The feeding ecology of certain larvae in the genus tipula (Tipulidae, Diptera), with special reference to their utilisation of Bryophytes

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THE FEEDING ECOLOGY OF CERTAIN LARVAE IN THE GENUS *TIPULA* (TIPULIDAE, DIPTERA), WITH SPECIAL REFERENCE TO THEIR UTILISATION OF BRYOPHYTES

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Abstract

Bryophytes are rarely used as a food source by any animal species, but the genus *Tipula* (Diptera, Tipulidae) contains some of the few insect species able to feed, and complete their life-cycle, on bryophytes.

Vegetation particle volumes ingested by larvae of eleven *Tipula* species increased only marginally between instars and not to the extent expected from the rate of growth of body mass. Early and late instars within a species frequently ingested similar sized particles.

The overall efficiency of digestion of vegetation particles was low and similar between the four instars of each of the eleven species. Generally, the only method by which later larval instars can obtain a higher proportion of nutrients is by feeding on a larger number of smaller vegetation particles and not by ingesting large particles.

In feeding choice experiments, *Tipula confusa* preferred moss species from woodland habitats, whereas *Tipula subnodicornis* did not show an overall preference for either woodland or moorland moss species. *Tipula subnodicornis* also showed a less extensive hierachical preference/avoidance than *Tipula confusa* for the ten moss species investigated.

The moss species *Campylopus paradoxus* and *Sphagnum papillosum* accumulated Pb^{2+} ions and Zn^{2+} ions to high concentrations. There was some evidence that *Tipula subnodicornis* larvae were deterred from feeding on these mosses with high levels of introduced heavy metal ions.

Tipula montana was able to thrive and complete its life-cycle in Britain at lower altitudes than had been previously thought. Individuals of this species show a combination of one-year and two-year life-cycles at Waskerley Common.

The feeding methods employed by *Tipula* species can explain why some of them have remained as consumers of bryophytes.

I certify that all material in this thesis which is not my own work has been identified and that no material is included for which a degree has previously been conferred on me

For my mother and father

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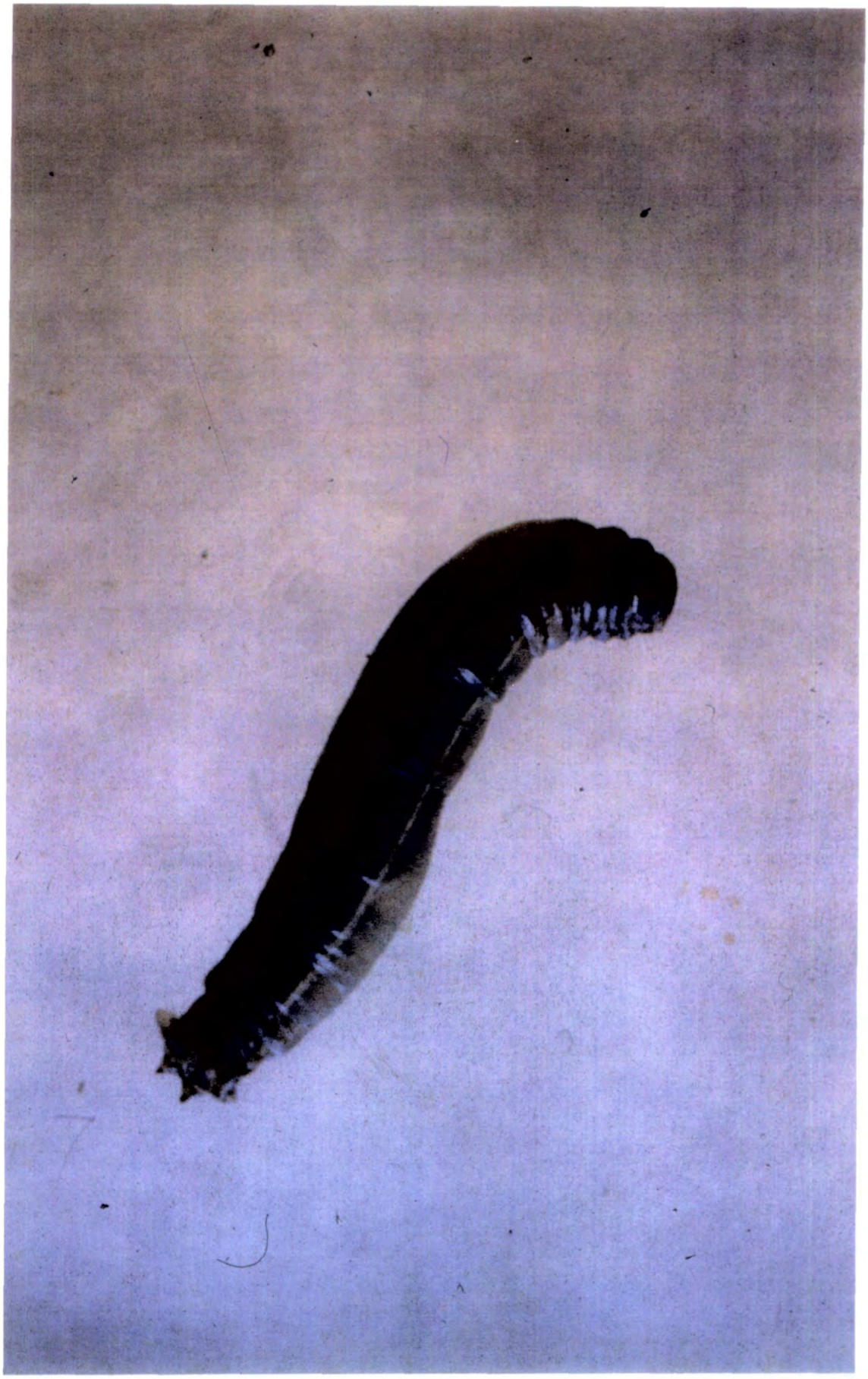


A fourth instar larva of *Tipula montana*.

Contents

Chapter 1. General Introduction

Bryophytes are not used to any great extent by insect herbivores, either as a habitat in which to live or as a food source. Examples from the literature of insects utilising and feeding on mosses are not extensive and include: Coleoptera, many of which are associated with mosses and, although most use them as refugia rather than as a food source, some staphylinids are known to be moss feeders (Mani 1962); the ant species *Myrmica ruginosis* and *Formica picea* which form nests in *Sphagnum* spp. (Matthey 1971) and there are observations of these insects feeding on mosses (Plitt 1907); crickets and grasshoppers (Vickery 1969); moth caterpillars (Chapman 1894); mayflies nymphs (Richardson 1981); aphids from the genera *Myzodium* and *Muscaphis* (Gerson 1969); biting midges from the genus *Forcipomyia* (Oldroyd 1964); the tipulid *Phalacrocera replicata*, which feeds on *Sphagnum* spp. (Clymo and Hayward 1982), and other tipulid species have been observed burrowing into *Sphagnum* spp.

Despite the ancient origin of insects, larvae of species in the genus *Tipula* are some of the few insects to feed on, and complete their life cycle on, mosses. Feeding only occurs in the larval stages of these species, the adults only taking in liquid.

The present study arose initially from two observations: firstly that the *Bryophyta* are infrequently utilised as a food source by vertebrate and invertebrate animals, and secondly species in the genus *Tipula* have been observed feeding on mosses (Coulson 1962, Freeman 1968), as well as members of other genera, including *Dolichopeza* (Byers 1961)

The family Tipulidae, the largest family in the order Diptera, comprises the sub-families Tipulinae, Limoniinae and Cylindrotominae, (Alexander and Byers 1981) and consists of 14 000 described species. Mosses may even have played a part

in the evolution of these flies. Considered by Oldroyd (1964) to be one of the most ancestral of the fly families, the Tipulidae have members living and feeding on mosses, and some assume the form and colour of their habitat, *e.g. Liogma trisulcata* and *Triogma* sp. and he suggests that the origin of these flies was from an ancestor whose larvae lived in wet mosses. The larvae of the three sub-families in general feed on mosses and higher plants (Cylindrotominae and Limoniinae) although some limoniids are predatory, and algae, higher plants, mosses and liverworts (Tipulinae).

Based on Brindle (1960), seven main larval habitat types can be recognised for the Tipulinae in Britain. These are: wood/detritus; damp soils; stream and river edges; marshy soils; pasture soils; terrestrial mosses and semi-aquatic or aquatic mosses. Coulson (1959) states that tipulids generally are more abundant in wet areas, with very few species on better drained soils. The sub-family Tipulinae contains the genus *Tipula*, and species in this genus are used in this investigation. There are over 2000 species in the genus *Tipula* world-wide, 59 of which are represented in Britain (Freeman 1967), and these are dispersed through the seven habitats; approximately a quarter of these species are moss feeders. Moss feeders apparently always have four pairs of short anal papillae, at their posterior end, and never long papillae such as found in species from wetter environments, (Brindle 1960), and the vast majority of these moss feeders are from the same sub-genus, *Savtshenkia*.

Here eleven species of *Tipula* are studied. Eight of these species are moss-feeders, and seven of these, *Tipula rufina*, *T. confusa*, *T. pagana*, *T. staegeri*, *T. limbata*, *T. alpium* and *T. subnodicornis*, are from the same subgenus *Savtshenkia*, and one, *T. montana*, is from the subgenus *Vestiplex*. Two of the species studies are grass-feeders, *T. paludosa* and *T. oleracea*, and these are both from the subgenus *Tipula* and one is an algal-feeder, *T. lateralis*, form the subgenus *Yamatotipula*. The authorities and subgenera are taken from Stubbs (1992).

The larvae of *Tipula* are commonly known as leatherjackets and some larvae of the terrestrial species live in tunnels which open on the soil surface (Milne *et al.* 1958). Many are herbivorous, feeding on vegetation just above or below the soil

surface. *Tipula paludosa* is such a species, well known as a pest of various crop species in Britain and Northern Europe and *Tipula simplex*, which feeds on the roots of its food plants, can denude hills of grass and forage in California (Hartman and Hynes 1977).

Species in the genus *Tipula* feed on a range of food items: the aquatic cranefly *Tipula sacra* feeds on diatoms, filamentous algae, mineral particles and detritus from the sediments, (Hall and Pritchard 1975); *Tipula signata* is found in fast running streams (Hemmingsen 1965) and the larvae feed on aquatic mosses, and larval stages of the aquatic cranefly *Tipula abdominalis* feed on partially decomposed particulate detritus (Cummins 1973). *Tipula cheethami* is another aquatic cranefly, (Brindle 1960), whose larvae feed on aquatic mosses.

Tipulid larvae can be important in the decomposition of leaf litter in many soils, *e.g. Tipula peliostigma*; and when it occurs in high numbers in forest-steppe zones, it can also increase the movement of organic material through the litter layer (Perel *et al.* 1971). Tipulids, as well as diplopods, are the main decomposers, and determine the turnover rate, of pistachio leaf litter in Tadshikistan, (Strigarova and Valiachmedov 1975). They can also increase the microbial activity of soils by the action of breaking down the leaf litter (Standen 1978) and insect herbivores, including tipulids, accelerate the release of nutrients from plants to the saprovore food chain by disrupting soil material and also by excreting them in the faeces, (Hodkinson and Hughes 1982).

Musci (mosses) and Hepaticae (liverworts) both make up the major group Bryophyta, but here the term bryophyte has been used to refer only to mosses. The biomass of mosses in many vegetational zones of the world is considerable, being most noticeable in tundra areas, *e.g.* in the Russian tundra over 50% of the ground cover is made up of mosses. In Britain, there are approximately 600 moss species, compared to over 1500 flowering plant species.

The rates of decomposition of mosses are low, and they generally accumulate as humus or peat (Clymo and Hayward 1982). The energy content tends to slightly

lower than that of associated flowering plants from the same area, (Wielgolaski and Kjelvik 1975, Gorham and Sanger 1967). This arises from lower concentrations of carbohydrates and proteins (Skre *et al.* 1975) and possibly lipids.

Mosses are one of the first colonisers of bare areas in many habitats and so normally constitute one of the initial stage in plant succession. Therefore invertebrates associated with these mosses, feeding on them and living in them, possibly constitute a similar stage in the faunal succession, (Gerson 1969).

Mosses may modify soil conditions and enable certain arthropods to improve survival under extreme environmental conditions, (Gerson 1969). They also have attributes which can make them acceptable as habitats and food sources for invertebrate animals (Gerson 1987). They are capable of holding large amounts of water, but can also withstand desiccation and can provide insulation for animals within them, particularly relevant in polar environments. Mosses usually have unprotected leaves, only one cell thick, apart from the midrib, which facilitates consumption of them. However, relatively few moss-animal associations have been described (Richardson 1981), and Chapter 2, Section 2.1. describes some of these. Mosses comprise 32-39% of the rumen content of Svalbard reindeer in winter, and Lemmings feed on *Polytrichum* sp. in the Canadian Arctic (Longton 1980, Pakarinen and Vitt 1974). However, most evidence for the consumption of bryophytes outside polar regions *i.e.* temperate and tropical regions is from invertebrate, and not vertebrate, herbivores.

There is a widely held view that mosses are under used as a food source, and whilst this may hold true, the present study sets out to determine, i) how they are consumed, ii) the method by which this is achieved, and iii) how efficiently the essential nutrients for growth and development are obtained when mosses are used as a food source in one insect group: members of the genus *Tipula*. The results are compared with the results of similar investigations carried out on different members of the same genus feeding on grasses and on algae.

The herbivorous tipulids are unable to digest all of the plant material, *e.g.*

Martin *et al.* (1980) showed that *Tipula abdominalis*, a shredder of leaf litter, is unable to digest the major cell wall polysaccharides, cellulose, hemicellulose and pectins, and similar difficulties would probably be encountered feeding on mosses, grasses and algae. So far few studies have been carried out on the suitability of moss as a food source in invertebrates. Also, few laboratory studies have been carried out on insects feeding on mosses generally, and little is known about the preferences for moss species in any herbivorous animal and this investigation attempts to go some way to determine the preferences in these insect species for different moss species.

Mosses are known to easily accumulate heavy metals ions to high concentrations in their tissues, (Tyler 1990). Therefore a further aspect of this study is to determine if these insects feeding on mosses with artificially high concentrations of heavy metals were adversely affected, as shown by selective avoidance of these mosses.

Tipula montana is a species normally associated with mountain plateaux, feeding on higher plant debris and dead plant material, at altitudes of 700m a.s.l. and above . In this study this species was found in a upland moorland habitat, at altitudes between 350m and 550m a.s.l., feeding exclusively on mosses, and so it was of interest to determine if the life cycle of this species from a 'new' lower altitude habitat which utilised a different food source had a similar life cycle to the montane individuals.

Lawrey (1987) states the reasons for the low numbers of moss herbivores have as yet not been adequately investigated and that more intensive laboratory studies on their feeding specificity needs to be carried out, which this study aims to do.
The study was carried out between June 1988 and May 1991, and the field work carried out in the Northern Pennines.

Chapter 2: An investigation into the size of vegetation particles ingested in larvae of the genus *Tipula*

2.1. Introduction

Bryophytes are an unusual source of food for any animal and very few animals (invertebrate or vertebrate) complete their life-cycle by feeding solely on them. Frankland (1974), Davis (1981) and Longton (1988) hold the view that consumption of bryophytes by animals is negligible. One possible reason for this is the high concentration of lignin-like polyphenolic compounds present in bryophytes, and these can have an antibiotic action (*e.g.* McCleary *et al.* 1960, McCleary and Wallington 1966).

There are a number of invertebrate animals which live and oviposite on mosses (Gerson 1982): for example Collembola and Protura are frequently associated with bryophytes: mites in the genus *Ledermuelleria* feed exclusively on mosses (Gerson 1972) and larvae of one family of the Mecoptera (Insecta; scorpion flies), the Boridae, occur frequently in and possibly feed on mosses, (Gerson 1982), as does an Australian mecopteran of the genus *Choristes* (Tillyard 1926), although most other Mecoptera are predatory. Caterpillars of a few species in the primitive lepidopteran suborder Zeugloptera have also been found to feed on mosses (Chapman 1894, Tillyard 1923).

However the Diptera are the group most strongly associated with bryophytes. These associations include the Psychodidae (sandflies), Culicidae (mosquitoes) and Tipulidae (craneflies), (Quate 1955; Fantham and Porter 1945). Byers (1961) states that bryophytes are the larval habitats of at least some larvae of certain species of craneflies from the genera *Tipula, Dolichopeza, Liogma, Limonia, Erioptera* and

Gonomyia and that at least some do feed on the moss.

Tipula larvae are unable to deal effectively with polysaccharides (*e.g.* cellulose) and this is demonstrated by the fact that very little reduction in the size of leaf particle or cell structure occurs as it passes through the gut (Caspers 1980, Priesner 1961), although carbohydrate degrading bacteria may occur in the hindgut (Klug and Kotarski, 1980). Mosses generally contain low concentrations of the more easily digestible soluble carbohydrates and also hemicelluloses (polysaccharides forming the matrix of the cell wall in which the cellulose is embedded) and higher concentrations of the less easily digestible cellulose and lignin-like compounds (Lawrey 1987). Mosses do not posses true lignin (Erikson & Miksche 1974, Miksche & Yasuda 1978). However, a lignin-like compound does occur which protects cellulose, hemicellulose and other polysaccharides in the cell wall against hydrolytic attack (Harkin 1973, Schreier 1973). This, in turn, makes the cellulose contents less accessible to digestive enzymes. As a result, the food particles present in the faeces of the larvae are virtually intact - the physical structure of the particles remaining almost identical to the physical structure of the particles after ingestion. Most of the damage to the plant material is caused by the action of the mandibles cutting the particle from the food plant.

