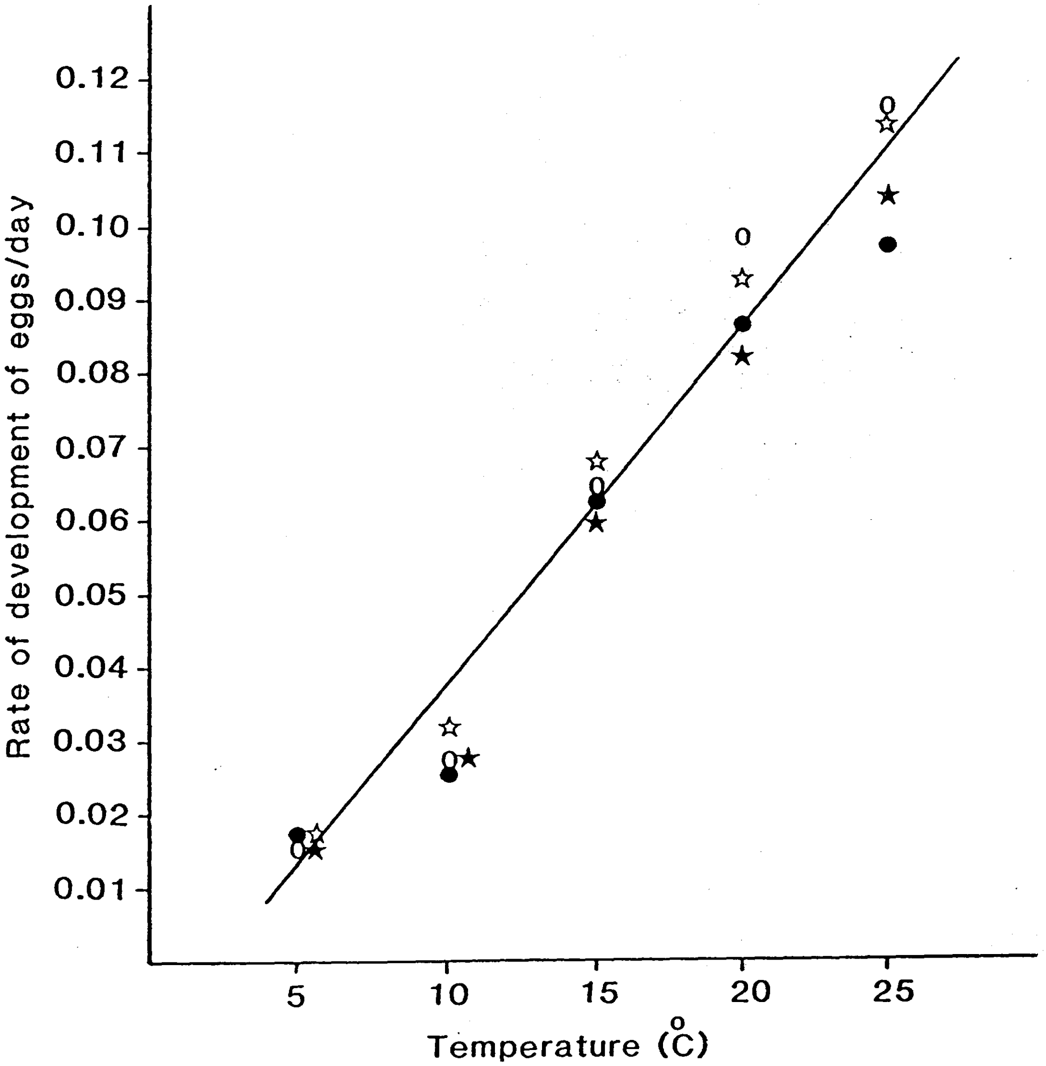
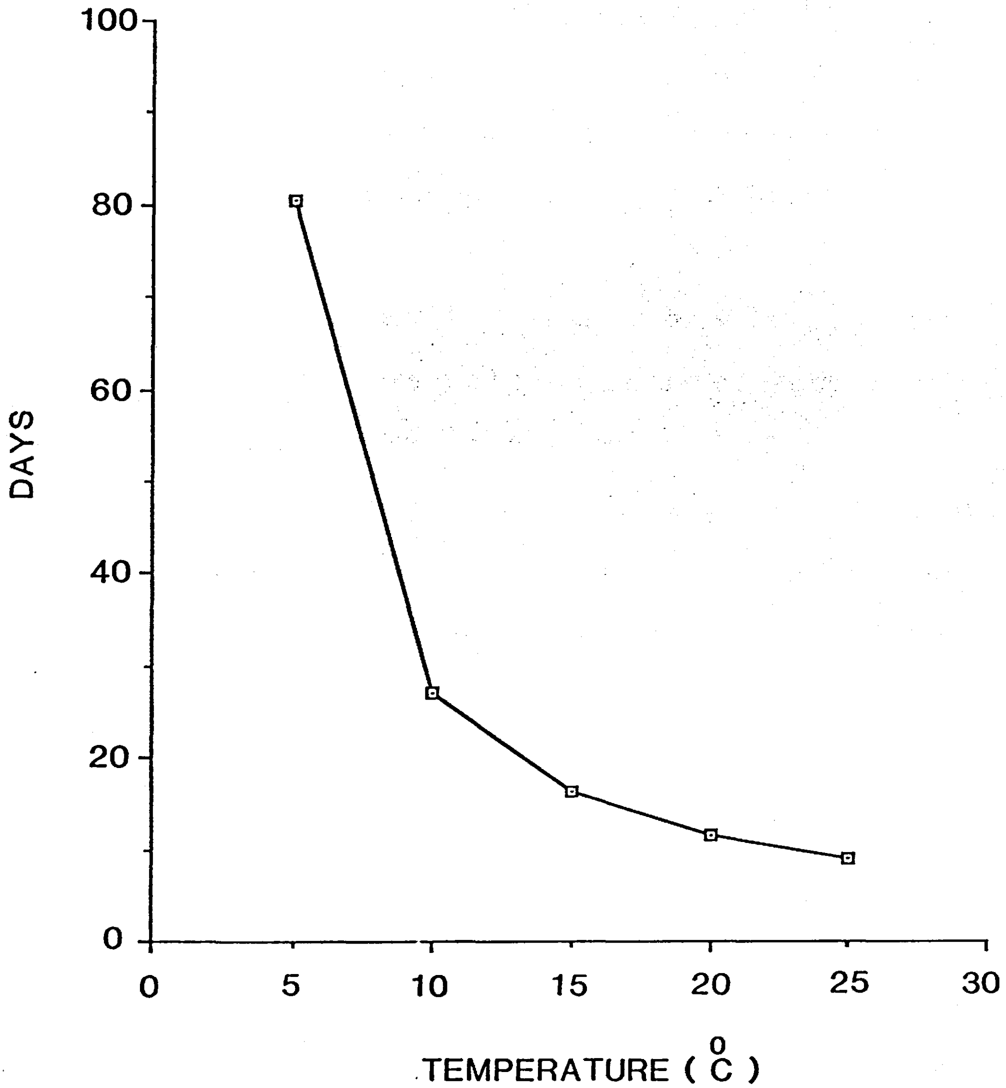

Rate of development of eggs/day
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
5
10
15
20
25
Temperature (°C)

Figure 19. The relationship between time of development of eggs and temperature (based on regression line Figure 18).


100
80
60
40
20
0
DAYS
0
5
10
15
20
25
30
TEMPERATURE (^{0}C)

SECTION 7

EFFECTS OF REDUCED HUMIDITY ON EGGS AND FIRST INSTAR LARVAE OF TIPULA PALUDOSA

7.1. Introduction

The summer of 1984 was particularly hot and dry and the following year the populations of adults of T.paludosa in the areas under observation were very low. This phenomenon has been observed and investigated by several workers and has been attributed to the susceptibility of the eggs and young larvae to desiccation.

Most species of Tipulidae live in wet habitats (Coulson, 1959; Hemmingsen,1959) and, although T.paludosa is found in drier conditions than many other species, its early stages are quite susceptible to drought (Brindle, 1960; Coulson, 1962; Freeman, 1967).

Maercks (1939a,b), working in Germany, showed that soil moisture was important to the larvae of T.paludosa and that dry autumns could cause drastic reductions in populations; he concluded that the young larvae were susceptible to desiccation. Later, (1941, 1943a, 1953) he correlated high densities of T.paludosa larvae in the spring with high rainfall during the previous autumn when the insects were small.

Milne et al. (1965) made similar observations in the N.E. of England and devised an experiment in which turves artificially "seeded" with eggs and young larvae were subjected to simulated late summer and autumn rainfalls of previous years. They found that with regimes simulating "dry" years mortalities amongst eggs and larvae were highest and concluded that, in nature, eggs which had been laid in such dry turf would probably fail to hatch. Their conclusion that prolonged drought during and after the oviposition period would have a catastrophic effect on population levels was supported by Meats (1968).

Very young eggs of T.oleracea L. (less than 15 minutes old) will start to shrivel within 2-4 minutes if kept in unsaturated air (Laughlin, 1958a) but older eggs of this species and of T.paludosa become more resistant to desiccation (Maercks, 1939b; Coulson, 1962). The former considered that eggs required a relative humidity of 100% to enable them to develops but Meats (1968) found that after they had passed the mid-incubation swelling they could continue their development and hatch at 80% R.H.

In the present observations two simple experiments were carried out in order to investigate the effects of reduced relative humidities on eggs and first instar larvae kept at constant temperature under laboratory conditions.

7.2. Materials and Methods

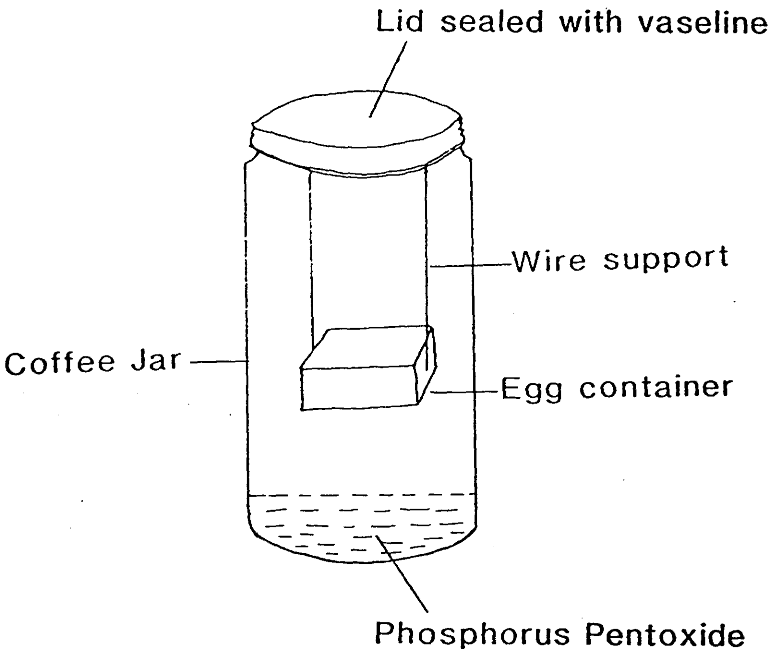
7.2.1. Observations on eggs

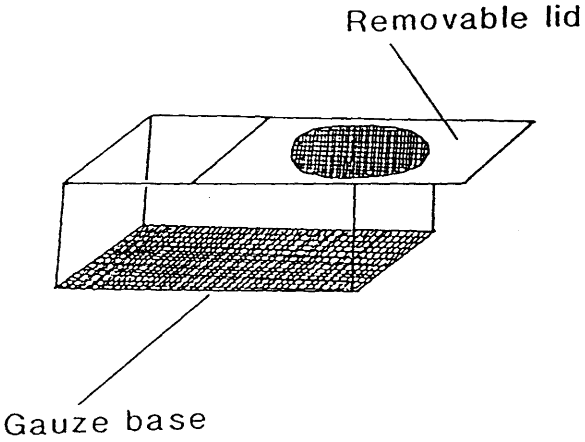
40 newly laid, 40 one-day old and 40 four-day old eggs were exposed to a relative humidity of 0% at 20°C. for 3, 9, 27, 81 and 243 minutes and for 24 hours. The newly laid eggs were obtained by a method based on that of Laughlin (1958b) in which legless, wingless, gravid females were floated on the surface of water. (Laughlin decapitated his flies also, but in the present observations decapitated females laid less readily than intact ones). Under these conditions eggs were laid rapidly and sufficient numbers could be collected within a few minutes.

Exposure chambers, made from 500 g. screw-topped coffee jars, were prepared by adding a layer of fresh phosphorus pentoxide and sealing them for 24 hours. A duplicate set of screw tops, fitted with platforms holding the eggs were prepared and then rapidly transferred to the jars (Figure 20). The exchange could be made in five seconds ensuring that the low humidities established in the jars were disturbed as little as possible.

Figure 20. Egg desiccating jar.

Figure 21. Plastic container for desiccating larvae.


Lid sealed with vaseline
Wire support
Coffee Jar
Egg container
Phosphorus Pentoxide


Removable lid
Gauze base

Immediately after exposure the eggs were transferred to moist filter paper in petri dishes and were incubated at 20°C. They were checked daily and the numbers hatching were recorded. A control batch of eggs remained on moist filter paper at 20°C. throughout the observations (Figure 22 shows <u>Tipula paludosa</u> eggs).

7.2.2. Observations on larvae

Batches of 50 or 100 unfed first instar larvae less than two days old were exposed to relative humidities of 76, 55, 33 and 0% at 20°C. in 5 x 4 x 2.5 cm. plastic-lidded boxes with gauze-covered "windows" (Figure 21). These were suspended in 2 lb Kilner jars containing desiccating agents for 30, 60, 120 or 240 minutes. After exposure the larvae were removed to moist filter paper in petri dishes and allowed to recover at 20°C. Those failing to recover were classed as dead.

The humidities in the jars were established by the addition of saturated solutions of the following salts: Sodium chloride (R.H. 76%), Magnesium nitrate (R.H. 55%) and Magnesium chloride (R.H. 33%) (Winston and Bates, 1960). 0% R.H. was established by the use of fresh Phosphorus pentoxide. Figures 23 and 24 show young larva emerging from egg and first instar larvae of <u>T.paludosa</u> respectively.

7.3. Results

7.3.1. Eggs

Table 15 shows the numbers of eggs which survived and eventually hatched after exposure to each humidity regime and Table 16 the analysis of these data. Exposure to 0% R.H. for 24 hours was fatal to all eggs but considerable differences in survival occurred at shorter exposure periods. Newly laid eggs were very susceptible and almost all failed to survive even a three-minute exposure. The one-day old eggs showed a more variable response but were probably no less susceptible

TABLE 15

Survival to hatching of eggs exposed to 0% relative humidity: 40 eggs in each batch

Period of exposure	Newly laid eggs	One day old eggs	Four day old eggs
3 mins	1	12	31
9 "	2	0	31
27 "	0	0	33
81 "	1	1	24
243 "	0	5	34
24 hours	0	0	0

(N.B. 30 out of 40 eggs hatched in a control batch)

TABLE 16

Analysis of Variance on Egg Survival/humidity Experiment: (3-243 minutes exposures only)

Source of Variance	D.F.	Mean Square	F value	P
Between exposure times	4	15.50	1.16	>0.05
Between egg ages	2	1354.06	101.05	<0.001
Residue	8	13.4		

than the newly-laid ones (t = 1.21, 8 d.f., P > 0.1). In contrast, the four-day old eggs were markedly resistant at all exposure periods and their mean survival, 30.6 eggs, was almost identical to that of the unexposed controls (30 eggs).

It is evident that, within the first four days at least, the age of the egg is much more important in determining its fate than the length of the period to which it is exposed to the hostile humidity.

7.3.2. Larvae

Percentage mortalities of first instar larvae exposed to the range of relative humidities for increasing periods are shown in Table 17. As might be expected exposure of the larvae to reduced humidities increases mortality and even at the relatively high relative humidity of 75% no larvae survived for as long as four hours. Although the figures for 30 minutes exposure seemed not to conform to any definite pattern, for both 60 minutes and 120 minutes exposure there was an observed increase in mortality with each decrease in relative humidity. When examined by 2-way Analysis of Variance, using angular transformation of the percentages, this trend was not statistically significant (F = 4.1; 3/3 d.f.; $0.20>P>0.05$). Examination of the entire data by the same technique suggested that the length of time that larvae were exposed to the lowered humidities seemed more important than the actual humidity levels to which they were exposed (Table 18) but to investigate further and to test for interactions a repeat experiment would be necessary.

These results generally confirm those of Maercks (1939a,b) and Coulson (1962) in that young eggs and 1st instar larvae of <u>T.paludosa</u> are very susceptible to desiccation and, as they occur at or near the soil surface, would be likely to succumb during hot dry weather. The comparative resistance of the older eggs (Meats, 1968) was also confirmed. No experiments were carried out on older larvae, but general observations suggest that they might be comparatively resistant to desiccation. On several occasions examples were seen crawling on the soil surface in full sunlight. Their escape responses

TABLE 17

Percentage mortalities amongst 1st instar larvae exposed to differing relative humidities for increasing periods

Times of exposure (minutes)	Relative Humidities (Nos. larvae used)			
	76(100)	55(50)	33(50)	0(50)
30	6	18	0	10
60	25	26	30	86
120	39	76	96	98
240	100	100	100	100

TABLE 18

Analysis of Variance on relative humidity experiment with first instar larvae (Angular Transf.)

Source of Variance	D.F.	Mean Square	F value	P
Between exposure times	3	4199.1	23.9	<0.001
Between humidities	3	309.3	1.8	>0.20
Residue	9	175.5		

might also enable them to avoid damage - Hadley (1971a) noted that they would move up and down in their burrows in order to avoid unfavourable conditions (see also p. 117, experiments with freezing).

Figure 22 shows Tipula paludosa eggs

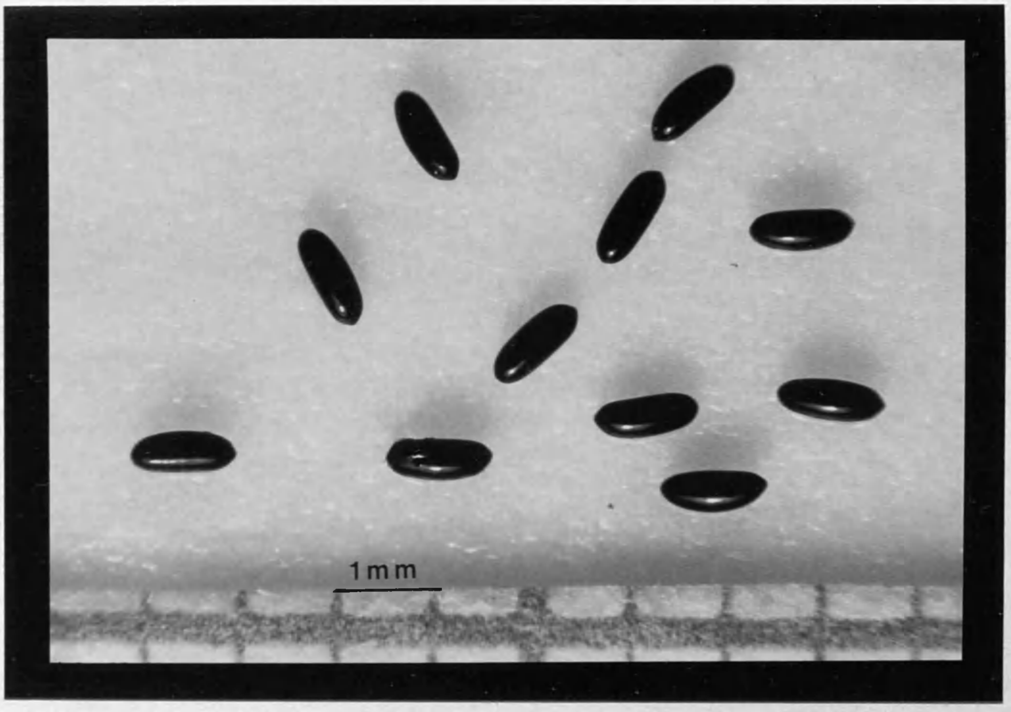
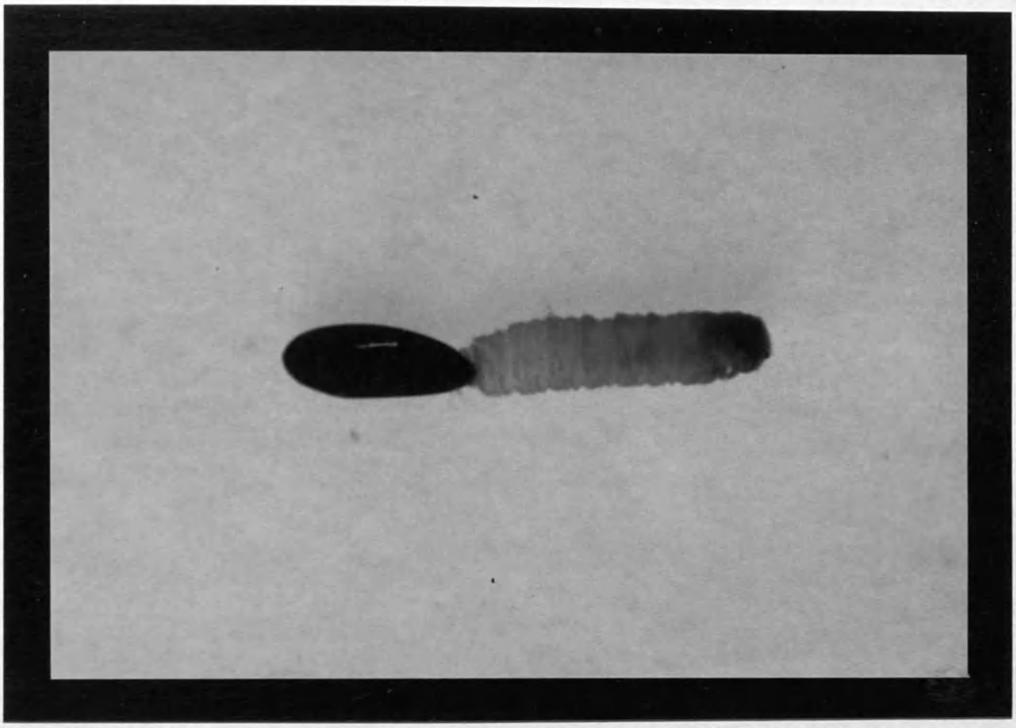
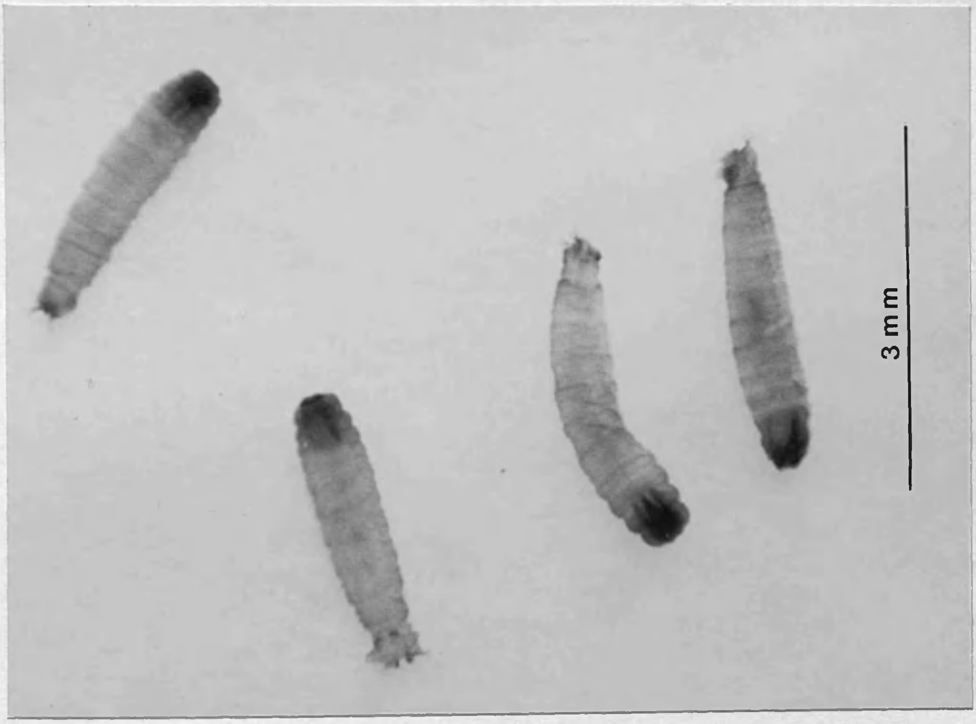

1mm

Figure 23. Young larva emerging from T.paludosa egg.

Figure 24. First instar larvae of T.paludosa.




3 mm

SECTION 8

STUDIES ON LARVAE

8.1. Extraction of larvae from the soil

The larvae of Tipulidae occur in the superficial layers of the soil and rarely penetrate to depths of more than 5cm. As they are of economic importance numerous methods have been devised for extracting them quantitatively and these include expulsion by means of chemicals, e.g. the "St Ives" method which uses an emulsion based on orthodichlorobenzene (Dawson, 1932), flooding the soil with sodium chloride solution (Stewart and Kozicki, 1987). "wet sieving" (Stewart, 1969) and methods which use "wet heat" (Milne et al., 1958) or "dry heat" (Blasdale, 1974). None of these methods is perfect and none works with equal efficiency at all times of the season. The various techniques have been reviewed by Stewart (1969).

The method used in the present study was essentially that devised by Blasdale (1974). Soil samples of uniform size, 6.5cm. in diameter and 7.5cm. deep were taken in individual cutting cylinders 10cm. in length, fashioned out of a standard plastic drain pipe. During the process of cutting the samples these cylinders were held in a special holder which supported them and prevented them from penetrating too deeply (Figure 25). On returning to the laboratory, the samples, still within their cylinders were placed in an inverted position, i.e. grass side down, in 8cm. diameter glass crystallizing dishes containing 2cm. of cold tap water. The samples, in their dishes, were then placed in the extraction apparatus in batches of 20 at a time (Figure 26) and heat was applied from above until the temperature reached 60°C. at the upper surface of the sample and 40°C. within it. The larvae were expelled from the sample by the heat and fell into the water where they could be recovered. A coil of stout wire, approximately 2cm.

Figure 25. Plastic trays with cutting cylinders and the soil sampler used in the field.

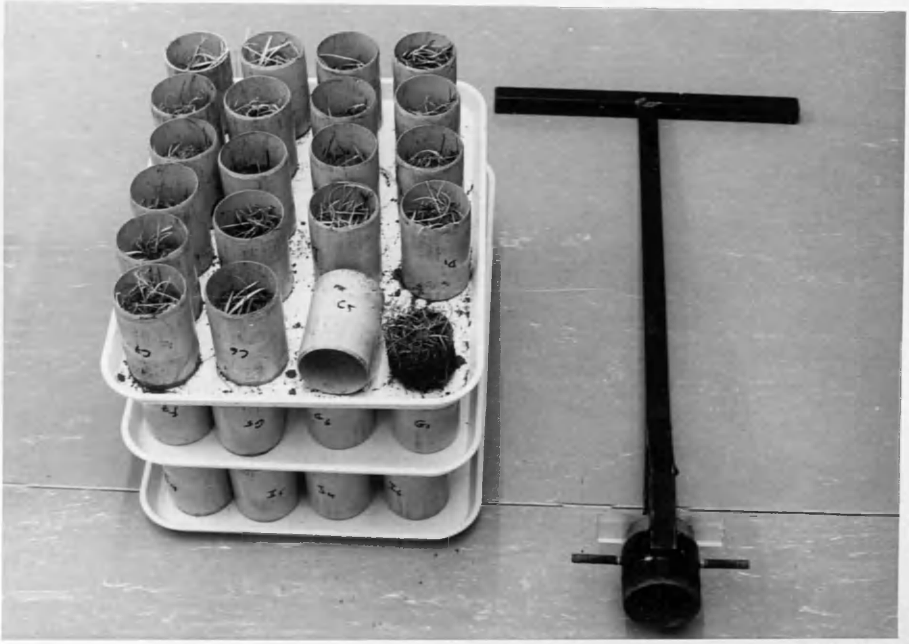


Figure 26. Leatherjacket extractor used in this study.


LEATHERJACKET
EXTRACTOR
A5
A4
A3
A2
A1

high, standing in each dish prevented the samples from falling in the water if, due to shrinkage during the heating process, they became detached from the cylinders.

The time required for complete extraction varied somewhat according to the condition of the samples being processed. Usually an hour was sufficient but it was found advisable to check the temperatures about half an hour after the start and, especially where material was required alive for experimental or observational purposes, at least every 15 minutes subsequently and to remove any larvae which had emerged.

Two sets of apparatus were available enabling 40 samples to be extracted at a time, but there were occasions when the number of samples taken were too great to enable extraction to be completed on one day. In such instances the unextracted samples were left overnight standing over water in a cool room at approximately 10°C. and were dealt with first thing next morning. Where such samples came from a quantitative survey or a balanced experiment care was taken to ensure that the samples from the various strata or treatments were extracted in similar proportions on the two days so that no confounding was introduced by this procedure. Care was also taken to ensure that samples were allocated to the two extractors in such a way as to introduce no bias.

8.2. Sampling for larvae in the field

Larvae were sought in the field for four purposes:

1. To obtain data on population numbers throughout the season,
2. To obtain data on growth in the field,
3. To obtain material for laboratory and insectary studies,
4. To obtain data on the prevalence of parasites and diseases.

8.2.1. Studies on population densities of larvae in the field

Observations were made over three successive seasons, 1984-85, 1985-86 and 1986-87 on the experimental pasture at Lawmuir.

In the first year, to ensure even coverage of the field, a rectangle 75 x 30m was marked out and divided into two rows of five plots each 15 x 15m and sampling was carried out on each of these plots at random at intervals varying from 2 to 8 weeks between September 1984 and April 1985.

The sample was taken in the form of 6.5cm diameter and 7.5cm deep cores which were extracted by means of the Blasdale apparatus described elsewhere. On the first and third occasions, a scatter of four cores was taken from each plot but on the second, fourth, fifth and sixth occasions eight cores were taken. (This variation was at first connected with assessing the errors associated with sampling and in the early stages four cores gave satisfactory results. Later as populations declined, eight cores were needed.)

8.2.2. Results

Counts from individual cores were recorded separately and showed markedly asymmetry in their distribution, there being a high and increasing proportion of zeros as the season progressed. Original counts were therefore subjected to square root transformation ($\sqrt{x + 0.5}$) for estimates of population densities and their errors. The original and transformed values are shown in appendix V tables E1-E10 and transformed and detransformed mean nos./core are shown in Tables 19 and 20.

A high density was found in the first two instars in September but numbers declined progressively throughout the season so that only about 14% of the September population survived until March. These population changes are summarised in Table 21.

Causes of death are numerous and include diseases, parasites, predation and probably cannibalism. Density independent factors of possible importance include freezing and drowning in water-logged soil. These factors will be discussed separately in this thesis.

Comparable observations made in 1985-86 and 1986-87 were combined with investigations on the effects of bird predation and will be discussed later.

TABLE 19

Transformed mean nos. of larvae/core ($\bar{x}$) with standard deviations (S.D.), no. of observations (N) and standard errors (S.E.)

Date	$\bar{X}$	S.D.	N	S.E.
23.9.1984	1.693	0.4857	40	0.0768
23.10.1984	1.396	0.4847	80	0.0542
6.11.1984	1.195	0.3441	40	0.0544
4.12.1984	1.092	0.4257	80	0.0476
5.2.1985	1.001	0.3667	80	0.0410
27.3.1985	0.911	0.3291	80	0.0368

TABLE 20

Detransformed mean nos. of larvae/core and detransformed $\bar{x} \pm$ S.E.

Date	Mean	Detr. $\bar{x}$ − S.E.	Detr. $\bar{X}$ + S.E.
23.9.1984	2.366	2.112	2.632
23.10.1984	1.449	1.300	1.603
6.11.1984	0.928	0.801	1.061
4.12.1984	0.692	0.591	0.799
5.2.1985	0.502	0.422	0.586
27.3.1985	0.330	0.264	0.398

TABLE 21

Summary of population change in the field

Date	Mean	Nos. of larvae/m^2	Percentage
23.9.1984	2.366	712	(100)
23.10.1984	1.449	436	61
6.11.1984	0.928	279	39
4.12.1984	0.692	208	29
5.2.1985	0.502	151	21
27.3.1985	0.330	99	14

8.3. Test of technique - The efficiency of the extracting equipment

On six occasions during the extraction of soil samples checks were made on the efficiency of the apparatus. After extraction had been completed the samples were broken up by hand in a white tray and examined for residual larvae. The results (Table 22) suggested an efficiency of extraction of 96.86%, a figure almost identical with that obtained by Blasdale (1974) in his original description of the technique.

The superiority of this method over the "St. Ives" technique was clearly demonstrated by Shaw et al. (1974) and for the present purposes it was clearly superior to the salt solution, "on the site", method of Stewart and Kozicki (1987). It was considered, therefore, that the larval counts obtained by it in the present work could be accepted without correction.

TABLE 22

The Efficiency of the Larval Extractors

Date	No. of samples examined	No. of larvae extracted by machine	No. of larvae remaining in samples
11/12/85	80	40	0
24/12/85	80	34	2
15/01/86	80	25	1
18/02/86	80	28	2
16/03/86	80	27	1
18/05/86	160	31	0
Total	560	185	6

Overall efficiency 96.86%

8.4. Rearing *Tipula paludosa* in the laboratory

8.4.1. Introduction

T.paludosa is a univoltine species whose life-cycle normally occupies a complete year, the larval stages lasting some nine months. Laboratory studies on its physiology etc. are somewhat hindered by the various stages only being available at certain times, so the following simple method, based partially on the techniques of Laughlin (1958b) and Carter (1975), was devised and enabled thousands of larvae to be produced with two generations in a single year.