Accordingly, it might be expected more damage, such as the loss of chloroplasts, would occur in the edge cells (those cells in which the perimeter makes up part of the margin of the vegetation particle) of the ingested particle, rather than in the more central (mid) cells (*i.e.* all other cells of the particle). This is investigated in Chapter 2.

The nutrients, water, mineral salts, carbohydrates and proteins required by the animal, are therefore removed from within the damaged cells. As a result of the low digestive activity, the size of food particles present in the faeces of larvae gives a good indication of their size when ingested by the larva and this has been confirmed by examining particle size in the fore gut with those in the faeces.

In a comparison between grasses and herbaceous plants, and mosses:

Wielgolaski *et al.* (1975) found that the mineral concentrations were similar, except that mosses were significantly lower in K^+ and Mn^{2+} ions; Margaris and Kalaizakis (1974) found that the same sugars were present and Rastofer (1976) found that the caloric value, at 3800-4500 cal g^{-1}, was similar to that of herbs and grasses. With protein levels, however, Skre *et al.* (1975) found that the levels in mosses were a constant 10% of the dry weight throughout the year, whereas the levels in herbaceous plants and grasses, fluctuated from over 20% in the summer months to 10% in autumn.

In this chapter the sizes of vegetation particles ingested by eight species of *Tipula* which are primarily moss-feeders have been investigated and are compared with three other *Tipula* species which feed on other food plants and two other insect herbivores. It is also concerned with a comparison of the sizes of the vegetation particles which are ingested by each larval instar of a series of species of *Tipula*, allowing comparisons to be made:

i) between instars within each species,

ii) between species which primarily feed on bryophytes,

iii) between the bryophyte feeding species and those species which primarily feed on other food plants, such as grasses and filamentous algae.

In addition, the extent of damage to vegetation particles of higher plants by the mandibles in the locust, *Locusta migratoria*, a species of Lepidoptera, *Hydriomena furcata* and in several *Tipula* species is compared.

2.2. Methods

2.2.1. Larvae in the genus *Tipula*

The methods used here are original. The species in the genus *Tipula* used in the investigation were the moss feeders *Tipula rufina*, *T. confusa*, *T. pagana*, *T. staegeri*, *T.limbata*, *T. alpium*, *T. subnodicornis* and *T. montana*; the grass-feeders *T. paludosa* and *T. oleracea* and the algae-feeder *T. lateralis*. Vegetation particles, in the faeces of certain species of *Tipula* have been found to have the same structure as when ingested (Pritchard 1983), and confirmed in this study. Therefore to determine the sizes of vegetation particles ingested the vegetation particles present in the faeces were sampled and examined.

2.2.1.1. Methods by which vegetation particles were obtained

The larvae under investigation were allowed to feed on their food plant, mosses (*e.g. Brachythecium rutabulum*), grasses (*e.g. Deschampsia flexuosa*) or filamentous algae, for two days (first instar larvae), and for a maximum of seven days for later instars. The larvae were removed from their food plant, the spiracular disc diameter (to determine the larval stage) and mandible length (so a comparison could be made between the length of mandible and vegetation particle volume) and the weight of each larva were determined. Each larva was then transferred to a separate pot containing damp tissue paper to prevent desiccation. They were kept at 10^{o}C. If no frass was produced in a pot after 12 hours for first instars and up to 24 hours for later instars, the larva was removed, returned to its food plant for at least two days and the procedure repeated.

The frass is produced in cylindrical masses containing numerous particles of vegetation. Once a larva had defaecated, the frass was removed, placed on a microscope slide, and the material was spread out so that the individual vegetation particles were distinct on the slide. The specimen was covered with a coverslip and

sealed using clear nail varnish to reduce evaporation, and examined under a microscope under suitable magnification.

2.2.1.2. Determination of vegetation particle volumes ingested

For all species and all instars, the area of the vegetation particles were measured with a graticule, under a 100x magnification. To obtain the area of the vegetation particles, the shape was approximated to a simple two dimensional shape: rectangle, triangle, oval or semicircle and two appropriate measurements were taken so that the surface area could be calculated. The particles were one cell thick in almost all cases, and with the measurement of the mean thickness of the particle, the volume of the particle could readily be determined. A sample of 25 vegetation particles per slide gave an acceptable estimate of the mean vegetation particle size.

2.2.2. Detailed study on *Tipula rufina*

A more detailed study was carried out on the particles in the frass of *Tipula rufina*. The vegetation particles in the frass collected from each larva were measured as described above and all four instars were investigated. In addition, all vegetation particles measured were divided into distinct size classes and, for each instar, the number of particles in each size class was determined.

2.2.3. Additional studies on *Hydriomena furcata* and *Locusta migratoria*

Samples of ingested food material were taken from immature stages of two other insect herbivores, the locust, *Locusta migratoria* and the caterpillars of the

moth, *Hydriomena furcata* (July High-Flyer), as a comparison with the tipulids.

The locusts used were obtained from the Durham University insectary. They were fed on grass for at least one week prior to examination. Much more damage and digestion of the grass particles occurred along the gut tract than in the case of tipulids, and as vegetation particles cut by the action of the mandibles only were required (as a comparison to the method used by *Tipula* species), food particles were taken from the extreme anterior end of the gut. Gut contents from five individuals were sampled in both the first and the fifth instars. The food particles so obtained were treated in the same way as described above for the *Tipula* species.

The vegetation particles present in the frass of the lepidopteran larvae were compared with those which are present in the frass of *Tipula* species. The data for this work were collected and measured by Karen Haysom at my suggestion, on caterpillars of *Hydriomena furcata*. Frass from instars II, III IV and V was collected and treated in the same way as for the *Tipula* species.

2.3. Results

2.3.1. Comparison of size of vegetation particles ingested through the instars in species of *Tipula*

Table 2.1 presents the mean vegetation particle volumes in μm^3 for each instar in the species studied. F-values, from analysis of variance to test for differences in volume of vegetation particle ingested, are given in the footnote of the Table for each of the instars. In the case of a significant difference, the least significant difference multiple comparison test has been used to determine which species made most contribution to the significant difference (at $p<0.05$ level) in each group tested, the results of which are discussed in the text.

Table 2.1 Mean vegetation particle volume ingested by larvae of *Tipula* species in μm^3 x100 (±S.E.), n being the number of larvae investigated.

	Species	Instar I	II	III	IV
1	*T. rufina*	307 (±47)	357 (±42)	496 (±64)	1215 (±76)
	n	7	11	9	29
2	*T. confusa*	792 (±202)	968 (±124)	1228 (±110)	1586 (±136)
	n	4	9	23	7
3	*T. pagana*	677 (±45)	945 (±76)	1364 (±133)	2798 (±264)
	n	10	12	10	5
4	*T. staegeri*	782 (±84)	1129 (±32)	1381 (±20)	2711 (±125)
	n	5	10	9	9
5	*T. limbata*	737 (±44)	990 (±33)	1284 (±97)	1847 (±143)
	n	9	10	10	10
6	*T. alpium*	730 (±61)	1039 (±60)	1270 (±120)	1782 (±112)
	n	10	10	10	11
7	*T. subnodicornis*	383 (±54)	734 (±82)	1162 (±137)	2692 (±233)
	n	11	10	10	10
8	*T. montana*	654 (±85)	1036 (±272)	1520 (±126)	4719 (±535)
	n	6	11	10	10

/cont'd

Table 2.1 (cont'd) Mean vegetation particle volume ingested by larvae of *Tipula* species in μm^3 x100 (±S.E.), n being the number of larvae investigated.

Species	Instar I	II	III	IV
9. *T. paludosa*	693 (±85)	1675 (±84)	2825 (±239)	3462 (±275)
n	11	11	9	28
10. *T. oleracea*	609 (±14)	1574 (±236)	2788 (±416)	6698 (±531)
n	8	9	11	9
11 *T. lateralis*	247 (±11)	768 (±54)	2054 (±151)	2515 (±185)
n	11	9	10	12

Notes:

Instar	Species	ANOVA		
1	1 to 11	$F_{(10,82)}$	= 9.86	$p<0.001$
2	1 to 11	$F_{(10,107)}$	= 8.94	$p<0.001$
3	1 to 11	$F_{(10,110)}$	= 14.22	$p<0.001$
4	1 to 11	$F_{(10,130)}$	= 30.81	$p<0.001$

Significant differences were found within both the moss-feeding and the non-moss feeding species, when these were tested separately, as well as between all the species. As there were no obvious or recurrent differences between the two feeding groups, the following is an overview of the differences found throughout all the species.

2.3.1.1. Instar I

The significant variability in the particle volumes ingested between species, see Table 2.1, was found to be mainly due to *Tipula rufina*, *T. lateralis* and *T. subnodicornis*, the vegetation particles they ingested being significantly smaller ($p<0.05$) than those ingested by the other species.

2.3.1.2. Instar II

In instar II, the significant difference between the species in the size of particle ingested was due to particle sizes taken in four species being significantly different from all, or some of, the other species at the $p<0.05$ level (Table 2.1). Firstly, *T. rufina* again took smaller particles than any other species. Secondly, *T. subnodicornis* ingested significantly smaller particles than did *T. staegeri*, *T. paludosa* and *T. oleracea*. Lastly, *T. paludosa* and *T. oleracea* both ingested significantly larger particles than all other species, but not from each other. This probably reflects the greater size of their larvae.

2.3.1.3. Instar III

In instar III, the significant variation in size of particle taken by the different species was again due to differences in several species, (Table 2.1). Firstly, *T. rufina* continued to ingest significantly smaller particles than all other species; secondly,

T. lateralis took significantly larger sized particles than all the moss-feeders, but significantly smaller sized particles than *T. paludosa* and *T. oleracea*. *T. paludosa* and *T. oleracea* took significantly larger sized particles than all other species.

2.3.1.4. Instar IV

In instar IV, there was considerable variation in the sizes of vegetation particles taken by the different species (Table 2.1). *T. oleracea* took larger particles than all other species. *T. montana* and *T. paludosa* took larger sized particles than all other species except *T. oleracea*. *T. rufina* continued to take significantly smaller vegetation particles than all species, except *T. confusa*, *T. limbata* and *T. alpium*. The last three species also ingested significantly smaller sized particles than *T. pagana*, *T. staegeri*, *T. subnodicornis* and *T. lateralis*.

2.3.2. Mandible lengths in *Tipula* species

2.3.2.1. Comparison of mandible length between the species

The mean mandible lengths are given in Table 2.2. Mandible length was used as this could be readily measured, whereas the cutting edge length of the mandible was difficult to measure due to its orientation. The cutting edge of the mandible increased directly with the mandible length. The F-value (from the analysis of variance to test for differences in mandible length) showed that there is considerable variation between all the species. The following is a general overview of the differences.

Overall *T. paludosa* had significantly larger mandibles than any other species in each instar and *T. rufina* had significantly smaller mandibles than all species in each instar, except instar I. In the moss feeders, *T. rufina* generally had the shortest mandibles in each instar, except in instar I where the size in *T. staegeri* and *T. limbata*

Table 2.2. Mean mandible length of larvae of *Tipula* species in m (±S.E.), n being the number of larvae investigated.

Species		Instar I	II	III	IV
1	*T. rufina*	0.058 (±0.004)	0.104 (±0.006)	0.166 (±0.003)	0.244 (±0.004)
	n	7	11	9	29
2	*T. confusa*	0.070 (±0.003)	0.109 (±0.003)	0.194 (±0.008)	0.356 (±0.025)
	n	4	9	23	7
3	*T. pagana*	0.062 (±0.001)	0.142 (±0.008)	0.198 (±0.011)	0.310 (±0.021)
	n	10	12	10	5
4	*T. staegeri*	0.052 (±0.004)	0.107 (±0.005)	0.198 (±0.008)	0.302 (±0.009)
	n	5	10	9	9
5	*T. limbata*	0.057 (±0.003)	0.122 (±0.004)	0.217 (±0.006)	0.318 (±0.009)
	n	9	10	10	10
6	*T. alpium*	0.080 (±0.004)	0.141 (±0.005)	0.208 (±0.010)	0.281 (±0.012)
	n	10	10	10	11
7	*T. subnodicornis*	0.096 (±0.004)	0.186 (±0.006)	0.210 (±0.009)	0.272 (±0.008)
	n	11	10	10	10
8	*T. montana*	0.090 (±0.001)	0.155 (±0.002)	0.256 (±0.005)	0.388 (±0.026)
	n	6	11	10	10

/cont'd

Table 2.2. (cont'd) Mean mandible length of larvae of *Tipula* species in mm (±S.E.), n being the number of larvae investigated.

	Species	Instar I	Instar II	Instar III	Instar IV
9	*T. paludosa*	0.112 (±0.004)	0.347 (±0.007)	0.384 (±0.025)	0.738 (±0.023)
	n	11	11	9	28
10	*T. oleracea*	0.084 (±0.003)	0.148 (±0.008)	0.276 (±0.016)	0.468 (±0.001)
	n	8	9	11	9
11	*T. lateralis*	0.081 (±0.003)	0.133 (±0.003)	0.222 (±0.004)	0.375 (±0.007)
	n	11	9	10	12

Notes:	Instar	Species	ANOVA	
	1	1 to 11	$F_{(10,82)} = 26.59$	$p<0.001$
	2	1 to 11	$F_{(10,107)} = 132.14$	$p<0.001$
	3	1 to 11	$F_{(10,110)} = 24.49$	$p<0.001$
	4	1 to 11	$F_{(10,132)} = 109.44$	$p<0.001$

were not significantly different. *Tipula montana* generally had the longest mandibles in each instar. In the non-moss feeders *T. paludosa* had the largest and *T. lateralis* the shortest in each instar.

2.3.2.2. Relationship between mandible length and the vegetation particle volume ingested

The mean vegetation particle volume in μm^3 (y) plotted against the mean mandible length in mm (x) per instar for each of the moss-feeding species, gives the regression equation:

$y = 76508\ (\pm 10044\ \text{S.E.})x - 834\ (\pm 2033\ \text{S.E.})$.

The same variables for the non-moss feeding *Tipula* species give the regression equation:

$y = 65455\ (\pm 19690\ \text{S.E.})x + 3219\ (\pm 6637\ \text{S.E.})$.

There is no significant difference between the slopes of these two lines: therefore the two plots were combined, and are presented on Fig. 2.1. The standard errors of the means have been omitted on the graph, for clarity of presentation: they are given in Tables 2.1 and 2.2 for vegetation particle volume and mandible length respectively. The regression equation for the combined plot is:

$y = 71640\ (\pm 8690\ \text{S.E.})x + 4224\ (\pm 2142\ \text{S.E.})$,

where 62% of the variation is explained by the regression equation. There is a strong positive correlation between mean vegetation particle size and mandible length.

2.3.2.3. Relationship between mandible length and the weight of the larvae

When the mean weight in mg (y) is plotted against the mean mandible length in mm (x) per instar for ten of the *Tipula* species (Fig. 2.2), the regression equation is:

$y = 231.4\ (\pm 1.8\ \text{S.E.})x - 15.3\ (\pm 0.4\ \text{S.E.})$,

where 92% of the variation is explained by the regression equation. The slope and the

Fig. 2.1

The relationship between the mean vegetation particle volume and mean mandible length in each instar of the eleven *Tipula* species studied. $y = 71640x + 422$, $r_{42} = 0.79$, $p<0.001$.