8.4.2. Materials and Methods

All stages of the life-cycle were reared in a "phytotron" room kept at 20°C. with a daily light cycle of 16 hours light and 8 hours dark.

Adult crane-flies, collected from the field during the summer were allowed to mate and eggs were obtained from the females by the method used by Laughlin (1958b) in which decapitated wingless and legless females were floated on water in which their eggs, laid by reflex action, were collected. The eggs were then incubated in batches of 50 on moist filter paper in covered 9.0 cm. diameter petri dishes.

The newly hatched larvae were transferred to plastic trays 20 cm. long, 20 cm. wide and 7 cm. deep containing a 5 cm. deep layer of John Innes potting compost. These culture trays were placed in larger trays containing water and the compost was kept moist by water passing through small gauze-covered holes drilled in their bases. A layer of fine gauze was placed over the culture trays to prevent larvae from escaping.

Food, in the form of finely powdered dried grass ("green meal") was added to the surface of the compost at intervals which varied according to the numbers of larvae in each culture. It was essential to avoid over-feeding as excess food soon became mouldy to the detriment of the culture (cf. Carter, 1975).

From time to time the larvae were extracted by hand sifting and the survivors were transferred to new cultures. They are noticeably cannibalistic when kept at high density so care was taken to avoid overcrowding. About 30 mature individuals seemed optimal for cultures of the size stated.

When the pupae appeared they were washed, sexed (females are larger and more sharply pointed posteriorly than males, see p. 147) and placed on moist double filter paper in 1 lb. Kilner jars with perforated lids to allow aeration. When the adults appeared they were allowed to pair and the life cycle recommenced with the laying of their eggs. N.B. to avoid possible effects of inbreeding in restricted populations eggs from several different cultures were mixed to set up the new cultures.

8.4.3. Results

The first experiment was started in September 1986 and by mid-February 1987 both male and female adults had emerged and eggs had been laid. These, in turn, produced a second generation of adults by July 1987. Thus two complete generations were reared within 10 months. It is thought that this method, which is less elaborate than that used by Laughlin (1958b) and Carter (1975), could readily be adapted to produce continuous supplies of insects for experimental purposes.

8.5. The Effects of Population Density on Survival of Larvae of *Tipula paludosa*

8.5.1. Introduction

Young larvae of *T.paludosa* kept together in culture were often seen to bite one another and, in a typical encounter, one larvae would sieze another in its mandibles and after a period of writhing the bitten larva would become quiescent as though exhausted. The encounter would then be broken off and would not be resumed. The ultimate outcome of these encounters was not determined as the observations were incidental to other studies but it seems likely that some deaths ensued because dead, chewed larvae were frequently found. Sometimes the soft parts of the bodies had been completely consumed so that nothing was left but the head capsule.

Black spots were often observed on the cuticles of larvae and were particularly numerous in densely populated cultures. Coulson (1962) attributed these to fungal lesions but in the present studies microscopical examination showed only small melanised nodules resembling wound reactions and there were no traces of fungi. It seems probable that many of these were healed bite scars following sublethal attacks. An attempt to simulate the condition by puncturing the cuticle of a few larvae proved unsuccessful.

Laughlin (1958b) observed that there was much cannibalism amongst the larvae of T.oleracea L. and Pritchard (1983) found that mortality amongst the larvae of T.subnodicornis Zetterstedt and T.sacra Alexander was density dependent. It seemed likely that, in view of the mutual antagonism of the young larvae of T.paludosa, their mortality might also be affected by the degree to which they were crowded.

Experiments were, therefore, devised so as to investigate the effects on the survival of larvae of T.paludosa reared in the insectary at widely differing population densities.

Two different types of experiment were carried out. In the first, standard sized containers were used and the numbers of larvae/container were varied and in the second, a fixed number of larvae were used but the size of the container was varied.

8.5.2. Materials and Methods

8.5.2.1. Experiment 1

The larvae were reared in 152 mm. "dwarf" plastic plant pots containing a measured 1175.5 cc. of soil which came to within 5 cm. of their tops. An excess of food, in the form of dry powdered grass sprinkled on the soil surface, was maintained at all times and the pots were kept adequately watered. Their tops were covered with fine terylene netting to prevent escape.

The experiment was set up on 10 September 1985 when newly hatched larvae were added to the pots as detailed in Appendix VI Table F. After being labelled the pots were arranged at random in a well-ventilated, shaded insectary and were left undisturbed, apart from watering and the addition of food, until 9 December 1985 when their contents were examined.

8.5.2.2. Experiment 2

To check on the results of Experiment 1, and to avoid differing experimental errors due to using unequal numbers of larvae in the various densities, an experiment was set up in which the same number of larvae were used in each regime to be compared but the sizes of the containers were varied.

Four different sizes of 7 cm. deep plastic tray were used; 80 x 80 cm., 40 x 40 cm., 20 x 20 cm. and 10 x 10 cm. Each was filled with soil and 200 newly hatched larvae of T.paludosa were added. As before, ample powdered grass was used as food, and the tops were covered with fine terylene netting.

Each treatment was replicated three times and the trays were arranged at random standing in water-filled larger trays in the insectary, the soil being kept moist by allowing water to percolate throughout small, gauze-covered holes bored in the base of each.

The experiment was set up on 5 September 1986 and remained undisturbed until assessed on 8 Decmber 1986.

8.5.3. Results

8.5.3.1. Experiment 1

The results are shown in full Appendix VI Table F and are summarised in (Text) Table 23. Clearly there was a progressive decrease in the survival rate as the density of larvae increased. This was examined by calculating the correlation between the probit of the percentage survival (y) and the logarithm of the number of larvae/pot (x) and the regression of the former on the latter (Fig. 27) thus: $r = -0.9916$ (5 df.), $p<0.001$

$$y = 7.3833 - 1.1333x \text{ (S.D., 0.1051)}$$

The Coefficient of Determination (r^2), indicated that over 98.3% of the total variance in the data could be explained by the correlation.

TABLE 23

Experiment 1 - The Relationship between the numbers of larvae/culture and their survival

Number/culture (N.)	Log. No.	% Survival	Probit
10	1.00	88.0	6.17
20	1.30	85.5	6.06
40	1.60	71.5	5.57
80	1.90	59.9	5.25
160	2.20	41.0	4.77
320	2.50	34.3	4.59
640	2.80	23.4	4.27

The calculations were based on the overall %s observed, which were derived from total numbers of larvae varying between 100 and 1280, depending on the densities being used. No weighting was used and it is thought that in this case there would be no useful purpose served in carrying out a more refined computational procedure as the results were sufficiently clear cut.

8.5.3.2. Experiment 2

The data are shown in Table 24 and the results of an examination by Analysis of Variance in Table 25. As before there was a clear and progressive decrease in survival as population density increased.

Figure 27. Relationship between the numbers of larvae/culture and their survival.

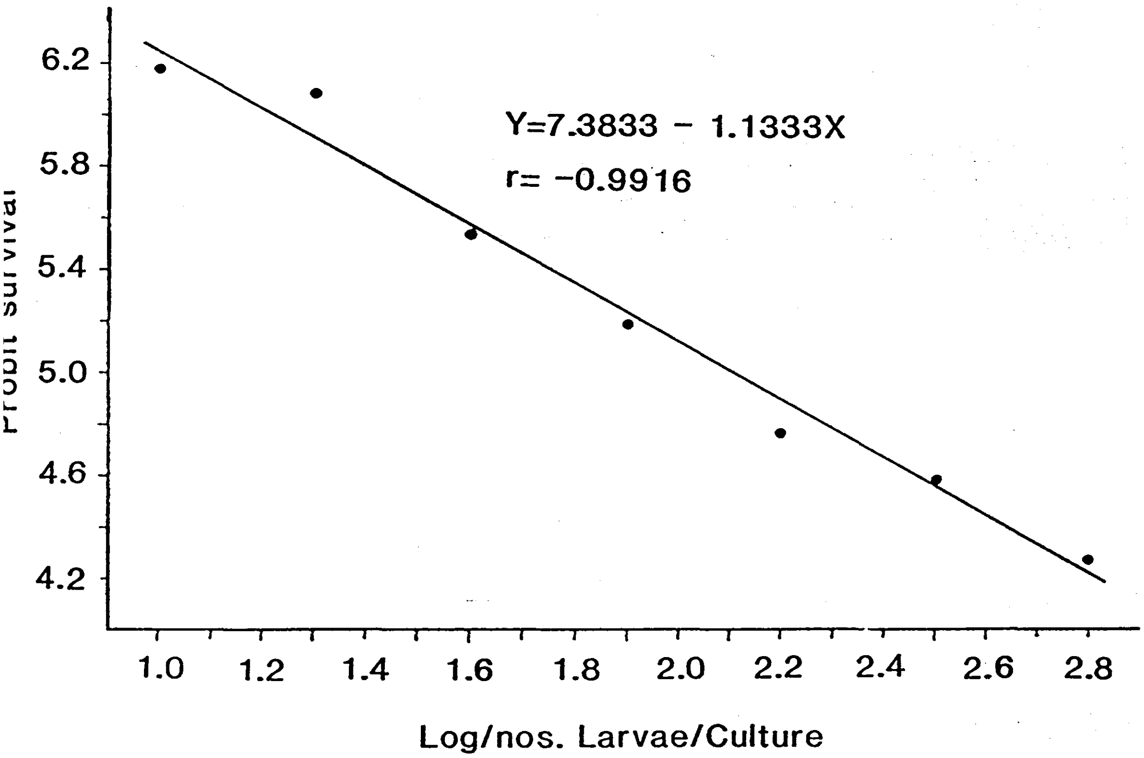

Y=7.3833 - 1.1333X
r= -0.9916
Probit survival
6.2
5.8
5.4
5.0
4.6
4.2
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
Log/nos. Larvae/Culture

TABLE 24

Using a constant number of larvae with different sizes of rearing area

Replicate number	200 larvae/ culture Area I (80x80x7 cm)	200 larvae/ culture Area II (40x40x7 cm)	200 larvae/ culture Area III (20x20x7 cm)	200 larvae culture Area IV (10x10x7 cm)
1	147	124	94	08
2	133	137	71	53
3	161	95	84	30
Total survived	441	356	249	91
% survived	73.5	59.3	41.5	15.2

TABLE 25

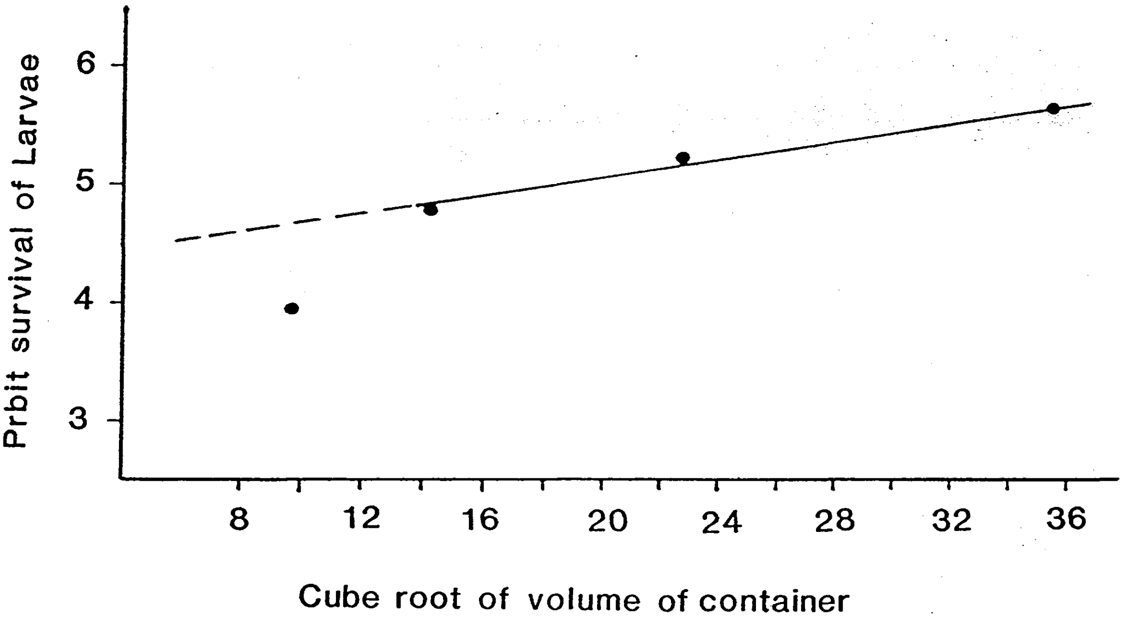
Analysis of Variance on Results of Density/Survival Experiment

Using Different Sized Enclosures

Source	df.	Sum of Sq.	Mean Sq.	Var.Rat.	P
Between areas	3	22768.92	7589.64	18.14	<0.01
Between Replicates	2	85.50	42.75		
Residue	6	2509.83	418.31		
Total	11	25364.25			

The results, with considerable variation between replicates and only four contrasting regimes, do not merit more sophisticated analysis but it is interesting to note that when the probits of the overall percentage survivals are plotted against the cube roots of the volumes of the regimes (a measure of the inter-larval distance) there

Figure 28. Relationship between the numbers of larvae surviving using different sized enclosures.


Prbit survival of Larvae
6
5
4
3
8
12
16
20
24
28
32
36
Cube root of volume of container

is nearly a straight-line relationship between the values for the three lower densities. At the highest density, however, survival is much lower than might be expected by extrapolation of this line (Figure 28). Possibly the effect of crowding becomes enhanced at high density but further experimentation would be needed to investigate this.

8.5.4. Discussion

These results show that, within the range studied, the mortalities amongst young larvae of T.paludosa in insectary soil cultures became higher as their density increased and this accords with the observations of Pritchard (1983) on the density dependence of mortality in T.subnodicornis and T.sacra.

Ample food was provided in the cultures and there was no evidence of diseases, parasites or predators so the most likely cause of the increase of mortality with density was the increase in intra-specific competition resulting in a greater number of encounters and fights between larvae.

Such behaviour in the field could result in the thinning out of dense populations and prevent over-exploitation of the environment which could prove deleterious to the whole population.

8.6. The Effects of Submergence and Oxygen Depletion on the Third Instar Larvae of T.paludosa

8.6.1. Introduction

The larvae of Tipulidae are adapted to a wide variety of habitats (Brindle, 1960) ranging from the intertidal zone to leaves of terrestrial plants and from running water to quite dry soil (Alexander, 1920). Most species live in damp soil, but some are aquatic, others live in moss or dead wood and a few live on agricultural land where they may become pests.

Pasture soils may be subject to periodic saturation or flooding, for example, in February 1987, there was an exceptionally heavy fall of snow at Lawmuir and the ground was covered for several weeks. The soil must have been inundated with water for a considerable part of this time. Also, after heavy rains, soil often becomes water-logged or covered with surface water.

According to Oldroyd (1964), the only cranefly larvae that can stay continuously submerged in water without access to atmospheric oxygen are those of the genus Antocha, but several other species e.g. Tipula abdominalis (Say), T.caloptera Loew (Rogers, 1933) and T.sacra Alexander are quite independent of air in well aerated water (Pritchard and Hall, 1971). In terrestrial species such as T.paludosa respiratory exchange takes place through aeropyles in the stigmatic ring in all larval stages but they also have a well-developed "tracheal lung" emanating from the spiracular atrium which is thought to act as a tracheal gill when terrestrial habitats become flooded (Pritchard and Stewart, 1982).

In wet soils oxygen becomes depleted (Russell, 1961; McKee, 1962) and, in order to survive, tipulid larvae must either come to the surface to breathe air directly or move into the comparatively well-aerated water near the surface. Their capacity to survive when kept in aerated water without access to atmospheric oxygen and under conditions of severe oxygen deficiency was tested by the following two experiments.

8.6.2. Materials and Methods

8.6.2.1. Survival in aerated water

Three replicate batches of 15 third instar larvae were immersed in tap water in crystallizing dishes (150 mm. wide, 75 mm. high) which was aerated for three hours daily. The larvae remained submerged throughout the observations and were unable to obtain gaseous air from either the surface or from the aerator outlet. A few stones were provided as a "retreat". The temperature was maintained at 10°C.

Observations were made daily and the numbers of survivors were noted. Larvae were regarded as dead when they showed no response to prodding of the sensitive spiracular discs.

8.6.2.2. Survival in oxygen-deficient water

Six water-filled crystallizing dishes, similar to those used in the previous experiment, were first prepared by covering them with close-fitting white expanded polystyrene discs as lids, so that no air was entrapped and then bubbling nitrogen into the water until the oxygen tension, as measured by an oxygenometric meter, registered zero (Fig. 29). Fifteen third instar larvae were then introduced into each dish, the lids were quickly re-instated and the flow of nitrogen was continued. The temperature was maintained at 10°C. and observations were made hourly.

8.6.3. Results

The times survived by larvae kept in aerated and in oxygen-deficient water differed greatly, but for consistency the periods of exposure will be given in hours for both sets of data.

8.6.3.1. Aerated water

The numbers of larvae surviving after each day (24 hours) of immersion, totalled for the three replicate dishes, are shown on Table 26 along with the accumulated % mortalities, and equivalent probits*. No deaths occurred during the first day (24 hours) but 8 larvae had died by day 2 (48 hours). Two larvae survived for 26 days (624 hrs.) but all were dead by day 27 (648 hrs.).

TABLE 26

Number of larvae surviving after each day (24 hrs) of immersion

Day No.	Day Hours	No. Survived	Accumulated No. died	Accumulated % mortality	Probit
1	24	45	-	-	-
2	48	37	8	18	4.08
3	72	34	11	24	4.29
4	96	32	13	29	4.45
5	120	30	15	33	4.56
6	144	25	20	44	4.85
7	168	22	23	51	5.03
8	192	21	24	53	5.08
9	216	21	24	53	5.08
10	240	18	27	60	5.25
11	264	16	29	64	5.36
12	288	14	31	69	5.50
13	312	13	32	71	5.55
14	336	13	32	71	5.55
15	360	12	33	73	5.61
16	384	8	37	82	5.92
17	408	8	37	82	5.92
18	432	8	37	82	5.92
19	456	7	38	84	5.99
20	480	6	39	87	6.13
21	504	5	40	89	6.23
22	528	3	42	93	6.48
23	552	3	42	93	6.48
24	576	3	42	93	6.48
25	600	2	43	96	6.75
26	624	2	43	96	6.75
27	648	0	45	100	-

Figure 29. Oxygenometric meter used in experiment No.B.

3
3
2
2

For further analysis the probit mortalities (y) (excluding 0% and 100%) were plotted against hours (x), Figure 30, and the regression of x on y was calculated to enable confidence limits to be placed around the estimate of the period required to produce 50% mortality.

The equation:

$$x = 220.10y - 895.7 \text{ with S.D.} = 26.9$$

gave 50% mortality after 204.8 ± 15.2 hrs. (95% Conf. Int.)

8.6.3.2. Oxygen-deficient water

Here the larvae succumbed rapidly (Table 27).

All survived for 3 hours, but by 4 hours 26 had died. None survived for more than 20 hrs. Probit analysis was carried out as before (Figure 31) and the regression equation

$$x = 3.16y - 10.2 \text{ with S.D.} = 0.38$$

gave 50% mortality after 5.6 ± 0.5 hrs. (95% Conf. Int.)

* Probits gave a better fit to the data (Coefficient of Determination = 0.977) than %s (C.D. = 0.944).

Figure 30. Probit mortality of larvae after each day (24 hrs) of immersion in oxygenated water.

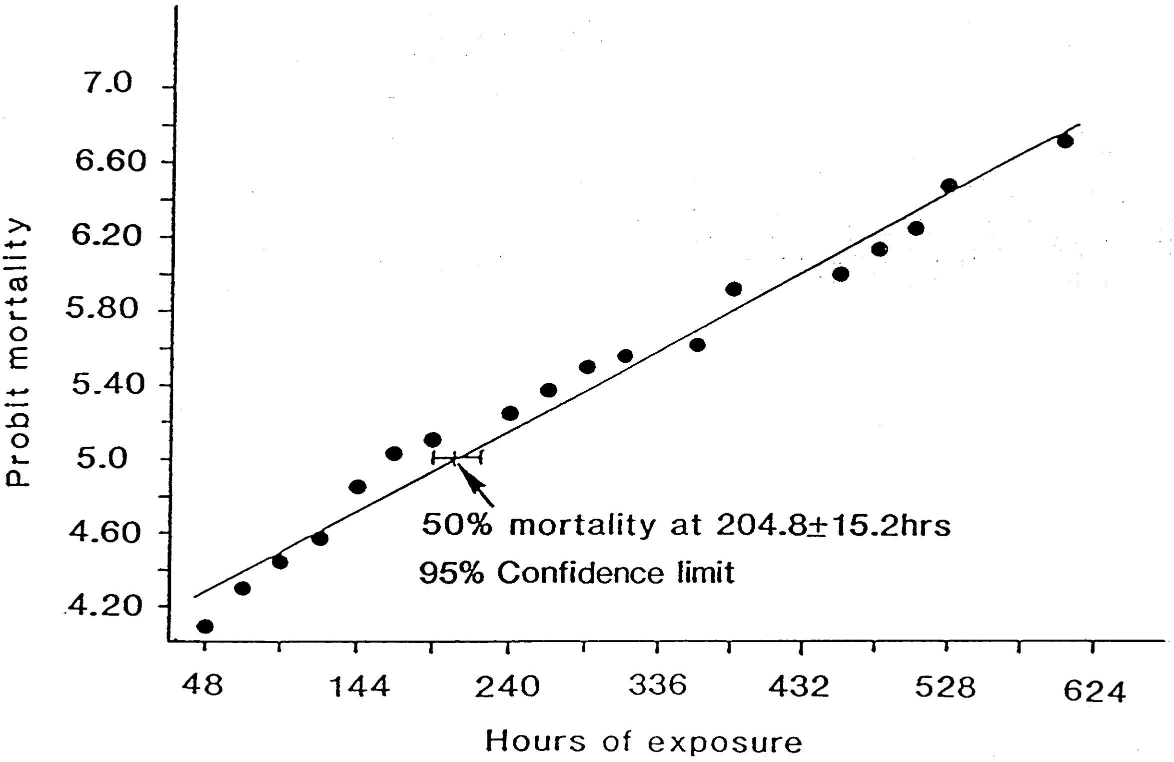
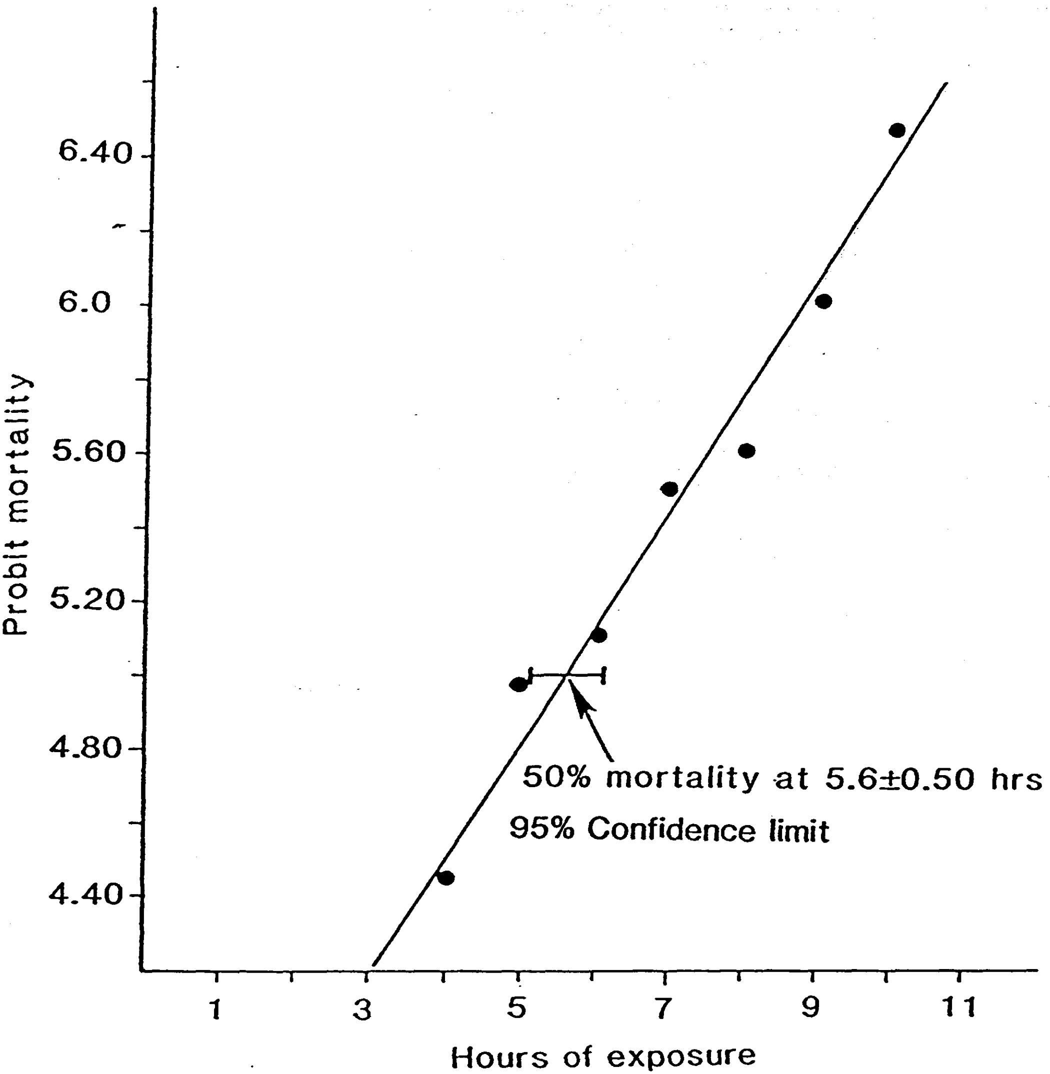

7.0
6.60
6.20
5.80
5.40
5.0
4.60
4.20
Probit mortality
48
144
240
336
432
528
624
Hours of exposure
50% mortality at 204.8±15.2hrs
95% Confidence limit

Figure 31. Probit mortality of larvae after each hour in oxygen-deficient water.



Probit mortality
6.40
6.0
5.60
5.20
4.80
4.40
1
3
5
7
9
11
Hours of exposure
50% mortality at 5.6±0.50 hrs
95% Confidence limit

TABLE 27

The effect of submersion on larvae in water deficient oxygen

No. of Hours	No. of dead larvae in each hour	Accumulated No. died	Accumulated % mortality	Probit
0	0			
1	0			
2	0			
3	0	0	0	
4	26	26	28.9	4.44
5	18	44	48.9	4.97
6	5	49	54.4	5.11
7	13	62	68.9	5.49
8	4	66	73.3	5.62
9	10	76	84.4	6.01
10	8	84	93.3	6.49
11	6	90	100.0	

8.6.4. Discussion

The purpose of the present experiments was to investigate the effects on T.paludosa larvae of simplified "drowning" regimes where they were immersed without access to atmospheric oxygen in de-oxygenated and oxygenated water. As expected, larvae succumbed rapidly in de-oxygenated water, but showed considerable tenacity of life in oxygenated water and more than half of them survived 8 days under the conditions of the experiment.

While soil flooding has been regarded as an important factor in the mortality of larvae and pupae (Rogers, 1942; Ricou, 1967b) it is not easy to apply the results of these simple laboratory experiments to field events.

The larvae of T.paludosa live in many different types of soils including clay, sand, peat, marl, mineral soils, alluvium and marshes (Brindle, 1957, 1959, 1960; Coulson, 1959) and their nature will partly determine the effects of flooding on tipulid populations. Peat soils, for example, are almost permanently water logged and already have low oxygen tensions so that any further deprivation of oxygen might well cause deaths among its inhabitants.

Meats (1970) pointed out that deaths of larvae in the field were not so much due to submergence itself, but to submergence operating with other factors such as wind, temperature, soil type and the turbidity of the water. The stage of the larvae is also of importance, e.g. first instar larvae of T.paludosa have hair tufts on their spiracular discs, not developed in other instars, which help respiration under conditions of sumbergence (Pritchard, 1983).

Clearly much more experimentation will be needed before the results of flooding can be fully appreciated.

8.7. Effects of Cold on Third Instar Larvae of Tipula paludosa

8.7.1. Introduction

During frosty weather the upper part of the soil may freeze but the cold rarely penetrates far as stored heat will be conducted upwards and counteract it so that the subsurface minimum temperatures remain higher than those of the air or at the surface. Coutts (1955) showed that in observations carried out over two years in a forest ride in Aberdeenshire, although a minimum air temperature of -11.1°C. was recorded, the temperature of the soil at a depth of 2.5 cm. never fell below -1.7°C. and at greater depths never fell below zero. Russell (1957) commented on the effects of surface vegetation in protecting the soil against frost. During a cold spell at Rothamsted

(Hertfordshire) during the winter of 1939-40 the temperature at a depth of 10 cm. under turf never fell below -2.8°C. whereas that under bare soil fell to about -5.8°C. Freshly fallen snow has a considerable insulating effect: Oke and Hannell (1966) recorded the temperatures of wet soils under grass plots in Canada over a 24 hour period with and without a 12.5 cm. covering of fresh snow. When the air temperature was -10.2°C. the temperature of the snow surface reached -17.3°C. but the soil remained at 0°C. throughout the top 30 cm. With no snow cover, however, when the air temperature reached -11.3°C. the soil temperature just below the surface was -7.2°C., whereas at depths of 5 and 10 cms. it was -5.0°C. and -3.0°C. respectively.

Tipula paludosa usually overwinters as a third instar larva in the water-saturated upper layers of the soil which, in cold weather, may be subjected to sub-zero temperatures for prolonged periods and, in order to survive the winter, at least part of its population must be adapted to tolerate the lowest temperature likely to occur in its habitat. It would be expected, therefore, that these third instar larva would show appropriate adaptations, either of a physiological or behavioural nature.

In most years it is probable that the soil temperatures in lowland Britain will not fall to a sufficiently low level to cause appreciable harm to the larvae of T.paludosa and, as pointed out by Larsen (1949), the species normally feeds whenever temperatures are suitable and has no protective winter diapause. In exceptionally cold winters, however, such as that observed by this authoress in Denmark in 1940-41 however, serious depletion of the population may occur. In this connection Maercks (1943a) noted that in Germany cold winters are unfavourable to populations of T.paludosa but favour those of T.czizeki de Jong. a species which is normally less common but which overwinters as an egg in diapause.

The cold resistance of insects, apart from that associated with diapause has been much studied in recent years and various mechanisms have been demonstrated. One cause of death is the production of ice crystals in the body as shown for Tipulidae by Freeman (1967) and means to prevent this include the production of various substances, cryoprotectives, which allow supercooling or postpone the formation of ice. Such substances include glycerol and other polyols, sugars, inorganic phosphates, unsaturated fatty acids, free amino acids, proteins and glycoproteins (Salt, 1961; Danks, 1978; Somme, 1982). Another method of avoiding freezing conditions is by showing an escape response and numerous soil-dwelling micro-arthropods show seasonal changes in their vertical distribution, which may be attributable to such factors as adverse surface temperatures (Strickland, 1947; Macfadyen, 1952; Belfield, 1956; Peachey, 1963). Some soil-dwelling species have been shown to burrow to greater depths, where temperatures are less severe, at the onset of freezing conditions. Barnes (1980) and Ricou (1967a) found that under freezing conditions the larvae of <u>T.paludosa</u> moved to a position 1 cm. below the frost level; they did not, however, penetrate to depths below 7 cm.

Detailed studies of the cold-hardiness mechanisms of the larvae of <u>T.paludosa</u> are outside the scope of the present work but some investigations on the ability of the larvae to survive or to escape from sub-zero conditions were carried out. These were devised to test:

1) The ability of the larvae to survive sub-zero temperatures in an environment from which they could not escape.
2) Their ability to avoid being frozen by moving into more favourable conditions.

8.7.2. The Ability of T.paludosa Third Instar Larvae to Survive Exposure to Sub-zero Temperatures

8.7.2.1. Materials and Methods

The larvae used in these experiments were all reared from the egg in cultures kept in an outside insectary at ambient temperatures. They were placed in batches of 10 on damp filter paper in 9.0 cm. diameter petri dishes and in each instance 10 replicate batches were exposed to a range of sub-zero temperature (regulated to within ± 1°C.) for fixed periods as shown in Table 28. At the end of each exposure the larvae were transferred to an environment at 10°C. where, after allowing them to defrost for several, hours the numbers of survivors were counted. Larvae were regarded as dead when they showed no responses to prodding of the sensitive spiracular discs. The work was carried out during the last week of December 1986.

TABLE 28

The percentage of larvae surviving prolonged freezing experiment

Temperature °C	-2.5°					-5.0					-7.5			
Period of Exposure (hours)	10	20	30	40	60	10	20	30	40	60	10	20	30	40
Number of larvae used	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Percentage survived	100	61	46	31	18	37	32	30	5	0	24	0	1	0

8.7.2.2. Results

The results are presented in full in Appendix VII Table G and in summary form in text Table 28. They also appear in graphic form as Figure 32. Larvae were rigid, motionless and shrunken when first removed from the sub-zero regimes, but the survivors relaxed and resumed activity after several hours. A slight frost (-2.5°C.) caused no deaths within the first ten hours but progressively longer exposures caused increasingly greater mortalities. More severe frost (-5.0°C.) caused substantial numbers of deaths even after ten hours and, again, mortality increased steeply with further exposure. At -7.5°C. mortality was heavy at ten-hours and only one individual survived a longer exposure.