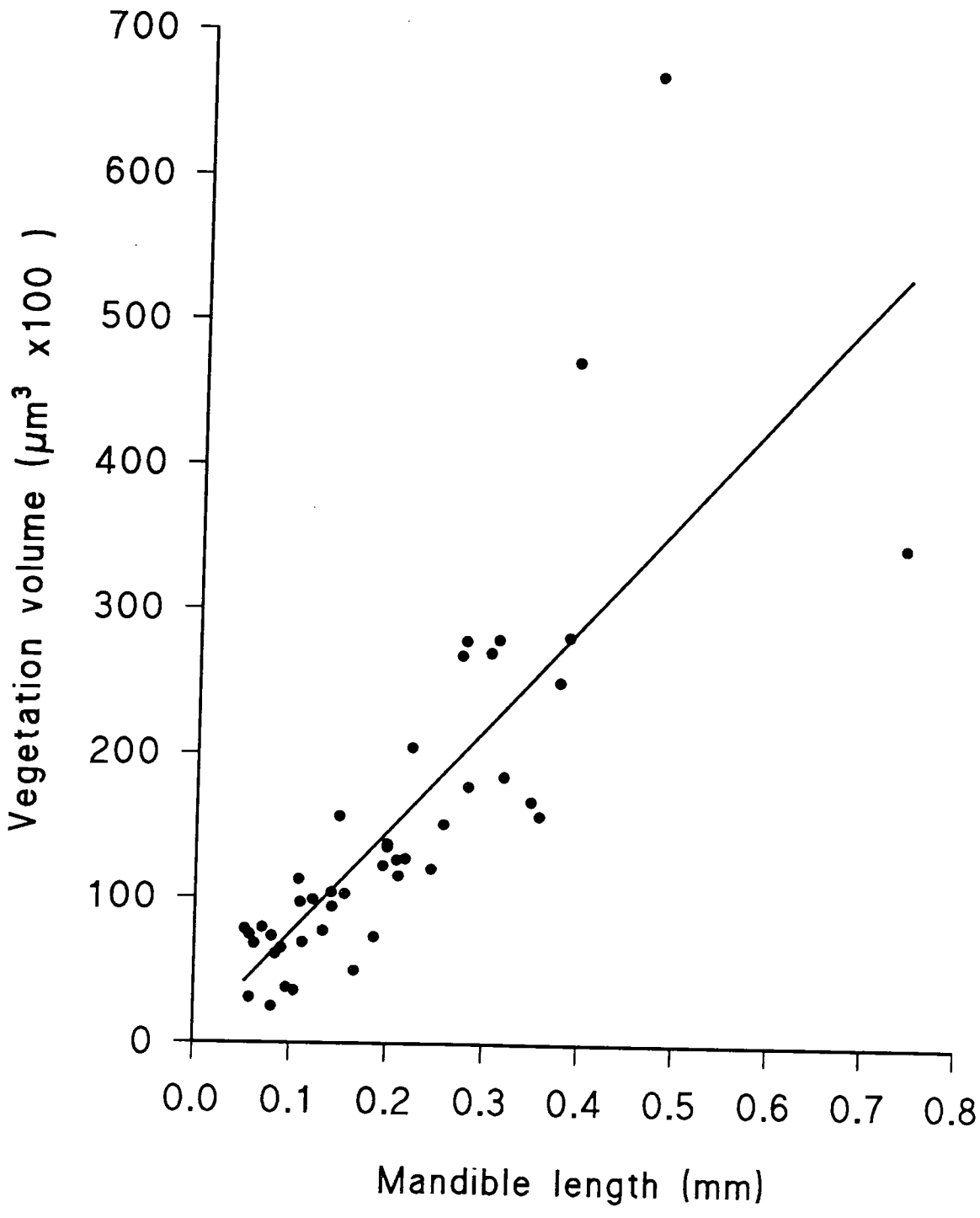
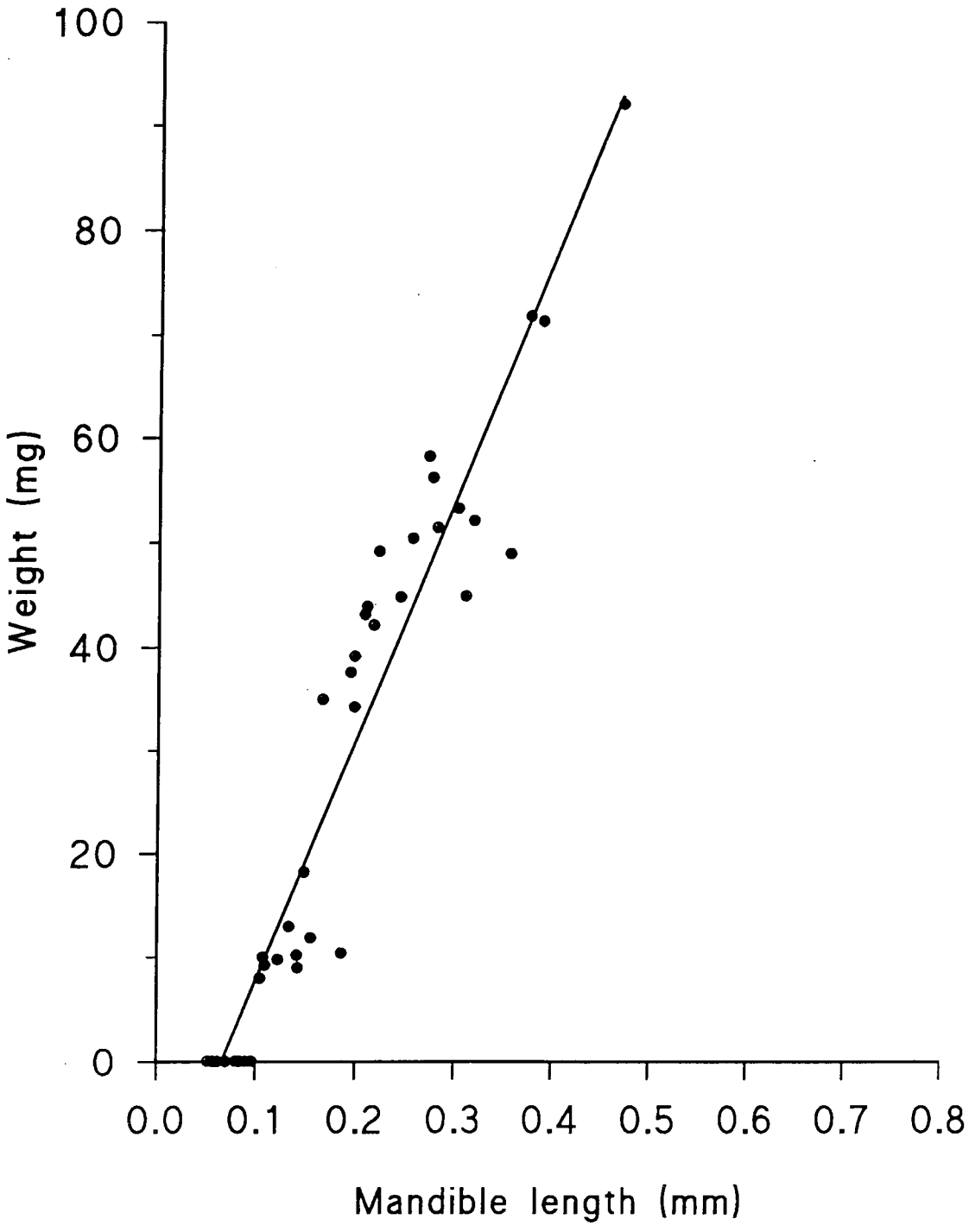


Fig. 2.2

The relationship between the mean weight and the mean mandible length in each instar of ten of the Tipula species studied. $y = 231.4x - 15.3$, $r_{38} = 0.96$, $p<0.001$.



intercept are presented with their standard errors. The grass feeder, *T. paludosa* is omitted from the plot and the regression equation, as this is the unusual species, having greater mandible lengths in each instar than any other species in the same weight range.

There is a significant positive correlation between the weight of a larva and its mandible length; smaller animals consistently have shorter mandibles.

2.3.3. Spiracular disc diameters in *Tipula* species

2.3.3.1. Comparison of spiracular disc diameter through the species

The mean spiracular disc diameters in each instar (Table 2.3) for all species, shows *T. rufina* and *T. subnodicornis* overall had the smallest spiracular disc diameters and *T. paludosa* and *T. montana* the largest. *T. rufina* and *T. subnodicornis* had the smallest spiracular disc diameters amongst the moss-feeders (apart from instar IV where *T. pagana* has the smallest), and *T. montana* had larger spiracular disc diameters than the other moss-feeding species in each instar. This may be explained by the fact that *T. montana* is from a different subgenus (*Vestiplex*) than all the other moss-feeders. In the non-moss feeding species *T. paludosa* had the largest spiracular disc diameter and *T. lateralis* the smallest in all instars.

2.3.3.2. Relationship between the vegetation particle volume ingested and spiracular disc diameter

Graphs of the mean vegetation particle volume ingested by the larvae plotted against the spiracular disc diameter in a sample of species are presented in Figs. 2.3-2.6, to determine if larger instars of a species ingested larger particles. Table 2.4 gives the regression equations and correlation coefficients for these and the other

Table 2.3 Mean spiracular disc diameter of larvae of *Tipula* species in m (±S.E.), n being the number of larvae investigated.

	Species	Instar I	II	III	IV
1	*T. rufina*	0.020 (±0.000)	0.076 (±0.005)	0.170 (±0.008)	0.337 (±0.004)
	n	7	11	9	29
2	*T. confusa*	0.033 (±0.003)	0.098 (±0.005)	0.196 (±0.005)	0.313 (±0.013)
	n	4	9	23	7
3	*T. pagana*	0.022 (±0.001)	0.115 (±0.008)	0.216 (±0.004)	0.284 (±0.017)
	n	10	12	10	5
4	*T. staegeri*	0.032 (±0.004)	0.107 (±0.008)	0.242 (±0.010)	0.409 (±0.013)
	n	5	10	9	9
5	*T. limbata*	0.041 (±0.004)	0.107 (±0.005)	0.225 (±0.010)	0.331 (±0.009)
	n	9	10	10	10
6	*T. alpium*	0.029 (±0.004)	0.103 (±0.004)	0.191 (±0.005)	0.288 (±0.006)
	n	10	10	10	11
7	*T. subnodicornis*	0.022 (±0.002)	0.082 (±0.005)	0.164 (±0.003)	0.297 (±0.007)
	n	11	10	10	10
8	*T. montana*	0.048 (±0.002)	0.129 (±0.003)	0.264 (±0.010)	0.520 (±0.019)
	n	6	11	10	10

/cont'd

Table 2.3 (cont'd) Mean spiracular disc diameter of larvae of *Tipula* species in mm (±S.E.), n being the number of larvae investigated.

	Species	Instar I	Instar II	Instar III	Instar IV
9	*T. paludosa*	0.040 (±0.004)	0.136 (±0.002)	0.334 (±0.003)	0.636 (±0.016)
	n	11	11	9	28
10	*T. oleracea*	0.035 (±0.005)	0.117 (±0.003)	0.275 (±0.005)	0.576 (±0.006)
	n	8	9	11	9
11	*T. lateralis*	0.023 (±0.001)	0.113 (±0.004)	0.226 (±0.007)	0.408 (±0.017)
	n	11	9	10	12

Notes:

Instar	Species	ANOVA
1	1 to 11	$F_{(10,82)} = 8.70$ $p<0.001$
2	1 to 11	$F_{(10,107)} = 12.73$ $p<0.001$
3	1 to 11	$F_{(10,110)} = 48.52$ $p<0.001$
4	1 to 11	$F_{(10,132)} = 140.92$ $p<0.001$

Fig. 2.3

The relationship between the mean vegetation particle volume and mean spiracular disc diameter in individual larvae of *Tipula rufina*. $y = 31503x + 12590$, $r_{54} = 0.80$, $p<0.001$.

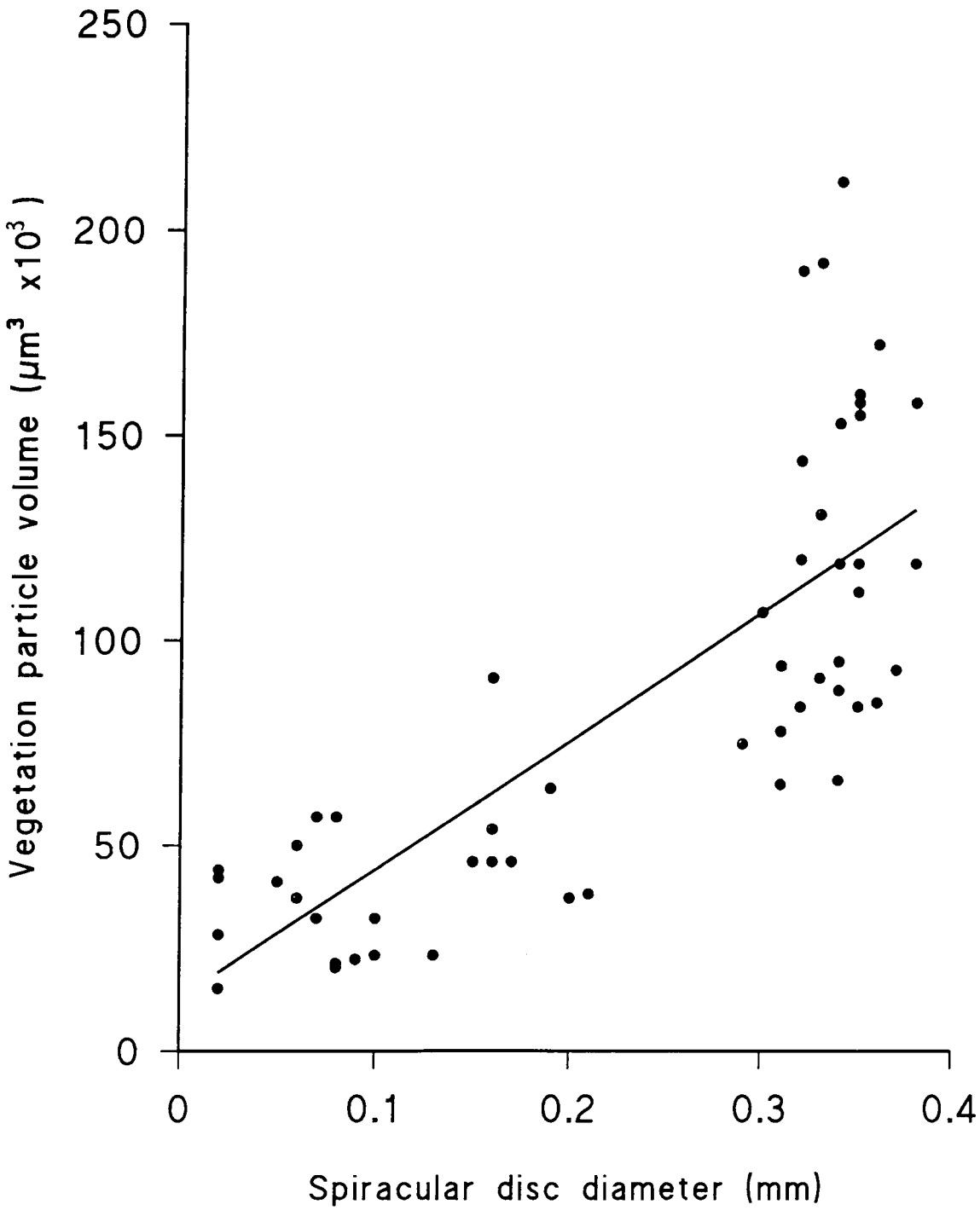


Fig. 2.4

The relationship between the mean vegetation particle volume and mean spiracular disc diameter in individual larvae of *Tipula staegeri*. $y = 48663x + 52930$, $r_{31} = 0.92$, $p<0.001$.

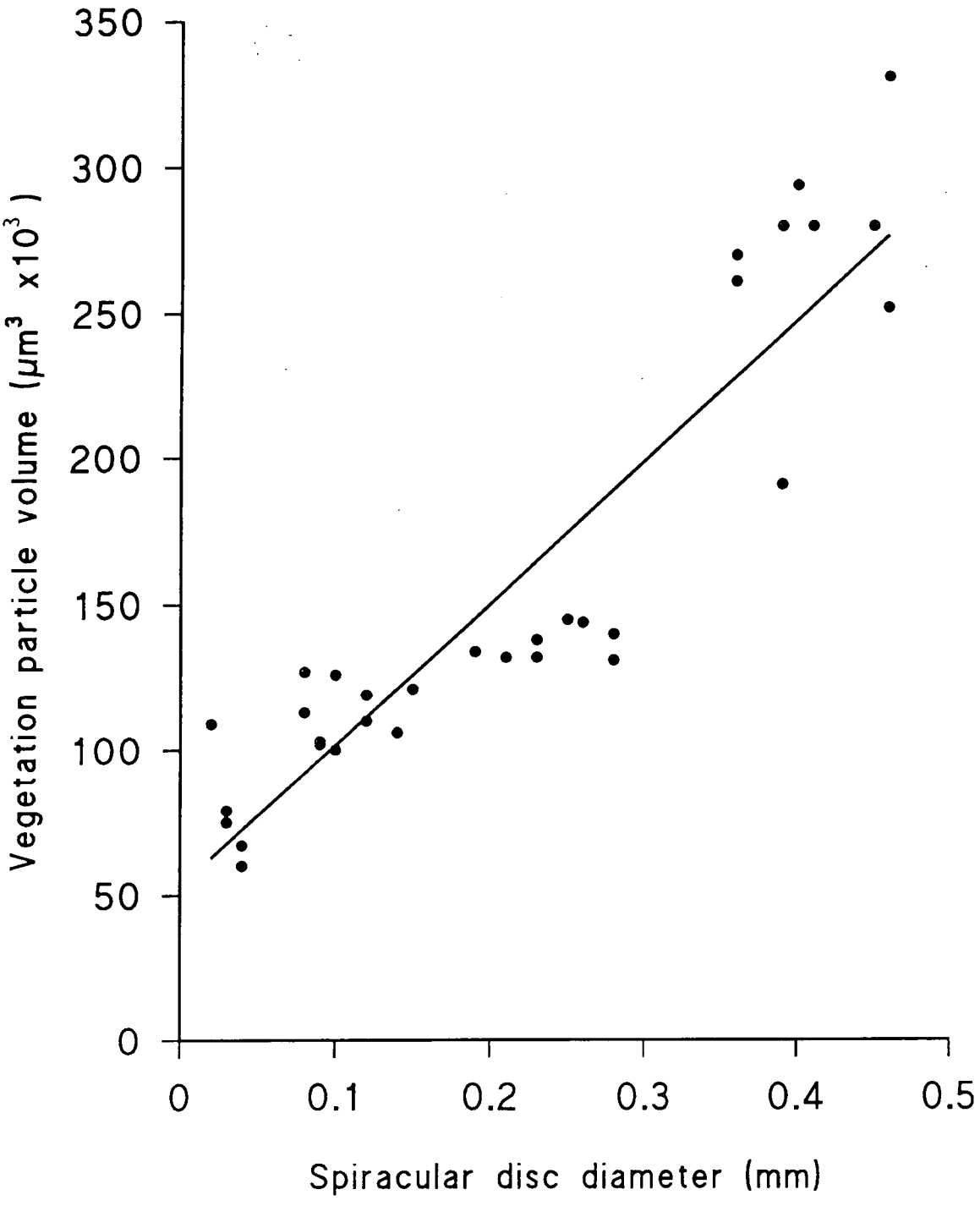


Fig. 2.5

The relationship between the mean vegetation particle volume and mean spiracular disc diameter in individual larvae of *Tipula montana*. $y = 91595x - 26440$ $r_{35} = 0.86$, $p<0.001$.