8.7.3. The Ability of the Larvae to Move from Freezing Conditions

8.7.3.1. Materials and Methods

The apparatus is shown in Figure 33. Exposure chambers were made from Perspex cylinders, 10 cms. in diameter and 15 cms. long, the cut ends forming the top and bottom and the curved walls the sides. The bottom of each was covered with fine terylene netting, to prevent the larvae from escaping, and the sides were covered with black paper and were heavily lagged with insulating material. The cylinders were filled with soil from the Lawmuir site and ten laboratory-reared third instar larvae were placed in each. The following day, after the larvae had had time to bury themselves, the cylinders were placed in cold environments at 0.0°C., -2.0°C. and -4.0°C. in such a way that only the upper surface was exposed to the cold. Six cylinders were used at each temperature and half were examined after 3 hours and the rest after 24 hrs.

Figure 32. Percentage of larvae survived at each time (hrs) during exposure to sub-zero temperature (^{o}C).

Vertical bars = standard error.

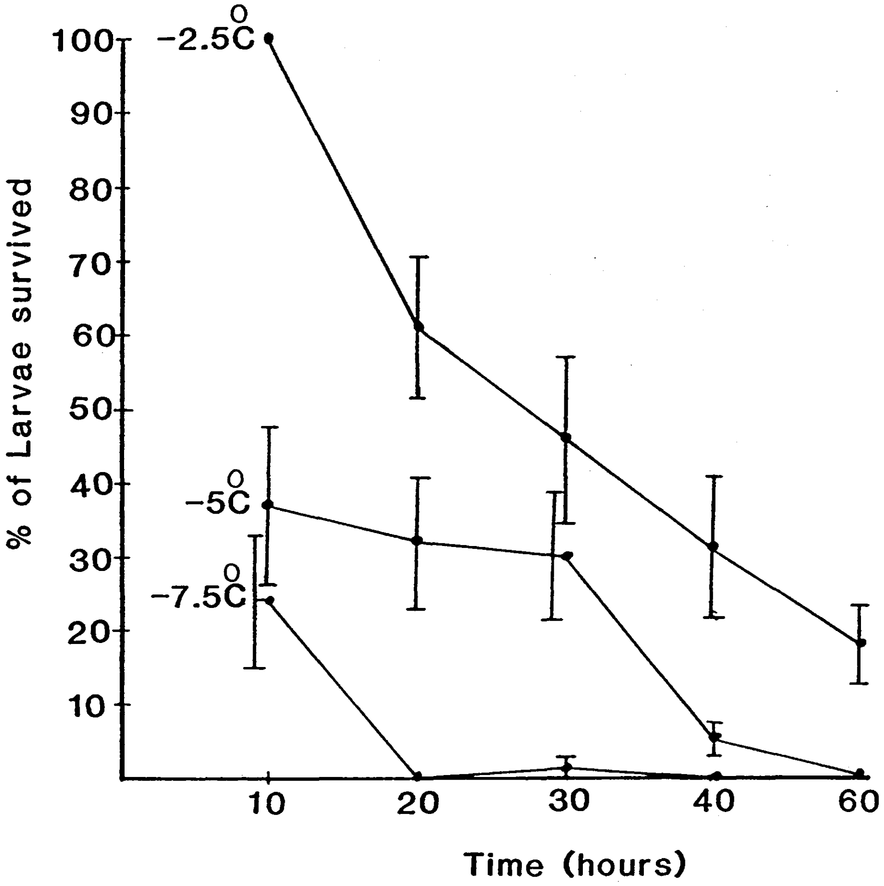

% of Larvae survived
100
90
80
70
60
50
40
30
20
10
-2.5°C
-5°C
-7.5°C
10
20
30
40
60
Time (hours)

Figure 33. View of exposure chambers which were used to investigate the movement of larvae from freezing conditions.


-2 (3hrs)
-4 (24hrs)
-2 (24hrs)
4 (3hrs)

8.7.3.2. Results

Table 29 shows the distribution of larvae at each temperature after 3 and 24 hours exposure. All the larvae, except for one in the -4.0°C./24hr. treatment combination (cell) survived. To test the significance of the differences in distribution observed the mean depth of larvae in each cell was calculated and a two-way Analysis of Variance was carried out, the numbers in the cells being first equalised, to compensate for the death noted above, by withdrawing one reading at random from each of the unaffected cells.

TABLE 29

Vertical distribution of larvae in the soil at different temperatures and time

Temperature °C	0		-2		-4	
Time (hrs)	3	24	3	24	3	24
Depth (cm)						
1	6	8	0	0	0	0
2	15	11	0	0	0	0
3	9	11	2	0	1	0
4			13	7	3	1
5			15	14	6	1
6				9	10	2
7					6	7
8					4	15
9						3
10						1 dead

A marked interaction between Time and Temperature was evident (F = 10.2, 2/168 d.f., P < 0.001) in this preliminary analysis so the whole of the data in each level of treatment were examined separately by one-way Analyses of Variance. The means and the results of these analyses are shown in Table 30.

TABLE 30

Mean Depths of Larvae and Results of Analyses in Cold-escape Response Experiment

Period of Exposure (Hrs.)	Mean Depths of Larvae (cms.) at Temperatures of Exposure (^{o}C)			F. value (horiz. comparison)	D.F.	P
	0.0	-2.0	-4.0			
3	2.10	4.43	5.97	131.8	2/87	<0.001
24	2.10	5.07	7.48	264.4	2/86	<0.001
F values vertical comparison	0.0	12.81	22.9			
D.F.		1/58	1/57			
P		<0.001	<0.001			

Clearly there were no differences in the distribution of larvae at 0^{o}C. after 3 and 24 hours and they were probably not reacting to this temperature. At -2.0^{o}C., however, some downwards movement had occurred by 3 hours and this had increased considerably by 24 hrs. At -4.0^{o}C., the movements away from the surface followed the same pattern but were more rapid and to a greater depth. Evidently larvae are well able to protect themselves from freezing by moving deeper into the soil.

8.8. Studies on the Growth of Larvae

The growth of larvae was investigated in two ways, by weighing larvae taken from the field at intervals throughout the season, and by rearing them in pot cultures under constant temperature conditions.

8.8.1. Studies on Field Populations

8.8.1.1. Materials and Methods

The growth of larvae in the field was investigated during the season 1985-86 by periodically taking random samples from the field at Lawmuir and extracting them by the "Blasdale" apparatus. The extracted larvae were first washed, to remove adhering soil particles, quickly dried by rolling them on filter paper and then weighed immediately on an electro-magnetic balance before any losses due to desiccation could occur.

8.8.1.2. Results

The mean weights of the larvae extracted on each occasion are shown in Table 31 along with their standard errors. The % increases in weight from each sampling date to the next are also shown along with estimates of the mean air temperatures during each intervening period. These data are also presented graphically in Figure 34.

Larvae grew throughout the period October to June when they reached their maximum weight. At first, during the autumn, growth appeared to be fairly rapid, but as winter approached the rate of weight gain decreased until it reached a minimum in February. After this, growth accelerated rapidly until shortly before the June maximum. There was then a marked fall in weight which corresponded with the prepupal diapause characteristic of the species.

There were also, during this period, marked changes in the environmental temperatures and it is interesting to see to what extent these changes in growth rate can be related to temperature. To do this,

Figure 34. Growth curve of T.paludosa larvae under field conditions in the season 1985-1986.

Vertical bars = standard error.

Stars = temperature of period °C.

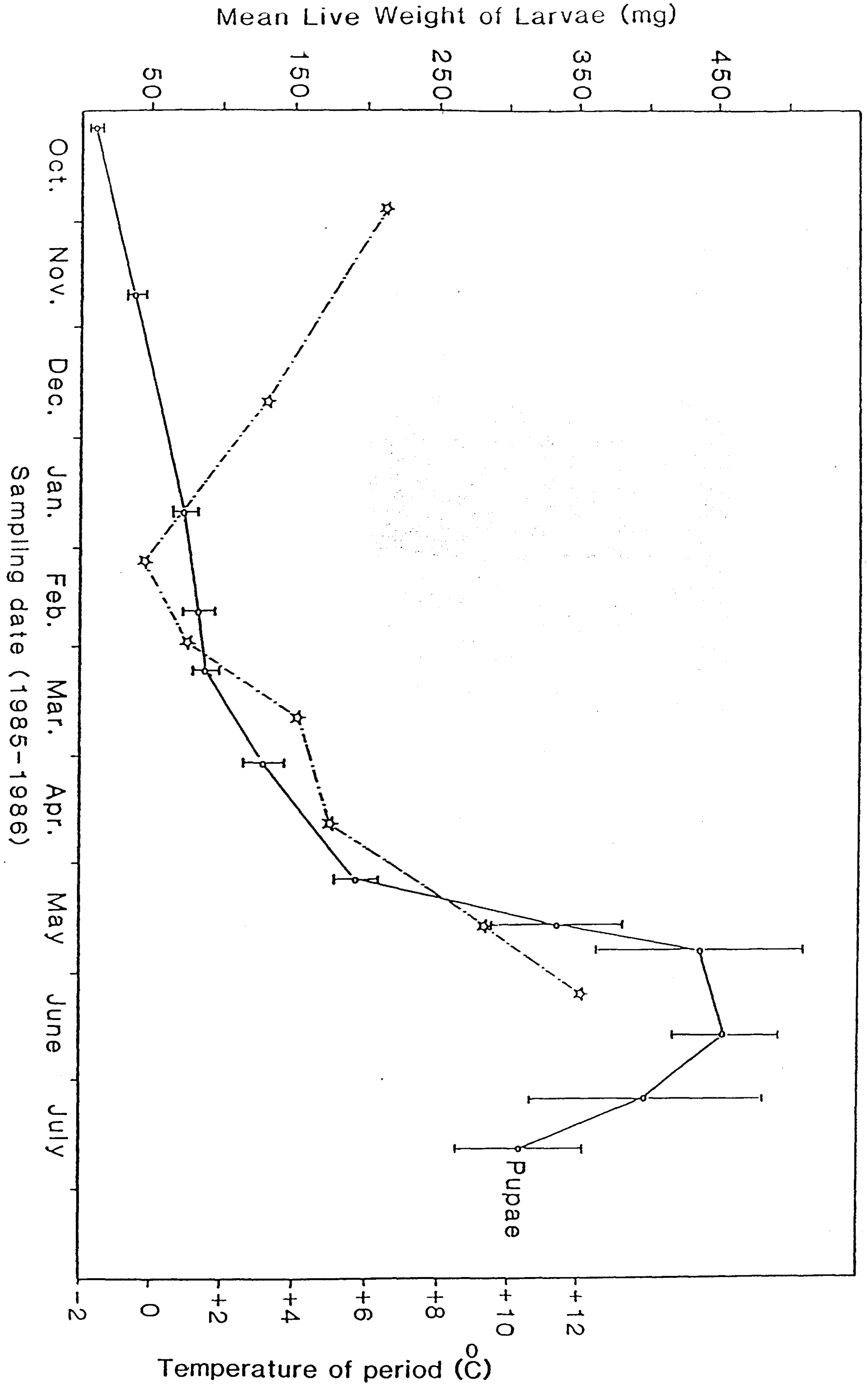

Mean Live Weight of Larvae (mg)
50
150
250
350
450
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Sampling date (1985-1986)
Pupae
-2
0
+2
+4
+6
+8
+10
+12
Temperature of period (0C)

TABLE 31

Mean live weights of larvae (mg.) collected from the field in 1985-86

Date	Mean weight (mg) ± S.E.	N	Mean air temp. of period °C
5 October 1985	10.3 ± 1.30	17	—
21 November 1985	39.7 ± 3.2	48	6.5
21 January 1986	73.0 ± 3.8	77	3.2
18 February 1986	84.4 ± 4.9	32	-0.2
7 March 1986	88.9 ± 3.7	48	1.0
2 April 1986	129.2 ± 6.8	37	4.1
5 May 1986	194.4 ± 7.7	56	5.0
18 May 1986	329.8 ± 23.01	27	9.3
25 May 1986	437.14 ± 36.62	14	9.3
18 June 1986	453.7 ± 17.48	65	12.0
6 July 1986	398.8 ± 41.07	9	12.9
20 July 1986	307.9 ± 22.94	21	13.2

the mean larval weights on each sampling date were converted to logarithms (base 10) and the differences between the logarithms between successive dates were taken as representing the ratios of the weights on these days. These values, divided by the number of days elapsing between observations represented daily growth rates. Clearly, this process can only be applied while the larvae are growing so no observations after June 18 are included.

These data are shown in Table 32 and the log. growth rates are plotted against temperature in Figure 35. From Figure 35 it can be seen that there was a fairly close correlation between growth rate and temperature throughout the period except for the last observation when

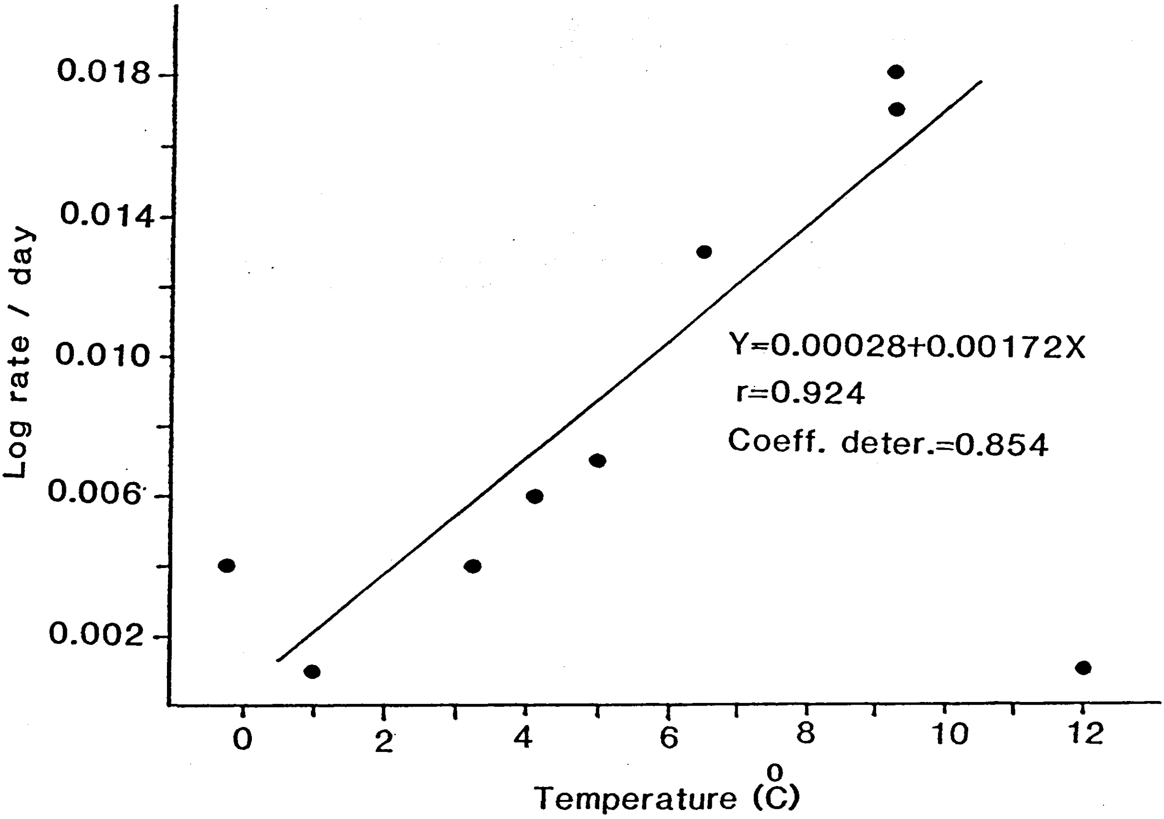
TABLE 32

Relation between Growth Rate of Larvae in Field and Temperature

Date	Mean Wt. mg.	Log.Wt.	Log.Wt. increase (A)	No.of days between obs. (B)	Rate of increase (A/B)	Mean Temp. of period
5 Oct.	10.3	1.01				
21 Nov.	39.7	1.60	0.59	47	0.013	6.5
21 Jan.	73.0	1.86	0.26	61	0.004	3.2
18 Feb.	84.4	1.93	0.07	28	0.0025	-0.2
7 Mar.	88.9	1.95	0.02	17	0.001	1.0
2 Apr.	129.2	2.11	0.16	26	0.006	4.1
5 May	194.4	2.29	0.18	33	0.005	5.0
18 May	329.8	2.52	0.23	13	0.018	9.3
25 May	437.1	2.64	0.12	7	0.017	9.3
18 Jun.	453.7	2.66	0.02	23	0.001	12.0
6 Jul.	398.8	2.60	-			
20 Jul.	307.9	2.49	-			

it was very much less than might be expected at the prevailing temperature. It must be concluded that at this stage growth was decelerating because it was almost complete and its rate was no longer related to temperature. The strength of the correlation of growth rate on temperature for the eight observations between the period 5 October to 25 May was measured by product-moment correlation analysis, and gave the highly significant "r" value of 0.924 (r value, 6 d.f. for $P<0.001 = 0.925$). The Coefficient of Determination (r^2) suggested that 85.4% of the total variation observed in growth rate could be explained by the observed variations in temperature.

Figure 35. Log rates of growth/day at different temperatures in field observations.


0.018
0.014
0.010
0.006
0.002
Log rate / day
0
2
4
6
8
10
12
Temperature (°C)
Y=0.00028+0.00172X
r=0.924
Coeff. deter.=0.854

In interpreting these results it must be borne in mind that the measurements of temperatures and larval weights are only estimates and that, especially in the case of temperature, where the values were obtained by averaging air temperatures over long periods, may be subject to errors of unknown magnitude. However, they do give some indication of the likelihood of temperature being the most important climatic factor involved in regulating the speed of development and invite further investigation. The temperature and precipitation records from October 1983-86 are shown in Appendix VIII Table H and Fig. A.

8.8.2. The Effects of Temperature on the Growth of Larvae in the laboratory

8.8.2.1. Materials and Methods

Larvae were collected from the field and kept in insectary cultures until needed for the experiments. To investigate the effects of temperature on their growth, larvae were placed separately in 6 cm. diameter labelled plant pots containing moist compost. Powdered grass was added for food and the pots were kept at the following constant temperatures: 5, 10, 20 and 25 degrees C. Eight pots were set up at each temperature and were kept in darkness, either by using a closed incubator, or, in the case of those kept in the 20°C. "Phytotron", by covering them with black paper.

Each larva was weighed to the nearest milligram at the outset of the observations and, thereafter at intervals varying from one to three weeks, all measurements being made on the same days. Before weighing each larva was washed to remove all soil and was then quickly rolled on filter paper to remove surface moisture. Dead larvae were replaced as necessary.

8.8.2.2. Results

The weights of individual larvae kept at each temperature are shown against weighing dates in Tables 33-36. Deaths are indicated by the letter "D" and the weights of replacement larvae are given in parentheses.

Most larvae increased in weight with time although there was much variation between individuals and decreases between successive weighings were frequent. Numerous deaths occurred especially at the lowest and highest temperatures.

To investigate growth rates, the weights of all larvae that survived for four or more weighings were converted to logarithms (base 10) and the logged values for each individual are entered against weeks in Tables 37-40. The initial weighing is entered for Week 1, and a repeat weighing the next week is entered for Week 2 and so on until all values had been entered.

To investigate and compare the rates of growth of larvae at different temperatures the logged values in Table 37-40 were combined to give a regression line of log. weight on temperature for each temperature and the four regressions were compared by Analysis of Covariance (Table 41). The value of the variance ratio for differences between b-values (F = 8.34, 3/148 d.f., P<0.001) indicated clear differences in the rates of growth of larvae at different temperatures.

The b-values given in Table 41 represent the rates of growth of the larvae in logarithmic terms and their antilogs. give the factors by which their weight increases each week. These factors and their values - and + one standard deviation are also shown in Table 41 and in graphical form in Figure 36. The large standard deviations associated with each b are indicative of the high variability of the larvae: some individuals developed much more quickly than others even at the same temperature.

Figure 36. Growth rates of larvae at different temperatures ($\pm$ 1 S.D.).

Vertical bars = standard deviation

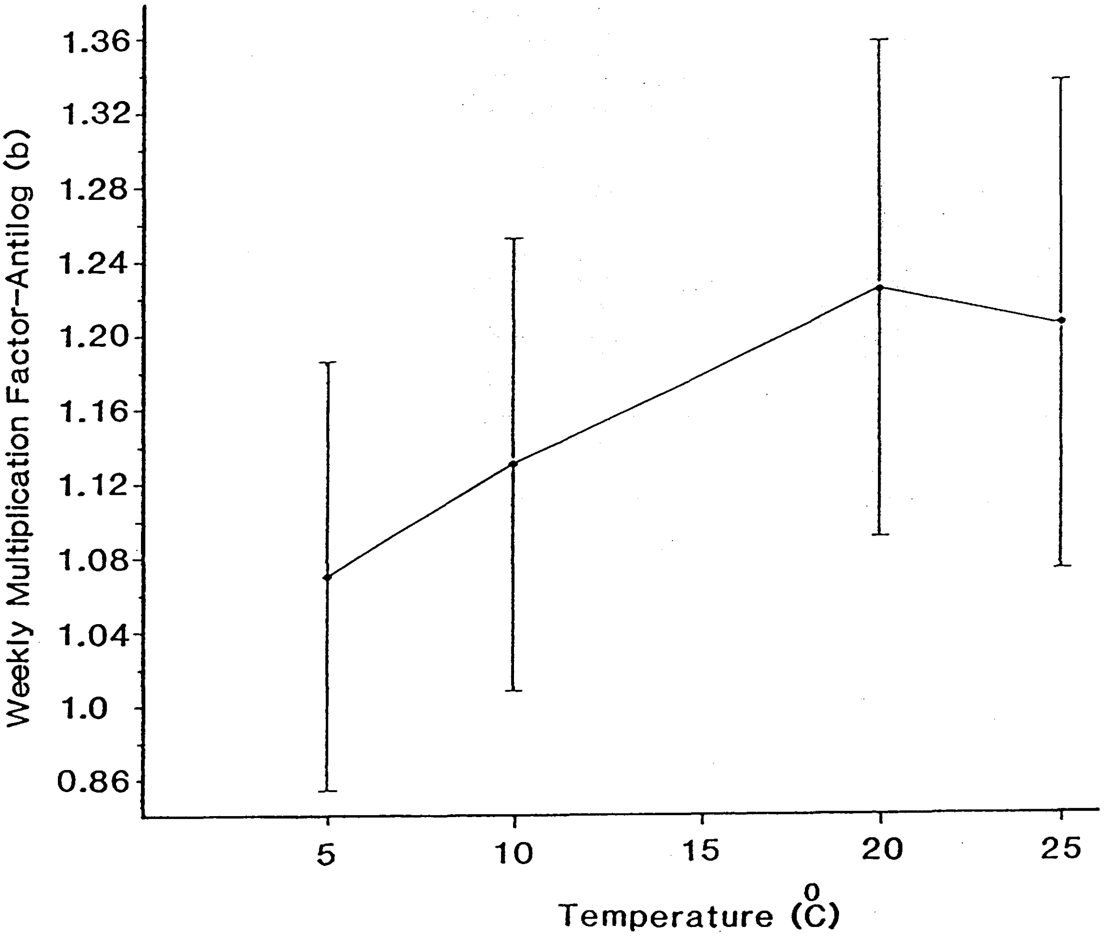

1.36
1.32
1.28
1.24
1.20
1.16
1.12
1.08
1.04
1.0
0.86
Weekly Multiplication Factor-Antilog (b)
5
10
15
20
25
Temperature (°C)

TABLE 33

Weights of Leatherjackets (mg.) at 5.0°C

Date of weighing	Individual Pot Number							
	1	2	3	4	5	6	7	8
20.11.85	60	16	21	14	44	30	20	12
27.11.85	D (61)	D (30)	D (65)	18	34	19	53	20
4.12.85	54	53	81	D (27)	27	26	45	22
18.12.85	D (49)	74	81	D 63	27	31	D (81)	D (40)
7.1.86	D (83)	91	100	93	49	47	104	31
22.1.86	D (121)	107	116	98	40	70	108	D (103)
5.2.86	141	120	108	103	42	90	129	D (60)
19.2.86	D	118	155	D	100	134	136	80

TABLE 34

Weights of Leatherjackets (mg.) at 10.0°C

Date of weighing	Individual Pot Number							
	1	2	3	4	5	6	7	8
20.11.85	73	10	18	60	70	11	22	21
27.11.85	64	13	40	70	D (15)	43	32	D (40)
4.12.85	116	82	80	48	45	30	41	50
18.12.85	120	87	D (60)	50	D (34)	30	36	52
7.1.86	114	100	36	44	35	31	74	80
22.1.86	152	136	D (147)	D (116)	D (111)	D (118)	84	91
5.2.86	196	145	150	160	114	130	110	100
19.2.86	226	159	156	204	147	130	119	110

TABLE 35

Weights of Leatherjackets (mg.) at 20.0°C (covered)

Date of weighing	Individual Pot Number							
	1	2	3	4	5	6	7	8
4.12.85	21	91	42	72	90	80	47	50
18.12.85	D (80)	105	49	103	92	96	70	60
7.1.86	91	204	123	312	215	225	186	140
22.1.86	230	264	180	317	282	255	D (100)	140
5.2.86	450	411	436	546	550	382	162	370
19.2.86	670	526	454	628	750	386	354	623

TABLE 36

Weights of Leatherjackets (mg.) at 25.0°C

Date of weighing	Individual Pot Number							
	1	2	3	4	5	6	7	8
20.11.85	63	40	35	62	54	51	41	40
27.11.85	D (52)	D (50)	56	49	D (50)	D (70)	40	D (46)
4.12.85	58	82	88	D (57)	53	62	D (45)	125
18.12.85	110	226	73	93	D (110)	225	64	298
7.1.86	263	333	120	175	250	424	132	357
22.1.86	342	370	99	D (150)	348	418	144	D (146)
5.2.86	477	412	D (55)	123	426	502	180	230
19.2.86	D	420	D	105	414	555	379	450

Table 37. Log_{10} of individual larval weight per week at 5.0°C.

Group	Pot No.	WEEK													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Y 1	2	1.48	1.72		1.87			1.96		2.03		2.08		2.07	
Y 2	3	1.81	1.91		1.91			2.00		2.06		2.03		2.19	
Y 3	5	1.64	1.53	1.43		1.43			1.69		1.60		1.62		2.00
Y 4	6	1.48	1.28	1.42		1.49			1.67		1.85		1.95		2.13
Y 5	4	1.80		1.97		1.99		2.07							
Y 6	7	1.90		2.01		2.03		2.11		2.13					

N = 39
Mean $\bar{X}$ = 6.2821
Mean $\bar{Y}$ = 1.8292
a = 1.6371

b = 0.0306
S.D. about Regression = 0.9285
S.D. of b = 0,0357

Table 38. Log_{10} of individual larval weight per week at 10.0°C.

Group	Pot No.	WEEK													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Y 1	1	1.86	1.81	2.03		2.08			2.06		2.18		2.29		2.35
Y 2	2	1.00	1.11	1.91		1.94			2.00		2.13		2.16		2.20
Y 3	4	1.78	1.85	1.68		1.70			1.64						
Y 4	6	1.04	1.63	1.48		1.48			1.49						
Y 5	7	1.34	1.51	1.61		1.56			1.87		1.92		2.04		2.08
Y 6	8	1.60	1.70		1.72			1.90		1.96		2.00		2.04	

N = 41
Mean $\bar{X}$ = 6.0976
Mean Y = 1.7983
a = 1.4736

b = 0.0532
S.D. about Regression = 1.0099
S.D. of b = 0.0368

Table 39. $\log_{10}$ of individual larval weight per week at 20.0°C.

Group	Pot No.	WEEK													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Y 1	2	1.96		2.02		2.31			2.42		2.61		2.72		
Y 2	3	1.62		1.69		2.09			2.26		2.64		2.66		
Y 3	4	1.86		2.01		2.49			2.50		2.74		2.80		
Y 4	5	1.95		1.96		2.33			2.45		2.74		2.88		
Y 5	6	1.90		1.98		2.35			2.40		2.58		2.59		
Y 6	8	1.70		1.78		1.45			1.45		2.57		2.79		
Y 7	1	1.90		1.96			2.36		2.65			2.83			

N = 41
Mean $\bar{X}$ = 6.4146
Mean Y = 2.2680
a = 1.7107

b = 0.0869
S.D. about Regression = 1.0295
S.D. of b = 0.0420

Table 40. $\log_{10}$ of individual larval weight per week at 25.0°C.

Group	Pot No.	WEEK													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Y 1	1	1.72	1.76		2.04			2.42		2.53		2.68			
Y 2	2	1.69	1.91		2.35			2.52		2.57		2.61		2.62	
Y 3	3	1.54	1.75	1.94		1.86			2.08		2.00				
Y 4	5	2.04			2.40		2.54		2.63		2.62				
Y 5	6	1.84	1.79		2.35			2.63		2.62		2.70		2.74	
Y 6	8	1.66	2.10		2.47			2.55							

N = 35
Mean $\bar{X}$ = 5.7143
Mean Y = 2.2363
a = 1.7754

b = 0.0807
S.D. about Regression = 0.8998
S.D. of b = 0.0402

TABLE 41

Comparison of rates of growth of larvae at different temperatures by Analysis of Covariance

Temp. °C	N	b-value	S.D.of b	Growth rates Antil. b	Antil. (b -S.D.)	Antil. (b + S.D.)
5	39	0.0306	0.0357	1.073	0.988	1.165
10	41	0.0532	0.0368	1.130	1.038	1.230
20	41	0.0869	0.0420	1.222	1.109	1.345
25	35	C.0807	0.0402	1.204	1.098	1.321

F for diff. between b-values = 8.34, 3/148 d.f., $P<0.001$.

The rate of growth is low at 5°C. but becomes progressively higher through 10°C. to 20°C. At 25°C., however, there was a diminution of growth rate and this, combined with a sharp observed increase in mortality (see below), suggests that this temperature may be approaching the limits of tolerance for this species.

8.9. Proportions of Larvae Pupating when Cultured at Different Temperatures

To investigate the effects of culturing temperature on the proportions of larvae pupating successfully larvae taken from soil samples in the field at Lawmuir on 30 June 1986 were transferred to "pot" cultures and kept at 10, 15, 20 and 25°C. They were kept under intermittent observation until October, by which time they had either pupated or died. The results are indicated in Table 42.

Complete and partial analyses of these data suggest that the proportions of larvae which failed to pupate were much higher at 10 and 25°C than at 15 and 20°C. The results lend support to the hypothesis that 25°C. is nearing the limits of tolerance of this species.

TABLE 42

The Fate of Mature Larvae Kept in Culture at Different Temperatures

	Temperature, C.			
	10	15	20	25
Nos. Pupated	7	23	12	2
Nos. Failed to pupate	18	15	13	23

Comparisons: All Data: Chi-square = 19.63, 3 d.f. $P < 0.001$
10/15/20 C. " " = 6.39, 2 d.f. $P < 0.05$
15/20/25 C. " " = 17.58, 2 d.f. $P < 0.001$
10/15 C. " " = 5.15, 1 d.f. $P < 0.05$
15/20 C. " " = 0.51, 1 d.f. $P > 0.10$
20/25 C. " " = 8.03, 1 d.f. $P < 0.01$

8.10. The Weights of Mature Larvae and Pupae in Relation to Their Sex and the Fecundity of the Females

8.10.1. Introduction

Adult Tipulidae vary greatly in size and, in general, the fecundity of the females is related to size: e.g. Molophilus ater Meigen is a small species laying about 78 eggs (Hadley, 1969) whereas the large Eriocera spp. may lay more than 1000 (Alexander, 1920). A similar relationship between size and fecundity has also been found within individual species (Hadley, 1971b; Meats, 1974a; Pritchard, 1976) and it has been shown for T.paludosa (Maercks, 1939b), T.oleracea (Laughlin, 1967) and M.ater (Hadley, 1971b) that fecundity in the adult depends on the weight of the pupa. This, of course, depends on the weight of the larva before pupation as imagines are fully fed at eclosion.

Female adults are generally larger than males and the differences are evident during development. Laughlin (1960) was able to determine the sex of the last instar larvae of T.oleracea by referring to growth curves which differed significantly in the two sexes.

In the present work observations were made on the weights of mature larvae and pupae of T.paludosa and these were related to sex and to the fecundity of the females.

8.10.2. Materials and Methods

To investigate the weights of male and female mature larvae and pupae of T.paludosa, cores of soil, taken from the Lawmuir site on 30 June 1986, were processed by means of the Blasdale apparatus. The larvae extracted were immediately washed and weighed, placed individually in plastic cups containing a shallow layer of soil and transferred to an incubator at 15°C. Observations were made daily and, on pupation, each individual was weighed again and its sex was recorded. (The method used to distinguish between sexes is illustrated in the photograph, Fig. 37).

To determine whether the fecundity of the female depended on the weight of pupa, larvae collected from the field were reared in the laboratory and, on pupation, the females were weighed. As soon as the adults emerged they were dissected and the eggs were counted.

8.10.3. Results

Fifteen male and thirteen female mature larvae were reared to pupae and their weights, as both larvae and pupae, are recorded in Table 43. There was a wide range of weight amongst the larvae, the heaviest being some 2.57 times as heavy as the lightest and there was a clear difference between the sexes, the males, ($\bar{x} = 363.3 \pm 14.5$) being smaller than the females ($\bar{x} = 539.2 \pm 24.4$) (t = 6.39, 26df. P<0.001).

Figure 37. Female and male T.paludosa pupae.

♀
♂
1 cm

TABLE 43

The Relationship Between the Weight of the Larva and the Weight and Sex of the Pupa

	Weights in mg.				
	Male			Female	
No.	Larva	Pupa	No.	Larva	Pupa
1	320	180	1	630	340
2	280	160	2	450	250
3	370	160	3	520	230
4	320	180	4	480	250
5	320	170	5	470	240
6	330	160	6	460	190
7	340	190	7	560	320
8	300	180	8	460	230
9	390	220	9	660	320
10	480	240	10	480	250
11	430	210	11	520	220
12	360	170	12	600	330
13	400	190	13	720	365
14	370	190			
15	440	190			
Means	363.3	186.0		539.2	271.9
S.E	14.5	5.9		24.4	15.3

There was a similarly wide range amongst the weights of the pupae (1:2.28) and males ($\bar{x}$ = 186.0 $\pm$ 5.9) were much smaller than females ($\bar{x}$ = 271.9 $\pm$ 15.3) (t = 5.51, 26df. $P<0.001$).

The relationships between the weights of the larvae and of the pupae they produced are shown in Fig. 38. In both sexes there was a close correlation between the weight of a larva and its pupa (r for males, 0.758, 13df., $P<0.01$; r for females, 0.887, 11df., $P<0.001$). the relevant regression equations of pupal/larval weights were:

Male: $Y = 73.722 + 0.309X$ (S.E. = 15.508)

$r = 0.7584$

r^2 (c.d.) = 0.5752

Female: $Y = -28.950 + 0.558X$ (S.E. = 26.662)

$r = 0.8870$

r^2 (c.d.) = 0.7868

The mean % loss of weight on pupation was similar in both sexes (males = 48.8; females = 49.6).

Nine female pupae matured into adults and their weights and the numbers of eggs found by dissection are shown in Table 44 and Fig. 39. There was a close correlation between the weight of the pupa and the egg burden of the adult ($r = 0.869$, $P<0.05$) and the regression equation for No. of eggs/pupal weight was:

$Y = -91.189 + 1.757X$

Several workers estimated the fecundity of T.paludosa by counting eggs from dissected females. These data are summarized in Table 45.

Figure 38. The relationship between the weight of the larvae and the weight and sex of the pupae.

Closed circles = Males

Open circles = Females

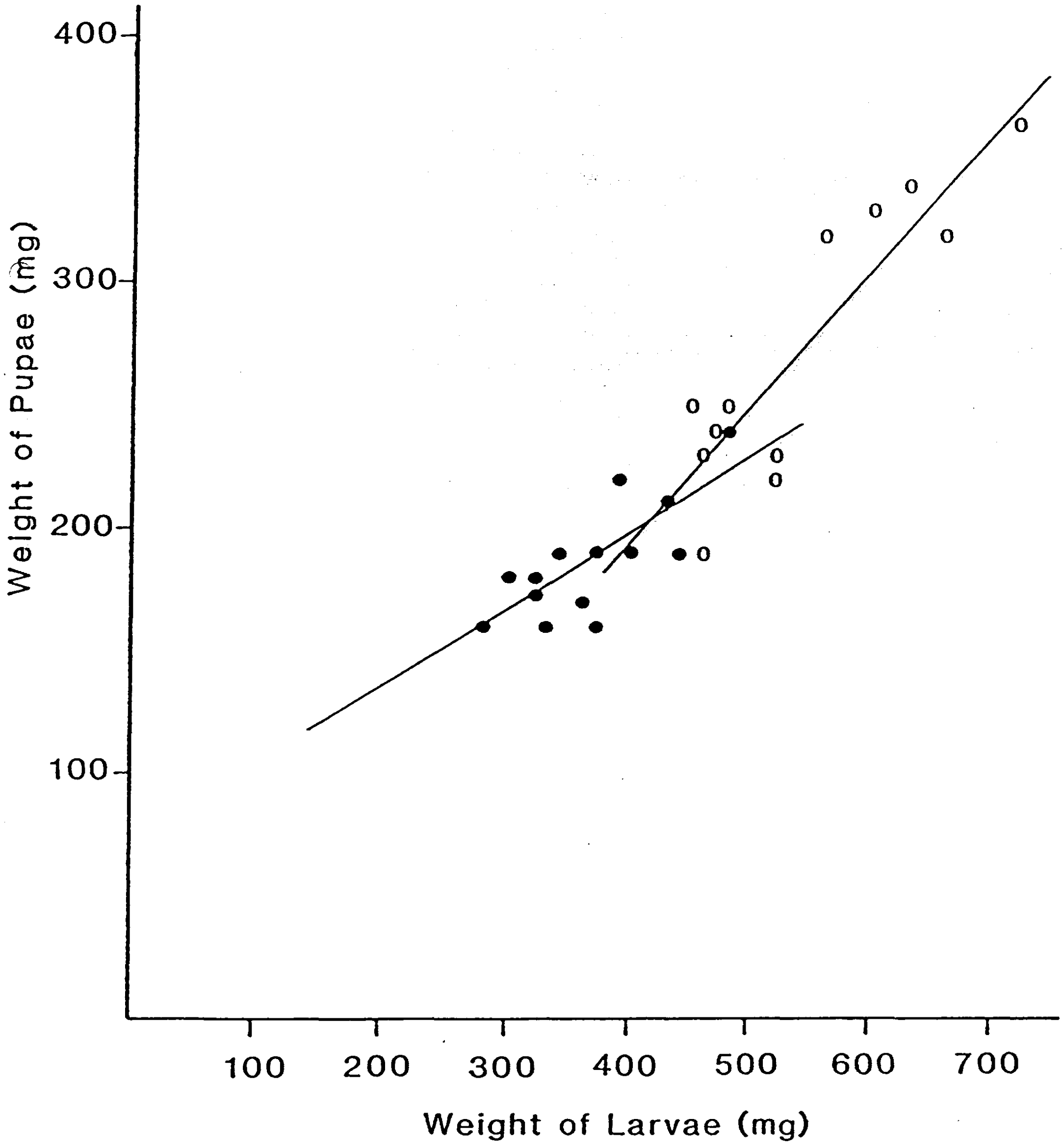
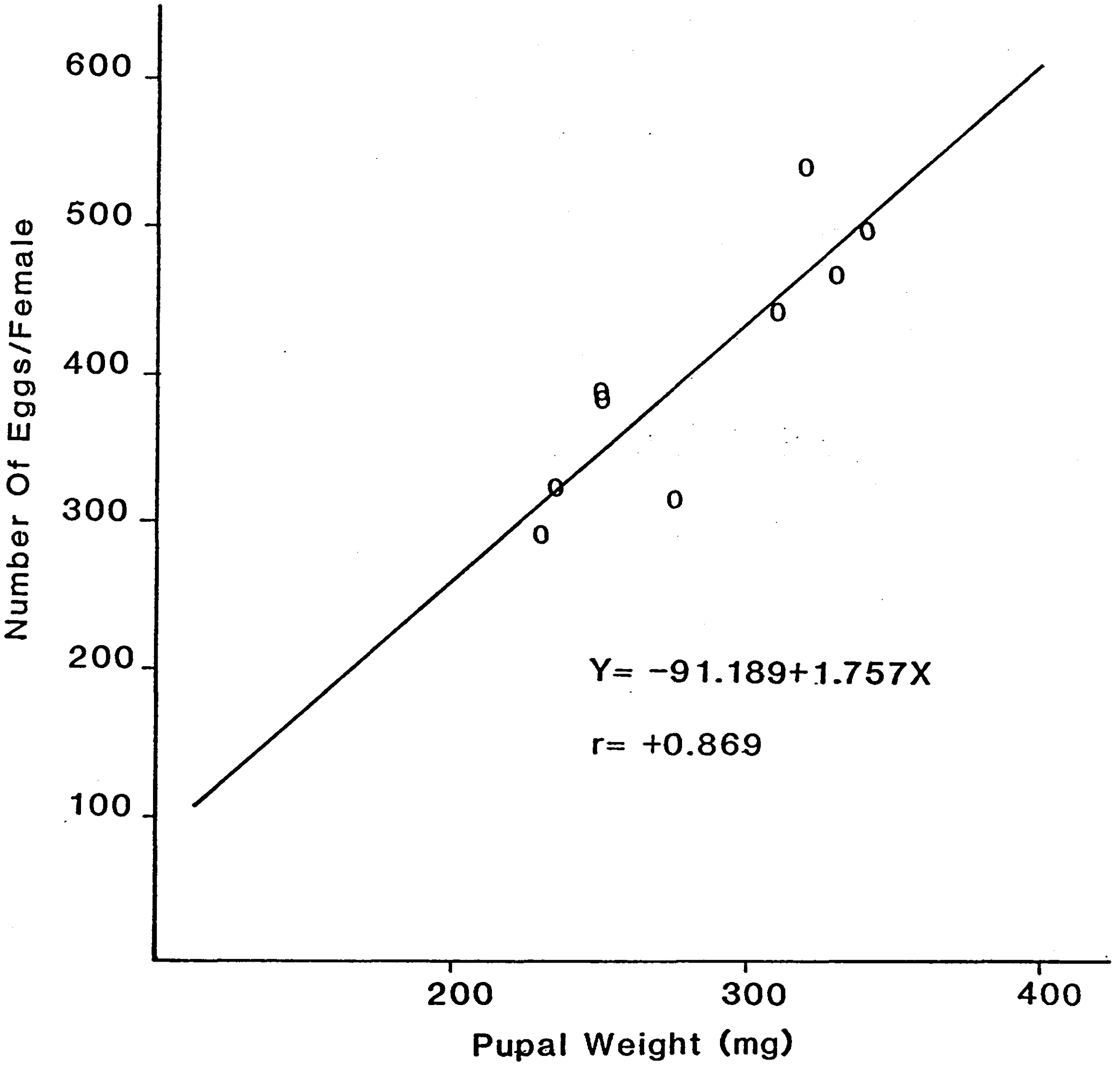

400
300
200
100
Weight of Pupae (mg)
100
200
300
400
500
600
700
Weight of Larvae (mg)

Figure 39. The relationship between the weight of the female pupae and the egg burden of the adults.



600
500
400
300
200
100
Number Of Eggs/Female
200
300
400
Pupal Weight (mg)
Y= −91.189+1.757X
r= +0.869

TABLE 44

The Relationship Between the Weights of the Female Pupae and the Egg Burden of the Adults

No.	Weight of Pupa (mg.)	Egg Burden of Adult
1	340	496
2	230	290
3	250	387
4	250	385
5	310	442
6	275	315
7	320	540
8	330	467
9	235	321
Mean	282.2	404.8
S.E.	14.4	29.1

TABLE 45

Tipula paludosa fecundity estimates of various workers

Author	Mean ($\bar{x}$)	S.D.	N	Range
Present observation	371.4	94.0173	25	130 - 540
Jackson (1975)	337.6	136.6	35	147 - 759
Coulson (1962)	360	-	24	-
Laughlin (1958b)	-	-	-	Max. 971
Thompson (1946)	400	-	-	-
Maercks (1939a)	550	-	-	-
(1939b)	350	-	-	Max. 1300
Barnes (1937)	273.4	110.2	28	48 - 487
Lovibond (1937)	272	-	-	48 - 429
Sellke (1936)	-	-	-	36 - 500
Rennie (1917)	397.7	125.3	3	255 - 490

SECTION 9

DISEASES, PARASITES AND INVERTEBRATE PREDATORS OF TIPULIDAE

9.1. Introduction

Many workers have recorded the occurrence of fatal and debilitating pathogenic organisms in natural populations of Tipulidae and, in particular, in those of T.paludosa from Scotland (Ahmed, 1968), England (Sherlock, 1973; Carter, 1976, Carter et al., 1983; Beesley, 1977) and France (Ricou and Douyer, 1975). These pathogens include viral, bacterial, protozoal and fungal diseases, parasitic Nematoda, parasitoid Diptera and various types of invertebrate predators. Although there is much detailed knowledge of these separate factors few workers (apart from Carter et al., 1983, who demonstrated total mortalities, due to viruses, bacteria and insect parasitoids, of 10.7% and 7.7% amongst populations of larvae of T.paludosa in a field near Liverpool for the years 1977-78 and 1978-79 respectively) have attempted to assess the separate and combined effects of them in terms of the mortality they produce and their influence in population regulation.

One of the aims in the present work has been to identify and to evaluate the pathogens affecting populations of T.paludosa in the Glasgow Area of S.W. Scotland and, towards this end large numbers of larvae collected in the field, mainly at Lawmuir, Garscube and Cochno during the years 1984-86 were first examined externally for obvious signs of infection or damage and were then dissected in Insect Ringer Solution and examined for parasites and parasitoids. Special studies were made of the body wall, fat body and alimentary canal either with the aid of a light microscope or, where necessary, by means of a scanning electron microscope. The incidence of diseases, parasites and parasitoids was noted in each case and the results are summarised in Table 46.

TABLE 46

Summary of Parasite Species Recorded From *T. paludosa* larvae in this study

Phylum	Subphylum	Class	Subclass	Order	Suborder	Family	Genus & Species
Protozoa	Sporozoa	Telosporea	Gregarinia	Eugregarinida	Cephalina	Gregarinidae	*Gregarina longa*
"	"	"	"	"	"	"	*H. ventricosa*
"	"	"	"	"	"	Actinocephalidae	*Actinocephalus tipula*
		Arthropodophaga		Arthropodophagales		(TIV)	*Pseudomorator virus tipula*
		"		"		(NPV)	*Borrelinavirus tipula*
Nematoda					Suberfamily: Mermithoidea	Mermithidae	Unidentified
Arthropoda		Insecta	Pterygota	Diptera		Tachinidae	*Siphona geniculata* De Geer

The separate pathogens are discussed in detail below.

9.2. Virus Diseases

9.2.1. Previous work

Two types of fatal virus disease have been described from the larvae of Tipulidae: Tipula Iridescent Virus (TIV) and Tipula Nuclear Polyhedrosis Virus (NPV).

TIV was first described by Xeros (1954). It is a free virus which produces a characteristic purple-blue iridescence of the affected tissues. A relatively non-specific infection, it naturally affects several species of the genus Tipula L. including T.paludosa Mg., T.pagana Mg., T.marmorata Mg. and T.hortulana Mg. (Stewart, 1969). In addition T.livida v. de Wulp, T.oleracea L. and two other, unspecified, species of the genus have been infected experimentally (Smith et al., 1961).

Many detailed studies have been made on TIV but these mostly deal with its structure, chemical composition and development in the host and comparatively little is known about its mode of transmission and its incidence in natural populations.

Xeros (1954), recorded 15% infection amongst larvae in a low density population of T.paludosa in England whereas Ahmed (1968) and Stewart (1969), working in South-East and South-West Scotland respectively, found from 4.7 to 6% infection of the populations they studied.

NPV affects the haemocytes and fat cells of its host (Smith & Xeros, 1954) and infected individuals gradually become pallid and eventually chalky white before they die. The disease was first noticed in T.paludosa from Scotland by Rennie (1923), who described the presence of the polyhedra, although he could not, at that time, associate them with any causative organism. It was later reported from

France by Meynadier _et al._ (1964) and Ahmed (1968) found a 1% infection amongst a population of _T.paludosa_ which he observed in South-East Scotland.

9.2.2. Present observations

In the West of Scotland populations studied, virus infections, as noted by Ahmed (1968) in the East of Scotland were generally infrequent. Both TIV and NPV were found, as recorded in Tables 47, 48 and 49.

TIV

Infected larvae were readily recognised by the characteristic blue-purple iridescence of the infected tissues (Figures 40 and 41). In every instance the fat showed the infection and in many instances the muscles of the body wall also showed iridescence. However, in no instance was there evidence of infection of the skin as observed by Anderson _et al._ (1959). In the early stages, infected larvae retained much of their activity, but they became progressively more lethargic with time.

Most of the infected larvae were in third or fourth instar and were found between October and June. Although the proportions of infected individuals in the separate samples fluctuated greatly at each site, much of this variation was probably due to the small sizes of samples taken, so the best estimates of incidence of infection should be made by taking the overall figures for each locality. Of the principal sites investigated, Cochno showed the highest rate of infection with 9.3% of individuals infected (4 out of 43), Garscube showed 3.8% (2 out of 53) and Lawmuir showed only 0.5% (2 out of 444). Clearly natural infection of TIV plays little part in regulating populations at these sites.

Figure 40 showing the colour blue-purple iridescence of TIV in dissected larva.

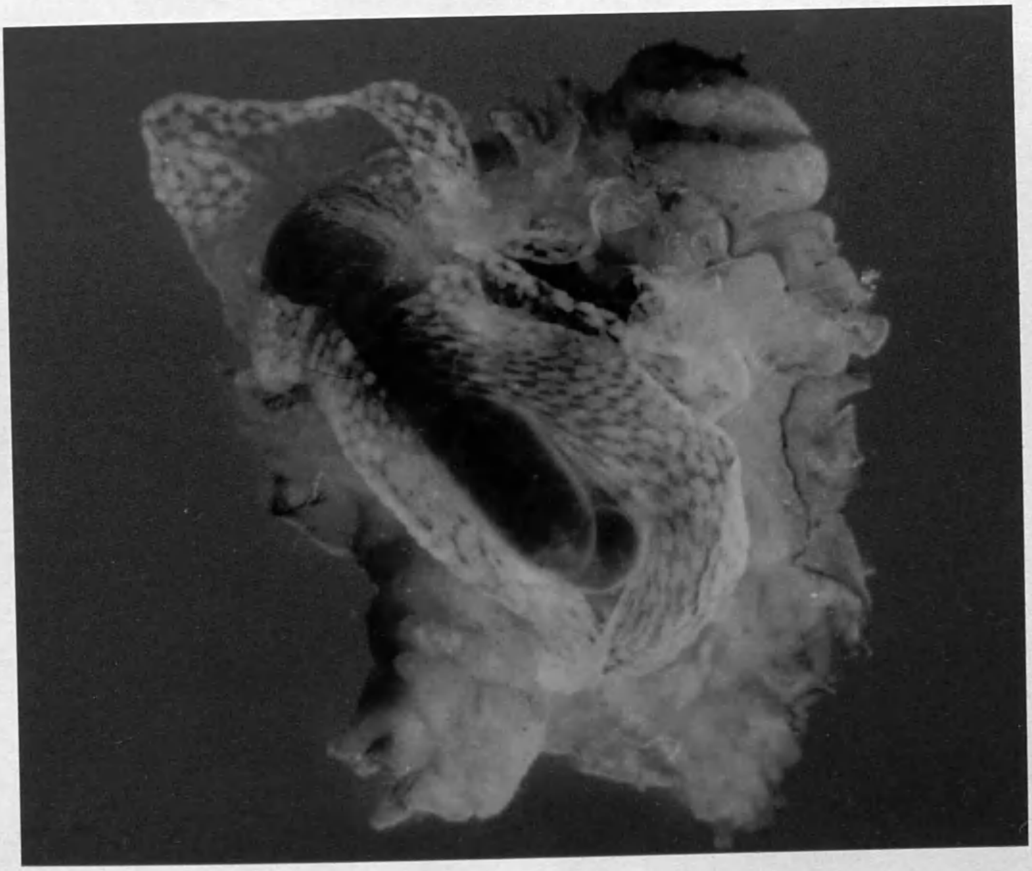


Figure 41. Dissected T.paludosa larva displaying (TIV) in the fat body and muscles of the body wall (above), non-dissected and normal larva of T.paludosa (below).

As TIV seems of possible use as an agency for the biological control of tipulid populations an attempt was made to introduce it artificially into healthy larvae by inoculation. This attempt was unsuccessful but a brief account is given in Appendix IX.

NPV

Throughout the entire sampling operations only 6 larvae were found to be infected by NPV. The incidence of infection at Lawmuir was 1.1% (5 out of 444 larvae examined) and at Garscube it was 1.9% (1 out of 53). No larvae showing this disease were found at Cochno.

Diseased larvae were characterized by their having a milky-white colour in contrast to the usual earthy grey-brown. Some individuals appeared to be heavily infected and were lethargic.

This disease appears to be of little importance in the area under study.

9.3. Bacterial Diseases

Little is known about these in Tipulidae. Ricou (1967b), however, reported an unspecified bacterial epizootic amongst T.paludosa in northern France.

No infections attributable to pathogenic bacteria were found in the present survey.

9.4. Infections by Fungi

9.4.1. Previous work

Several types of fungi have been reported to infect Tipulidae but none is common. Müller-Kögler (1965) first recorded the well-known ascomycete Cordyceps militaris (Fr.) as affecting larvae of T.paludosa and Stewart (1969) found an unidentified Cordyceps-like fungus in his laboratory cultures of T.hortulana Mg. Coulson (1962) commented on an unidentified fungal infection which affects the tracheal system of the larvae of *Tipula subnodicornis* Zetterstedt in north-east England.

9.4.2. Present observations

Several larvae were found infected by unidentified fungi most of which were white and mainly affected the areas round the spiracles. Affected larvae appeared moribund and, after death, decomposed rapidly.

Adults affected by *Empusa*-like fungi were frequently seen in field populations particularly in wet weather. As these do not affect the immature stages and the flies only succumb after several days of incubation, by which time they have laid all their eggs, their occurrence is unlikely to have any influence in population regulation.

9.5. Protozoal Infections

9.5.1. Previous work

Infections of the larvae of Tipulidae by large and conspicuous species of Gregarinidea (Sporozoa) were first noted by Leger (1892) who decribed three species, *Gregarina longa* (Leger) Labbe, *Hirmocystis ventricosa* (Leger) Labbe and *Actinocephalus tipula* (Hammerschmidt) Leger from *T.oleracea* L. in France. These organisms, attributable to the suborder Eugregarinaria, inhabit the alimentary tract and are commonly observed in the large sporont stage. Although the trophozoite develops in and damages individual cells of the mid-gut little damage is caused and the organisms have low pathogenicity.

Ricou (1967b) found infection rates of 75 and 100% of *Gregarina longa* in populations at two localities in France and Stewart (1969) found eugregarines, possibly of this species, infecting *T.paludosa*, *T.vernalis* Mg. and *Nephrotoma quadrifaria* (Mg.) in south-west Scotland.

Other Sporozoa affecting larvae of Tipulidae are an unnamed coccidian, described by Ricou (1967b) in T.paludosa from northern France which infected the fat body and caused 70% mortality and a microsporidian which affected the nervous tissue of three individuals of T.paludosa from England examined by Carter (1976).

The only record of a non-sporozoan protozoan parasite of Tipulidae is that of Vickerman (1960) who found Herpetomonas ludwigi (Krámer), a flagellate, infesting the gut.

9.5.2. Present observations

Three species of intestinal Eugregarinaria, identical to those reported by Leger (1892), were found and identification of these was confirmed by Professor K. Vickerman. They were:

(1) Gregarina longa (Leger) Labbe. This species was characterized by having a flattened bun-shaped protomerite and a long, cylindrical deutomerite (Figures 42-44). Its colour is less opaque than in the other species.

(2) Hirmocystis ventricosa (Leger) Labbe. Here the deutomerite is bulging and squat while the epimerite is expanded anteriorly (Figures 45-48). Its surface, as revealed by the scanning electron microscope, shows a characteristic longitudinal lamellar structure.

(3) Actinocephalus tipula (Hammerschmidt) Leger. This species is white, the deutomerite is tapered and the epimerite is conical.

The surface structure of this gregarine is unique in Gregarinidea (Figure 49) and this alone would justify a special study of the species (Vickerman, pers. comm.).

Gregarinidia were found in instar III and IV larvae only and most of those examined were infected. A greater proportion of larvae were infected and these showed a greater intensity of individual infection at Lawmuir, where the population density of larvae was higher, than at either Cochno of Garscube where populations were lower.

Figure 42. Scanning electron microscope view of immature stages of *Gregarina longa* from gut of *T.paludosa* larva. x 768.

Figure 43. SEM of immature stage of G.longa before pairing (SYZYGY).

x 1536.

A - Protomerite

B - Deutomerite

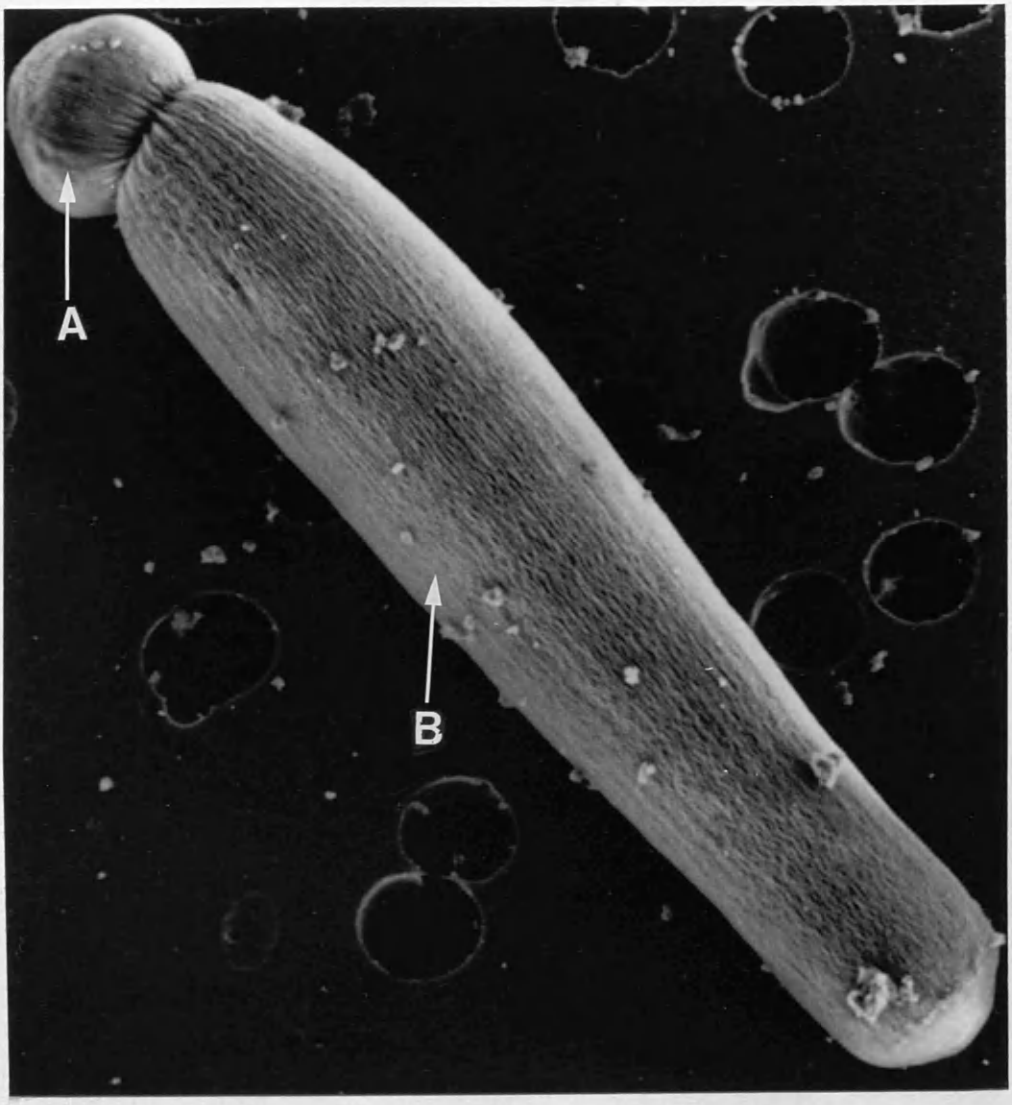

A
B

Figure 44. SEM view of adult G.longa in SYZYGY (associating gamontes).

x 384.

C - Primite individual

D - Satellite individual

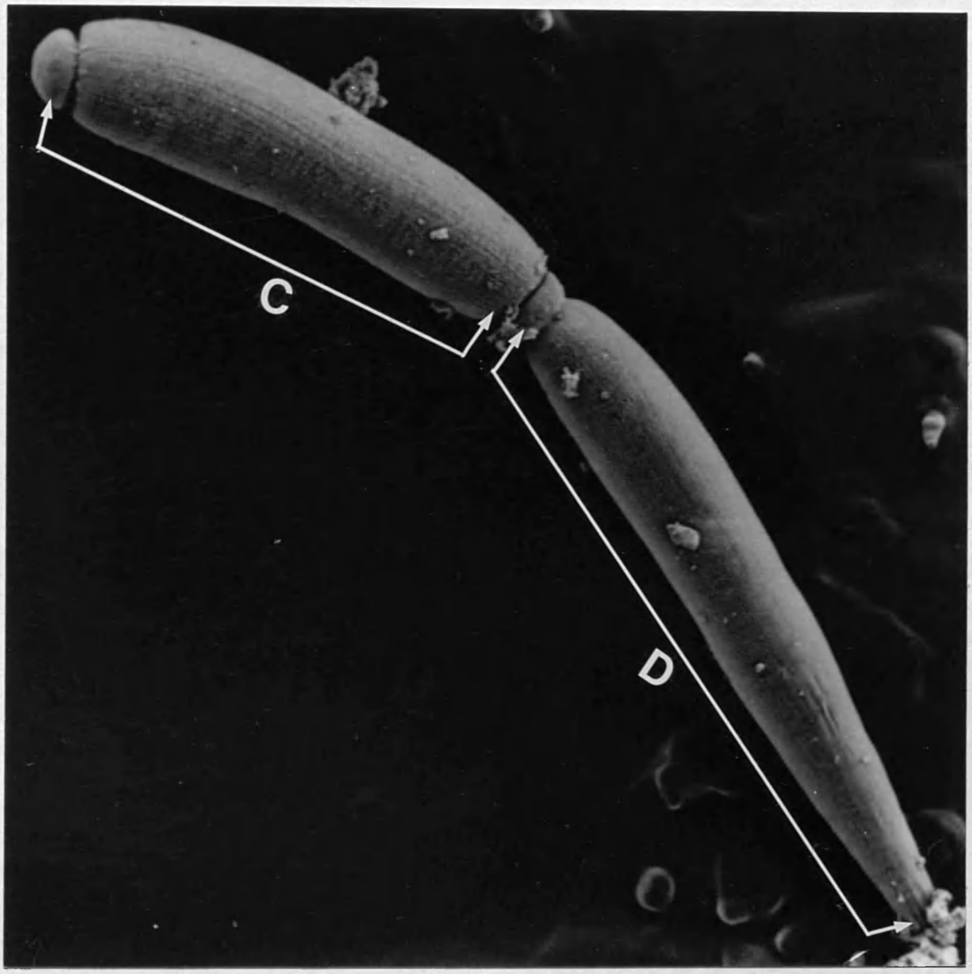
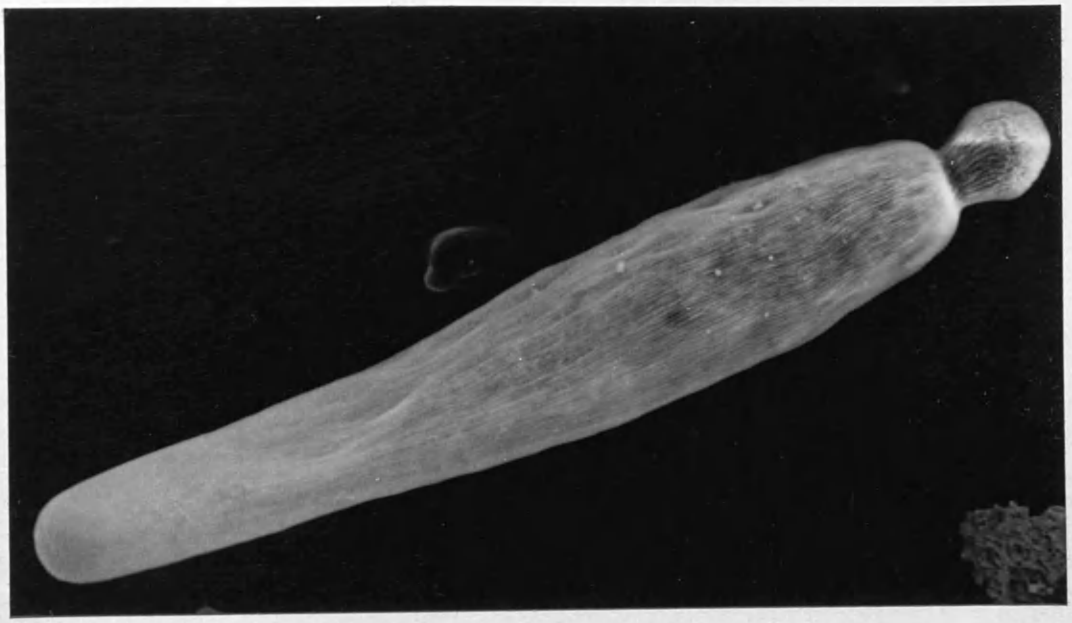

C
D

Figure 45. SEM of immature stage of *Hirmocystis ventricosa* before pairing (SYZGY). x 768.

Figure 46. SEM view of *H.ventricosa* in SYZYGY (associating gamont). x 768.