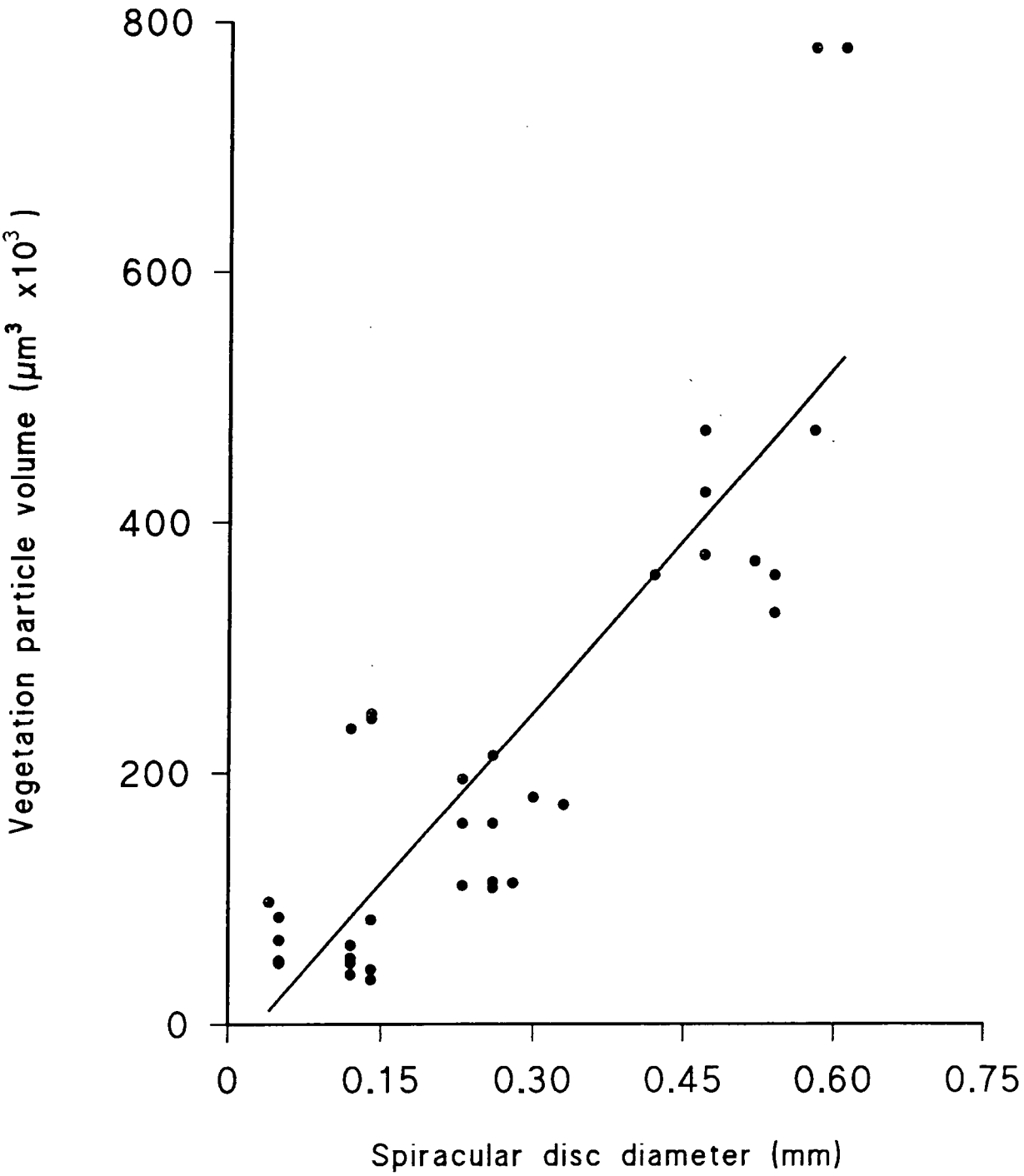
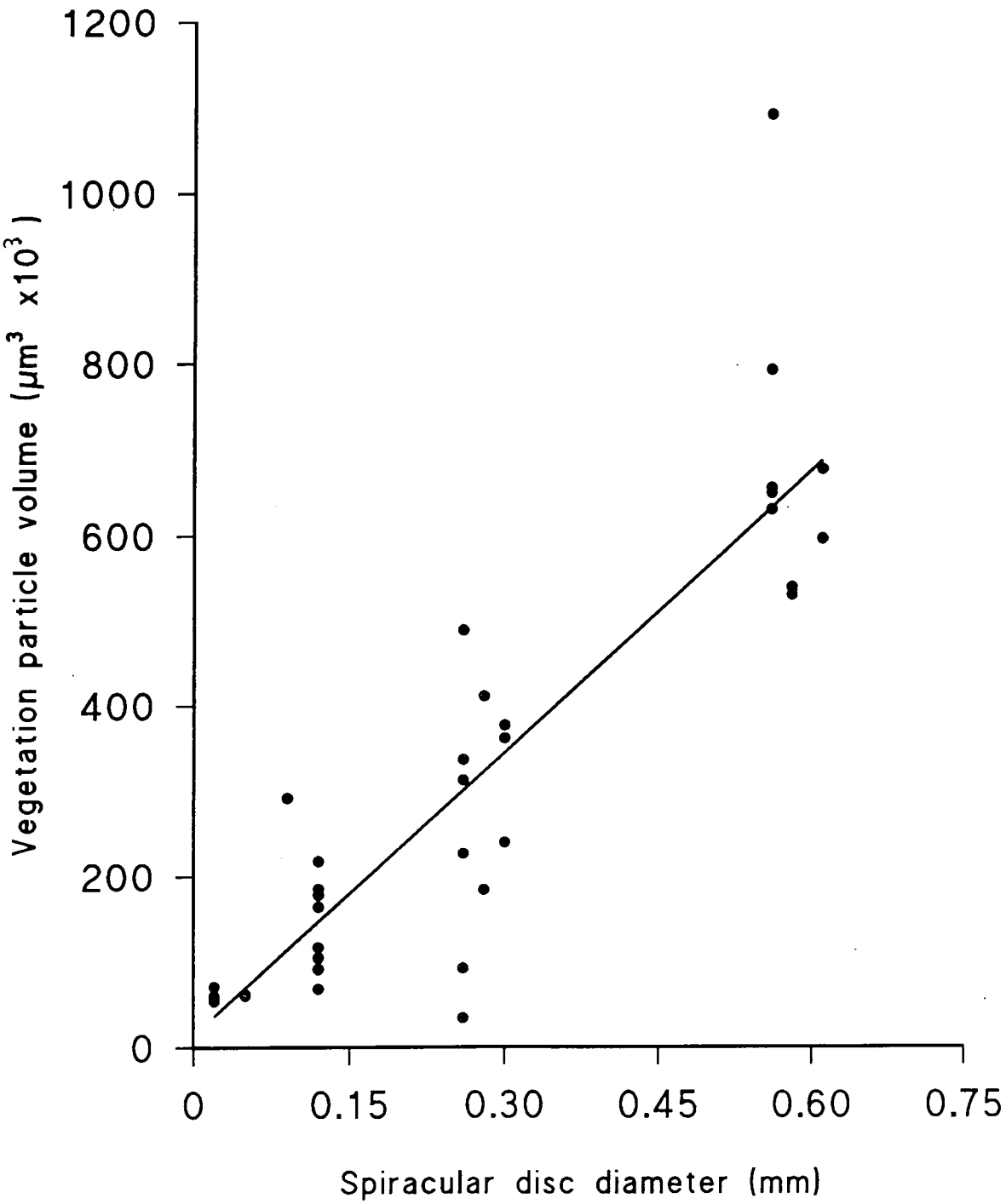


Fig. 2.6

The relationship between the mean vegetation particle volume and mean spiracular disc diameter in individual larvae of *Tipula oleracea*. $y = 110360x + 13810$, $r_{35} = 0.89$, $p<0.001$.



Tipula species studied. For each species, there is a significant positive correlation between the mean vegetation particle size per individual and the spiracular disc diameter. The correlation is weakest in *T. confusa* (r=0.46) and strongest in *T. staegeri* (r=0.92). Considering the slope of the regression line; if the spiracular disc diameter doubles, the vegetation particle volume ingested also approximately doubles, ranging from 1.6 times in *T. paludosa* through to 2.1 fold in *T. montana*.

2.3.4. Determination of the variability of vegetation particles ingested

For the vegetation particle sizes the coefficients of variation (CV) have been calculated, using the equation:

$$CV = \frac{S.D.}{x} x100$$

where S.D. is the standard deviation of the sample and x is the sample mean. The CV's are presented in Table 2.5. The correction suggested by Haldane (1955) and Sokal and Rohlf (1981) has not been used as Sokal and Braumann (1980) state it only makes appreciable differences for sample sizes less than 5. The coefficient of variation is easily interpreted and is widely used as a measure of variability, in relation to the mean, *i.e.* to show the variation in proportion to the size of the mean (McArdle *et al.* 1990). Both the moss feeders and the non-moss feeders appear to have a similar range of coefficients of variations for the mean vegetation particle size in the instars of each species. This has also been found by Bernays and Janzen (1988) for saturniid caterpillars feeding on leaves of herbaceous species, in contrast to the range of coefficients of variation found by the same authors for caterpillars of the Sphingidae.

When the standard deviation of the mean vegetation particle volume (y) is

Table 2.4. Regression equations and correlation coefficients (r) for *Tipula* species for plots of vegetation particle volume (y) in μm^3 against spiracular disc diameter (x) in mm.

Species	n	Regression equation	r	p
Tipula rufina	56	y = 51303x + 1259	0.80	0.001
Tipula confusa	43	y = 28270x + 6839	0.46	0.05
Tipula pagana	37	y = 62986x + 3689	0.79	0.001
Tipula staegeri	33	y = 48706x + 5329	0.92	0.001
Tipula limbata	39	y = 36458x + 5724	0.83	0.001
Tipula alpium	41	y = 38814x + 6134	0.80	0.001
Tipula subnodicornis	41	y = 81898x + 764	0.87	0.001
Tipula montana	37	y = 91631x - 2648	0.86	0.001
Tipula paludosa	59	y = 41336x + 9280	0.70	0.001
Tipula oleracea	37	y =110360x + 1392	0.89	0.001
Tipula lateralis	42	y = 56964x + 2907	0.85	0.001

Table 2.5. Coefficient of variation (%) of mean vegetation particles ingested by larvae of *Tipula* species.

Species	Instar I	II	III	IV
T. rufina	40	39	39	34
T. confusa	51	36	44	23
T. pagana	21	29	28	21
T. staegeri	24	9	4	14
T. limbata	18	11	24	24
T. alpium	27	18	30	21
T. subnodicornis	44	45	39	27
T. montana	32	87	29	40
T. paludosa	40	17	25	40
T. oleracea	7	45	50	25
T. lateralis	16	21	23	25

plotted against the mean vegetation particle volume (x) for the moss feeding species of *Tipula* the regression equation is:

$y = 0.266\ (\pm 0.039)x + 2825\ (\pm 6221)$.

The slope and intercept are presented with their standard errors.

For the same plot for non-moss feeders the regression equation is:

$y = 0.274\ (\pm 0.049)x + 5512\ (\pm 13348)$.

The slopes of these two lines were not significantly different: therefore they were combined and the resultant plot is presented in Fig. 2.7. The regression equation of the combined plot is:

$y = 0.276\ (\pm 0.026)x + 3796\ (\pm 505)$,

where 74% of the variation was explained by the regression equation.

The coefficient of variation (CV) along the regression line was constant at 29%. Therefore for both groups of animals 29% is the relative standard deviation of the vegetation particle volumes ingested by *Tipula* larvae.

2.3.5. Comparison of the increase in the biometric measurements and vegetation particle volumes ingested in *Tipula* species

2.3.5.1. In moss-feeders.

In the majority of moss-feeders (the exceptions being *T. subnodicornis* and *T. montana*) the increase in mandible length from instar I to instar IV, is greater than the corresponding change in vegetation particle volume in the food (Table 2.6); *e.g.* if mandible length increased by five fold (as for instar I to IV in *T. pagana*) the vegetation particle volume increased only by four fold. In all cases the increases in both vegetation particle volume and mandible length were of the same order of magnitude. However, in the functional increase for spiracular disc, *i.e.* the spiracular disc area - as area determines the increase in respiration rate by the larvae - the increases were more than ten times greater than that of the vegetation particle

Fig. 2.7

The relationship between the standard deviation of the mean vegetation particle volume and the mean vegetation particle volume ingested in each instar of the eleven species of *Tipula* studied. $y = 0.276x + 3796$, $r_{42} = 0.85$, $p<0.001$.

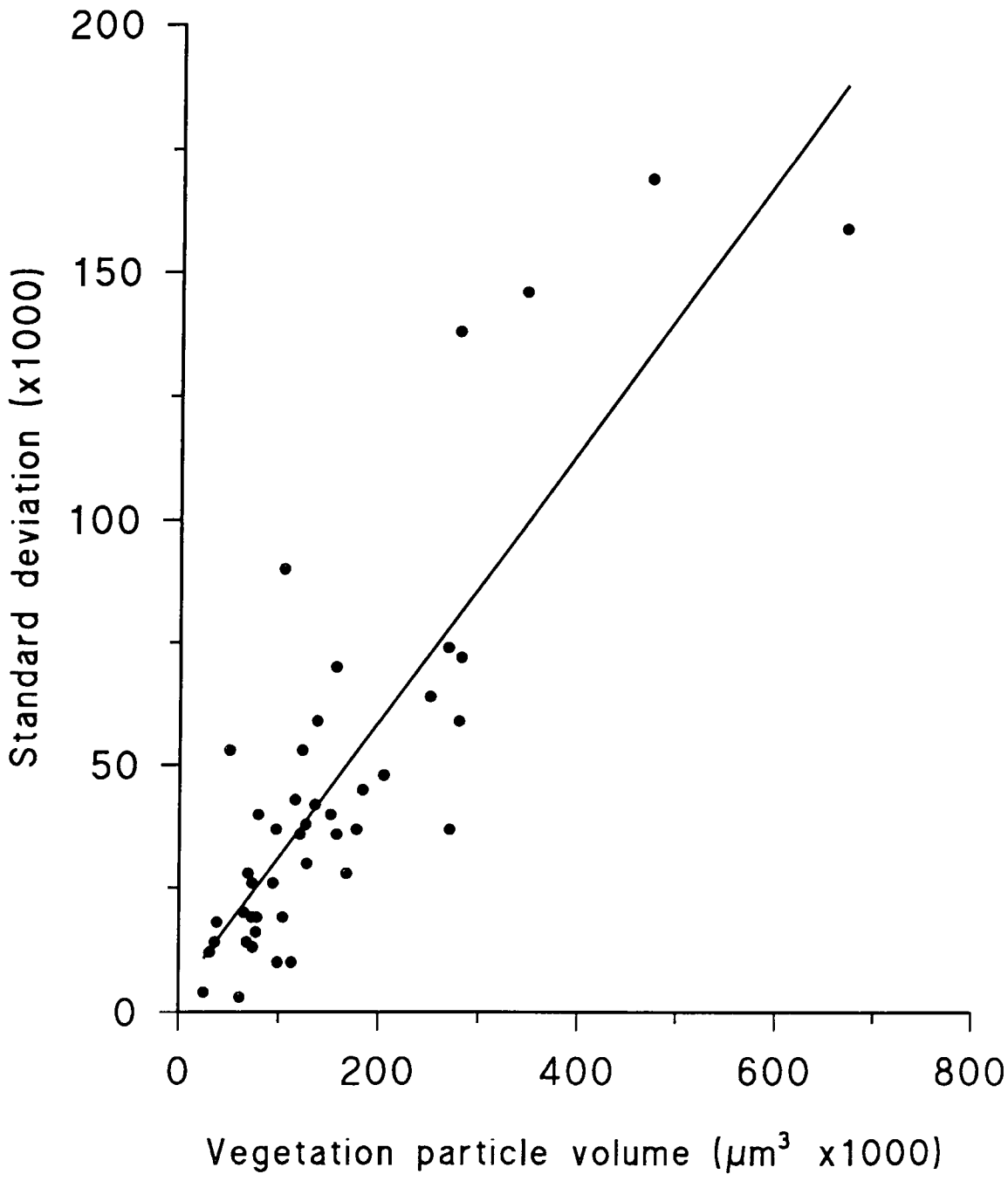


Table 2.6 Increases in mean measurements from 1st to 4th instars. The values are the multipliers to obtain the size in the fourth instar from that in the first instar.

Species	Measurement			
	Vegetation particle volume	Mandible length	Spiracular disc area	Weight
Tipula rufina	3.9	4.2	284	676
Tipula confusa	2.0	5.1	90	696
Tipula pagana	4.1	5.0	168	602
Tipula staegeri	3.5	5.8	163	651
Tipula limbata	2.5	5.6	65	662
Tipula alpium	2.4	3.5	99	660
Tipula subnodicornis	7.0	2.8	182	674
Tipula montana	7.2	4.3	117	820
Tipula paludosa	5.0	6.6	253	926
Tipula oleracea	11.0	5.6	271	931
Tipula lateralis	10.1	4.6	315	789

volumes between instars I and IV, *e.g.* the spiracular disc area increased 100 fold in *T. alpium* whereas the increase in vegetation particle volume was only 2.4 times greater. The increases in weight was 100 fold greater than the increases in vegetation particle volume between instars I and IV: for a 676 fold increase in weight in *T. rufina*, the corresponding increase in vegetation particle volume was only 3.9 times. The increase in vegetation particle volume ingested and in mandible length between the first and last instars was at least an order of magnitude less then the increase in spiracular disc area and weight

2.3.5.2. In non-moss feeders

Within the non-moss feeders, the increases in spiracular disc area and weight between instars I and IV were also much greater than the corresponding vegetation particle volume, being considerably greater than 10 fold in both cases (Table 2.6). However it was the increase in mandible lengths where they differed from the moss-feeders - in that these increased to a lesser extent than did the vegetation particle volumes from instars I to IV (except *T. paludosa*). For example, the mandible length in *T. oleracea* increased more than five fold, whereas the vegetation particle volume increased eleven times. However, the increases in mandible lengths and vegetation particle volume were again of the same order of magnitude; unlike the other two biometric measurements. Although the increase in vegetation particle volume ingested between the first and last instars was greater than the increase in mandible length, the increases in both these measurements were again at least an order of magnitude less than the increase in spiracular disc area and weight.

2.3.6. Results of the detailed study on *Tipula rufina*

The extended study on *T. rufina* was carried out to determine the range of

vegetation particle volumes taken within each instar. The results are presented on Fig. 2.8. First instar larvae took particles less than 19,0000μm^3, second instars less than 30,0000μm^3, third instars less than 47,0000μm^3 and fourth instars less than 106,0000μm^3. The proportion of particle sizes in the same range which were taken in each of the four instars, *i.e.* particles with volumes less than or equal to 19,0000μm^3 was also determined. The results are given in Table 2.7. More than 50% of the particles consumed in each instar were within the same size range. The percentage of particles with volumes of 19,0000μm^3 or less decreased with increasing instar, but it was always greater than 50%. Therefore the majority of vegetation particles taken are from the same size range, irrespective of instar.

2.3.7. Results of the studies on *Hydriomena furcata* and *Locusta migratoria*

Table 2.8 gives the vegetation particle sizes taken by, and the biometric measurements of, the additional insect species investigated, *Hydriomena furcata* and *Locusta migratoria.*

The instars of the caterpillars of *H. furcata* were identified by measurement of the head capsule diameter. The vegetation particles in the frass of *H. furcata* were very similar in structure to that in the frass of *Tipula* species; *i.e.* the particles were distinct and contained intact cell contents. The mean vegetation particle volume taken by each caterpillar investigated was plotted against the head capsule diameter for that individual (Fig. 2.9). The regression equation can explain 84% of the variation, and the vegetation particle sizes are closely correlated with head capsule diameter, (r=0.91). Considering the slope of the regression line; the vegetation particle volume increased at approximately the same rate as the head capsule diameter, *e.g.* if the head capsule diameter doubled, the vegetation particle volume increased by 2.3 fold.

The actual increases in vegetation particle volume ingested and head capsule

Fig. 2.8.

Frequency histograms to show the range of vegetation particle volumes ingested by each instar of *Tipula rufina*.

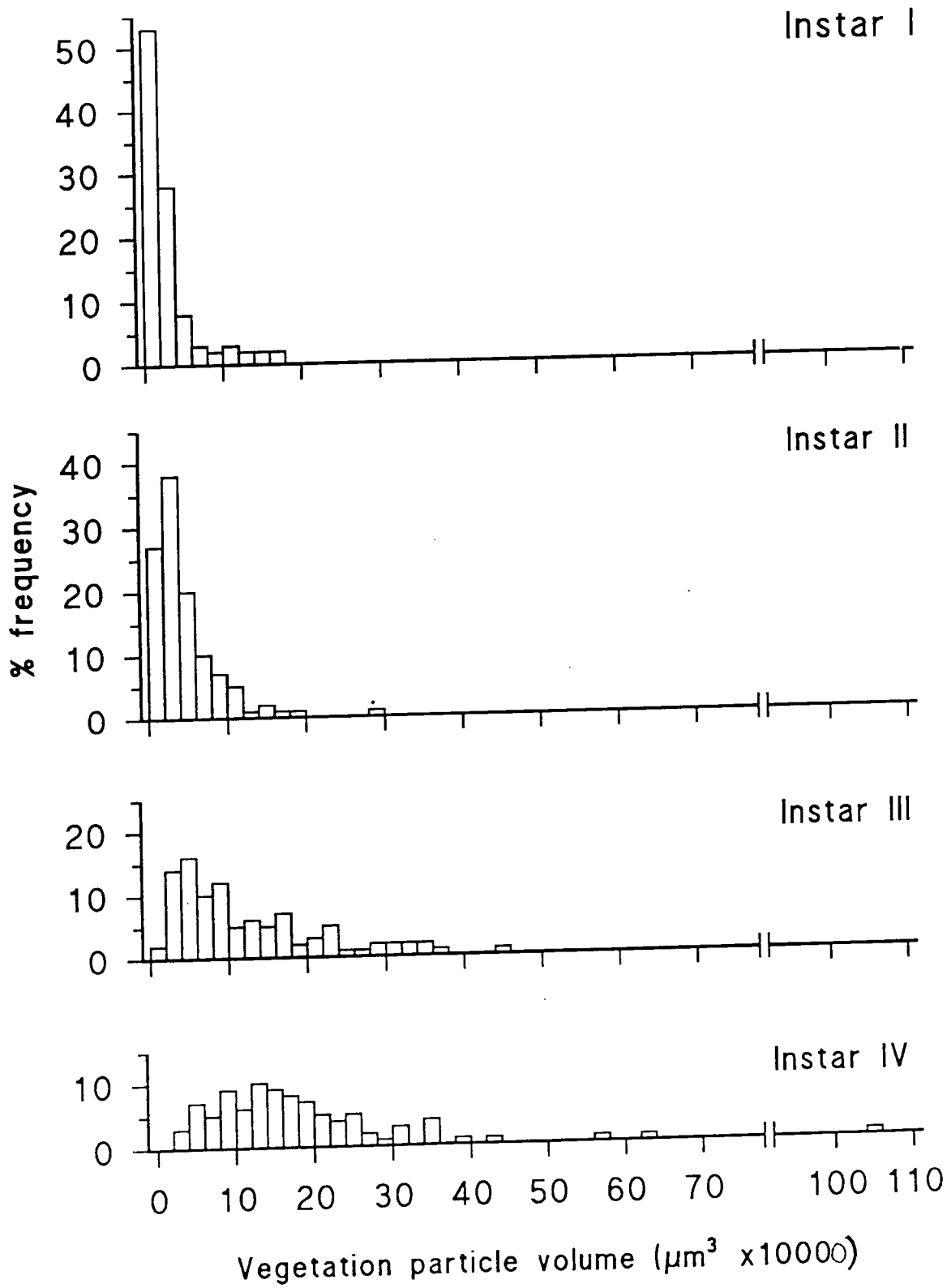


Table 2.7 The proportion of vegetation particles from the same volume range found in the frass of each instar of *Tipula rufina*.

Instar	Number of larvae	Total number of particles examined	Particles less than 18000μm^3
I	7	175	175 (100%)
II	11	275	267 (97%)
III	9	225	160 (71%)
IV	29	725	435 (60%)

Table 2.8 Mean vegetation particles size ingested and biometric measurements of the additional species, *Hydriomena furcata* and *Locusta migratoria migratoria*

Species	Measurement	Instar				
		I	II	III	IV	V
Hydriomena furcata	Head capsule diameter mm (±S.E.)	-	0.568 (±0.010)	0.856 (±0.008)	1.116 (±0.051)	1.692 (±0.018)
	Vegetation particle volume μm^3 (±S.E.)	-	30880 (±3575)	41688 (±541)	57847 (±6458)	96835 (±13948)
	n		4	3	5	8
Locusta migratoria	Mandible length (±S.E.)	1.286 (±0.083)	-	-	-	5.100 (±0.187)
	Vegetation particle volume μm^3 (±S.E.)	21705 (±2997)	-	-	-	145761 (±24504)
	Weight (g)	0.413 (±0.040)	-	-	-	15.546 (±2.196)
	n	5	-	-	-	5

Fig. 2.9

The head capsule diameter of *Hydriomena furcata* against the mean vegetation particle volume in the frass.

$y = 71167x - 15205$, $r_{18} = 0.91$, $p<0.001$.

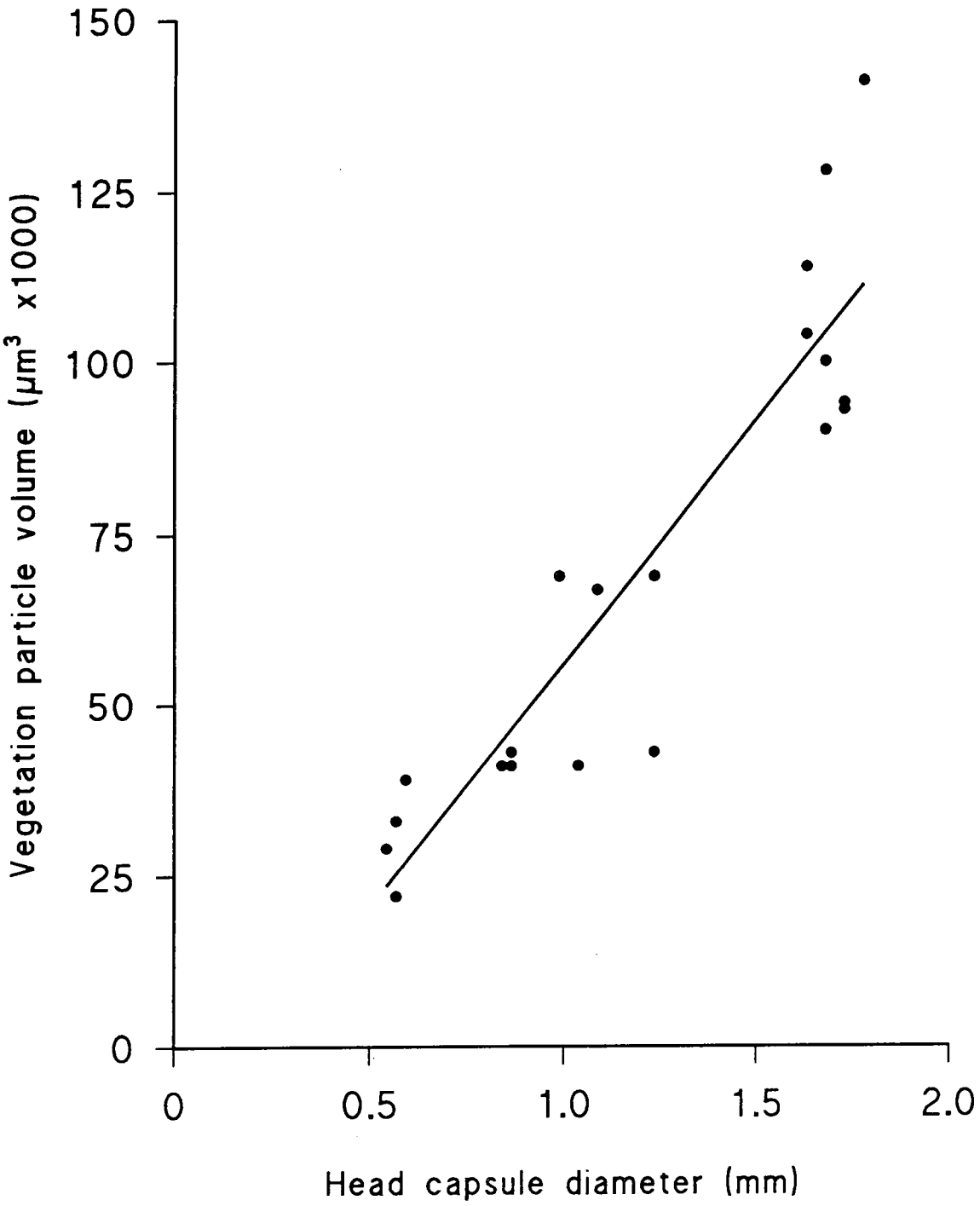
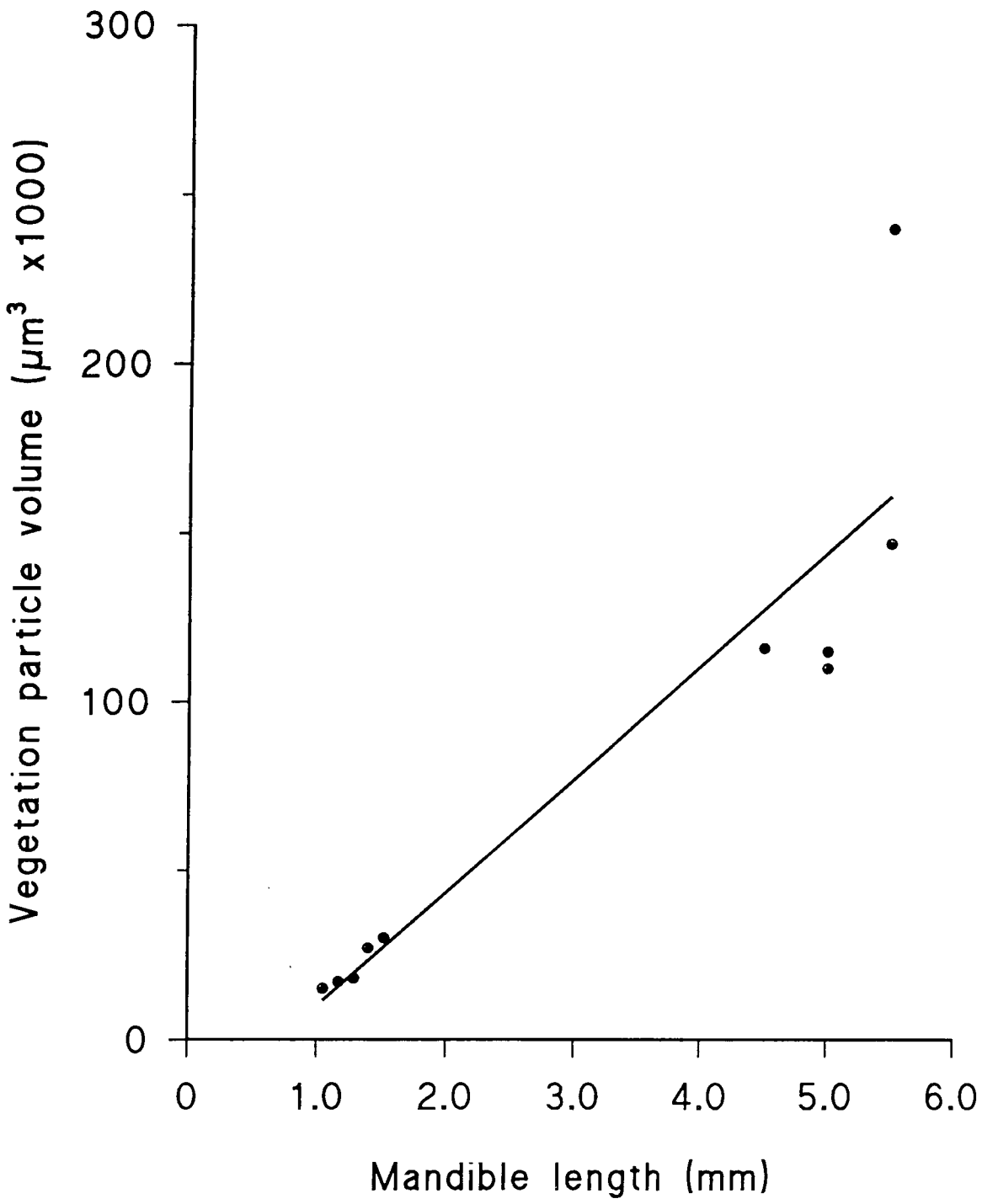


Fig. 2.10

The mean mandible length of *Locusta migratoria* against the mean vegetation particle volume in the gut.

$y = 33642x - 23920$, $r_8 = 0.91$, $p<0.001$.



diameter from instar II to instar IV were 3.0 fold and 3.1 fold respectively. This was the only biometric measurement available for comparison with the vegetation volume ingested, and the increases were of the same order of magnitude, as were the mandible lengths and vegetation particle volume ingested in tipulids.

The relationship between the mean vegetation particle size against individual mandible length (which can be used as a means of identifying the instars) for the locust is given in Fig. 2.10. Only two instars, the first and the fifth, were investigated, and the vegetation particle size is closely correlated to the mandible lengths in these instars ($r=0.91$), and 83% of the variation is explained by the regression equation. Considering the slope of the regression line, the vegetation particle volume increased by approximately the same rate as the mandible length, *e.g.* if the mandible length doubled the vegetation particle volume increased by 2.5 fold.