F - Epimerite with expanded anterior region



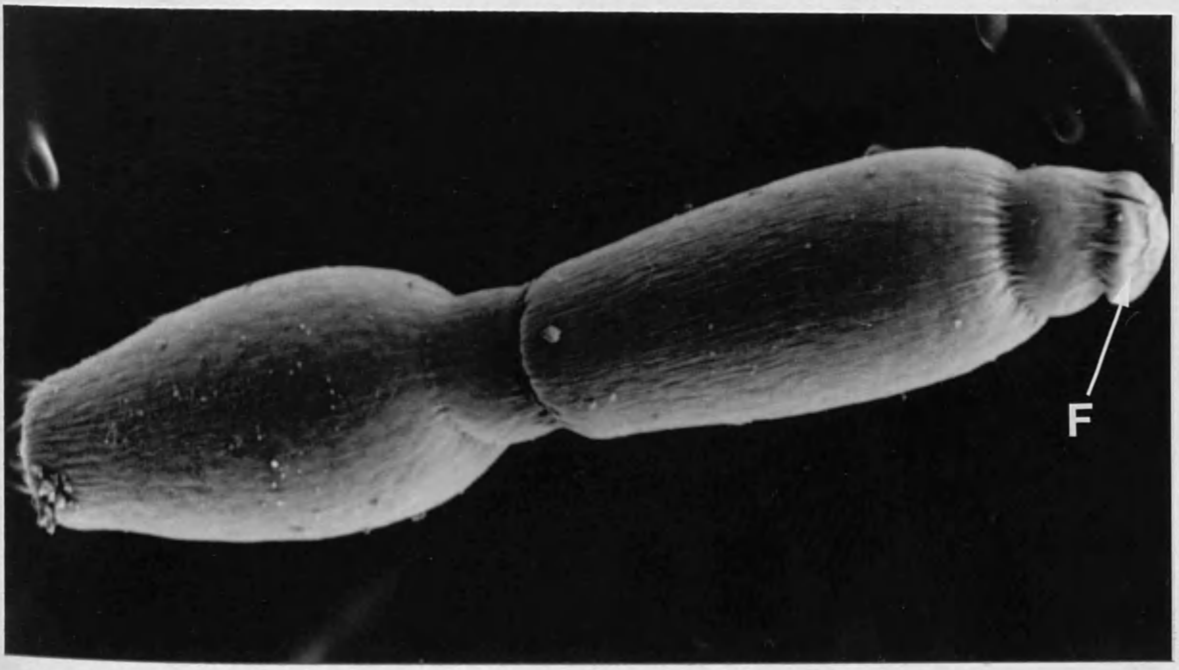

F

Figure 47. SEM view of <u>H.ventricosa</u> body surface. x 768.

Figure 48. Scanning electron microscope of Hirmocystis ventricosa showing the longitudinal fine body folds (lamellae). x 3000.

Figure 49. SEM of Actinocephalus tipulae. x 384.

E - Conical epimerite

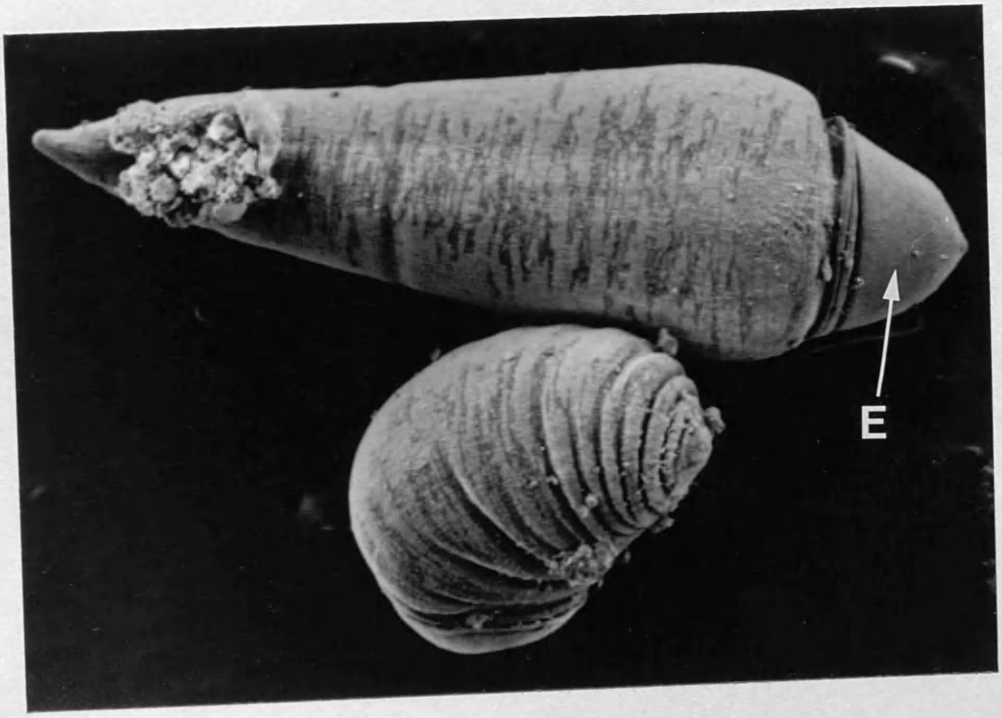
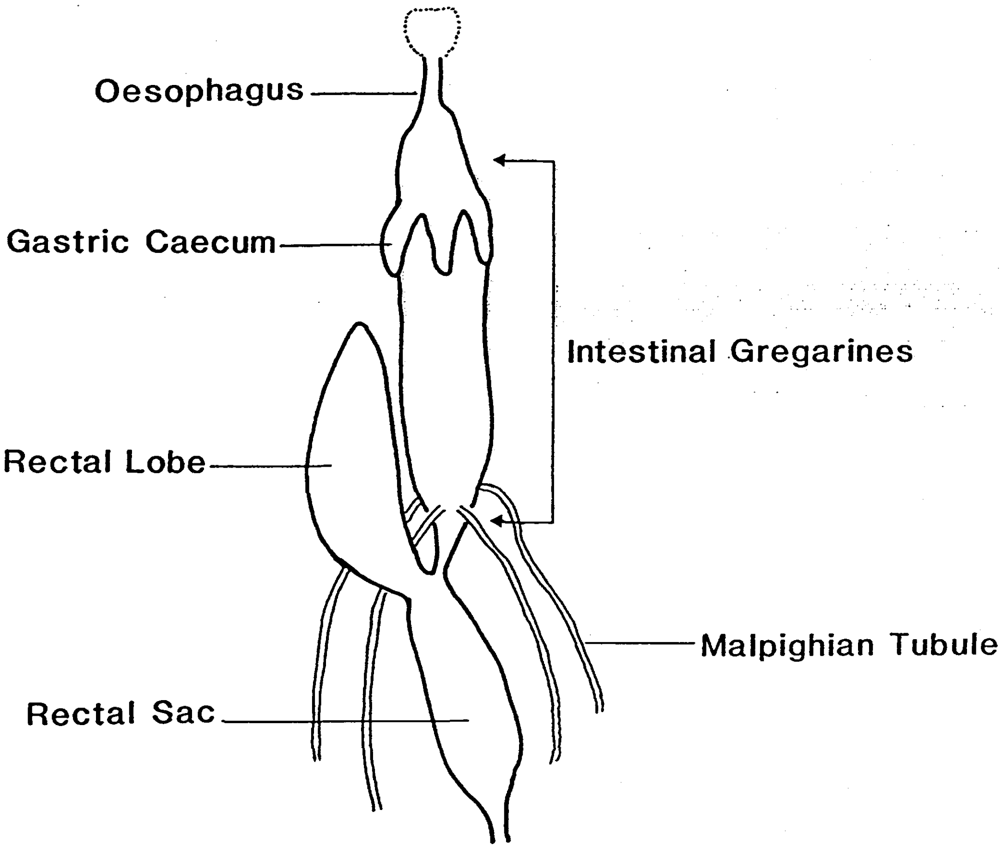

E

Figure 50. Location of intestinal Gregarines in the gut of *Tipula paludosa* larvae.


Oesophagus
Gastric Caecum
Intestinal Gregarines
Rectal Lobe
Malpighian Tubule
Rectal Sac

Infection was mainly centred on the mesenteron (Figure 50) but in heavily infected individuals the parasites also occurred in the mesenteric caeca and in the rectum. Although Gregarinidea are not regarded as acute pathogens, in heavily infected individuals they were sometimes packed so tightly that it seems likely that they must have caused some hindrance to the passage of food. Whether this affected nutrition or the behaviour of the larvae would have to be investigated experimentally. Tipulids obtain all the nutriment needed for reproduction in their larval state and female larvae which are heavy when fully fed produce adults with greater numbers of eggs (see p. 149). It would be interesting to investigate the effects of gregarine larval infections on adult fecundity.

9.6. Parasitic Nematoda

9.6.1. Previous work

Both Rennie (1924) and Sherlock (1973) recorded infections of T.paludosa larvae in the U.K. with unidentified species of mermithid nematodes, large fatal obligatorily parasitic species which live in the haemocoele of various insects. Bovien (1937) recorded two species of saprozoic and parasitic species (Neoaplectana bibionis n.sp. and N.affinis n.sp. from populations of T.paludosa in Denmark and more recently Lam and Webster (1971) two new species were described from the cadavers of T.paludosa larvae in British Columbia, Canada : Panagrolaimus tipula Lam & Webster and Rhabditis (Rhabditella) tipula Lam & Webster. In a recent trial in Newfoundland (Canada) Finney and Bennett (1984) used Heterorhabditis heliothidis (Khan, Brooks and Hirschmann) to control T.paludosa. They achieved a mortality of 96.7%.

9.6.2. Present observations

Larvae infected by unidentified species of mermithid nematodes were found at all three of the main sites (Tables 47, 48 and 49). Only third and fourth instar larvae were found infected and the rates of infection varied from 17% at Garscube (9 out of 53 larvae examined) to 4.7% at Cochno (2 out of 43) and 1.8% at Lawmuir (8 out of 444). Rennie (1924) stated that each larva contained only a single mermithid but Sherlock (1973) found between one and seven to be supported by a single host. In the present study the numbers varied from one to twelve per host.

Mermithid larvae are white and are usually found coiled up in a ring- or figure of eight-shape within the haemocoele of the host (Figure 51). The largest found was 42 mm. long.

According to Carter (1976) the mermithids generally emerged after several days when their hosts were kept at 20°C. Here they emerged within a month when larvae were kept at that temperature. Infected larvae sometimes pupated but none survived to the imaginal stage.

The emerged mermithids survived for several weeks in the soil at 20°C.

Mermithid nematodes, although fatal parasites, may be of dubious value as biological control agencies although they indubitably reduce the numbers of individuals surviving to maturity. Larvae affected by them retain much of their activity but it is not known whether or not they feed normally. It is possible that they cause as much damage as uninfected ones and that the effect of the nematode is only felt after the damage is done. More attention could be given to the bionomics of these parasites.

TABLE 47

Total number of larvae collected from Garscube and the percentage of parasites found

			No. larvae parasitised or infected with			
Date	Instar	No. larvae collected	TIV	NPV	Nematode	Tachinid
23.3.84	III	13	0	0	5	0
19.4.84	III	6	0	0	1	0
22.5.84	III	6	0	0	1	0
13.11.84	III	8	0	0	1	0
27.11.84	III	6	2	0	0	0
26.2.85	III	5	0	0	1	0
16.4.85	III	9	0	1	0	1
Total		53	2	1	9	1
Percentage			3.8	1.9	17	1.9

TABLE 48

Total number of larvae collected from Cochno and the percentage of parasites found

			No. larvae parasitised or infected with			
Date	Instar	No. larvae collected	TIV	NPV	Nematode	Tachinid
22.5.84	IV	8	1	0	1	0
28.5.84	IV	27	1	0	1	0
26.6.84	IV	8	2	0	0	0
Total		43	4	0	2	0
Percentage			9.3	0	4.7	0

TABLE 49

Total number of larvae collected from Lawmuir and the percentage of parasites found

			No. larvae parasitised or infected with			
Date	Instar	No. larvae collected	TIV	NPV	Nematode	Tachinid
6.11.84	III	42	0	0	1	0
13.11.84	III	35	0	0	0	1
4.12.84	III	70	0	2	2	0
9.12.84	III	33	0	0	1	2
5.2.85	III	51	0	3	0	1
15.10.85	III	40	1	0	0	0
22.10.85	III	29	0	0	1	0
3.11.85	III	27	0	0	1	0
27.11.85	III	39	0	0	1	0
11.12.85	III	40	0	0	1	0
18.6.85	IV	38	1	0	0	0
Total		444	2	5	8	4
Percentage			0.5	1.1	1.8	1.0

Figure 51. T.paludosa 3rd instar larva with Mermithid after dissecting.

9.7. Insect parasitoids

9.7.1. Previous observations

The larvae of Tipulidae are host to several species of parasitoid Diptera, including representatives of the families Tachinidae, Phoridae and Muscidae. Other types of parasitoids seem, however, to be of little or no significance.

9.7.1.1. Tachinidae

The first record of a tachinid parasitizing tipulid larvae was that of Roubaud (1906) (quoted by Rennie and Sutherland, 1920) who found *Siphona* (= *Crocuta* = *Buccentes*) *cristata* Fab., affecting *Tipula maxima* Poda. This species was later reported from *T.maxima* by Audcent (1942).

A closely allied species, *Siphona* (=*crocuta*) *geniculata* de Geer, has been recorded from tipulid larvae on numerous occasions. First noted by Rennie (1912) it is now known to parasitize *T.paludosa* and *T.oleracea* L. (Rennie and Sutherland, 1920), *T.maxima*, *T.oleracea* and *T.paludosa* (Chiswell, 1956), *T.lateralis* Mg., *T.fulvipennis* Deg. and *T.vittata* Mg. (Audcent, 1942), *T.subnodicornis* Zetterstedt and *T.montium* Egger in England (Coulson, 1962).

In Scotland, both *Siphona cristata* and *S.geniculata* have been reported from *T.paludosa*, in the Clyde Area where 7.5% of 3rd instar larvae, out of 200 examined, were infected with Tachinidae (Stewart, 1969).

The proportion of larvae affected by *S.geniculata* varies greatly between different populations. Rennie and Sutherland (1920) found from 6 to 17% parasitism in *T.paludosa* and *T.oleracea* in Aberdeenshire, Scotland and Alma (1975) in his studies of 3rd and 4th instar larvae at White Knights (Reading, Berks.) and Turk's Wood (Hants.) found from 15.2% to 34.1% infection of *T.maxima* (33 & 91 larvae examined respectively), 2.8% of *T.fulvipennis* (145 examined) and 4.0% of *T.vittata* (50 examined). Carter (1976) recorded 2.9% parasitism of *T.paludosa* (34 examined) in Northumberland, England, and Carter *et al.* (1981) recorded the highest level to date, 8 out of 11 larvae affected (73%) in the Liverpool area.

S.geniculata lays up to 9 eggs on the stigmatic crown of the host larva. On hatching, the larvae enter the main tracheal trunks and bore into the haemocoele but retain a respiratory connection with the tracheae by means of a characteristic "tachinid sheath" (Wilkinson and MacCarthy, 1967). There are two generations per year and the parasite overwinters in the host (Rennie, 1912; Rennie and Sutherland, 1920). The species is not entirely confined to the larvae of tipulidae but has also been bred out of larvae of the noctuid moth *Mamestra brassicae* (L.) (Chiswell, 1956).

The potential of *S.geniculata* as a biological control agent was recognised by Wilkinson (1971) who released the species in British Columbia in an attempt to control *T.paludosa*. So far no recoveries have been made.

A third species of tachinid, *Trichopareia maculisquama* Zetterstedt, has been recorded from the larvae of *T.hortulana* Mg. and *Nephrotoma quadrifaria* Mg. in S.W. Scotland (Stewart, 1969) but there appears to be no further information on this species.

9.7.1.2. Phoridae

There are few records of this family affecting the larvae of Tipulidae. *Megaselia paludosa* (Wood) was reported from *T.paludosa* in England by Coggins (1970) and by Carter (1977). It has also been reported from N.Germany by Carl (1972).

9.7.1.3. Muscidae

Phaonia signata Mg. was reported from the larvae of *T.paludosa* by Griffiths *et al.* (1984) in Liverpool and this is thought to be the

TABLE 50

Number of larvae collected from the field and examined for infection on each sampling date

Location	Date	No. samples	No. larvae	Nematode	Tachinid	TIV	NPV
Auchincruive	7.3.84	25	38				
Garscube	23.3.84	20	13	5			
"	19.4.84	28	6	1			
"	10.5.84	20	5				
"	22.5.84	20	6	1 (with 12 nematodes)			
Cochno	22.5.84	20	8	1		1	
Cochno	28.5.84	120	27	1		1	
"	26.6.84	20	8			2	
Lawmuir	23.9.84	40	104				
Garscube	9.10.84	40	24				
Lawmuir	16.10.84	40	80				
"	23.10.84	40	55				
Garscube	30.10.84	40	8				
Lawmuir	6.11.84	40	42	1			
"	13.11.84	40	35		1		
Garscube	13.11.84	40	8	1			
"	27.11.84	40	6			2	
Lawmuir	4.12.84	80	70	2			2
"	9.12.84	40	33	1	2		
"	5.2.85	80	51		1		3
Garscube	26.2.85	80	5	1			
Lawmuir	5.3.85	40	18				
"	27.3.85	80	35				
Bishopbriggs	2.4.85	80	5		1		
Garscube	16.4.85	80	9		1		1

Table 50 (Contd.)

Location	Date	No. samples	No. larvae	Nematode	Tachinid	TIV	NPV
Lawmuir	24.9.85	40	1				
"	8.10.85	60	23				
"	15.10.85	77	40			1	
Mains of Baguh apple Thornhill	13.10.85	40	8				
Lawmuir	22.10.85	80	29	1			
"	24.10.85	40	17				
"	3.11.85	40	27	1			
"	5.11.85	40	11				
"	8.11.85	80	35				
Garscube	13.11.85	80	4				
Lawmuir	20.11.85	80	33				
Mains of Baguh apple Thornhill	23.11.85	80	18				
Lawmuir	27.11.85	80	39	1			
Garscube	5.12.85	80	2				
Lawmuir	8.12.85	80	17				
"	11.12.85	80	40	1			
"	24.12.85	80	34				
"	15.1.86	80	25				
Cochno	19.1.86	60	4				
Lawmuir	21.1.86	80	20				
"	28.1.86	80	36				
"	4.2.86	80	11				
"	18.2.86	80	28				
"	9.3.86	80	27				
"	16.3.86	80	27				
"	30.3.86	120	47				
"	18.5.86	160	31				
"	25.5.86	120	14				
"	18.6.86	160	38			1	
"	6.7.86	160	9				
"	20.7.86	160	24 (20L.+4 pupae)				
"	3.8.86	136	18 (12L.+6 pupae)				
Total		3966	1436	19	6	8	6

Figure 52. T.paludosa 3rd instar larva with parasitic tachinid larva visible in the body cavity.

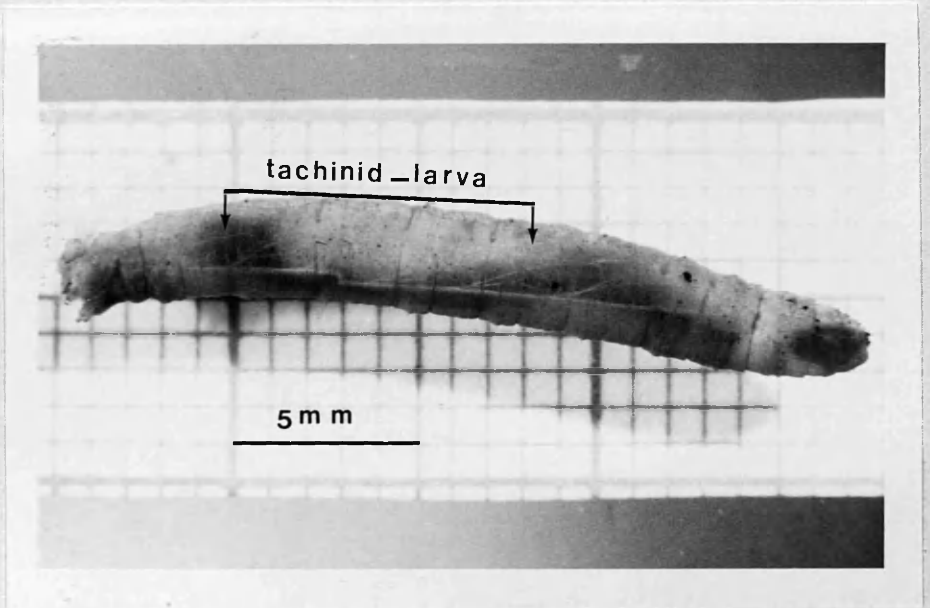

tachinid _larva
5 m m

Figure 53. Tachinid fly (*Siphona geniculata*) emerged from its pupa in the laboratory after parasitizing *T.paludosa* larva.

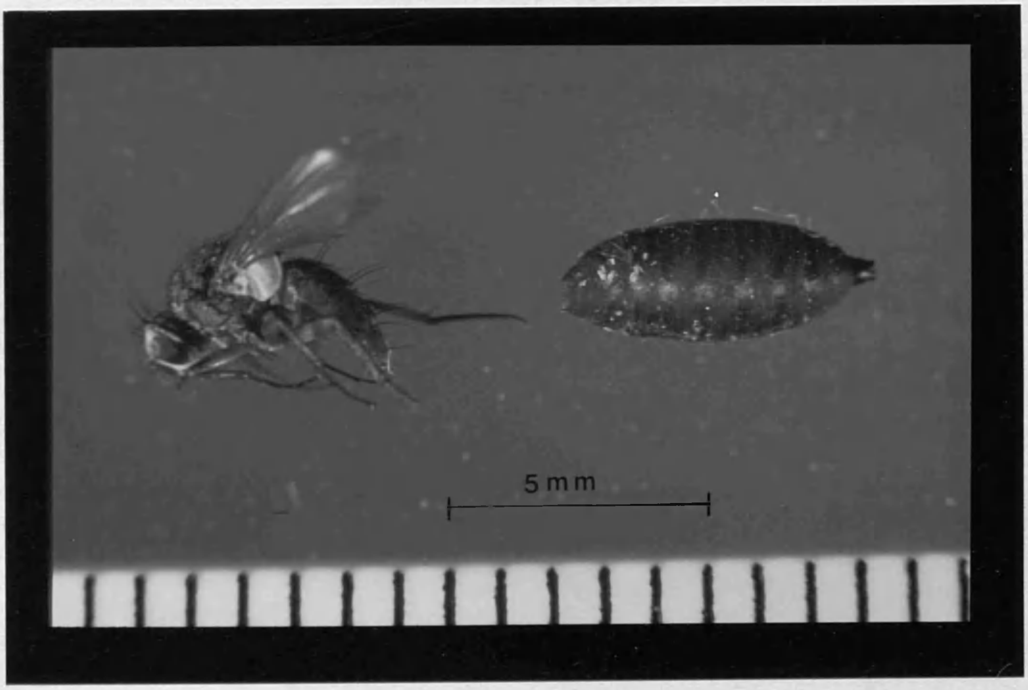
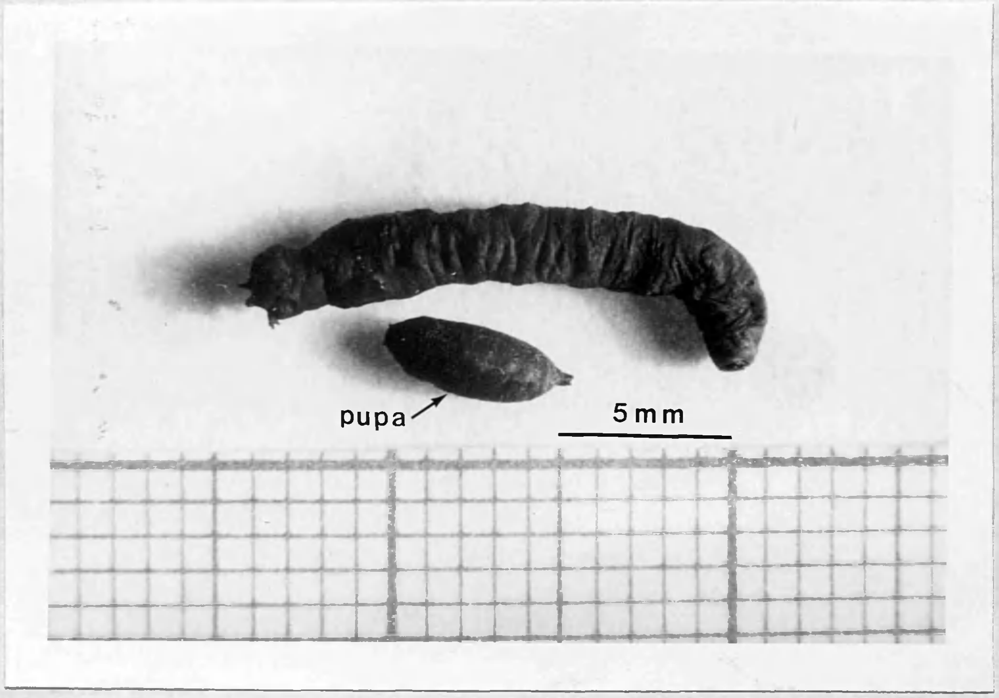

5 mm

Figure 54. Infected T.paludosa larva died shortly after the tachinid S.geniculata emerged to pupate.


pupa
5mm

first recorded instance of this species attacking larval Tipulidae. Members of the genus Phaonia are carnivorous and P.signata has been found inside a living adult of Carabus italicus Dejean (Keilin, 1917; quoted by Griffiths et al., 1984).

9.7.2. Present observations

The only parasitoid insects found affecting tipulid larvae in the present survey were Tachinidae and all occurrences are detailed in Table 50. All were found in 3rd instar larvae where they occurred in the middle segments (Figure 52) often attached to one of the main tracheal trunks by a respiratory funnel. In two instances the larvae were bred out to adults and proved to be Siphona geniculata (Figure 53). In each instance they emerged about a month after the larvae had been placed in an unheated insectary. The infected host larvae seemed almost normal but died shortly after the parasitoids emerged to pupate leaving holes in their integument (Figure 54). Only one parasitoid was found in each infected host larva. The overall rate of infection, 4.5% suggests that these parasitoids are not of great consequence in the Glasgow area.

9.8. Insect predators

9.8.1. Previous work

There have been few observations of predation by insects on the larvae of Tipulidae.

Carnivorous larvae of the Limoniine tipulid Trichyphona immaculata (Mg.) were observed to feed on another limoniine, Molophilus ater (Mg.) in the laboratory (Horobin, 1971) and Ahmed (1968) observed that a number of species of carabid beetles would feed on larvae. As there was little information available on the possible role of predaceous Coleoptera a few simple observations were made in the present study.

9.8.2. Present observations

Pitfall traps were set out in the field at the Lawmuir site from July to October during 1985 to investigate the principal species of predatory Coleoptera present and the following species were found.

Carabidae:	Nebria brevicollis (F.)
	Pterostichus madidus (F.)
	Notiophilus spp.
Staphylinidae:	Philonthus spp.

Of these, N.brevicollis and P.madidus were abundant and seemed likely to be the only species of importance. Their capacity to eat tipulid larvae was tested by offering them first instar larvae of T.paludosa in the laboratory: both species consumed them with avidity.

Although no alternative foods were available during these observations it seems possible that these hemiedaphic beetles may play some part in the natural mortality of tipulids and in particular, of the early stages which remain close to the soil surface.

9.9. Parasites and predators of Adult Tipulidae

9.9.1. Previous work

There appear to be no records of parasites affecting the adults of Tipulidae.

Insects recorded as preying on the adults include Odonata, Diptera of the families Asilidae, Empididae, Anthomyiidae, Scatophagidae and Rhagionidae (Alexander, 1920; Cuthbertson, 1927) and adults of the social wasp, Vespula sp., Hymenoptera (Parker, 1985).

9.9.2. Present work

Several hundreds of adults were collected during the course of the observations, mainly from Cochno, Garscube and Lawmuir and these were examined externally and internally for parasites. No signs of external parasites were found but on one occasion a small unidentified platyhelminth was found within the alimentary canal.

Parasitism and predation of adults was thought to be of minor importance because they have a short life span and most of the eggs are laid within a few hours of emergence. It was noted, however, that given the opportunity, adults of Nebria brevicollis would eat all the soft parts of the adults of T.paludosa leaving only the legs and wings.

9.10. Other abnormalities

Several apparently abnormal, possibly pathogenic conditions were found affecting Tipulidae and, although it was not possible to follow these up in detail, their existence is recorded below.

9.10.1. Affecting larvae

1. Groups of spherical white, cyst-like structures on the mid-gut and connected to tracheae.
2. Black patches in the fat body.
3. Blackening of the anal papillae and sometimes around the spiracular discs.
4. Numerous small circular black spots or larger black markings of the cuticle observed in many individuals. Such markings have been observed previously (Butterfield, 1973; Carter, 1976) and may be scars or wound responses following the attacks of predators, including other tipulid larvae.
5. Blackening of the main tracheal trunk in the middle of the body was seen in one larva out of a sample of six examined.
6. White granular bodies within the haemolymph.

9.10.2. Affecting adults

Black oval, cyst-like bodies were found attached to the alimentary canal of a number of individuals. These were mainly found in males.

9.11. Discussion

Data on the prevalence of diseases and invertebrate parasites and predators of Tipula paludosa in the Glasgow Area were obtained during the course of this work with a view to assessing their importance as mortality factors and their potential as possible biological control agencies.

Two virus diseases were recorded and, although these are fatal, their incidence in the populations was low. Carter (1978) carried out a field trial with the more common of these (Tipula Iridescent Virus) but failed to introduce a rate of infection sufficiently high to provide a useful degree of control. There could be a future in the use of virus as control agencies but the problems of obtaining sufficient infective material and of distributing and maintaining it in the environment economically would have to be overcome.

Three of the six species of parasitic Protozoa recorded by Sherlock (1973) from larvae were recorded, the gregarines Gregarina longa, Hirmocystis ventricosa and Actinocephalus tipula. These are not usually regarded as pathogens but it should be noted that Carl (1972), in Germany, reared gregarine-infected and non-infected larvae of T.paludosa to the adult and found 52% survival amongst infected individuals compared to 62.7% amongst healthy ones. Possibly infection with these parasites weakens their hosts but their effect would be insufficient to warrant their use in biological control.

Mermithids nematodes were common at the Garscube site and, as fatal parasites, probably caused appreciable mortality. However they do not kill their hosts until these are well-grown by which time the crops have been damaged. Moreover their use as biological control agencies is limited by the difficulty of mass-producing them (Poinar, 1971).

The incidence of the Tachinid parasitoid Siphona geniculata was low in this study but elsewhere high rates of infection have been recorded e.g. Alma (1975) found 34.1% infection at Reading, Berks. Attempts have been made to use this species as a biological control agent in Canada (Wilkinson, 1971) but these were unsuccessful. Tachinids, like mermithids have the disadvantage that their effect comes too late to save the crops.

Predation by carabid beetles on tipulids has been discussed by Ahmed (1968) and by Freeman (1967) but seems of little consequence in population regulation. Probably cannibalism by densely crowded populations is much more effective.

In conclusion it would seem that endemic diseases, and invertebrate parasites and predators offer little scope for the control of field infestations of Tipula paludosa by biological means in the area studied.

SECTION 10

PREDATION OF LARVAE BY BIRDS

10.1. Introduction

Birds are well known as predators of larval Tipulidae and their activities in this respect have been well-documented in various parts of the world e.g. U.S.A. - Alexander (1920), Hartman and Hynes (1977); Canada - Wilkinson and MacCarthy (1967); Switzerland - Matter (1982); Holland - Tinbergen and Drent (1980); U.K. - Dunnet (1955, 1956), Dunnet and Patterson (1968), Galbraith (1986).

The species most implicated are often omnivorous opportunisitic species which require a high protein diet and which will probe the upper parts of the soil to obtain insects, earthworms etc. In the U.K. the most important species is the European Starling, Sturnus vulgaris L. (Ministry of Agriculture, Fisheries and Food, 1984), and the feeding habits of this species in Aberdeenshire have been studied in detail by Dunnet (1955, 1956) who noted (1956) that, of the insect foods taken during autumn and winter, weevils (Coleoptera, Curculionidae) predominated during the autumn but from November tipulid larvae occurred with increasing intensity. On the average, tipulid larvae formed over 80% of the food brought to the nestlings and earthworms 16% (Dunnet, 1955).

The Rook, Corvus frugilegus L. also consumes large quantities of tipulid larvae and, second only to earthworms, these constitute their most important animal food in terms of bulk (Coombs, 1978). Amongst other species exploiting this resource are the Lapwing, Vanellus vanellus (L.), (Galbraith, 1986) and the Red-billed Chough, Pyrrhocorax pyrrhocorax (L.), (Holyoak, 1971).

Although much is known about the dietary requirements of these birds and the numbers of larvae they consume, there is little information on the effects their feeding has on the size of the populations of their prey in the field. Dunnet (1955) however obtained some data for Starlings in Aberdeenshire. He estimated that nestlings consumed about 1.86% and 7% of the total larvae available in 1951 and 1952 respectively. These figures are necessarily minimal because to them must be added unknown numbers taken by breeding adults, non-breeding cocks and first-brood juveniles. Jones and Jones (1984) consider that "it is doubtful whether the Rook or other birds with a similar diet have any appreciable effect upon populations of noxious insects" but do not substantiate this statement.

Clearly much remains to be discovered with regard to the influence of predatory birds on tipulid populations and to this end experiments were carried out during the 1985-86 and 1986-87 seasons.

10.2. Materials and Methods

10.2.1. The 1985-86 Experiment

During the period November-June, 1985-86, an experiment was carried out at the Lawmuir site, East Kilbride, to determine the influence of predatory birds on the population of larvae. The field used was permanent pasture and was kept ungrazed during the course of the observations. It was mowed shortly before the work was initiated.

The experimental area was a rectangle subdivided into 20 equal plots, each 5 x 10 metres in extent (Fig. 55). Within each plot two areas, each 1 x 2 metres were selected at random and marked out. One of these was covered with a cage designed to exclude birds, while the other was kept as a control. (Details of construction of the cages is given in Appendix X Figures B, C and D).

The cages were put in place on 8 November 1985 and, apart from being removed temporarily on sampling dates, were left in position until the end of the experiment.

Figure 55. The experimental field at Lawmuir.

The soil within these experimental areas was sampled by taking equal numbers of vertical cylinders (cores), 6.5cm. in diameter and 7.5cm deep, at random from each, using the sampling tool and extracting apparatus designed by Blasdale (1974) - see Section 8, Extraction of larvae from the soil.

Sampling dates were 7 March, 5 May and 18 June 1986 and, to compensate for gradually diminishing populations and to increase the precision of the estimates, the numbers of cores taken from each area were increased from four on 7 March to eight on both 5 May and 18 June.

10.2.2. The 1986-87 Experiment

The experiment was repeated, with some modifications, during the period November 1986 - May 1987 and I gratefully acknowledge the assistance of Miss Collette Coll who undertook the management of the repeat run under my guidance.

The same field was used as in 1985-86 but this time the area was divided into ten 10 x 10 metre plots, the observational areas were 2 x 2 metres and five cores were taken from each area on each sampling occasion. These changes reduced the total amount of work needed without materially affecting the precision of the experiment and the "pressure" of sampling on the areas was greatly reduced.

The plots were sampled on 1 November 1986 and immediately after this the cages were set up. Subsequent sampling dates were 18 December 1986, 1 March 1987 and 13 May 1987.

To check on the actual presence of feeding birds at the site, observations were made at irregular intervals about twice a month from November 1986 until May 1987 using binoculars from cover at distances of from 200 to 500 m. The observations were made at different times between 0500 hrs. and 1710 hrs. and varied in duration from 1hr. 15min. to 10hrs. 40min. On seven occasions exact counts were made. The depths of bird probe holes were measured on one occasion and droppings collected on plastic sheets placed under a roost of starlings were examined for traces of tipulid larvae.

10.3. Results

10.3.1. Results - 1985-86

The numbers of larvae found in the cores from each area on each sampling date are shown in Appendix X Tables J1, J3 and J5. The frequency distributions of larvae per core were markedly assymmetrical, there being a preponderance of zero counts on each occasion with the proportion of zeros increasing from date to date as the populations reduced (Table 51). The original counts were therefore subjected to square root transformation ($\sqrt{x + 0.5}$) (Appendix X Tables J2, J4 and J6) and after the transformed data had been examined by Variance Ratio tests (Table 52) and shown to be homoscedastic (i.e. the variances of the various groups being compared were similar) were examined by two-way Analysis of Variance.

The results of the analyses are shown in Tables 53, 54 and 55. In each case the variance components due to differences between plots and due to plot/treatment interaction were of no significance so they were pooled with the residual error to give a better assessment of treatment (i.e. cage v. control) effects.

On 7/3/86, more larvae were found on the caged areas than on the controls, but the difference just failed to reach significance ($0.05 < P < 0.10$). On 5/5/86 and 18/6/86 real differences were evident, the populations of larvae on the control areas being 64.6% and 62.8% of those on the caged areas on these dates respectively.

The transformed mean numbers/core ($\bar{x}t$) for caged and uncaged areas with their standard deviations and standard errors (S.E.) are shown in Table 56 and the detransformed means with the detransformed values of $\bar{x}t \pm$ S.E. are shown in Table 57 and Fig. 56.

TABLE 51

1985-86 Experiment

Frequency Distribution of larvae in individual cores

	Nos. of larvae/core				Date
	0	1	2	3	
% of cores with 1,2 or 3 larvae	54	38	8	0	7/3/86
	70	25	5	0	5/5/86
	78	21	1	0	18/6/86

TABLE 52

1985-86 Experiment

Variance Ratio Tests on Transformed Data

Date	Area	Variance	d.f.	F.ratio	P (2-tailed)
7/3/86	Cage	0.0987	79	1.22	> 0.1
	Cont.	0.0806	79		
5/5/86	Cage	0.0644	159	1.21	> 0.1
	Cont.	0.0644	159		
18/6/86	(Cage	0.0522	159)	1.19	> 0.1
	(Cont.	0.0438	159)		

TABLE 53

1985-86 Experiment

Analysis of variance on transformed data from first date of sampling, 7/3/86

Source	d.f.	Sum of Sq.	Mean Sq.	V.R.	P.
Treatment	1	0.2976	0.2976	3.28	>0.05
Plots	19	2.3567	0.1240	1.37	>0.10
Interaction	19	0.9172	0.0483	0.53	>0.75
Residual	120	10.8877	0.0907		
Total	159	14.4592			
Treatment	1	0.2976	0.2976	3.32	>0.05
Error (Pooled)	158	14.1616	0.0896		

TABLE 54

1985-86 Experiment

Analysis of variance on transformed data from second date of sampling, 5/5/86

Source	d.f.	Sum of Sq.	Mean Sq.	V.R.	P.
Treatment	1	0.3485	0.3485	4.85	<0.05
Plots	19	1.0538	0.0555	0.77	>0.50
Interaction	19	1.4894	0.0784	1.09	>0.25
Residual	280	20.1186	0.0719		
Total	319	23.0103			
Treatment	1	0.3485	0.3485	4.89	<0.05
Error (Pooled)	318	22.6618	0.0713		

TABLE 55

1985-86 Experiment

Analysis of variance on transformed data from third date of sampling, 18/6/86.

Source	d.f.	Sum of Sq.	Mean Sq.	V.R.	P.
Treatment	1	0.2050	0.2050	3.97	<0.05
Plots	19	0.4711	0.0248	0.48	>0.75
Interaction	19	0.3506	0.0185	0.36	>0.75
Residual	280	14.4433	0.0516		
Total	319	15.4700			
Treatment	1	0.2050	0.2050	4.27	<0.05
Error (Pooled)	318	15.2650	0.0480		

TABLE 56

1985-86 Experiment

Transformed mean nos of larvae/core ($\bar{x}t$) with standard deviations (S.D.), nos. of observations (n), and standard errors (S.E.)

Date	Treatment	$\bar{x}t$	S.D.	n	S.E.
7/3/86	Cage	1.018	0.314	80	0.035
	Contr.	0.932	0.284	80	0.032
5/5/86	Cage	0.910	0.279	160	0.022
	Contr.	0.844	0.254	160	0.020
18/6/86	Cage	0.850	0.228	160	0.018
	Contr.	0.800	0.209	160	0.017

TABLE 57

1985-86 Experiment

Detransformed mean nos. of larvae/core and detransformed $\bar{x}t \pm$ S.E. values

Date	Treatment	Mean	Detr $\bar{x}t$ - S.E.	Detr. $\bar{x}t$ + S.E.
7/3/86	Cage	0.536	0.466	0.609
	Contr.	0.369	0.310	0.429
5/5/86	Cage	0.328	0.288	0.369
	Contr.	0.212	0.179	0.246
18/6/86	Cage	0.223	0.192	0.253
	Contr.	0.140	0.113	0.167

Each core was almost exactly 1/300 sq. metre in area so these figures can readily be converted into nos./sq. metre or nos./hectare by multiplying by 300 or 3000000 respectively. The scales at the right of Fig. 56 indicate these values. The almost straight-line relationship between Log number of larvae/sq. metre and sampling date shown in Fig. 57 suggests that the population decreased at a fairly constant rate during the observation.

Figure 56. Density relationship of Leatherjackets in enclosed cages and control in 1985-1986.

Closed circles = enclosed cages

Open circles = control

Vertical bars = standard error.

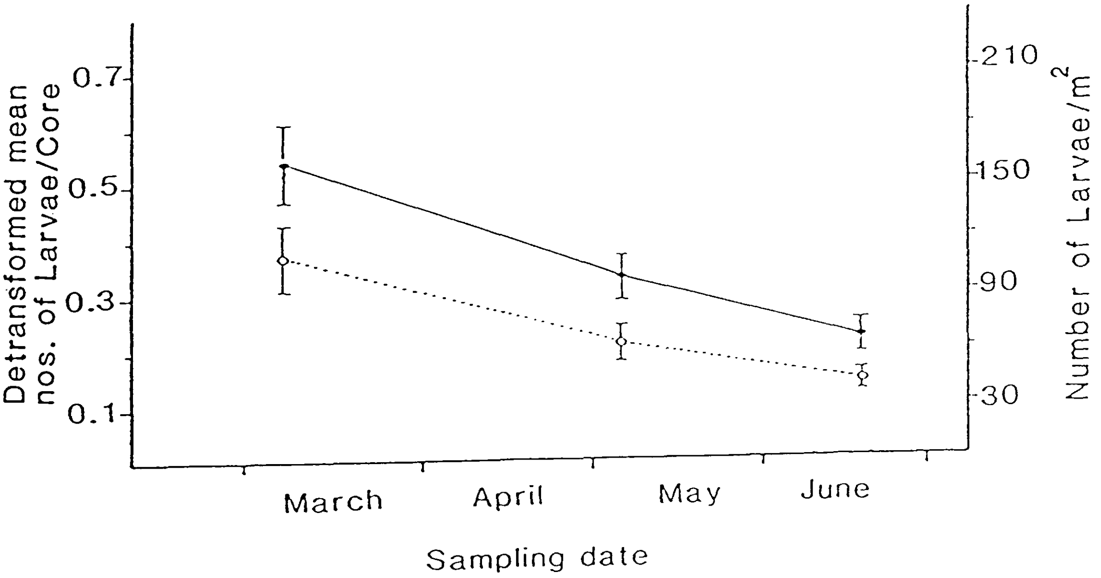
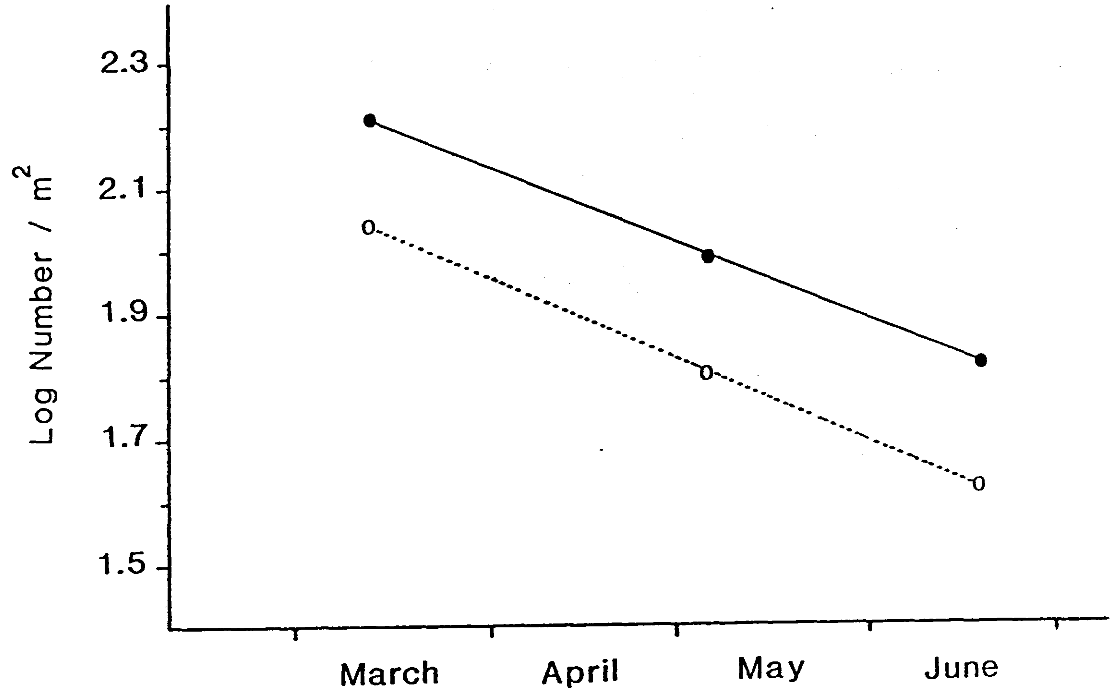

Detransformed mean nos. of Larvae/Core
0.7
0.5
0.3
0.1
Number of Larvae/m^2
210
150
90
30
March
April
May
June
Sampling date

Figure 57. The relationship between log number of larvae/m^2 and sampling date in enclosed cages and control in 1985-1986.

Closed circles = enclosed cages

Open circles = control


Log Number / m^2
2.3
2.1
1.9
1.7
1.5
March
April
May
June

10.3.2. Results - 1986-87

The data for the four sampling dates are shown in Table 58. As in the previous year, the counts showed markedly asymmetrical distributions (Appendix X Tables J7, J9, J11 and J13). They were, therefore, subjected to $\sqrt{x + 0.5}$ transformation (Appendix X Tables J8, J10, J12, J14 Pt. 1 & 2), and after homoscedasticity tests proved satisfactory, were examined by two-way Analysis of Variance. Effects due to plot differences and plot/treatment interactions were slight and were pooled with the residual as before.

TABLE 58

1986-87 Experiment

Analysis of Variance on Transformed Data

Date	Source	d.f.	SS	MS	F	P
1/11/86	Treat.	1	0.0441	0.0441	0.28	>0.5
	Error	98	15.1569	0.1547		
18/12/86	Treat.	1	0.1444	0.1444	1.10	>0.25
	Error	98	12.8331	0.1310		
1/03/87	Treat.	1	0.5491	0.5491	5.16	<0.05
	Error	98	10.4158	0.1063		
13/05/87	Treat.	1	2.0612	2.0612	29.39	<0.001
	Error	140	9.8179	0.0701		

The transformed means ($\bar{x}t$) with their standard deviations and standard errors (S.E.) are shown in Table 59 and the detransformed means and detransformed values of $\bar{x}t \pm$ S.E. are shown in Table 60 and Fig. 58. The relationship between Log number of larvae/sq. metre and sampling date is shown in Fig. 59. The fall off in numbers offered

TABLE 59

1986-87 Experiment

Transformed mean nos. of larvae/core, ($\bar{x}t$) , with standard deviations (S.D.), nos. of observations (n) and standard errors (S.E.)

Date	Treatment	$\bar{x}t$	S.D.	n	S.E.
1/11/86	Cage	1.197	0.387	50	0.054
	Contr.	1.239	0.404	50	0.057
18/12/86	Cage	1.176	0.367	50	0.052
	Contr.	1.100	0.357	50	0.050
1/03/87	Cage	1.145	0.326	50	0.046
	Contr.	0.997	0.326	50	0.046
13/05/87	Cage	1.095	0.349	80	0.039
	Contr.	0.868	0.267	80	0.029

TABLE 60

1986-87 Experiment

Detransformed mean nos. of larvae/core and detransformed $\bar{x}t$ ± S.E. values

Date	Treatment	Mean	Detr $\bar{x}t$ - S.E.	Detr. $\bar{x}t$ + S.E.
1/11/86	Cage	0.932	0.806	1.065
	Contr.	1.035	0.897	1.180
18/12/86	Cage	0.883	0.763	1.008
	Contr.	0.710	0.603	0.823
1/03/87	Cage	0.811	0.708	0.918
	Contr.	0.494	0.404	0.588
13/05/87	Cage	0.699	0.615	0.785
	Contr.	0.253	0.203	0.304

Figure 58. Density relationship of Leatherjackets in enclosed cages and control in 1986-1987.

Closed circles = enclosed cages

Open circles = control

Vertical bars = standard error

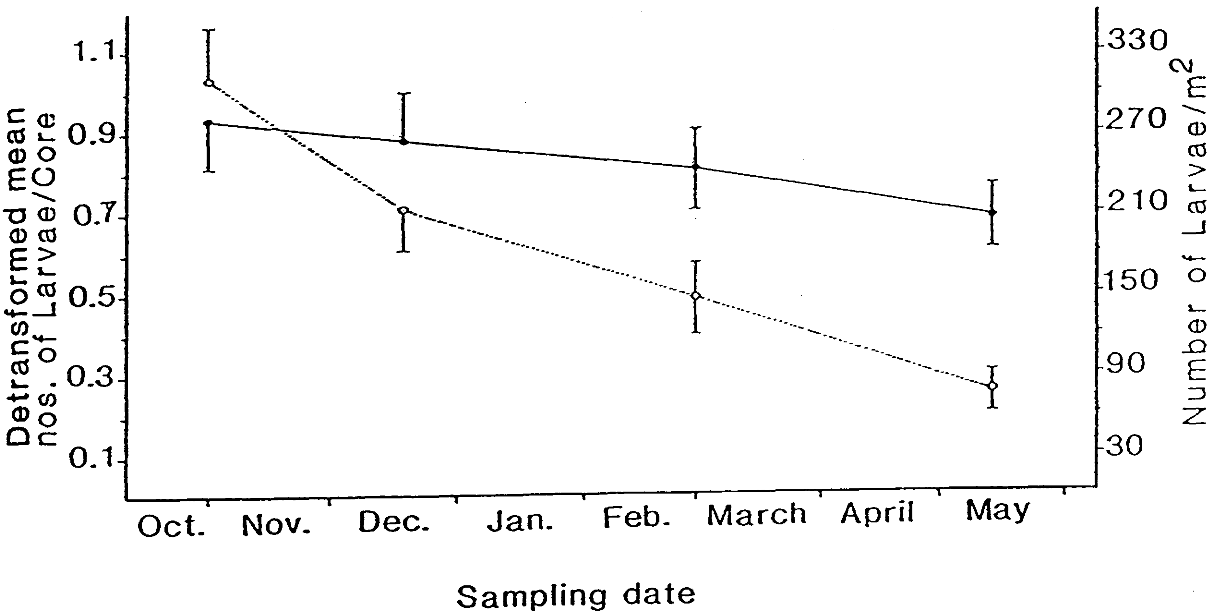
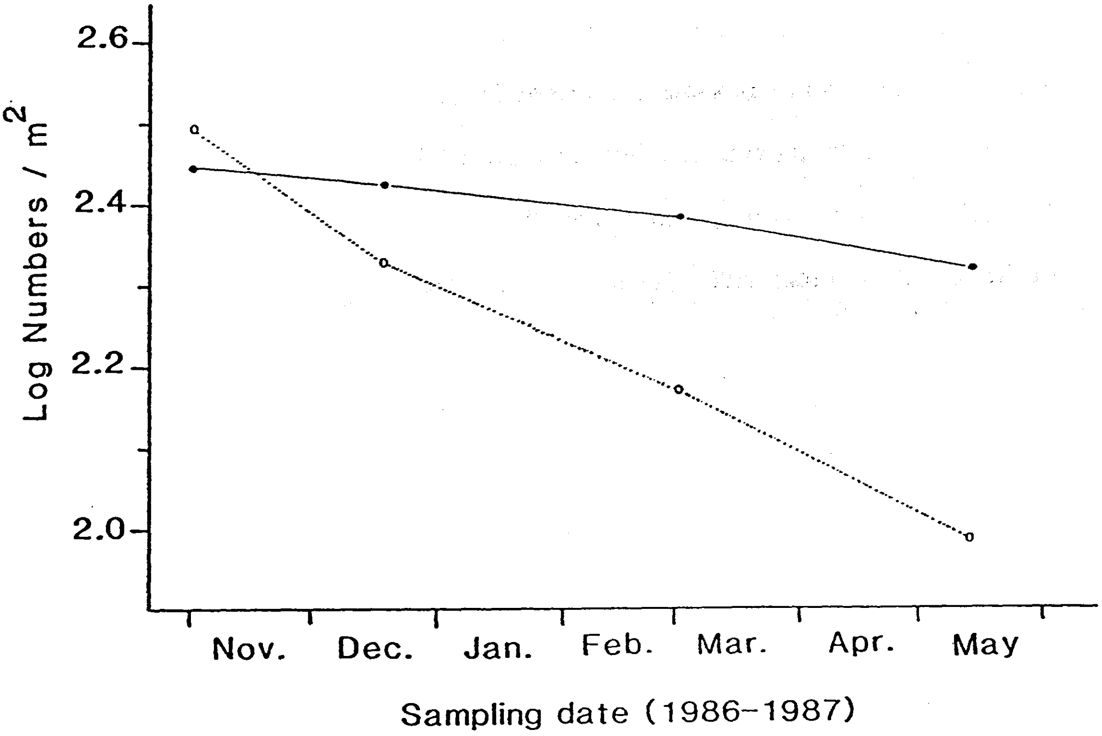

Detransformed mean nos. of Larvae/Core
1.1
0.9
0.7
0.5
0.3
0.1
Number of Larvae/m^2
330
270
210
150
90
30
Oct.
Nov.
Dec.
Jan.
Feb.
March
April
May
Sampling date

Figure 59. The relationship between log number of larvae/m^2 and sampling date in enclosed cages and control in 1986-1987.

Closed circles = enclosed cages

Open circles = control


2.6
2.4
2.2
2.0
Log Numbers / m^2
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
Sampling date (1986-1987)

fairly constant for the caged population but the steeper slope of the line during November, December on the bird predated plots suggested that predation may be heavier at this time than later.

At the outset of the experiment, on 1/11/86, a few more larvae were found in the control areas than in those about to receive the cages but this was clearly a chance occurrence. On 18/12/86 the greater number was found under the cages but the difference was slight; on 1/3/87, however, there was a marked difference, the control population being only 60.9% of that of the cages. This difference was further accentuated by 13/5/87 when the population on the control plots was 36. 2% of that in the cages. The mortalities of larvae between 1/11/86 and 13/5/87 were 75.6% in the controls compared to 25% in the cages, the difference of 50.6% representing the influence of bird predation between these dates.

In the direct observations on feeding, birds were frequently seen probing the soil with their beaks and also apparently catching small prey from the surface. The total counts for the seven occasions on which exact records were kept comprised 55 starlings, 17 rooks and 4 magpies (Pica pica L.), the maximum number observed at any one time being 20 starlings at 0815 hrs. on 7 March.

At the distances of observation it was not possible to identify the prey taken, although starlings could quite clearly be seen struggling with earthworms, but the probe holes had depths of from 2 - 3 cms. which accorded well with the distribution of tipulid larvae in the soil.

The examination of faeces produced disappointing results: only two tipulid fragments were identified out of numerous insect remains found.

SECTION 11

EFFECTS OF THE LARVAE OF TIPULA PALUDOSA ON THE GRASS OF THE BIRD-EXCLUSION EXPERIMENTS

11.1. Introduction

The harmful effects of the larvae of Tipulidae to grassland have been well documented since attention was brought to them by Reaumur (1734-43) whose observations in France were quoted by Kirby and Spence (1860). More recently their depredations have been noted by numerous authors, both in the British Isles and on the continent of Europe, several of whom have attempted to relate the degree of damage, in terms of crop loss, to the size of the populations present. It is probably not possible to correlate populations and losses at all precisely, except possibly in a limited area (see Newbold, 1981), because so many other factors such as rainfall, temperature and soil type exert their influences. Also it is necessary to know at what time during the season the counts took place and to use comparable measures of damage. Some general figures may, however, be given.

Blackshaw (1984), working in Northern Ireland, recorded damage to fields on many occasions where populations were less than 2 million/ha. and he suggested that application of control measures to a population of 1 million larvae/ha. in March could prevent a dry-weight loss of 400 kg./ha. in the first silage cut. Newbold (1981), in S.W. Scotland achieved a yield increase of 13% at the first silage cut by controlling a population of 1.14 million in early March.

From the economic standpoint it is, however, important to assess the probable costs of control in terms of the advantages gained. To this end Newbold (1981) derived an "economic threshold" for populations, i.e. the smallest population which would cause sufficient injury to justify the use of artificial control measures. To do this he calculated the value of the increase in grass yield which would be obtained by using control and compared this to the cost of control. The "break-even" increase in yield, at that time, was calculated as 300 kg. dry matter/ha. and this was equivalent to the amount of damage caused by 2 million larvae/ha. in December or January.

On the Continent of Europe, Lange (1963) considered that in N.W. Germany damage could be associated with a population of 1 million/ha. larvae in autumn and early winter and Vlug and Paul (1986) stated that in Holland control was worth while when populations exceeded 1.5 million/ha.

The bird-exclusion experiments carried out at Lawmuir during the winters 1985-86 and 1986-87 produced strong evidence that birds caused appreciable mortality amongst the larvae of T.paludosa and it seemed possible that this partial control might be reflected in increased yields of grass on the plots which were accessible to birds. Mean populations of larvae on caged and control plots were respectively 1.61 and 1.11 million/ha. in March 1986 and 2.43 and 1.48 million/ha. in March 1987.

In order to investigate this the yields from these plots were measured in both years.

11.2. Yields of Grass in Bird-exclusion Experiments

11.2.1. Materials and Methods

On 19 June, 1986, the day after the final samples of turf had been taken, all the twenty pairs of 2-square metre area caged and control plots were mowed close to the ground using a standardised procedure. The grass from each plot was weighed immediately to obtain the "wet-weight" and then left to dry in the insectary for three months when it was weighed again to obtain the "dry-weight".

In 1987 a similar procedure was adopted except that the individual caged and control areas now measured 4-square metres (2 x 2) and there were only ten pairs of them. The grass was mowed on 5 July, its wet weight was recorded immediately and its dry weight 6 weeks later.

11.3. Results

The results for 1986, along with those of 1-tailed "matched pairs" t-tests are shown in Tables 61 and 62. In each instance the mean weights of grass from the control plots exceeded those from the caged plots, the differences being attributable to greater damage caused by the larvae. The mean wet-weight yield on the caged areas (1,985.8g.) was 91% of that of the controls (2171.4g.), which amounts to a difference of 0.928 tonnes/hectare. The corresponding dry-weight difference was 68.5 kilograms.

The results for 1987 and those for similar analyses appear in Tables 63 and 64. Again the mean weights of grass from the control plots exceeded those from the caged plots, the wet-weight yield on the caged areas (1,674.6g.) being 82.8% of that of the controls (2021.9g.). This difference amountd to 0.868 tonnes/hectare with a corresponding dry-weight difference of 209.8 kilograms.

The results for both experiments were consistent and showed that even a partial reduction of the leatherjacket burden, such as that produced by bird predation, can lead to increased yields of grass.

TABLE 61

Weight of wet grass (gram) within caged and control areas
(Sunday 29 June 1986)

Plot Number	Caged Areas	Control Areas
A	2149.5	2373.5
B	2756.5	2083.5
C	2305.0	2203.0
D	1811.0	1732.5
E	2088.0	2396.0
F	1735.0	2393.0
G	1818.5	1847.5
H	1648.0	1968.0
I	1959.5	2152.5
J	1885.5	1758.0
K	1719.0	2097.5
L	1827.0	1906.5
M	1827.0	2000.0
N	1766.0	1721.0
O	1877.0	2922.5
P	1834.0	2156.5
Q	2358.0	2473.5
R	2219.5	2819.0
S	2287.5	1987.0
T	1844.5	2437.5
Total weight	39716.0	43428.5
Mean ($\bar{x}$ ± S.E.)	1985.8 ± 62.58	2171.4 ± 75.16

N = 20
Standard deviation = 377.555
T = 2.1987
(one tail test)
P = < 0.025

Mean difference = 185.625
Degrees of freedom = 19

TABLE 62

Weight of dry grass (gram) within caged and control areas (29/6/1986)

Plot Number	Caged Areas	Control Areas
A	211.0	189.0
B	239.5	221.0
C	228.0	237.5
D	158.0	150.0
E	180.5	183.0
F	156.0	245.0
G	163.5	176.0
H	149.0	198.0
I	158.0	189.5
J	173.5	152.0
K	167.0	223.0
L	169.0	188.5
M	155.0	173.0
N	165.5	168.5
O	179.0	194.0
P	193.0	266.0
Q	231.0	212.5
R	227.5	231.0
S	243.0	188.0
T	189.0	224.5
Total weight	3736.0	4010.0
Mean ($\bar{x} \pm$ S.E.)	186.8 ± 7.0	200.5 ± 6.9

N = 20
Standard deviation = 34.95
T = 1.75
(one tail test)
P = < 0.05

Mean difference = 13.70
Degrees of freedom = 19

TABLE 63

Weight of wet grass (gram) within caged and control areas
(Monday 6/7/1987)

Plot Number	Caged Areas	Control Areas
A	1774.5	1232.5
B	1744.0	1753.5
C	2178.0	2512.0
D	1780.0	2335.0
E	1701.0	2047.5
F	1634.0	2265.0
G	1464.5	2094.0
H	1560.0	2010.0
I	1302.5	2300.0
J	1607.0	1669.0
Total weight	16745.5	20218.5
Mean ($\bar{x} \pm$ S.E.)	1674.55± 73.18	2021.85±120.20

N = 10

Standard deviation = 424.67

T = 2.59 (one tail test)

P = $0.01 < P < 0.025$

Mean difference = 347.30

Degrees of freedom = 9

TABLE 64

Weight of dry grass (gram) within caged and control areas (6/7/1987)

Plot Number	Caged Areas	Control Areas
A	359.0	271.0
B	386.0	414.0
C	456.5	526.0
D	400.5	509.0
E	327.5	450.5
F	367.5	526.0
G	331.0	435.5
H	340.0	471.0
I	311.5	543.5
J	388.0	360.0
Total weight	3667.5	4506.5
Mean ($\bar{x}$ ± S.E.)	366.75± 13.61	450.65± 27.05

N = 10

Standard deviation = 92.903

T = 2.855 (paired tail test)

P = < 0.01

Mean difference = 83.900

Degrees of freedom = 9

1 tail test

11.4. Discussion

Although many species of birds are known to be predaceous on insects there is little information on the effect these have on the populations of their prey. The available information has been discussed by Askenmo <u>et al</u> (1977) and by Dickson <u>et al</u> (1979). Clearly the effect of birds will be related to the numbers present and East and Pottinger (1975) stated that predation is only likely to be effective in areas where the populations of birds are high. Lundberg (1987) in his work on the breeding season of the starling in Sweden noted that the success of a predator in utilizing its prey depended both on the biomass of the prey and on its availability to the predator. Tinbergen and Klomp (1960) demonstrated that the percentage mortality inflicted on an insect population by a vertebrate predator increases with increasing prey density up to a peak and then declines with further increase in prey density. However, density-dependent predation by birds on insect pests at endemic levels and on insects whose populations tend to remain permanently at low levels has been reported by Le Roux _et al_. (1963) and by Furuta (1972).

The results of the two bird exclusion experiments carried out in 1985-86 and 1986-87 were consistent and showed that, at the experimental site, bird predation was a significant factor influencing the overwintering mortality of _T.paludosa_ larvae. Moreover, the influence of birds on the tipulid populations was reflected in increased yields of grass on the plots where predation took place. Although bird predation alone could hardly be expected to control a population of tipulids its contribution is worthy of recognition and an understanding of its role in relation to that of other density-dependent factors such as invertebrate predators, parasitoids and diseases is desirable.

Varley _et al_. (1973) considered that population regulation depends on the consecutive action of density-dependent mortality factors, basically a succession of predators and parasites operating at different times. It should, however, be noted that the effects of birds and other mortality factors are unlikely to be additive because birds would be unlikely to be able to discriminate between healthy and parasitised or diseased larvae of Tipulidae.

In the present work birds were seen probing the ground and their faeces were examined and showed traces of tipulid larvae. It was not possible, however, to examine the crops of birds directly. However, the predatory role of starlings and rooks, the commonest birds observed, has been well-documented (Dunnet & Patterson, 1968; Feare, 1984).