A comparison of the actual increases from instar I to instar V in vegetation volume ingested (7 fold) and mandible length (4 fold) revealed that the locust is more similar to the non-moss feeding tipulids than to the moss-feeding ones, in the increases from instar I to instar IV, at this stage of the locust's digestive processes. However the increase in weight between the two instars, at 38 fold, was very much less than for the non-moss feeding tipulids.

2.4. Discussion

Tipula rufina differed from all the other species studied in that it consistently ingested the smallest sized vegetation particles in each instar. *T. rufina* had the shortest mandibles in instars II III and IV of the moss-feeding species and in all instars of the non moss-feeding species studied. There was found to be a positive correlation between the weight of a larva and its mandible length and so *Tipula rufina* has the smallest mandibles because of its small size.

The grass feeders, *Tipula paludosa* and *T. oleracea* ingest the largest sized particles in all instars except instar I, and this is possibly due to the mandibles at that small stage not being developed enough to break off larger sized particles from the grass blade. The grass-feeders, as they are generally the larger larvae in each instar of the species studied, have longer mandibles than the moss-feeders, which in turn allows larger particles to be ingested, and these species fit on the vegetation particle volume/mandible length regression line, apart from instar IV in both *T. paludosa* and *T. oleracea*.

From the contents of the faecal material, it was evident that very little, if any, processes which digest the cell contents occurred in the gut after the vegetation particle had been cut from the food plant, and mechanically damaged by the action of the mandibles. Therefore the only part of the plant which is readily available for rapid digestion and removal of nutrients is that around the margins of the leaf particle.

The material in the faeces could be easily distinguished as distinct particles of the vegetation ingested, with intact cell contents, as found in saturniid caterpillars (Bernays and Janzen 1988). In sphingid caterpillars the same authors found the faecal material contained extremely small and unrecognisable mushy tissue, suggestive of further digestive breakdown of the ingested material after the cutting action. However Griffiths and Cheshire (1987), claim that in *T. paludosa* feeding on rye grass (*Lolium perenne*), 50% of the cellulose and 86% of the hemicellulose was assimilated, suggesting that the cell walls of the food particle ingested were at least partially broken down whilst passing through the gut. However this work needs confirmation as there was no evidence of these processes occurring here. Cellulases and hemicellulases were not detected in *Tipula abdominalis*, (Martin *et al.* 1980) and it was suggested that microbes ingested with the food items contained these enzymes (Lawson *et al.* 1984, Martin 1984, Sinsabaugh *et al.* 1985).

In this study, there was no evidence that the cell wall in the particles had been broken down during transport through the gut. Possibly partial breakdown of cellulose is not sufficient to break down the cell wall completely and destroy its

structure. It is clear that the great majority of cell contents pass through the gut and are not available to the tipulid larvae. In effect *Tipula* larvae are bulk feeders, ingesting much more food than would be necessary if they were efficient feeders, in order to obtain sufficient nutrients from the ingested food. Therefore nutrients from within the cell contents are unavailable except for the damaged edge cells. Also the digestion of cellulose and hemicellulose would be dependent upon which microbes are ingested with the food.

This produces mean particle sizes which are closely correlated to mean mandible lengths, similar to that found in saturniid caterpillars by Bernays and Janzen (1988), and a strong positive relationship has been found in both cases.

Overall, smaller increases occurred in the volume of vegetation particles ingested in the later instars of *Tipula* spp., compared to the increases in most biometric measurements of the larvae. It is more efficient for the larvae to feed on smaller particles than on larger ones, as there are proportionally more edge cells in smaller particles than in larger particles. Therefore by ingesting smaller particles, a higher number of cells can be damaged by the action of the mandibles; the most important method by which *Tipula* larvae obtain the cell contents. Also by doing this the bulk of food material passing through the animal will be reduced.

It is not advantageous for animals, with this type of digestion, to take larger sized food particles as they grow. This is well illustrated in the distribution of particle sizes taken by the four instars of *T. rufina*, where the majority of the vegetation particles in all instars were taken from the same size range.

The mouthpart morphology in the larvae of *Tipula sacra*, an aquatic cranefly species feeding on diatoms, filamentous algae and debris, has been studied by Hall and Pritchard (1975). They suggest that the upper limit of the size of particle that can be ingested is governed by the size of larvae and the elasticity of the mouthparts, but the lower limit cannot be well defined. Houston (1973) found that small Common frogs feed almost entirely on small food items; whereas larger frogs feed on a wider range of food items. In their consideration of body sizes of animal predators and

animal prey in food webs, Cohen *et al.* (1993) also found that larger predators eat prey with a wider range of body size than do smaller predators. If this idea is extended to herbivorous animals, the same situation is found to be true in *T. rufina*, where as the animal become larger the range of vegetation particle volumes ingested increases. Such a pattern of feeding puts considerable constraints on the size of the final stage larvae. This is because there is no advantage for tipulid larvae to ingest the largest particles that they are physically able to ingest.

The results of the plot of vegetation particles size against mandible length for *Locusta migratoria* appear to agree with those for species which mainly use mechanical digestion by the mouthparts. The vegetation particles had only undergone mandibular break-up, as they were collected in the extreme anterior end of the gut of the locust, and therefore can give a indication of the size of vegetation particle ingested, and the damage occurring by this method. In the moth species *Hydriomena furcata* a similar type of digestive process occurs in the larvae as for *Tipula* species, as virtually intact vegetation particles were present in the frass.

Although the tipulids studied here fed on a variety of vegetation types; mosses, grasses, algae and dead plant material, the methods used to obtain nutrients from their particular food items were basically similar. The methods used could probably be employed to a greater or lesser extent in a number of insect herbivores.

Chapter 3. The efficiency of digestion of vegetation particles by larvae from the genus *Tipula*

3.1. Introduction

Chapter 2 concluded that the vegetation particle size ingested by larvae of species of *Tipula* did not increase between instars to the extent expected from the rate of growth of the body. In fact, early and late instars within a species frequently ingest similar sized particles. It is appropriate to consider if the efficiency of digestion of the vegetation particles ingested changes between instars, and this has been determined by measuring the proportion of damaged cells in each instars.

The gross cellular structure of vegetation particles appeared to be intact after passing through the gut of *Tipula* species with, for example, chloroplasts remaining in place. As there appeared to be no additional breakdown of the cellular structure of the particles in the gut, the only nutrients readily available for immediate digestion are the damaged cells around the margins of the particles where the action of the mandibles cut the particles and damaged the cell walls of the food plants. Therefore, it might be expected that i) more damage would occur in cells on the margins of the particle than in the remaining cells ii) that the proportion of the total damaged cells per particles would decrease with particle size and iii) as a consequence, damage would be less in the later instars, as a result of their ingesting larger particles. These hypotheses are investigated here.

No breakdown of the cellular structure of the particles in the gut was evident, although Griffith and Cheshire (1987) suggested that some cellulose digestion occurs

in the gut of *Tipula paludosa*. This is probably dependent upon the number of cellulase-carrying microbes present (Martin 1984).

Some species of *Tipula* are known to be partial detritivores, ingesting dead plant material, possibly feeding on bacteria, and therefore the proportion of dead cells in the food was investigated.

3.2. Methods

The methods used to obtain the vegetation particles for this investigation were described in Chapter 2, Section 2.2.1.1. The same eleven species of *Tipula* were used: eight moss-feeders; *Tipula rufina, T. confusa, T. pagana, T. staegeri, T. limbata, T. alpium, T. subnodicornis* and *T. montana*, two grass-feeders; *T. paludosa* and *T. oleracea* and one algae-feeder, *T. lateralis*.

Food particles were divided into two regions: the 'edge cell' region - defined as those cells on the perimeter of the particle, and the 'mid-cell' region, which encompassed all other cells in the particle.

In this investigation, the total number of cells was determined in each zone of the particle considered, as was the number of cells which had been damaged (defined as having no cell contents, particularly chloroplasts, in the cell). In addition the number of long 'dead' cells, (*i.e.* those without contents prior to being cut by the mandibles, obvious by the brown colour of the cell wall), were determined.

By summing the figures, the numbers and percentages of damaged cells in the whole particle could be determined and the percentage of the long dead cells ingested in the vegetation particles for each instar was calculated. Similar measures were made for the edge and the middle cells and these results were analysed for each instar of

each species.

3.3. Results

3.3.1. Total damage per particle caused by larvae

3.3.1.1. Comparison with the spiracular disc diameters

For each species, to ascertain the proportion of damaged cells occurring through the instars of each species, the regression equation and correlation coefficient were calculated for the mean percentage of damaged cells per particle against the spiracular disc diameter (used to identify the instars) for each individual (Table 3.1). Within all of the species there was no significant relationship between the mean percentage of damaged cells per particle and spiracular disc diameter *i.e.* similar proportions of damaged cells were produced by animals of the same species through the four instars. Although the majority of the correlations were positive (8 to 3), this difference is not significant and, on existing evidence, there is no indication that cell damage is inversely related to instar.

3.3.1.2. Comparison with the vegetation particle volume

Plots of the mean percentage damaged cells against the mean vegetation particle volumes in Instar I are presented on Figs. 3.1 and 3.2 for *T. confusa* and *T. paludosa* respectively. There is no significant relationship between the two variables in either species; for *T. confusa* r=0.14 (df=43 NS) and for *T. paludosa* r=0.20 (df=59 NS). Thus the mean percentage of damaged cells per particle in larger particles was no less

Table 3.1 Regression equations and correlation coefficients (r) in *Tipula* species for the mean percentage of damaged cells per vegetation particle against spiracular disc diameter.

Species	n	Regression equation	r	p
Tipula rufina	56	y = 9.1x + 47.7	0.05	NS
Tipula confusa	43	y = -21.6x + 55.7	-0.08	NS
Tipula pagana	37	y = -35.2x + 47.7	-0.22	NS
Tipula staegeri	33	y = 46.2x + 42.8	0.22	NS
Tipula limbata	39	y = 16.9x + 50.2	0.01	NS
Tipula alpium	41	y = 16.7x + 51.4	0.07	NS
Tipula subnodicornis	41	y = 73.1x + 31.7	0.29	NS
Tipula montana	37	y = -10.1x + 45.1	-0.08	NS
Tipula paludosa	59	y = 16.8x + 51.6	0.20	NS
Tipula oleracea	37	y = 21.4x + 41.4	0.23	NS
Tipula lateralis	42	y = 10.6x + 52.2	0.07	NS

NS = $p>0.05$

Fig. 3.1

The relationship between the percentage of damaged cells per vegetation particle and the mean vegetation particle volume in Instar I of *Tipula confusa*. The correlation is not significant: $r_{41} = 0.14$.

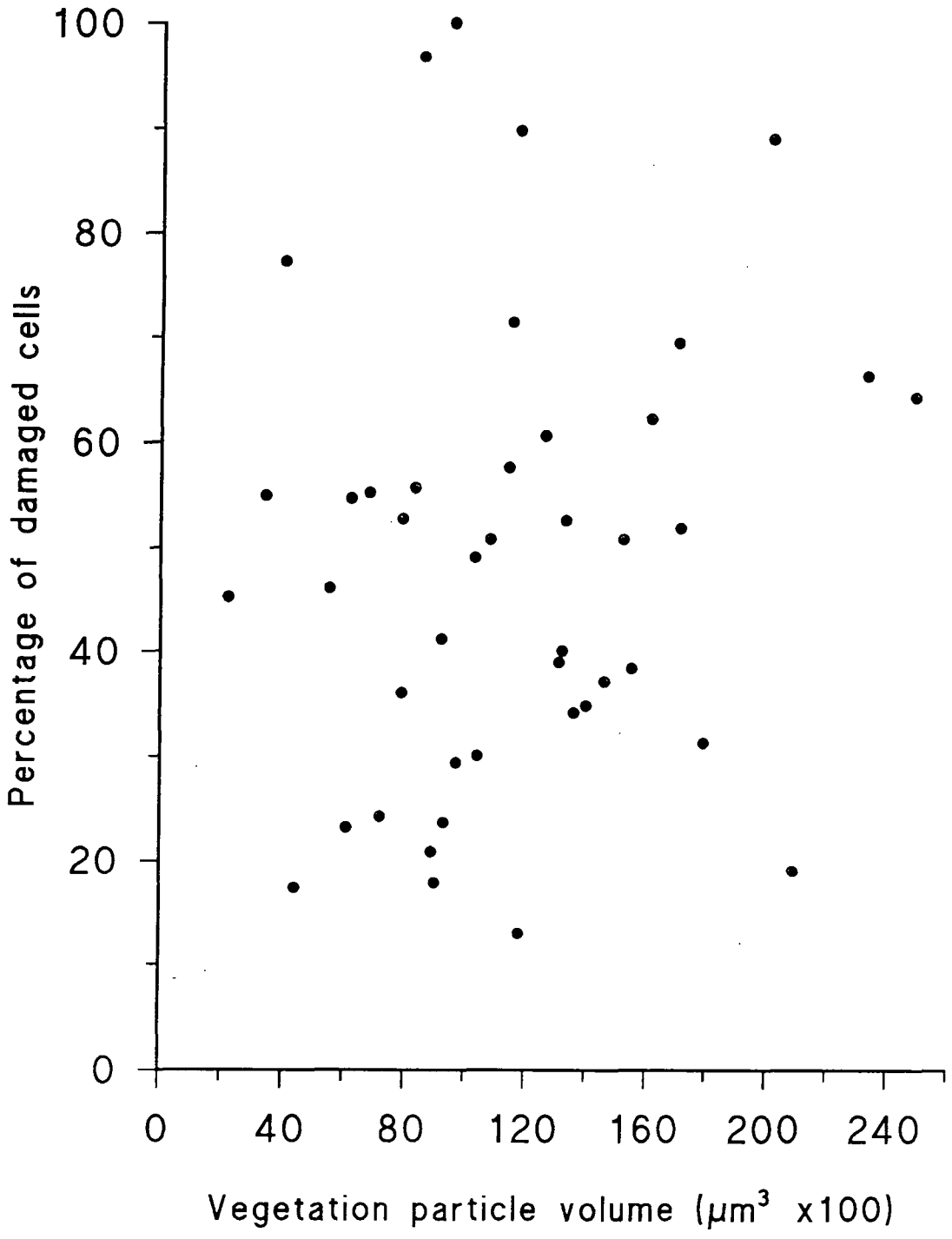
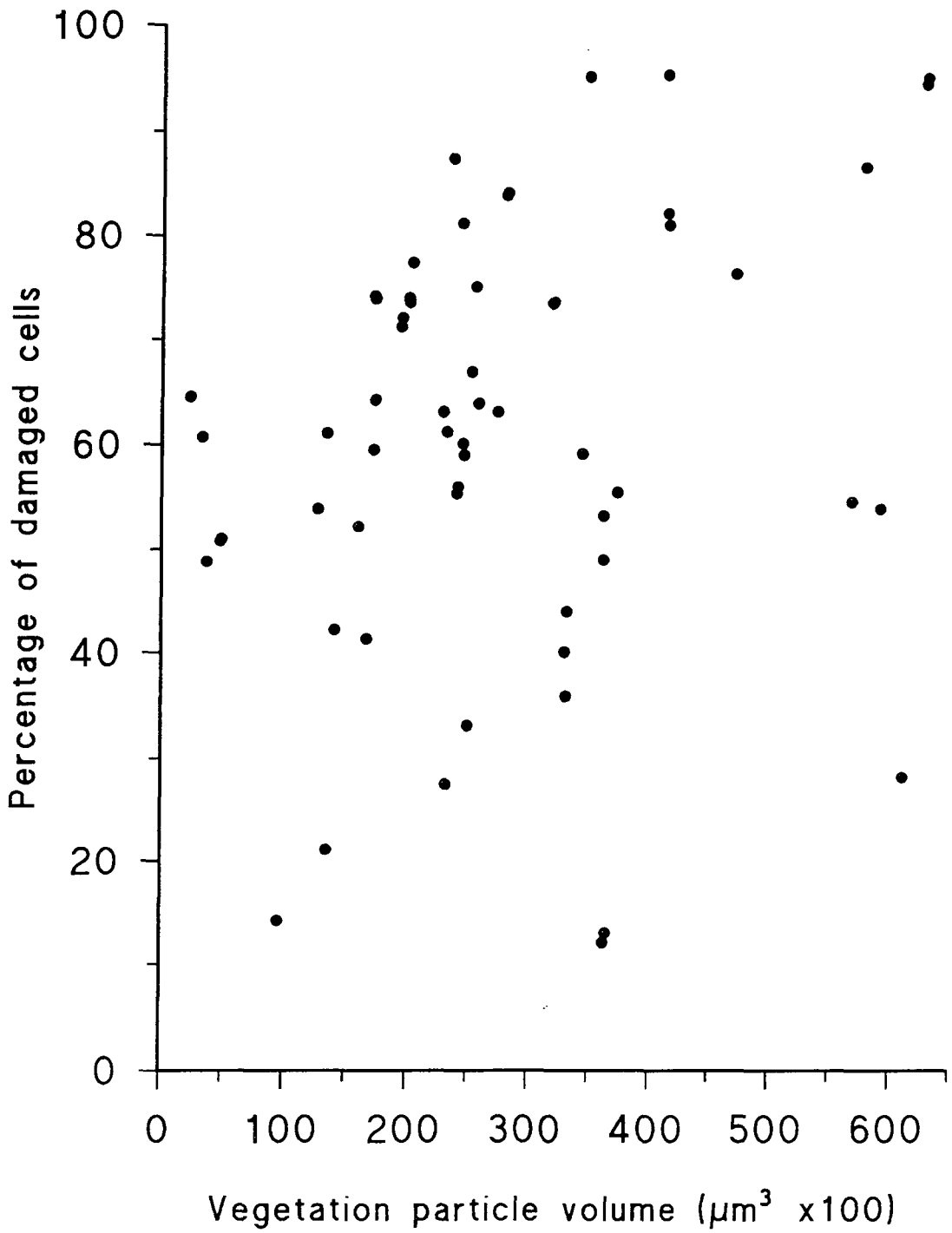


Fig. 3.2

The relationship between the percentage of damaged cells per vegetation particle and the mean vegetation particle volume in Instar I of *Tipula paludosa*. The correlation is not significant: $r_{57} = 0.20$.



than that in smaller particles, or vice versa. This was true in the species examined (Table 3.2); the graphs for *T. confusa* and *T. paludosa* being presented as examples. This does not agree with the hypothesis.