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APPENDIX I

TABLE A1

The numbers, accumulative numbers and accumulative percentages of males and females extracted daily from 10 emergence traps in 1984

Date	No.of Males	Accumu-lated No.	Accumulated Percentage	No.of Females	Accumu-lated No.	Accumulated Percentage
24.7.84	0	0	0	0	0	0
25.7.84	1	1	1	0	0	0
26.7.84	1	2	2	0	0	0
27.7.84	0	2	2	0	0	0
28.7.84	1	3	4	0	0	0
29.7.84	4	7	8	2	2	4
30.7.84	0	7	8	0	2	4
31.7.84	0	7	8	0	2	4
1.8.84	0	7	8	0	2	4
2.8.84	1	8	10	0	2	4
3.8.84	1	9	11	0	2	4
4.8.84	1	10	12	0	2	4
5.8.84	0	10	12	1	3	6
6.8.84	0	10	12	0	3	6
7.8.84	4	14	17	1	4	9
8.8.84	0	14	17	2	6	13
9.8.84	1	15	18	0	6	13
10.8.84	0	15	18	1	7	15
11.8.84	1	16	19	0	7	15
12.8.84	3	19	23	0	7	15
13.8.84	1	20	24	1	8	17
14.8.84	0	20	24	1	9	19
15.8.84	4	24	29	2	11	23
16.8.84	3	27	32	2	13	28
17.8.84	9	36	43	0	13	28
18.8.84	2	38	45	2	15	32
19.8.84	7	45	54	5	20	43
20.8.84	3	48	57	3	23	49
21.8.84	1	49	58	2	25	53
22.8.84	0	49	58	0	25	53
23.8.84	0	49	58	0	25	53
24.8.84	1	50	60	0	25	53
25.8.84	7	57	68	4	29	62
26.8.84	4	61	73	1	30	64
27.8.84	9	70	83	8	38	81
28.8.84	1	71	85	0	38	81
29.8.84	1	72	86	0	38	81
30.8.84	1	73	87	0	38	81
31.8.84	0	73	87	0	38	81
1.9.84	5	78	93	3	41	87
2.9.84	3	81	96	4	45	96
3.9.84	1	82	98	0	45	96
4.9.84	0	82	98	0	45	96
5.9.84	0	82	98	1	46	98
6.9.84	1	83	99	0	46	98
7.9.84	1	84	100	0	46	98
8.9.84	0	84	100	0	46	98
9.9.84	0	84	100	0	46	98
10.9.84	0	84	100	0	46	98
11.9.84	0	84	100	0	46	98
12.9.84	0	84	100	0	46	98
13.9.84	0	84	100	0	46	98
14.9.84	0	84	100	1	47	100
15.9.84	0	84	100	0	47	100
TOTAL	84			47		

TABLE A2

The numbers, accumulative numbers and accumulative percentages of males and females extracted daily from 20 emergence traps in 1985

Date	No.of Males	Accumu-lated No.	Accumulated Percentage	No.of Females	Accumu-lated No.	Accumulated Percentage
4.8.85	0	0	0	0	0	0
5.8.85	2	2	10	0	0	0
6.8.85	1	3	14	0	0	0
7.8.85	0	3	14	0	0	0
8.8.85	0	3	14	0	0	0
9.8.85	0	3	14	0	0	0
10.8.85	1	4	19	1	1	11
11.8.85	1	5	24	0	1	11
12.8.85	1	6	29	0	1	11
13.8.85	0	6	29	0	1	11
14.8.85	2	8	38	0	1	11
15.8.85	0	8	38	0	1	11
16.8.85	0	8	38	0	1	11
17.8.85	1	9	43	0	1	11
18.8.85	2	11	52	2	3	33
19.8.85	2	13	62	0	3	33
20.8.85	0	13	62	0	3	33
21.8.85	4	17	81	1	4	44
22.8.85	0	17	81	0	4	44
23.8.85	0	17	81	0	4	44
24.8.85	0	17	81	0	4	44
25.8.85	0	17	81	0	4	44
26.8.85	1	18	86	1	5	56
27.8.85	0	18	86	0	5	56
28.8.85	1	19	90	0	5	56
29.8.85	0	19	90	0	5	56
30.8.85	2	21	100	1	6	67
31.8.85	0	21	100	0	6	67
1.9.85	0	21	100	0	6	67
2.9.85	0	21	100	0	6	67
3.9.85	0	21	100	1	7	78
4.9.85	0	21	100	0	7	78
10.9.85	0	21	100	2	9	100
11.9.85	0	21	100	0	9	100
TOTAL	21			9		

TABLE A3

The numbers, accumulative numbers and accumulative percentages of males and females extracted daily from 20 emergence traps in 1986

Date	No.of Males	Accumu-lated No.	Accumulated Percentage	No.of Females	Accumu-lated No.	Accumulated Percentage
19.7.86	0	0	0	0	0	0
20.7.86	1	1	0.6	0	0	0
21.7.86	0	1	0.6	0	0	0
22.7.86	1	2	1	0	0	0
23.7.86	0	2	1	0	0	0
24.7.86	0	2	1	0	0	0
25.7.86	0	2	1	0	0	0
26.7.86	0	2	1	0	0	0
27.7.86	0	2	1	0	0	0
28.7.86	0	2	1	0	0	0
29.7.86	0	2	1	1	1	2
30.7.86	0	2	1	1	2	3
31.7.86	0	2	1	0	2	3
1.8.86	2	4	2	2	4	6
2.8.86	0	4	2	1	5	8
3.8.86	1	5	3	1	6	9
4.8.86	1	6	3	2	8	12
5.8.86	0	6	3	1	9	14
6.8.86	0	6	3	0	9	14
7.8.86	8	14	8	0	9	14
8.8.86	3	17	10	0	9	14
9.8.86	3	20	11	1	10	15
10.8.86	2	22	12	4	14	22
11.8.86	4	26	15	2	16	25
12.8.86	1	27	15	2	18	28
13.8.86	0	27	15	4	22	34
14.8.86	6	33	19	0	22	34
15.8.86	6	39	22	2	24	37
16.8.86	10	49	28	2	26	40
17.8.86	15	64	36	4	30	46
18.8.86	9	73	41	2	32	49
19.8.86	15	88	49	4	36	55
20.8.86	12	100	56	6	42	65
21.8.86	23	123	69	6	48	74
22.8.86	10	133	75	4	52	80
23.8.86	8	141	79	2	54	83
24.8.86	3	144	81	2	56	86
25.8.86	14	158	89	2	58	89
26.8.86	10	168	94	2	60	92
27.8.86	3	171	96	3	63	97
28.8.86	4	175	98	0	63	97
29.8.86	0	175	98	2	65	100
30.8.86	1	176	99	0	65	100
31.8.86	1	177	99	0	65	100
1.9.86	0	177	99	0		
2.9.86	0	177	99	0		
3.9.86	0	177	99	0		
4.9.86	1	178	100	0		
5.9.86	0	178	100	0		
6.9.86	0	178	100	0		
7.9.86	0	178	100	0		
TOTAL	178			65		

APPENDIX II

TABLE B1

The number of eggs collected weekly in 1984

Week	Date	Eggs collected	Number hatched	% Hatched	% Mortality
1	5/8/84	12	-	-	100
2	12/8/84	47	10	21.3	78.7
3	19/8/84	646	376	58.2	41.8
4	26/8/84	698	477	68.3	31.7
5	2/9/84	250	110	44.0	56.0
6	9/9/84	64	30	46.9	53.1
7	16/9/84	29	17	58.6	41.4
8	23/9/84	7	4	57.1	42.9
9	30/9/84	6	4	66.7	33.3
10	7/10/84	0	0	-	-
Total		1759	1028	58.4	41.6

TABLE B2

The number of eggs collected weekly in 1985

Week	Date	Eggs collected	Number hatched	% Hatched	% Mortality
1	11/8/85	33	18	54.5	45.5
2	18/8/85	50	30	60	40
3	25/8/85	81	62	76.5	23.5
4	1/9/85	129	63	48.8	51.2
5	8/9/85	109	34	31	69
6	15/9/85	55	26	47.2	52.8
7	22/9/85	32	11	34.4	65.6
8	29/9/85	14	5	35.7	64.3
9	6/10/85	0	0	-	-
Total		503	249	49.5	50.5

TABLE B3

Number of eggs collected from 10 plots (10x10m) on each sampling date (1985)

Week ending	A	B	C	D	E	F	G	H	I	J
11/8/85	5	2	3	2	4	3	2	2	4	6
18/8/85	6	4	3	3	2	2	9	7	6	8
25/8/85	4	6	6	2	3	4	22	11	14	9
1/9/85	6	4	9	4	5	3	16	6	18	58
8/9/85	4	2	2	2	3	2	9	6	4	75
15/9/85	4	2	4	2	4	3	6	3	6	21
22/9/85	3	0	2	3	2	3	4	3	5	7
29/9/85	0	1	0	3	0	0	0	0	6	4
6/10/85	0	0	0	0	0	0	0	0	0	0
TOTAL	32	21	29	21	23	20	68	38	63	188

TABLE B4

Number of eggs hatched from 10 plots (10x10m) in the insectary (1985)

Week ending	A	B	C	D	E	F	G	H	I	J
11/8/85	4	0	3	1	2	2	1	2	1	2
18/8/85	3	2	3	3	0	1	8	2	2	6
25/8/85	2	2	5	0	3	4	20	11	9	6
1/9/85	3	4	5	2	2	3	14	6	9	15
8/9/85	0	1	0	1	2	0	6	2	2	20
15/9/85	2	0	1	0	2	1	3	3	5	9
22/9/85	0	0	0	1	0	1	1	3	2	3
29/9/85	0	1	0	0	0	0	0	0	2	2
6/10/85	0	0	0	0	0	0	0	0	0	0
TOTAL	14	10	17	8	11	12	53	29	32	63

TABLE B5

The number of eggs collected weekly in 1986

Week	Sampling date	Eggs collected	Number hatched	% Hatched	% Mortality
1	27/7/86	10	6	60.0	40.0
2	3/8/86	19	7	36.8	63.2
3	10/8/86	27	15	55.5	44.5
4	17/8/86	222	153	68.9	31.1
5	24/8/86	379	294	77.6	22.4
6	31/8/86	268	230	85.8	14.2
7	7/9/86	148	113	76.3	23.7
8	14/9/86	159	115	72.3	27.7
9	21/9/86	22	18	81.8	18.2
10	28/9/86	11	8	72.7	27.3
11	5/10/86	0	0	0	0
Total		1265	959	75.8	24.2

TABLE B6

Number of eggs collected from 10 plots (10x10m) on each sampling date (1986)

Week ending	A	B	C	D	E	F	G	H	I	J
27/7/86	0	0	0	1	0	5	3	0	0	1
3/8/86	3	0	1	0	2	1	3	1	2	6
10/8/86	3	1	5	0	1	0	3	2	11	1
17/8/86	17	3	32	23	22	34	26	25	25	15
24/8/86	24	30	24	27	43	50	42	58	30	51
31/8/86	10	54	28	23	30	12	23	31	16	41
7/9/86	12	7	16	24	23	7	10	21	11	17
14/9/86	16	12	14	19	20	12	18	18	12	18
21/9/86	0	2	3	2	7	3	2	1	0	2
28/9/86	0	0	1	2	3	0	0	2	2	1
5/10/86	0	0	0	0	0	0	0	0	0	0
TOTAL	85	109	124	121	151	124	130	159	109	153

TABLE B7

Number of eggs hatched from 10 plots (10x10m) in the insectary (1986)

Week ending	A	B	C	D	E	F	G	H	I	J
27/7/86	0	0	0	0	0	2	3	0	0	1
3/8/86	2	0	1	0	1	0	3	0	0	0
10/8/86	2	1	1	0	1	0	1	1	7	1
17/8/86	14	3	22	18	14	32	11	16	13	10
24/8/86	24	33	12	24	31	33	36	37	22	42
31/8/86	11	50	21	20	25	8	18	26	14	37
7/9/86	11	2	12	16	18	5	10	20	8	11
14/9/86	9	10	9	12	13	8	14	14	9	17
21/9/86	0	2	2	2	5	3	1	1	0	2
28/9/86	0	0	1	2	2	0	0	1	1	1
5/10/86	0	0	0	0	0	0	0	0	0	0
TOTAL	73	101	81	94	110	91	97	116	74	122

APPENDIX III

TABLE C1

Analysis of variance of oviposition preference
between grasses and sand

Source	dF.	Sum of squares	Mean square	var. rat.
Blocks (B)	3	17626.67	5875.56	1.86
Grasses (H)	1	6800.67	6800.67	2.15
Expts (E)	2	24731.59	12365.80	3.91
BXH	3	5635.33	1878.44	0.59
BXE	6	39657.08	6609.51	2.09
EXH	2	1866.58	933.29	0.29
Residual	6	18985.41	3164.24	
Total	23	115303.33		

TABLE C2

Analysis of variance of oviposition preference between grasses and no sand

Source	dF.	Sum of squares	Mean square	var. rat.
Blocks (B)	3	28681.33	9560.44	5.42
Grasses (H)	1	38881.50	38881.50	22.06
Expts (E)	2	101892.25	50946.13	28.91
BXH	3	5669.83	1889.94	1.07
BXE	6	12382.42	2063.74	1.17
HXE	2	22494.25	11247.13	6.38
Residual	6	10574.41	1762.40	
Total	23	220575.99		

TABLE C3

Analysis of variance of oviposition preference between 20cm grasses (sand/no sand)

Source	dF.	Sum of squares	Mean square	var. rat.
Blocks (B)	3	18220.46	6073.48	3.35
Treat. (S)	1	26070.04	26070.04	14.39
Expts (E)	2	81452.33	40726.17	22.48
SXE	2	34892.33	17446.17	9.63
SXB	3	8532.79	2844.26	1.57
BXE	6	16167.67	2694.61	1.49
Residual	6	10868.34	1811.39	
Total	23	196203.96		

TABLE C4

Analysis of variance of oviposition preference between 5cm grasses (sand/no sand)

Source	dF.	Sum of squares	Mean square	var. rat.
Blocks (B)	3	4568.46	1522.82	0.43
Treat. (S)	1	13968.38	13968.38	3.96
Expts (E)	2	31383.25	15691.63	4.45
SXE	2	3256.76	1628.38	0.46
SXB	3	26291.46	8763.82	2.49
BXE	6	33418.42	5569.74	1.58
Residual	6	21144.90	3524.15	
Total	23	134031.63		

APPENDIX IV

TABLE D1

Analysis of egg hatches. Total and % hatched at 5.0°C.

Temp. °C	Age of Eggs	No. Eggs used	NUMBER OF DAYS AFTER INCUBATION																																																				Total hatch	Percentage hatched
			16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70		
5.0	1	50																																						2		14	11	8	8	2	2											47	94
		50																																								1	3	3	7	8	6	5	1	3	2	1						41	82
		50																																									5		7	6	11	5	4	3	1	3						45	90
		50																																											5	4	6	11	9	2	3	3		1				44	88
	Total	200																																						2		15	19	11	27	20	25	22	14	8	6	7		1				177	88.5
	Accum. Percentage																																							1		10	20	27	42	53	67	80	88	92	95	99		100					
	3	40																													1	11	4	6	3	1	1		1	1																		29	72.5
		40																													6	12	6	1	3	1	1																					30	75
		40																												2	6	5	1	3		1																						18	45
		40																													3	5	7	7	7	1			1																			31	77
	Total	160																												2	16	33	18	17	13	4	2		2	1																		108	67.5
	Accum. Percentage																													2	17	47	64	80	92	95	97		99	100																			
	6	50											2		8	3	9	3	3	2	3	2	1		2	1		1		1					1		1										1											44	88
		50											2		5		4	5	1	1	2			1	1	1				1		2		2			3			4			2	3	1		1											42	84
		50											2		2	1	2	4	6	3	3	1	2		4	1		1		1				3	1		2	1																				40	80
		50											2		1	8	4	6	5	6	2	4	3		3																																	44	88
	Total	200											8		16	12	19	18	15	12	10	7	6	1	10	3		2		3		2		5	2		6	1		4			2	3	1		2											170	85
	Accum. Percentage												5		14	21	32	43	52	59	65	69	72	73	79	81		82		84		85		88	89		92	93		95			96	98	99		100												
	10	50			17			5	4			2	3				2	1	1											1	1		1																									43	86
		50			8			7	12				2		1	2		2	2				2		1		2																													42	84
		50			21			9	7			3		2		1		1				1	1	2			3																													45	90
		50			21			7	11			2	2		2	1		1																																						46	92
	Total	200			67			28	34			7	7	2	3	4	2	5	3			1	3	2	1		5				1		1																									176	88
	Accum. Percentage				38			54	73			77	81	82	84	86	88	90	92			93	94	95	96		98				99		100																										

TABLE D2

Analysis of egg hatches. Total and % hatched at 10.0°C.

Temp. °C	Age of Eggs	No. Eggs used	NUMBER OF DAYS AFTER INCUBATION																													Total hatch	Percentage hatched
			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	33	34	35	36		
10.0	1	50																								1	9	22	10	2	2	46	92
		50																										16	22	3	6	47	94
		50																										16	22	3	3	44	88
		50																										20	15	4	6	45	90
	Total	200																								1	9	74	69	12	17	182	91
	Accum. Percentage																									0.5	5	46	84	91	100		
	3	50																						1	2	13	17	13				46	92
		50																						2	7	12	14	12	1	1		49	98
		50																					1	10	11	11	12					45	90
		50																						2	3	11	18	9	1			44	88
	Total	200																					1	15	23	47	61	34	2	1		184	92
	Accum. Percentage																						1	9	21	47	80	98	99	100			
	6	50										15		7	9	3	4	2		1	4											45	90
		50										24		7	2	1		7	1		2											44	88
		50									4	21		6	6	5	1	1	2		1											47	94
		50									4	21		3	2		1	4		5	5											45	90
	Total	200									8	81		23	19	9	6	14	3	6	12											181	90.5
	Accum. Percentage										4	49		62	72	77	81	88	90	93	100												
	10	50		6	17		6	7	4																							40	80
		50			7		5	7	20			3		3																		45	90
		50		5	6		11	4	4		3	2			1	1		1			5											43	86
		50		7	14		8	2	5				2	1	2	1		2			1											45	90
	Total	200		18	44		30	20	33		3	5	2	4	3	2		3			6											173	86.5
	Accum. Percentage			10	36		53	65	84		86	88	90	92	94	95		97			100												

TABLE D3

Analysis of egg hatches. Total and % hatched at 15.0°C.

Temp. °C	Age of Eggs	No. Eggs used	NUMBER OF DAYS AFTER INCUBATION																				Total batch	Percentage hatched
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
15.0	1	50														35	10						45	90
		50													1	39	8	1					49	98
		50														40	6	1					47	94
		50													8	26	10				1		45	90
	Total	200													9	140	34	2			1		186	93
	Accum. Percentage														5	80	98	99			100			
	3	50												11	32	3							46	92
		50											1	14	27	3			3				48	96
		50												18	21	6							45	90
		50											4	22	14	2		1	2				45	90
	Total	200											5	65	94	14		1	5				184	92
	Accum. Percentage												3	38	89	97		97	100					
	6	50							21	9		7	4	6				2					49	98
		50							13	9		18	1	4									45	90
		50							5	12		17	1	3		1							39	78
		50							8	13		17	1	5			1						45	90
	Total	200							47	43		59	7	18		1	1	2					178	89
	Accum. Percentage								26	51		84	88	98		98	99	100						
	10	50				1	6	22	5	4	5												43	86
		50				3	21	15	4		2												45	90
		50				8	11	19	4	3	1		2			1							49	98
		50				8	12	12	7	3													42	84
	Total	200				20	50	68	20	10	8		2			1							179	89.5
	Accum. Percentage					11	39	77	88	94	98		99			100								

TABLE D4

Analysis of egg hatches. Total and % hatched at 20.0°C.

Temp. °C	Age of Eggs	No. Eggs used	NUMBER OF DAYS AFTER INCUBATION																	Total hatch	Percentage hatched
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
20.0	1	50									23	21	4							48	96
		50									37	12		1						50	100
		50									33	11								44	88
		50									26	12	3					2		43	86
	Total	200									119	56	7	1				2		185	92.5
	Accum. Percentage										64	95	98	99				100			
	3	50							1		30	15	2							48	96
		50									26	15	1				1			43	86
		50									39	8	1							48	96
		50									36	10	1				1			48	96
	Total	200							1		131	48	5				2			187	93.5
	Accum. Percentage								0.5		71	96	99				100				
	6	50					3	25	10	1	2	1				3				45	90
		50					10	23	5	4			2				1			45	90
		50					8	23	5	4							3			43	86
		50					9	20	8	1	1	2								41	82
	Total	200					30	91	28	10	3	3	2			3	4			174	87
	Accum. Percentage						17	70	86	91	93	95	96			98	100				
	10	50			2	28	7	3	2	2			1							45	90
		50			4	24	6	6	3				1							44	88
		50			4	25	7	4	3				2							45	90
		50			6	21	5	8		3			1							44	88
	Total	200			16	98	25	21	8	5			5							178	89
	Accum. Percentage				9	64	78	90	94	97			100								

TABLE D5

Analysis of egg hatches. Total and % hatched at 25.0°C.

Temp. °C	Age of Eggs	No. Eggs used	NUMBER OF DAYS AFTER INCUBATION												Total hatch	Percentage hatched
			1	2	3	4	5	6	7	8	9	10	11	12		
25.0	1	50							5	40	2				47	94
		50							3	35	4	1			43	86
		50							4	33	5		2		44	88
		50							6	29	4				39	78
	Total	200							18	137	15	1	2		173	86.5
	Accum. Percentage								10	90	98	99	100			
	3	50							14		26				40	80
		50							22		22				44	88
		50							16		27				43	86
		50							7		34				41	82
	Total	200							59		109				168	84
	Accum. Percentage								35		100					
	6	50				4	22	13	5						44	88
		50				2	23	11	7						43	86
		50				7	23	10	2						42	84
		50				6	19	8	8		1				42	84
	Total	200				19	87	42	22		1				171	85.5
	Accum. Percentage					11	62	87	99		100					
	10	50			33	11									44	88
		50			26	16									42	84
		50			23	10		1	1	1					36	72
		50			25	15		3							43	86
	Total	200			107	52		4	1	1					165	82.5
	Accum. Percentage				65	96		98	99	100						

TABLE D6

Analysis of egg hatches. Total and % hatched in the Insectary

Temp. °C	Age of Eggs	No. Eggs used	NUMBER OF DAYS AFTER INCUBATION 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total hatch	Percentage hatched
INSECTARY	1	50										11	12							43	86
		50										10	34							44	88
		50										10	37							47	94
		50											39							39	78
	Total	200										31	142							173	86.5
	Accum. Percentage											18	100								
	3	40								27		5	1							33	82.5
		40								27		2	1							30	75
		40								26		4	3							33	82.5
		40										28	4							32	80
	Total	160								80		39	9							128	80
	Accum. Percentage									63		93	100								
	6	50							14	13	10	3	3							43	86
		50					6		20	8	9	3	2			1				49	98
		50					8		22	6	4	1	2							43	86
		50					12		15	10	8	1	2							48	96
	Total	200					26		71	37	31	8	9			1				183	91.5
	Accum. Percentage						14		53	73	90	95	99			100					
	10	50				17	21	9												47	94
		50				14	18	7	1	3			2							44	88
		50				4	23	10	5	3										45	90
		50				14	15	3	7	6										45	90
	Total	200				49	77	29	13	12			2							182	91
	Accum. Percentage					27	69	85	92	99			100								

APPENDIX V

TABLE E1

Number of larvae collected from 40 soil cores taken from 10 plots on 23/9/1984, and their transformed data

Plot No.	No.of larvae collected from each soil core					Transformed data				
	1	2	3	4	Total	1	2	3	4	Total
A	3	1	2	1	7	1.87	1.22	1.58	1.22	5.89
B	1	4	1	1	7	1.22	2.12	1.22	1.22	5.78
C	1	2	1	1	5	1.22	1.58	1.22	1.22	5.24
D	2	0	3	2	7	1.58	0.71	1.87	1.58	5.74
E	2	3	6	4	15	1.58	1.87	2.55	2.12	8.12
F	2	1	2	1	6	1.58	1.22	1.58	1.22	5.6
G	4	2	1	5	12	2.12	1.58	1.22	2.35	7.27
H	2	4	7	7	20	1.58	2.12	2.74	2.74	9.18
I	4	1	4	4	13	2.12	1.22	2.12	2.12	7.58
J	1	3	5	3	12	1.22	1.87	2.35	1.87	7.31
	Total				104	Total				67.71
	Mean				2.6	Mean				1.693

TABLE E2

Number of larvae collected from 80 soil cores
taken from 10 plots on 23/10/1984

Plot No.	No. of larvae collected from each soil core								Total
	1	2	3	4	5	6	7	8	
A	1	1	1	1	0	0	1	0	5
B	0	1	0	1	1	1	0	0	4
C	2	1	0	1	1	1	1	1	8
D	1	0	1	1	1	0	1	1	6
E	2	1	1	2	7	4	2	4	23
F	1	1	3	1	0	0	1	1	8
G	3	2	1	4	3	2	4	1	20
H	1	1	2	0	3	2	5	6	20
I	3	2	2	5	4	1	4	2	23
J	1	4	1	1	2	5	3	1	18
							Total		135
							Mean		1.7

TABLE E3

Transformed Data 23/10/1984

Plot No.	1	2	3	4	5	6	7	8	Total
A	1.22	1.22	1.22	1.22	0.71	0.71	1.22	0.71	8.23
B	0.71	1.22	0.71	1.22	1.22	1.22	0.71	0.71	7.72
C	1.58	1.22	0.71	1.22	1.22	1.22	1.22	1.22	9.61
D	1.22	0.71	1.22	1.22	1.22	0.71	1.22	1.22	8.74
E	1.58	1.22	1.22	1.58	2.74	2.12	1.58	2.12	14.16
F	1.22	1.22	1.87	1.22	0.71	0.71	1.22	1.22	9.39
G	1.87	1.58	1.22	2.12	1.87	1.58	2.12	1.22	13.58
H	1.22	1.22	1.58	0.71	1.87	1.58	2.35	2.55	13.08
I	1.87	1.58	1.58	2.35	2.12	1.22	2.12	1.58	14.42
J	1.22	2.12	1.22	1.22	1.58	2.35	1.87	1.22	12.8
							Total		111.73
							Mean		1.396

TABLE E4

Number of larvae collected from 40 soil cores taken from 10 plots on 6/11/1984, and their transformed data

Plot No.	No.of larvae collected from each soil core					Transformed data				
	1	2	3	4	Total	1	2	3	4	Total
A	1	0	0	0	1	1.22	0.71	0.71	0.71	3.35
B	0	1	1	1	3	0.71	1.22	1.22	1.22	4.37
C	2	0	1	1	4	1.58	0.71	1.22	1.22	4.73
D	1	2	1	1	5	1.22	1.58	1.22	1.22	5.24
E	2	2	0	3	7	1.58	1.58	0.71	1.87	5.74
F	0	1	1	0	2	0.71	1.22	1.22	0.71	3.86
G	1	1	1	0	3	1.22	1.22	1.22	0.71	4.37
H	2	1	2	3	8	1.58	1.22	1.58	1.87	6.25
I	1	1	3	1	6	1.22	1.22	1.87	1.22	5.53
J	1	0	1	1	3	1.22	0.71	1.22	1.22	4.37
	Total				42	Total				47.81
	Mean				1.05	Mean				1.195

TABLE E5

Number of larvae collected from 80 soil cores taken from 10 plots on 4/12/1984

Plot No.	No. of larvae collected from each soil core 1	2	3	4	5	6	7	8	Total
A	0	1	0	1	0	2	0	1	5
B	2	1	2	0	3	0	0	1	9
C	1	0	0	1	0	0	1	2	5
D	0	2	6	0	0	0	0	0	8
E	0	2	1	2	0	0	0	1	6
F	0	2	0	0	0	1	1	1	5
G	0	1	1	1	0	0	2	1	6
H	2	0	0	0	4	2	2	1	11
I	1	0	0	1	1	0	1	1	5
J	2	4	2	2	0	0	0	0	10
						Total			70
						Mean			0.875

TABLE E6

Transformed Data 4/12/1984

Plot No.	1	2	3	4	5	6	7	8	Total
A	0.71	1.22	0.71	1.22	0.71	1.58	0.71	1.22	8.08
B	1.58	1.22	1.58	0.71	1.87	0.71	0.71	1.22	9.6
C	1.22	0.71	0.71	1.22	0.71	0.71	1.22	1.58	8.08
D	0.71	1.58	2.55	0.71	0.71	0.71	0.71	0.71	8.39
E	0.71	1.58	1.22	1.58	0.71	0.71	0.71	1.22	8.44
F	0.71	1.58	0.71	0.71	0.71	1.22	1.22	1.22	8.08
G	0.71	1.22	1.22	1.22	0.71	0.71	1.58	1.22	8.59
H	1.58	0.71	0.71	0.71	2.12	1.58	1.58	1.22	10.21
I	1.22	0.71	0.71	1.22	1.22	0.71	1.22	1.22	8.23
J	1.58	2.12	1.58	1.58	0.71	0.71	0.71	0.71	9.7
							Total		87.4
							Mean		1.092

TABLE E7

Number of larvae collected from 80 soil cores taken from 10 plots on 5/2/1985

Plot No.	No. of larvae collected from each soil core 1	2	3	4	5	6	7	8	Total
A	0	1	0	0	0	1	0	0	2
B	0	0	0	1	0	0	2	1	4
C	1	1	0	0	0	0	0	0	2
D	2	0	0	1	1	0	1	1	6
E	1	1	0	0	0	0	0	0	2
F	0	0	3	1	0	0	0	0	4
G	1	3	2	1	0	1	0	4	12
H	0	1	1	3	1	1	0	0	7
I	1	1	1	1	0	0	3	0	7
J	0	1	0	0	2	0	1	1	5
							Total		51
							Mean		0.6375

TABLE E8

Transformed Data 5/2/1985

Plot No.	1	2	3	4	5	6	7	8	Total
A	0.71	1.22	0.71	0.71	0.71	1.22	0.71	0.71	6.7
B	0.71	0.71	0.71	1.22	0.71	0.71	1.58	1.22	7.57
C	1.22	1.22	0.71	0.71	0.71	0.71	0.71	0.71	6.7
D	1.58	0.71	0.71	1.22	1.22	0.71	1.22	1.22	8.59
E	1.22	1.22	0.71	0.71	0.71	0.71	0.71	0.71	6.7
F	0.71	0.71	1.87	1.22	0.71	0.71	0.71	0.71	7.35
G	1.22	1.87	1.58	1.22	0.71	1.22	0.71	2.12	10.65
H	0.71	1.22	1.22	1.87	1.22	1.22	0.71	0.71	8.88
I	1.22	1.22	1.22	1.22	0.71	0.71	1.87	0.71	8.88
J	0.71	1.22	0.71	0.71	1.58	0.71	1.22	1.22	8.08
							Total		80.1
							Mean		1.001

TABLE E9

Number of larvae collected from 80 soil cores taken from 10 plots on 27/3/1985

Plot No.	No. of larvae collected from each soil core								Total
	1	2	3	4	5	6	7	8	
A	4	0	1	0	1	0	1	1	8
B	0	0	1	2	0	2	0	1	6
C	1	0	0	0	0	0	0	0	1
D	2	0	0	1	0	0	0	0	3
E	1	0	0	1	0	0	0	0	2
F	0	0	0	1	1	1	1	0	4
G	0	2	0	0	0	0	0	1	3
H	1	0	0	0	0	0	0	0	1
I	0	0	1	3	2	0	0	0	6
J	0	0	0	0	0	0	0	1	1
							Total		35
							Mean		0.4375

TABLE E10

Transformed Data 27/3/1985

Plot No.	1	2	3	4	5	6	7	8	Total
A	2.12	0.71	1.22	0.71	1.22	0.71	1.22	1.22	9.13
B	0.71	0.71	1.22	1.58	0.71	1.58	0.71	1.22	8.44
C	1.22	0.71	0.71	0.71	0.71	0.71	0.71	0.71	6.19
D	1.58	0.71	0.71	1.22	0.71	0.71	0.71	0.71	7.06
E	1.22	0.71	0.71	1.22	0.71	0.71	0.71	0.71	6.7
F	0.71	0.71	0.71	1.22	1.22	1.22	1.22	0.71	7.72
G	0.71	1.58	0.71	0.71	0.71	0.71	0.71	1.22	7.06
H	1.22	0.71	0.71	0.71	0.71	0.71	0.71	0.71	6.19
I	0.71	0.71	1.22	1.87	1.58	0.71	0.71	0.71	8.22
J	0.71	0.71	0.71	0.71	0.71	0.71	0.71	1.22	6.19
							Total		72.9
							Mean		0.911

APPENDIX VI

TABLE F

The effects of population density on survival of *Tipula paludosa* larvae (Expt. 1)

Number of larvae per culture	10	20	40	80	160	320	640
Replicate Number							
1	8	17	25	50	54	136	164
2	8	18	37	55	57	94	136
3	10	16	17	51	65	99	-
4	7	17	28	50	72	-	-
5	8	18	22	47	68	-	-
6	10	17	25	49	78	-	-
7	10	16	29	43	64	-	-
8	10	18	29	50	63	-	-
9	8	18	35	36	-	-	-
10	9	16	39	-	-	-	-
Total No. of survivors	88	171	286	431	521	329	300
% survival	88	85.5	71.5	59.9	41	34.3	23.4
Mean No. of larvae per Replicate ± (S.E.)	8.8±0.36	17.1±0.28	28.6±2.17	47.9±1.83	65.1.±2.73	109.7±13.25	150±14

APPENDIX VII

Table G. The number of *T.paludosa* larvae surviving prolonged freezing in the laboratory

Temperature		-2.5°C					-5°C					-7.5°C			
	Time of Exposure	10hrs	20hrs	30hrs	40hrs	60hrs	10hrs	20hrs	30hrs	40hrs	60hrs	10hrs	20hrs	30hrs	40hrs
	1	10	5	8	0	1	7	1	5	1	0	8	0	0	0
	2	10	9	3	0	0	3	4	0	0	0	4	0	0	0
	3	10	1	7	8	0	0	4	8	0	0	0	0	0	0
	4	10	8	1	0	3	2	0	0	0	0	0	0	0	0
Replicate No.	5	10	2	7	7	2	9	6	4	1	0	3	0	1	0
	6	10	8	1	3	1	0	5	1	0	0	5	0	0	0
	7	10	4	2	0	4	8	0	4	2	0	0	0	0	0
	8	10	9	7	5	2	3	7	0	1	0	0	0	0	0
	9	10	9	0	5	0	5	5	3	0	0	0	0	0	0
	10	10	6	10	3	5	0	0	5	0	0	4	0	0	0
Total survived		100	61	46	31	18	37	32	30	5	0	24	0	1	0
St. error		0	0.948	1.127	0.971	0.554	1.075	0.854	0.856	0.224	0	0.897	0	0.1	0
Mean		10.0	6.1	4.6	3.1	1.8	3.7	3.2	3.0	0.5	0	2.4	0	0.1	0

APPENDIX VIII

Temperature and Rainfall Records

(Oct. 1983 - Oct. 1986)

TABLE H

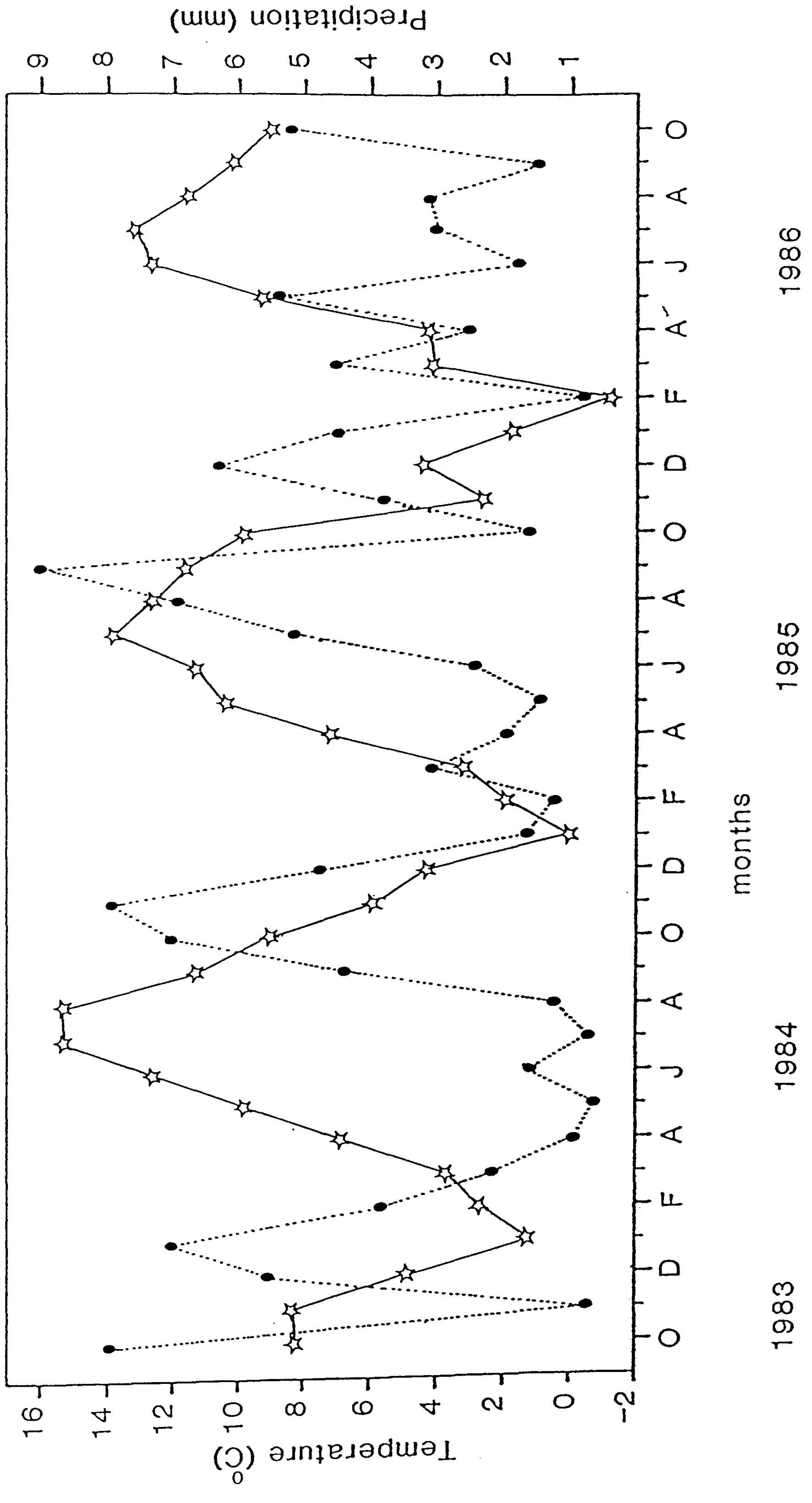
Mean monthly air temperature, rainfall, and days with rain from the period Oct. 1983 to Oct. 1986

Month	Ave. daily temp. (^oC)	Ave. daily rainfall (mm)	No. days with rain
1983 Oct.	8.2	7.9	27
Nov.	8.3	0.7	12
Dec.	4.8	5.5	25
1984 Jan.	1.2	7.0	27
Feb.	2.7	3.8	20
Mar.	3.7	2.1	17
Apr.	6.7	0.9	16
May	9.7	0.6	9
Jun.	12.5	1.6	14
Jul.	15.3	0.7	8
Aug.	15.3	1.2	8
Sep.	11.2	4.3	22
Oct.	9.0	7.0	27
Nov.	5.9	7.9	24
Dec.	4.2	4.7	26
1985 Jan.	0.0	1.6	12
Feb.	1.9	1.2	7
Mar.	3.1	3.0	19
Apr.	7.1	1.9	24
May	10.4	1.4	14
Jun.	11.3	2.4	22
Jul.	13.8	5.1	25
Aug.	12.5	6.9	31
Sep.	11.6	9.0	26
Oct.	9.7	1.6	11
Nov.	2.6	3.8	18
Dec.	4.4	6.3	25
1986 Jan.	1.7	4.5	21
Feb.	-1.2	0.8	11
Mar.	4.1	4.5	26
Apr.	4.2	2.5	22
May	9.3	5.4	29
Jun.	12.7	1.8	15
Jul.	13.2	3.0	23
Aug.	11.6	3.1	16
Sep.	10.2	1.5	16
Oct.	9.0	5.2	22

Figure A. Mean monthly air temperature and precipitation from the period October 1983 to October 1986.

Solid line with stars = temperature

Broken line with circles = rainfall


Temperature (°C)
16
14
12
10
8
6
4
2
0
-2
Precipitation (mm)
9
8
7
6
5
4
3
2
1
O
D
F
A
J
A
O
D
F
A
J
A
O
D
F
A
J
A
O
months
1983
1984
1985
1986

APPENDIX IX

A Trial with TIV

An attempt was made to introduce TIV infection into third-instar larvae. TIV virus was derived from dried dead infected larvae.

Homogenised piece of dead larva in Hill's insect saline, centrifuged at 1500g for 10 minutes to remove large particulate material.

Two petri-dishes each with 5 larvae cultured on moist filter paper.

All the healthy larvae were infected with 20ul supernatant using microsyringe.

The larvae were anaesthetized with carbon dioxide for few minutes.

Infected larvae were placed in petri-dishes with damp filter paper and dried powered grass was added, then kept in 20^{o}C.

All larvae were inspected regularly. On the second day, all the larvae inoculated were checked but no sign of TIV disease was evident. After 3 days from infection, all the larvae were found dead and NONE of them observed to be infected.

APPENDIX X

Figure B. Construction of the bird exclusion (1985-1986).

Figure C. The experimental field showing the bird exclusion experiment in 1985-1986.

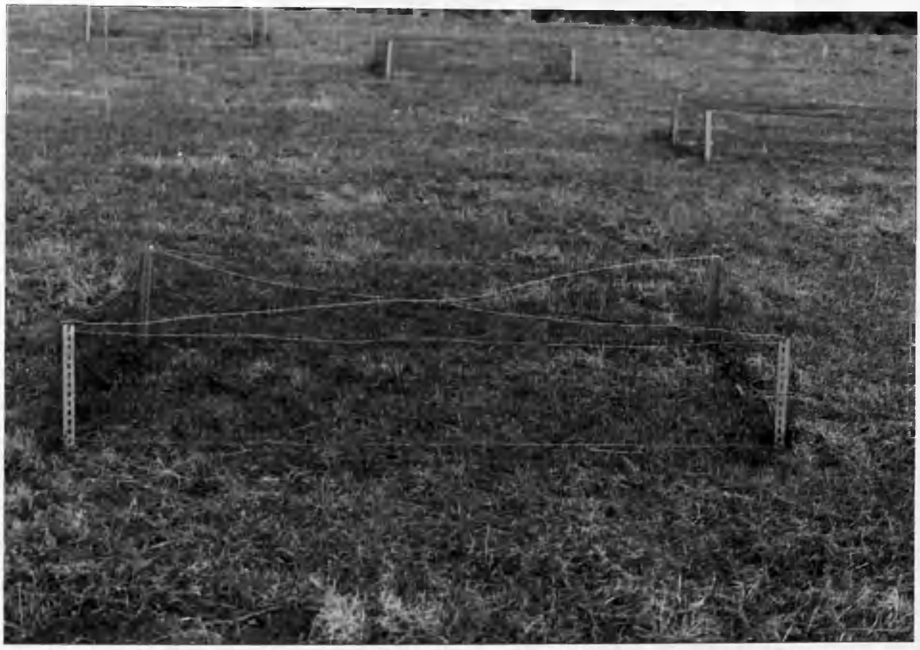


Figure D. The experimental field showing the bird exclusion experiment in 1986-1987 where the size of an exclusor doubled (4 m^2) and the number was reduced to 10 instead of 20.

TABLE J1

Number of larvae collected from 80 soil cores in 20 covered and control cages on 7.3.1986

Plot No.	No. of larvae collected from each soil core									
	COVERED CAGES					CONTROLS				
	1	2	3	4	Total	1	2	3	4	Total
A	0	0	1	0	1	0	1	0	0	1
B	1	1	0	2	4	0	0	0	1	1
C	0	1	0	1	2	0	0	1	0	1
D	1	0	2	1	4	1	0	0	1	2
E	0	0	1	0	1	0	0	1	0	1
F	1	0	1	0	2	0	1	0	1	2
G	1	0	1	0	2	0	0	0	0	0
H	0	1	0	0	1	0	1	0	1	2
I	1	0	0	1	2	0	2	0	2	4
J	0	0	1	0	1	1	0	1	1	3
K	0	1	0	1	2	1	0	0	0	1
L	1	2	0	2	5	1	0	0	2	3
M	1	1	0	2	4	1	1	0	1	3
N	0	0	1	0	1	0	0	0	0	0
O	0	0	2	1	3	0	0	1	0	1
P	0	1	0	1	2	0	1	1	0	2
Q	0	1	1	2	4	2	0	0	1	3
R	1	2	1	1	5	1	1	0	1	3
S	1	0	2	1	4	1	1	0	0	2
T	0	0	1	0	1	0	1	0	0	1
			Total		51		Total			36
			Mean		0.638		Mean			0.45

TABLE J2

Transformed Data (7.3.1986)

Plot	Covered Cages				Total	Controls				Total
A	0.71	0.71	1.22	0.71	3.35	0.71	1.22	0.71	0.71	3.35
B	1.22	1.22	0.71	1.58	4.73	0.71	0.71	0.71	1.22	3.35
C	0.71	1.22	0.71	1.22	3.86	0.71	0.71	1.22	0.71	3.35
D	1.22	0.71	1.58	1.22	4.73	1.22	0.71	0.71	1.22	3.86
E	0.71	0.71	1.22	0.71	3.35	0.71	0.71	1.22	0.71	3.35
F	1.22	0.71	1.22	0.71	3.86	0.71	1.22	0.71	1.22	3.86
G	1.22	0.71	1.22	0.71	3.86	0.71	0.71	0.71	0.71	2.84
H	0.71	1.22	0.71	0.71	3.35	0.71	1.22	0.71	1.22	3.86
I	1.22	0.71	0.71	1.22	3.86	0.71	1.58	0.71	1.58	4.58
J	0.71	0.71	1.22	0.71	3.35	1.22	0.71	1.22	1.22	4.37
K	0.71	1.22	0.71	1.22	3.86	1.22	0.71	0.71	0.71	3.35
L	1.22	1.58	0.71	1.58	5.09	1.22	0.71	0.71	1.58	4.22
M	1.22	1.22	0.71	1.58	4.73	1.22	1.22	0.71	1.22	4.37
N	0.71	0.71	1.22	0.71	3.35	0.71	0.71	0.71	0.71	2.84
O	0.71	0.71	1.58	1.22	4.22	0.71	0.71	1.22	0.71	3.35
P	0.71	1.22	0.71	1.22	3.86	0.71	1.22	1.22	0.71	3.86
Q	0.71	1.22	1.22	1.58	4.73	1.58	0.71	0.71	1.22	4.22
R	1.22	1.58	1.22	1.22	5.24	1.22	1.22	0.71	1.22	4.37
S	1.22	0.71	1.58	1.22	4.73	1.22	1.22	0.71	0.71	3.86
T	0.71	0.71	1.22	0.71	3.35	0.71	1.22	0.71	0.71	3.35
				Total	81.46				Total	74.56
				Mean	1.018				Mean	0.932

TABLE J3

Number of larvae collected from 160 soil cores in
20 covered and control cages on 5.5.1986

Plot No.	No. of larvae collected from each soil core																	
	COVERED CAGES									CONTROLS								
	1	2	3	4	5	6	7	8	Total	1	2	3	4	5	6	7	8	Total
A	0	1	0	0	0	1	1	0	3	0	0	0	2	0	0	0	0	2
B	0	0	1	0	1	1	0	0	3	0	0	1	0	0	1	0	0	2
C	0	0	1	0	1	0	2	0	4	0	0	0	1	0	0	1	1	3
D	0	1	0	0	2	0	0	1	4	1	1	0	0	0	1	1	0	4
E	0	1	0	0	0	0	0	1	2	0	0	2	0	2	0	0	1	5
F	1	1	1	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
G	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	2
H	1	0	0	0	0	0	0	0	1	0	0	2	0	1	0	0	0	3
I	0	1	0	0	1	0	1	0	3	0	0	0	0	1	0	0	0	1
J	0	1	0	0	1	0	2	1	5	0	0	0	1	1	0	0	2	4
K	0	1	1	1	1	1	0	0	5	0	1	0	0	0	1	0	0	2
L	1	0	1	0	0	0	0	2	4	0	0	0	0	0	0	0	0	0
M	1	0	1	0	0	2	0	0	4	0	1	0	0	1	0	0	0	2
N	1	0	0	1	0	1	0	0	3	0	1	0	0	1	0	0	0	2
O	0	0	1	0	0	0	1	1	3	0	1	1	0	0	0	2	0	4
P	0	0	1	1	0	0	0	2	4	0	0	0	0	0	0	0	0	0
Q	0	0	0	2	1	2	1	1	7	0	0	0	0	0	0	1	0	1
R	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2
S	0	1	1	0	1	1	1	0	5	0	1	0	0	1	0	0	0	2
T	0	0	0	0	0	0	1	0	1	0	0	0	0	1	2	0	0	3
					Total				65					Total				44
					Mean				0.406					Mean				0.275

TABLE J4 (Part 1)

Transformed Data (5.5.1986)

Plot	COVERED CAGES								Total
A	0.71	1.22	0.71	0.71	0.71	1.22	1.22	0.71	7.21
B	0.71	0.71	1.22	0.71	1.22	1.22	0.71	0.71	7.21
C	0.71	0.71	1.22	0.71	1.22	0.71	1.58	0.71	7.57
D	0.71	1.22	0.71	0.71	1.58	0.71	0.71	1.22	7.57
E	0.71	1.22	0.71	0.71	0.71	0.71	0.71	1.22	6.70
F	1.22	1.22	1.22	0.71	0.71	0.71	0.71	0.71	7.21
G	0.71	0.71	1.22	0.71	0.71	0.71	0.71	0.71	6.19
H	1.22	0.71	0.71	0.71	0.71	0.71	0.71	0.71	6.19
I	0.71	1.22	0.71	0.71	1.22	0.71	1.22	0.71	7.21
J	0.71	1.22	0.71	0.71	1.22	0.71	1.58	1.22	8.08
K	0.71	1.22	1.22	1.22	1.22	1.22	0.71	0.71	8.23
L	1.22	0.71	1.22	0.71	0.71	0.71	0.71	1.58	7.57
M	1.22	0.71	1.22	0.71	0.71	1.58	0.71	0.71	7.57
N	1.22	0.71	0.71	1.22	0.71	1.22	0.71	0.71	7.21
O	0.71	0.71	1.22	0.71	0.71	0.71	1.22	1.22	7.21
P	0.71	0.71	1.22	1.22	0.71	0.71	0.71	1.58	7.57
Q	0.71	0.71	0.71	1.58	1.22	1.58	1.22	1.22	8.95
R	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
S	0.71	1.22	1.22	0.71	1.22	1.22	1.22	0.71	8.23
T	0.71	0.71	0.71	0.71	0.71	0.71	1.22	0.71	6.19
							Total		145.55
							Mean		0.910

TABLE J4 (Part 2)

Transformed Data (5.5.1986)

Plot	CONTROLS								Total
A	0.71	0.71	0.71	1.58	0.71	0.71	0.71	0.71	6.55
B	0.71	0.71	1.22	0.71	0.71	1.22	0.71	0.71	6.70
C	0.71	0.71	0.71	1.22	0.71	0.71	1.22	1.22	7.21
D	1.22	1.22	0.71	0.71	0.71	1.22	1.22	0.71	7.72
E	0.71	0.71	1.58	0.71	1.58	0.71	0.71	1.22	7.93
F	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
G	1.22	0.71	1.22	0.71	0.71	0.71	0.71	0.71	6.70
H	0.71	0.71	1.58	0.71	1.22	0.71	0.71	0.71	7.06
I	0.71	0.71	0.71	0.71	1.22	0.71	0.71	0.71	6.19
J	0.71	0.71	0.71	1.22	1.22	0.71	0.71	1.58	7.57
K	0.71	1.22	0.71	0.71	0.71	1.22	0.71	0.71	6.70
L	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
M	0.71	1.22	0.71	0.71	1.22	0.71	0.71	0.71	6.70
N	0.71	1.22	0.71	0.71	1.22	0.71	0.71	0.71	6.70
O	0.71	1.22	1.22	0.71	0.71	1.58	0.71	0.71	7.57
P	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
Q	0.71	0.71	0.71	0.71	0.71	0.71	1.22	0.71	6.19
R	0.71	0.71	0.71	1.22	1.22	0.71	0.71	0.71	6.70
S	0.71	1.22	0.71	0.71	1.22	0.71	0.71	0.71	6.70
T	0.71	0.71	0.71	0.71	1.22	1.58	0.71	0.71	7.06
							Total		134.99
							Mean		0.844

TABLE J5

Number of larvae collected from 160 soil cores in
20 covered and control cages on 18.6.1986

Plot No.	No. of larvae collected from each soil core																	
	COVERED CAGES									CONTROLS								
	1	2	3	4	5	6	7	8	Total	1	2	3	4	5	6	7	8	Total
A	0	0	1	0	1	0	1	0	3	0	0	0	0	0	0	1	0	1
B	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1
C	0	0	0	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0
D	0	1	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0
E	1	1	0	1	0	0	1	0	4	1	0	0	0	0	0	0	1	2
F	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
G	1	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0
H	1	0	0	0	0	1	1	1	4	0	0	0	1	0	0	0	1	2
I	0	0	1	0	0	0	1	0	2	0	2	0	0	0	0	0	0	2
J	0	0	0	1	1	0	0	0	2	1	0	0	0	0	0	0	0	1
K	0	1	0	0	1	0	0	1	3	0	0	0	1	0	0	0	0	1
L	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	2
M	0	0	0	1	0	0	0	1	2	0	1	0	0	0	0	0	1	2
N	0	0	1	0	0	0	0	1	2	1	0	0	0	0	2	0	0	3
O	0	0	0	1	0	0	1	0	2	0	0	0	2	0	1	0	0	3
P	1	0	0	1	0	1	0	0	3	0	0	0	0	1	0	0	0	1
Q	0	1	0	0	0	1	0	0	2	0	0	0	1	0	0	1	0	2
R	0	0	0	0	0	1	0	1	2	0	0	1	0	0	0	0	1	2
S	0	1	0	0	0	1	0	0	2	0	0	0	0	1	0	0	1	2
T	0	0	1	0	1	0	0	0	2	0	0	0	1	0	0	0	0	1
					Total				44					Total				29
					Mean				0.275					Mean				0.181

TABLE J6 (Part 1)

Transformed Data (18.6.1986)

Plot	COVERED CAGES								Total
A	0.71	0.71	1.22	0.71	1.22	0.71	1.22	0.71	7.21
B	0.71	0.71	0.71	0.71	0.71	0.71	1.22	0.71	6.19
C	0.71	0.71	0.71	1.22	1.22	0.71	0.71	0.71	6.70
D	0.71	1.22	0.71	0.71	0.71	1.22	0.71	0.71	6.70
E	1.22	1.22	0.71	1.22	0.71	0.71	1.22	0.71	7.72
F	0.71	0.71	0.71	0.71	0.71	0.71	0.71	1.22	6.19
G	1.22	0.71	0.71	0.71	0.71	1.22	0.71	0.71	6.70
H	1.22	0.71	0.71	0.71	0.71	1.22	1.22	1.22	7.72
I	0.71	0.71	1.22	0.71	0.71	0.71	1.22	0.71	6.70
J	0.71	0.71	0.71	1.22	1.22	0.71	0.71	0.71	6.70
K	0.71	1.22	0.71	0.71	1.22	0.71	0.71	1.22	7.21
L	0.71	0.71	0.71	0.71	0.71	0.71	0.71	1.22	6.19
M	0.71	0.71	0.71	1.22	0.71	0.71	0.71	1.22	6.70
N	0.71	0.71	1.22	0.71	0.71	0.71	0.71	1.22	6.70
O	0.71	0.71	0.71	1.22	0.71	0.71	1.22	0.71	6.70
P	1.22	0.71	0.71	1.22	0.71	1.22	0.71	0.71	7.21
Q	0.71	1.22	0.71	0.71	0.71	1.22	0.71	0.71	6.70
R	0.71	0.71	0.71	0.71	0.71	1.22	0.71	1.22	6.70
S	0.71	1.22	0.71	0.71	0.71	1.22	0.71	0.71	6.70
T	0.71	0.71	1.22	0.71	1.22	0.71	0.71	0.71	6.70
							Total		136.04
							Mean		0.850

TABLE J6 (Part 2)

Transformed Data (18.6.1986)

Plot	CONTROLS								Total
A	0.71	0.71	0.71	0.71	0.71	0.71	1.22	0.71	6.19
B	0.71	0.71	0.71	0.71	1.22	0.71	0.71	0.71	6.19
C	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
D	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
E	1.22	0.71	0.71	0.71	0.71	0.71	0.71	1.22	6.70
F	0.71	0.71	0.71	0.71	0.71	0.71	1.22	0.71	6.19
G	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
H	0.71	0.71	0.71	1.22	0.71	0.71	0.71	1.22	6.70
I	0.71	1.58	0.71	0.71	0.71	0.71	0.71	0.71	6.55
J	1.22	0.71	0.71	0.71	0.71	0.71	0.71	0.71	6.19
K	0.71	0.71	0.71	1.22	0.71	0.71	0.71	0.71	6.19
L	1.22	0.71	0.71	0.71	1.22	0.71	0.71	0.71	6.70
M	0.71	1.22	0.71	0.71	0.71	0.71	0.71	1.22	6.70
N	1.22	0.71	0.71	0.71	0.71	1.58	0.71	0.71	7.06
O	0.71	0.71	0.71	1.58	0.71	1.22	0.71	0.71	7.06
P	0.71	0.71	0.71	0.71	1.22	0.71	0.71	0.71	6.19
Q	0.71	0.71	0.71	1.22	0.71	0.71	1.22	0.71	6.70
R	0.71	0.71	1.22	0.71	0.71	0.71	0.71	1.22	6.70
S	0.71	0.71	0.71	0.71	0.71	1.22	0.71	1.22	6.70
T	0.71	0.71	0.71	1.22	0.71	0.71	0.71	0.71	6.19
							Total		127.94
							Mean		0.800

TABLE J7

Number of larvae collected from 100 soil cores in 10 covered cages and controls on 1.11.1986

Plot No.	No. of larvae collected from each soil core											
	COVERED CAGES						CONTROLS					
	1	2	3	4	5	Total	1	2	3	4	5	Total
A	2	0	1	1	0	4	1	0	1	0	1	3
B	2	1	1	0	1	5	0	4	2	1	0	7
C	2	1	1	0	0	4	1	1	1	0	1	4
D	0	3	0	1	1	5	0	1	2	1	0	4
E	3	0	1	1	1	6	1	0	0	2	1	4
F	1	2	0	3	1	7	1	0	0	2	3	6
G	2	0	1	1	0	4	1	4	1	3	0	9
H	1	0	2	3	0	6	0	1	2	3	1	7
I	2	1	2	0	1	6	3	1	1	2	1	8
J	0	1	1	2	3	7	3	1	1	2	1	8
				Total		54				Total		60
				Mean		1.1				Mean		1.2

TABLE J8

Transformed Data 1.11.1986

Plot No.	Covered Cages 1	2	3	4	5	Total	Controls 1	2	3	4	5	Total
A	1.58	0.71	1.22	1.22	0.71	5.44	1.22	0.71	1.22	0.71	1.22	5.08
B	1.58	1.22	1.22	0.71	1.22	5.95	0.71	2.12	1.58	1.22	0.71	6.34
C	1.58	1.22	1.22	0.71	0.71	5.44	1.22	1.22	1.22	0.71	1.22	5.59
D	0.71	1.87	0.71	1.22	1.22	5.73	0.71	1.22	1.58	1.22	0.71	5.44
E	1.87	0.71	1.22	1.22	1.22	6.24	1.22	0.71	0.71	1.58	1.22	5.44
F	1.22	1.58	0.71	1.87	1.22	6.60	1.22	0.71	0.71	1.58	1.87	6.09
G	1.58	0.71	1.22	1.22	0.71	5.44	1.22	2.12	1.22	1.87	0.71	7.14
H	1.22	0.71	1.58	1.87	0.71	6.09	0.71	1.22	1.58	1.87	1.22	6.60
I	1.58	1.22	1.58	0.71	1.22	6.31	1.87	1.22	1.22	1.58	1.22	7.11
J	0.71	1.22	1.22	1.58	1.87	6.60	1.87	1.22	1.22	1.58	1.22	7.11
					Total	59.84					Total	61.94
					Mean	1.197					Mean	1.239

TABLE J9

Number of larvae collected from 100 soil cores in 10 covered cages and controls on 18.12.1986

Plot No.	No. of larvae collected from each soil core											
	COVERED CAGES						CONTROLS					
	1	2	3	4	5	Total	1	2	3	4	5	Total
A	2	0	1	0	0	3	0	1	1	0	1	3
B	1	0	1	1	2	5	0	1	1	1	0	3
C	0	2	3	0	0	5	0	1	1	0	0	2
D	0	1	1	1	2	5	1	0	2	1	0	4
E	0	1	2	1	1	5	1	0	1	0	1	3
F	1	0	2	2	2	7	0	0	1	2	1	4
G	0	2	1	1	0	4	1	0	3	2	0	6
H	1	2	0	1	1	5	1	0	4	2	1	8
I	2	2	1	1	0	6	2	1	1	1	0	5
J	0	1	2	3	0	6	2	0	0	1	1	4
					Total	51					Total	42
					Mean	1.0					Mean	0.84

TABLE J10

Transformed Data 18.12.1986

Plot No.	Covered cages						Controls					
	1	2	3	4	5	Total	1	2	3	4	5	Total
A	1.58	0.71	1.22	0.71	0.71	4.93	0.71	1.22	1.22	0.71	1.22	5.08
B	1.22	0.71	1.22	1.22	1.58	5.95	0.71	1.22	1.22	1.22	0.71	5.08
C	0.71	1.58	1.87	0.71	0.71	5.58	0.71	1.22	1.22	0.71	0.71	4.57
D	0.71	1.22	1.22	1.22	1.58	5.95	1.22	0.71	1.58	1.22	0.71	5.44
E	0.71	1.22	1.58	1.22	1.22	5.95	1.22	0.71	1.22	0.71	1.22	5.08
F	1.22	0.71	1.58	1.58	1.58	6.67	0.71	0.71	1.22	1.58	1.22	5.44
G	0.71	1.58	1.22	1.22	0.71	5.44	1.22	0.71	1.87	1.58	0.71	6.09
H	1.22	1.58	0.71	1.22	1.22	5.95	1.22	0.71	2.12	1.58	1.22	6.85
I	1.58	1.58	1.22	1.22	0.71	6.31	1.58	1.22	1.22	1.22	0.71	5.95
J	0.71	1.22	1.58	1.87	0.71	6.09	1.58	0.71	0.71	1.22	1.22	5.44
					Total	58.82					Total	55.02
					Mean	1.176					Mean	1.100

TABLE J11

Number of larvae collected from 100 soil cores in
10 covered cages and cages on 1.3.1987

Plot No.	No. of larvae collected from each soil core											
	COVERED CAGES						CONTROLS					
	1	2	3	4	5	Total	1	2	3	4	5	Total
A	1	0	2	0	0	3	0	1	0	1	0	2
B	0	2	0	2	1	5	1	1	0	0	0	2
C	1	1	1	1	1	5	1	1	0	0	0	2
D	0	1	0	1	3	5	0	2	0	0	0	2
E	0	2	2	1	0	5	0	1	0	1	0	2
F	2	1	1	0	1	5	1	2	0	1	0	4
G	2	1	2	0	1	6	0	1	0	3	1	5
H	1	1	1	1	0	4	0	1	1	1	0	3
I	1	2	0	1	1	5	0	1	1	2	0	4
J	0	0	1	1	1	3	1	2	0	1	0	4
					Total	46					Total	30
					Mean	0.92					Mean	0.60

TABLE J12

Transformed Data 1.3.1987

Plot No.	Covered cages 1	2	3	4	5	Total	Controls 1	2	3	4	5	Total
A	1.22	0.71	1.58	0.71	0.71	4.93	0.71	1.22	0.71	1.22	0.71	4.57
B	0.71	1.58	0.71	1.58	1.22	5.80	1.22	1.22	0.71	0.71	0.71	4.57
C	1.22	1.22	1.22	1.22	1.22	6.10	1.22	1.22	0.71	0.71	0.71	4.57
D	0.71	1.22	0.71	1.22	1.87	5.73	0.71	1.58	0.71	0.71	0.71	4.42
E	0.71	1.58	1.58	1.22	0.71	5.80	0.71	1.22	0.71	1.22	0.71	4.57
F	1.58	1.22	1.22	0.71	1.22	5.95	1.22	1.58	0.71	1.22	0.71	5.44
G	1.58	1.22	1.58	0.71	1.22	6.31	0.71	1.22	0.71	1.87	1.22	5.73
H	1.22	1.22	1.22	1.22	0.71	5.59	0.71	1.22	1.22	1.22	0.71	5.08
I	1.22	1.58	0.71	1.22	1.22	5.95	0.71	1.22	1.22	1.58	0.71	5.44
J	0.71	0.71	1.22	1.22	1.22	5.08	1.22	1.58	0.71	1.22	0.71	5.44
					Total	57.24					Total	49.83
					Mean	1.145					Mean	0.997

TABLE J13

Number of larvae collected from 160 soil cores in 10 covered cages and controls on 13.5.1987

Plot No.	No. of larvae collected from each soil core																	
	COVERED CAGES									CONTROLS								
	1	2	3	4	5	6	7	8	Total	1	2	3	4	5	6	7	8	Total
A	0	0	1	0	0	0	2	2	5	1	1	2	1	1	2	1	2	11
B	2	1	2	1	1	2	2	0	11	0	0	0	0	0	0	0	0	0
C	1	0	2	1	0	0	1	1	6	0	1	0	1	0	0	0	0	2
D	1	2	1	1	2	3	2	2	14	0	1	0	0	0	0	0	0	1
E	1	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0
F	0	1	1	1	0	1	0	1	5	1	0	1	0	0	0	0	0	2
G	0	1	1	2	1	0	0	2	7	0	2	1	0	1	1	1	0	6
H	1	0	1	1	1	0	3	0	7	0	0	0	1	0	1	1	0	3
I	0	0	1	0	1	0	0	1	3	0	0	0	0	0	0	0	0	0
J	0	1	1	0	2	1	0	1	6	0	0	0	1	0	0	0	0	1
						Total			66					Total				26
						Mean			0.825					Mean				0.325

TABLE J14 (Part 1)

Transformed Data (13.5.1987)

Plot	COVERED CAGES								
No.	1	2	3	4	5	6	7	8	Total
A	0.71	0.71	1.22	0.71	0.71	0.71	1.58	1.58	7.93
B	1.58	1.22	1.58	1.22	1.22	1.58	1.58	0.71	10.69
C	1.22	0.71	1.58	1.22	0.71	0.71	1.22	1.22	8.59
D	1.22	1.58	1.22	1.22	1.58	1.87	1.58	1.58	11.85
E	1.22	0.71	0.71	0.71	1.22	0.71	0.71	0.71	6.70
F	0.71	1.22	1.22	1.22	0.71	1.22	0.71	1.22	8.23
G	0.71	1.22	1.22	1.58	1.22	0.71	0.71	1.58	8.95
H	1.22	0.71	1.22	1.22	1.22	0.71	1.87	0.71	8.86
I	0.71	0.71	1.22	0.71	1.22	0.71	0.71	0.71	7.21
J	0.71	1.22	1.22	0.71	1.58	1.22	0.71	1.22	8.59
							Total		87.60
							Mean		1.095

TABLE J14 (Part 2)

Transformed Data (13.5.1987)

Plot No.	CONTROLS 1	2	3	4	5	6	7	8	Total
A	1.22	1.22	1.58	1.22	1.22	1.58	1.22	1.58	10.84
B	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
C	0.71	1.22	0.71	1.22	0.71	0.71	0.71	0.71	6.70
D	0.71	1.22	0.71	0.71	0.71	0.71	0.71	0.71	6.19
E	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
F	1.22	0.71	1.22	0.71	0.71	0.71	0.71	0.71	6.70
G	0.71	1.58	1.22	0.71	1.22	1.22	1.22	0.71	8.59
H	0.71	0.71	0.71	1.22	0.71	1.22	1.22	0.71	7.21
I	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	5.68
J	0.71	0.71	0.71	1.22	0.71	0.71	0.71	0.71	6.19
								Total	69.46
								Mean	0.868