3.3.2. Determination of the proportion of damaged cells in the vegetation particles

In no case were there significant differences between the proportion of damaged cells between the individuals of the same species and instar. Despite this, the number of larvae has been used as the sample size, rather than the number of cells examined, as a conservative estimate of the variance. If the cells were used many standard errors, using a binomial distribution, were less than 1%

3.3.2.1. Total damage to vegetation particles occurring between the instars

Table 3.3 shows the percentages of damaged cells in the food particles which had passed through the gut of larvae of the *Tipula* species. These percentages were compared between the instars of <u>each</u> species by analysis of variance to test for differences between the percentage of damaged cells through the instars of each species. No significant differences were found, except in *T. subnodicornis*. The mean percentage of damaged cells caused by *T. subnodicornis* increased significantly but the main difference was between instar I and the remaining instars. No obvious differences were found within, or between the moss-feeders and the non-moss feeders.

An unweighted mean, combining the four instars, for each species was calculated. There was no significant difference between the species, (Table 3.4A) $F_{(10,33)}=1.11$ NS. When the proportion of damaged cells was determined for each

Table 3.2. Regression equations and correlation coefficients (r) in *Tipula* species for mean percentage of cells damaged per particle against vegetation particle volume.

Species	n	Regression equation	r	p
Tipula rufina	56	$y = 0.001x + 41.9$	0.14	NS
Tipula confusa	43	$y = 0.001x + 41.9$	0.14	NS
Tipula pagana	37	$y = -0.0001x + 43.0$	-0.02	NS
Tipula staegeri	33	$y = 0.0002x + 47.6$	0.11	NS
Tipula limbata	39	$y = 0.001x + 42.4$	0.20	NS
Tipula alpium	41	$y = 0.001x + 43.0$	0.19	NS
Tipula subnodicornis	41	$y = 0.0005x + 35.7$	0.21	NS
Tipula montana	37	$y = -0.0002x + 47.2$	-0.18	NS
Tipula paludosa	59	$y = 0.0002x + 51.8$	0.20	NS
Tipula oleracea	37	$y = 0.0001x + 43.2$	0.18	NS
Tipula lateralis	42	$y = 0.0002x + 52.1$	0.08	NS

NS $p>0.05$

Table 3.3. Comparison of percentage of all cells damaged per larva in each instar in each *Tipula* species studied, n being the number of larvae examined.

Species	Mean percentage damaged cells (±S.E.) Instar I	II	III	IV	ANOVA
T. rufina	63 (±7)	41 (±7)	47 (±8)	52 (±8)	$F_{(3,42)} = 1.0$ NS
n	7	11	9	29	
T. confusa	65 (±9)	38 (±6)	43 (±4)	54. (±7)	$F_{(3,38)} = 1.3$ NS
n	4	9	23	7	
T. pagana	47 (±4)	36 (±4)	36 (±7)	43 (±8)	$F_{(3,31)} = 0.8$ NS
n	10	12	10	5	
T. staegeri	48 (±8)	56 (±6)	53 (±6)	55 (±9)	$F_{(3,28)} = 0.3$ NS
n	5	10	9	9	
T. limbata	33 (±8)	52 (±8)	51 (±7)	57 (±8)	$F_{(3,35)} = 0.1$ NS
n	9	10	10	10	
T. alpium	45 (±7)	45 (±8)	48 (±7)	61 (±6)	$F_{(3,37)} = 0.6$ NS
n	10	10	10	11	
T. subnodicornis	20 (±5)	53 (±7)	47 (±8)	48 (±8)	$F_{(3,36)} = 4.3$ *
n	11	10	10	10	
T. montana	45 (±10)	43 (±6)	46 (±8)	37 (±8)	$F_{(3,33)} = 0.3$ NS
n	6	11	10	10	
T. paludosa	48 (±6)	57 (±6)	56 (±9)	59 (±3)	$F_{(3,44)} = 0.4$ NS
n	11	11	9	28	
T. oleracea	37 (±8)	36 (±5)	47 (±6)	56 (±7)	$F_{(3,36)} = 1.6$ NS
n	8	9	11	9	
T. lateralis	38 (±6)	50 (±10	48 (±7)	59 (±8)	$F_{(3,38)} = 0.4$ NS
n	11	9	10	12	

NS $p>0.05$
* $p<0.05$

Table 3.4A. A comparison between each of the species of the unweighted mean percentage of damaged cells for the four instars.

Species	Mean percentage of damaged cells per larva (±S.E.)	
Tipula rufina	51±5	F(10,33)=1.1 NS
Tipula confusa	50±6	
Tipula pagana	41±3	
Tipula staegeri	53±2	
Tipula limbata	48±5	
Tipula alpium	50±4	
Tipula subnodicornis	42±8	
Tipula montana	43±2	
Tipula paludosa	55±2	
Tipula oleracea	44±5	
Tipula lateralis	49±4	

Table 3.4B. A comparison between each of the four instars of the unweighted mean percentage of damaged cells for the eleven species.

Instar	Mean percentage of damaged cells per larva (±S.E.)	
I	44±4	F(3,40)=1.8 NS
II	46±2	
III	48±2	
IV	53±2	

NS $p>0.05$

instar using an unweighted mean of the values for each species, again there was no significant difference, $F_{(3,40)}$=1.84 NS (Table 3.4B). There was a progressive trend for the percentage of damage to increase through each instar (Table 3.4B). Therefore, considering the total mean percentage of damaged cells which occurred in the particles, no significant difference was apparent either between the species or between the four instars (except instar I in *T. subnodicornis*). This was also not predicted from the hypothesis.

3.3.2.2. Damage in the edge cell region of the vegetation particles

Table 3.5 gives the mean percentage of damaged cells per larva in the edge cell region of the vegetation particles ingested in each instar for each species. From the analysis of variance across the four instars, four species (*T. limbata,* $F_{(3,35)}$=3.2 $p<0.05$, *T. alpium* $F_{(3,37)}$=12.2 $p<0.01$, *T. subnodicornis* $F_{(3,36)}$=7.6 $p<0.01$ and *T. lateralis* $F_{(3,38)}$=10.9 $p<0.01$,) showed a significant increase with instar, indicating that individuals in the later instars of these four species were able to cause a greater amount of damage in the edge cells of ingested vegetation particles.

An unweighted mean, combining the four instars, for each species was calculated (Table 3.6A). When these were compared using analysis of variance, no significant difference was found in the mean percentage of damaged edge cells: $F_{(10,33)}$=1.04 NS. However, when the unweighted means for each of the four instars was calculated by pooling the species and examined using analysis of variance, a significant difference between the means was obtained, $F_{(3,40)}$=6.4 $p<0.01$ (Table 3.6B). The percentage damage increased progressively with instar, from 49% in instar I to 68% in instar IV. This result suggests that the larger larvae induce a greater amount of damage when cutting their food from the plant.

Table 3.5 Comparison of percentage of edge cells damaged per larva in the vegetation particles ingested, in each instar in each *Tipula* species studied, n being the number of larvae examined.

	Mean percentage damaged edge cells (±S.E.)				
	Instar				ANOVA
Species	I	II	III	IV	
T. rufina	79 (±10)	49 (±6)	57 (±4)	72. (±7)	$F_{(3,42)} = 2.2$ NS
n	7	11	9	29	
T. confusa	67 (±15)	38 (±7)	48 (±4)	58 (±10)	$F_{(3,38)} = 2.7$ NS
n	4	9	23	7	
T. pagana	49 (±6)	37 (±2)	59 (±7)	51 (±11)	$F_{(3,31)} = 1.1$ NS
n	10	12	10	5	
T. staegeri	56 (±8)	66 (±2)	69 (±5)	79 (±6)	$F_{(3,28)} = 2.7$ NS
n	5	10	9	9	
T. limbata	44 (±4)	54 (±6)	64 (±6)	70 (±8)	$F_{(3,35)} = 3.2$ *
n	9	10	10	10	
T. alpium	37 (±6)	49 (±5)	57 (±7)	80 (±3)	$F_{(3,37)} = 12.2$ ***
	10	10	10	11	
T. subnodicornis	23 (±5)	56 (±6)	55 (±8)	68 (±8)	$F_{(3,36)} = 7.6$ ***
n	11	10	10	10	
T. montana	54 (±5)	55 (±4)	65 (±8)	64 (±12)	$F_{(3,33)} = 0.4$ NS
n	6	11	10	10	
T. paludosa	50 (±6)	66 (±8)	61 (±8)	69 (±3)	$F_{(3,44)} = 1.9$ NS
n	11	11	9	28	
T. oleracea	40 (±2)	46 (±6)	47 (±4)	58 (±7)	$F_{(3,36)} = 1.6$ NS
n	8	9	11	9	
T. lateralis	39 (±5)	56 (±6)	65 (±4)	71 (±4)	$F_{(3,38)} = 10.9$ ***
n	11	9	10	12	

NS $p>0.05$ * $p<0.05$ *** $p<0.001$

Table 3.6A. A comparison between each of the species of the unweighted mean percentage of damaged edge cells for the four instars.

Species	Mean percentage of damaged cells per larva (±S.E.)	
Tipula rufina	64±7	F(10,33)=1.0 NS
Tipula confusa	52±6	
Tipula pagana	49±4	
Tipula staegeri	68±5	
Tipula limbata	58±6	
Tipula alpium	56±9	
Tipula subnodicornis	50±10	
Tipula montana	59±3	
Tipula paludosa	61±4	
Tipula oleracea	48±4	
Tipula lateralis	58±7	

Table 3.6B. A comparison between each of the four instars of the unweighted mean percentage of damaged edge cells for the eleven species.

Instar	Mean percentage of damaged cells per larva (±S.E.)	
I	49±5	F(3,40)=6.4 **
II	52±3	
III	59±2	
IV	67±3	

NS $p>0.05$ ** $p<0.01$

Table 3.7. Comparison of percentage of mid cells damaged per larva in the vegetation particles ingested, in each instar in each *Tipula* species studied, n being the number of larvae examined.

Species	Mean percentage damaged mid cells (±S.E.) Instar I	II	III	IV	ANOVA
T. rufina	36 (±4)	30 (±5)	34 (±7)	40 (±6)	$F_{(3,42)}$= 1.6 NS
n	7	11	9	29	
T. confusa	58 (±20)	37 (±9)	30 (±4)	53 (±7)	$F_{(3,38)}$= 3.0 *
n	4	9	23	7	
T. pagana	29 (±7)	33 (±2)	24 (±2)	32 (±5)	$F_{(3,31)}$= 0.3 NS
n	10	12	10	5	
T. staegeri	27 (±5)	47 (±6)	26 (±4)	36 (±6)	$F_{(3,28)}$= 3.4 *
n	5	10	9	9	
T. limbata	25 (±3)	40 (±5)	29 (±4)	49 (±7)	$F_{(3,35)}$= 4.7 **
n	9	10	10	10	
T. alpium	50 (±7)	37 (±3)	32 (±7)	47 (±7)	$F_{(3,37)}$= 1.4 NS
	10	10	10	11	
T. subnodicornis	8 (±2)	20 (±4)	15 (±3)	12 (±2)	$F_{(3,36)}$= 3.1 *
n	11	10	10	10	
T. montana	16 (±4)	11 (±4)	28 (±4)	22 (±1)	$F_{(3,33)}$= 4.8 **
n	6	11	10	10	
T. paludosa	41 (±7)	38 (±5)	51 (±8)	53 (±5)	$F_{(3,44)}$= 0.8 NS
n	11	11	9	28	
T. oleracea	33 (±3)	14 (±2)	11 (±2)	22 (±2)	$F_{(3,36)}$=12.6 ***
n	8	9	11	9	
T. lateralis	17 (±3)	14 (±2)	21 (±2)	34 (±3)	$F_{(3,38)}$=12.7 ***
n	11	9	10	12	

NS $p>0.05$ * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Table 3.8A. A comparison between each of the species of the unweighted mean percentage of damaged mid cells for the four instars.

Species	Mean percentage of damaged cells per larva (±S.E.)	
Tipula rufina	35±2	F(10,33)=6.8 ***
Tipula confusa	44±6	
Tipula pagana	30±2	
Tipula staegeri	34±5	
Tipula limbata	36±6	
Tipula alpium	42±4	
Tipula subnodicornis	14±3	
Tipula montana	19±4	
Tipula paludosa	46±4	
Tipula oleracea	20±5	
Tipula lateralis	21±4	

Table 3.8B. A comparison between each of the four instars of the unweighted mean percentage of damaged mid cells for the eleven species.

Instar	Mean percentage of damaged cells per larva (±S.E.)	
I	31±4	F(3,40)=0.9 NS
II	29±4	
III	27±3	
IV	36±4	

NS $p>0.05$ *** $p<0.001$

Table 3.9A. Comparison of the percentage of edge cells and mid-cells damaged in the vegetation particles in instar I of each *Tipula* species studied, n being the number of larvae examined.

Species	Instar I: Mean percentage damage (±S.E.) in: Edge cells (E)	Mid-cells (M)	(E-M)	*t* (df)
T. rufina n=7	79 (±10)	36 (±4)	43	3.9 (12) **
T. confusa n=4	67 (±16)	58 (±21)	9	0.3 (6) NS
T. pagana n=10	49 (±6)	29 (±7)	20	2.8 (18) *
T. staegeri n=5	56 (±8)	27 (±5)	29	3.1 (8) *
T. limbata n=9	44 (±4)	25 (±3)	19	3.8 (16) **
T. alpium n=10	37 (±6)	50 (±7)	-13	1.4 (18) NS
T. subnodicornis n=11	23 (±5)	8 (±2)	15	2.8 (20) *
T. montana n=6	53 (±5)	16 (±4)	37	5.9 (10) ***
T. paludosa n=11	50 (±6)	41 (±7)	9	0.9 (20) NS
T. oleracea n=8	41 (±2)	33 (±3)	8	2.2 (14) *
T. lateralis n=11	39 (±5)	17 (±3)	22	3.8 (20) **

NS $p>0.05$ * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Table 3.9B. Comparison of the percentage of edge cells and mid-cells damaged in the vegetation particles in instar II of each *Tipula* species studied, n being the number of larvae examined.

Species	Instar II: Mean percentage damage (±S.E.) in: Edge cells (E)	Mid-cells (M)	(E-M)	*t* (df)
T. rufina n=11	49 (±6)	31 (±5)	18	2.4 (20) *
T. confusa n=9	38 (±7)	37 (±9)	1	0.1 (16) NS
T. pagana n=12	37 (±2)	32 (±2)	4	1.8 (22) NS
T. staegeri n=10	66 (±2)	47 (±6)	19	2.9 (18) **
T. limbata n=10	54 (±6)	40 (±5)	14	1.8 (18) NS
T. alpium n=10	49 (±5)	37 (±3)	12	1.9 (18) NS
T. subnodicornis n=10	56 (±6)	20 (±4)	36	4.6 (18) ***
T. montana n=11	55 (±4)	11 (±4)	44	8.3 (20) ***
T. paludosa n=11	66 (±8)	38 (±5)	28	3.5 (20) **
T. oleracea n=9	46 (±6)	14 (±2)	32	5.0 (16) ***
T. lateralis n=9	56 (±6)	14 (±2)	42	7.2 (16) ***

NS $p>0.05$ * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Table 3.9C Comparison of the percentage of edge cells and mid-cells damaged in the vegetation particles in instar III of each *Tipula* species studied, n being the number of larvae examined.

Species	Instar III: Mean percentage damage (±S.E.) in: Edge cells (E)	Mid-cells (M)	(E-M)	*t*	(df)
T. rufina n=9	57 (±4)	34 (±7)	23	2.8 *	(16)
T. confusa n=23	48 (±4)	30 (±4)	18	3.1 **	(44)
T. pagana n=10	59 (±7)	24 (±2)	35	4.9 ***	(18)
T. staegeri n=9	69 (±5)	26 (±4)	43	6.7 ***	(16)
T. limbata n=10	64 (±6)	29 (±4)	35	5.2 ***	(18)
T. alpium n=10	57 (±7)	32 (±7)	25	2.6 *	(18)
T. subnodicornis n=10	55 (±8)	15 (±3)	40	4.7 ***	(18)
T. montana n=10	65 (±8)	28 (±4)	37	4.1 ***	(18)
T. paludosa n=9	61 (±8)	51 (±8)	10	0.8 NS	(16)
T. oleracea n=11	47 (±4)	11 (±2)	36	7.7 ***	(20)
T. lateralis n=10	65 (±4)	21 (±2)	44	10.8 ***	(18)

NS $p>0.05$ * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Table 3.9D. Comparison of the percentage of edge cells and mid-cells damaged in the vegetation particles in instar IV of each *Tipula* species studied, n being the number of larvae examined.

Species	Instar IV: Mean percentage damage (±S.E.) in: Edge cells (E)	Mid-cells (M)	(E-M)	*t*	(df)
T. rufina n=29	72 (±7)	40 (±6)	32	3.6 ***	(56)
T. confusa n=7	58 (±10)	53 (±7)	5	0.4 NS	(12)
T. pagana n=5	51 (±10)	32 (±5)	19	1.8 NS	(8)
T. staegeri n=9	79 (±6)	36 (±6)	43	5.3 ***	(16)
T. limbata n=10	70 (±8)	49 (±7)	21	1.8 NS	(18)
T. alpium n=11	80 (±3)	47 (±7)	33	4.5 ***	(20)
T. subnodicornis n=10	68 (±8)	12 (±2)	56	6.7 ***	(18)
T. montana n=10	64 (±12)	22 (±1)	42	3.5 **	(18)
T. paludosa n=28	69 (±3)	53 (±5)	16	3.0 ***	(54)
T. oleracea n=9	58 (±7)	22 (±2)	35	4.9 ***	(16)
T. lateralis n=12	71 (±4)	34 (±3)	38	7.9 ***	(22)

NS $p>0.05$ ** $p<0.01$ *** $p<0.001$

3.3.2.3. Damage in the mid-cell region of the vegetation particles

Table 3.7. presents, for each species, the mean percentage of damaged cells per larva in the mid-cell region of the vegetation particles ingested in each instar. Analyses of variance was used to compare these percentages between the instars of each species. Significant differences were found in seven species, (*T. confusa*, ($F_{(3,38)}$=3.0 $p<0.05$), *T. staegeri*, ($F_{(3,28)}$=3.4 $p<0.05$), *T. limbata*, ($F_{(3,35)}$=4.7 $p<0.01$), *T. subnodicornis*, ($F_{(3,36)}$=3.1 $p<0.05$), *T. montana*, ($F_{(3,33)}$=4.8 $p<0.01$), *T. oleracea*, ($F_{(3,36)}$=12.6 $p<0.001$), *T. lateralis*, ($F_{(3,38)}$=12.7 $p<0.001$)), although no upward or downward trend through the instars was evident in these species.

Table 3.8A gives the unweighted mean for the percentage damage in the mid-cells across the four instars, in each species. These were compared, again using analyses of variance, and a significant difference was found between the species ($F_{(10,33)}$=6.8 $p<0.001$). When the unweighted means for each of the four instars was calculated for each of the species, (Table 3.8B), and compared using an analysis of variance, no significant difference was found. Damage to the mid-cells in the vegetation particles appears to be a random occurrence with no upward or downward trend through the species or instars, indicating that the extent of damage in mid-cells does not appear to be influenced by the size of the larvae.

3.3.2.4. Comparison of damage in the edge and mid cell regions

Within each instar of each species, the mean percentage of damaged cells in the edge cell region was compared to that in the mid-cell region (Table 3.9A - Table 3.9D). Apart from one out of 44 cases, this damage was greater in the edge cell region than in the mid-cell region, and significantly so in all but ten cases. Therefore in each instar the mean percentage damage in the edge cell region was significantly

greater than in the mid-cell region, which had been predicted in the hypothesis.

Using Wilcoxon's matched-pairs signed-rank two-tailed test, the non-parametric rank sum (T_s) obtained for the eleven species in instar I was $T_s=4$ $p<0.05$ $n=11$, and in instars II, III and IV $T_s=0$ $p<0.001$ $n=11$ in all cases.

The mean percentage difference (±S.E.) per instar in the proportion of damaged cells between the edge cell and mid-cell regions were as follows: for instar I 20±4%, for instar II 23±4% for instar III 31±3% and for instar IV 31±4%.

The only exception was in instar I of *T. alpium* where more damage occurred in the mid-cell region (50%) than in the edge cell region (37%), but this difference was not significant.

3.3.3. Determination of consumers of dead plant material

The mean percentage of dead cells per larva found in the vegetation particles in the frass of *Tipula* species are given in Table 3.10. The percentage of dead cells found ranged from 15.8±0.6%, in *T. montana* (instar III) down to 0% (in several species and instars). The F-values (from the analysis of variance to test for differences between the mean percentages of dead cells through the instars of each species) are also given. In the case of a significant result, the least significant difference multiple range test was used to elucidate which species contributed to the significant difference. The consistent feature from these results was that, within each instar, larvae of *T. montana* and *T. paludosa* ingested a significantly higher mean percentage of dead cells in the vegetation particles than the other species. The mean differences through the instars in the percentages of dead cells ingested in *T. montana* and the other species was 11±1% and in *T. paludosa* and the other species was 10±1% The proportions however did not differ significantly between *T. montana* and *T. paludosa* in each instar. Therefore *Tipula montana* and *T. paludosa* fed on

Table 3.10. Comparison of percentage of dead cells in the vegetation particles ingested per larva in each instar of each *Tipula* species studied, n being the number of larvae examined.

Species	Instar I		II		III		IV
	Mean percentage of dead cells (±S.E.)						
T. rufina	2.2 (±0.6)		1.5 (±0.4)	*	2.7 (±0.4)	*	0.0 (±0.0)
n	7		11		9		29
T. confusa	0.0 (±0.0)	*	6.3 (±1.3)	*	1.3 (±0.6)	*	3.2 (±0.4)
n	4		9		23		7
T. pagana	1.4 (±0.4)	*	6.8 (±0.9)		9.4 (±0.3)	*	6.3 (±0.1)
n	10		12		10		5
T. staegeri	7.8 (±0.8)	*	0.0 (±0.0)		0.0 (±0.0)		0.0 (±0.0)
n	5		10		9		9
T. limbata	3.4 (±0.6)	*	0.0 (±0.0)	*	1.1 (±0.2)	*	0.0 (±0.0)
n	9		10		10		10
T. alpium	1.3 (±0.2)		1.3 (±0.3)	*	5.0 (±0.1)	*	1.0 (±0.2)
	10		10		10		11
T. subnodicornis	1.6 (±0.4)	*	8.9 (±0.8)		10.3 (±0.4)	*	3.9 (±0.4)
n	11		10		10		10
T. montana	14.7 (±1.4)		13.5 (±0.7)	*	15.8 (±0.6)	*	12.9 (±0.7)
n	6		11		10		10
T. paludosa	12.9 (±1.2)		12.3 (±0.3)	*	15.7 (±0.5)	*	12.8 (±0.7)
n	11		11		9		28
T. oleracea	0.0 (±0.0)	*	10.0 (±0.9)	*	4.9 (±0.1)	*	0.4 (±0.1)
n	8		9		11		9
T. lateralis	0.0 (±0.0)		9.9 (±0.8)		4.4 (±0.5)		0.0 (±0.0)
n	11		9		10		12
ANOVA ($F_{(10,33)}$)	59.6 ***		78.8 ***		203.6 ***		197.0 ***

*** $p<0.001$

* significant difference between the mean percentage of dead cells in one instar and the succeeding one, at $p<0.05$ level, using Student's t-test

significantly greater amounts of dead plant material than did any of the other species.

Significant differences existed in the proportion of dead material present in the particles ingested between one instar and the succeeding instar of individual species (Table 3.10). However there were no progressive and significant upward or downward trends in these proportions of dead material consumed through the instars of any species.

3.4. Discussion

A survey of the literature has revealed few references on the efficiency of digestion of insect herbivores feeding on bryophytes, and Lawrey (1987) states that few laboratory studies have been made on insects feeding on mosses. Therefore it is not possible to compare the results of this work with other studies.

In this study no apparent differences existed in the digestive capacities of tipulids consuming the moss, grass or alga. The overall efficiency of digestion of the ingested particle, determined by the mean percentage of damaged cells per particle, was similar both between the four instars in each of the eleven species and between the eleven species in each instar.

In Chapter 2, it was suggested that more damage to the cells of vegetation particles would be expected to occur at the margins of the particles, as this is where the mandibles of the larvae cut the particles from the food plant. Considering all eleven species, significantly more damage did occur overall around the margin of the particle than in the middle section in each of the instars. Therefore, it would appear that these larvae are dependent on the edge cells of each vegetation particle ingested to provide them with the necessary nutrients for growth and development.

This being so, and with the fact that a high proportion of similar sized particles were ingested in each instar in the *Tipula* species studied (Chapter 2), suggests that the only way the later instars can obtain a higher proportion of nutrients is by actually feeding on a larger number of smaller sized vegetation particles rather than feeding on larger sized vegetation particles. There would be little advantage for these animals to feed on larger sized particles as there would be relatively less marginal cells available. In fact no more damage to cells does occur in the larger sized particles in any species.

However there was a significant increase in the mean proportion of edge cell damaged through the instars in each species, suggesting that larger instars are more efficient in feeding on vegetation particles than smaller instars. Therefore, later instars perhaps require an increase in relatively fewer particles of similar size than earlier instars to obtain the higher amount of nutrients required. Maxwell (1972) states that differences in food consumption in insects can be caused by a great variety of factors, *e.g.* proteins, sugars, lipids, organic salts *etc* and these can function both as feeding stimulants and being essential for growth and development.

All the species ingested dead material in at least one stage of their life cycle, but *T. montana* and *T. paludosa* ingested a higher total proportion of dead material through its larval stages than all other species. Within each species the proportion of dead material in the diet throughout the instars appeared to be completely random.

An obvious study which leads on from the present one would be to determine the amount of extra food material successive instars require. Laughlin (1960), working on *T. oleracea*, suggested that there is basically exponential growth in the early instars which then becomes a constant daily weight increase in the first part of the fourth instar. He also states that the principal purpose of the last instar feeding is to gather reserve substances for metamorphoses and reproduction, since all organs have been increasing in weight up to this time (from Laughlin 1957), and have reached the maximum weight, except for the fat body, by the last larval moult.

Therefore it would be interesting to determine the number of extra vegetation particles which need to be consumed in successive instars in order to provide sufficient nutrients for the required growth and development to occur in these *Tipula* species.

Scriber (1978) states that larvae of smaller species complete their growth sooner than most larvae of larger species *e.g.* the herb feeding lepidopteran *Pieris rapae*, with a mean dry weight of 20mg, consumed one sixth of the biomass which another herb feeding lepidopteran *Papilio polyxenes*, with a mean dry weight of 120mg consumed to ensure a similar relative growth rate, assuming equal efficiencies. Therefore another future study could compare the growth rate and quantity of vegetation consumed between different sized species in the same instar *e.g. T. paludosa* and *T. rufina*.

Chapter 4. Food choice experiments on *Tipula subnodicornis* and *Tipula confusa*

4.1. Introduction

Few food preference experiments have been described in the literature on invertebrate animals and fewer still on the moss-feeding invertebrates (Lawrey 1987). The experiments involving invertebrates have in most cases used slugs or mites (*e.g.* Davidson and Longton (1987) and Gerson (1972)). Food plant selection by herbivores is considered to be of major importance in relation to plant selection in natural ecosystems (Molgaard 1986, Grime 1979). In this study, food choice experiments were carried out using fourth instar larvae of *Tipula subnodicornis* and *Tipula confusa* to determine if preferences for particular moss species existed, and whether palatability of a moss species could be predicted. Both these cranefly species are known to be moss-feeders (Coulson 1962, Freeman 1967) and their preferences were tested using the same ten moss species which were taken from moorland and woodland habitats.

The habitats of the moss species are described in Section 4.2, in relation to the distribution of the two tipulid species. *T. subnodicornis* is characteristically a species associated with semi-aquatic mosses *e.g. Sphagnum* spp. and *Hypnum* spp. on moorland (Brindle 1960) but has also been found associated with liverworts (Coulson 1962) and higher plants *e.g. Eriophorum vaginatum* (in this study). It is found almost exclusively on upland moorland and bog habitats - but has been found in the low altitude New Forest bogs (Freeman 1967). *Tipula confusa* is a species associated with much drier habitats *e.g.* its larvae are found in *Brachythecium rutabulum* on walls and buildings as well as being associated with *Campylopus introflexus*, an introduced species, and other mosses on moorland. It is found in both woodland and moorland habitats. This study

aimed at determining if the distribution of the two *Tipula* species reflected preferences for particular moss species as food.

An additional experiment was carried out, using *Tipula subnodicornis*, to investigate whether feeding preferences existed in this species for the three main plant species found at a site on Chapel Fell Co. Durham, (NY 899315) which contained the highest densities of *T. subnodicornis* larvae. The plant species involved were the sedge 'cotton grass' *Eriophorum vaginatum*, and two bryophytes, *Campylopus paradoxus* and *Sphagnum papillosum*. In addition to feeding on mosses at this field site, *Tipula subnodicornis* was found in close association with *Eriophorum vaginatum*, as well as the two moss species and so all three species were considered to be potential food plants. The general impression has been formed that most moss-feeding *Tipula* species do not feed on higher plants and it was appropriate to determine what proportion of the diet of *Tipula subnodicornis* was *Eriophorum vaginatum*, and if a preference was shown for the sedge.

Tipula subnodicornis was also used in three preliminary experiments to test if:

i) the compaction of moss species or

ii) light and dark

affected preferences in laboratory experiments; and

iii) to determine the frequency that larvae changed to different moss species in choice experiments under laboratory conditions.

Throughout this Chapter, *Tipula subnodicornis* and *Tipula confusa* will be referred to as *T. subnodicornis* and *T. confusa* respectively whilst, to avoid confusion, the food plants used in the experiment will be referred to by their full scientific names.

4.2. Description and habitats of the moss species used in the experiments.

4.2.1 Moorland mosses

The moorland moss species that were used in these experiments were collected from Chapel Fell, a blanket bog in the northern Pennines. The descriptions and habitats stated first in the information and in quotation marks on each species are from Smith (1978) and Watson (1968). The authorities are taken from Smith (1978).

1. *Campylopus paradoxus* (Wils.) is 'a tuft-forming moss and is most commonly found on bare peat'. In this study it was found in association with *Sphagnum papillosum* (Lindb.) and *Eriophorum vaginatum* on fully vegetated peat.

2. *Dicranum scoparium* (Hedw.) is 'found in patches and is generally a species of a variety of habitats: as well as being found on moorland, it occurs in woodlands, grasslands and on sand dunes - most commonly being found on acidic or leached soils'. On Chapel Fell, it was found amongst *Sphagnum* spp. and *Eriophorum vaginatum.*

3. *Sphagnum papillosum* (Lindb.), 'a tussock forming moss, prefers strongly acidic habitats and is known to be the main hummock-former in all major kinds of bogs and prefers strongly acidic habitats'. This species was collected mainly from amongst *Eriophorum vaginatum* on Chapel Fell.

4. *Sphagnum recurvum* (Rush. Warns.), a 'lawn-forming moss species, is generally found at the margins of pools in moorland or in ditches in woodland, in moderately acidic areas'. In this study it was taken from waterlogged areas dominated by *Juncus effusus* and around pools in the blanket bog on Chapel Fell.

5. *Hypnum cupressiforme* (Hedw.) occurs 'as patches in a wide variety of habitats: from grassy heathlands and woodlands to open moorlands, and can grow on both calcareous and acidic soils in sheltered or exposed habitats'. This species was collected from sheltered areas of the Chapel Fell blanket bog.

6. *Polytrichum commune* (Hedw.) is 'a species which forms dark green tufts, which can be of considerable size and is abundant on wet highly acidic moorland and its

presence is indicative of strongly acidic soils'. It is the largest British moss species and the leaves are relatively large and possess a thick, impenetrable cuticle. It was collected from large swards on Chapel Fell.

4.2.2. Woodland mosses

The woodland moss species which were used in these series of experiments were collected from Hollinside Wood, Durham (NZ 277408). The descriptions and habitat stated first in the information and in quotation marks on each species are again from Smith (1978) and Watson (1968).

1. *Brachythecium rutabulum* (Hedw.), generally 'a ubiquitous species which can be found on rocks, by streams, as well as in most woodlands, and forms green patches on its substratum' It was obtained from bare ground in the woodland.

2. *Dicranella heteromalla* (Hedw.) is 'a moss species generally forming yellowish-green patches which can be extensive, on acidic or neutral soils in hedgerows, ditches and woods'. In this study it was collected from around the roots of beech *Fagus sylvatica*.

3. *Mnium hornum* (Hedw.) 'is a moss of acidic soils, which forms dark green tufts on its substratum, and can be found on bark, tree stumps and in woods generally'. It was collected from bare ground in Hollinside Wood.

4. *Eurhynchium praelongum* (Hedw.) 'is a moss species which is extremely shade tolerant, and is most abundantly found in hedgerows and woods'. It was collected beneath sycamore *Acer pseudoplatanus*.

4.3. Methods for studying food preferences

Two main methods were used to study the food preferences of *T. subnodicornis* and *T. confusa*:

i) Determination of the choice by larvae for a particular plant species by recording their presence on a selection of food plants in a choice experiment, using a series of instantaneous 'spot' observations, (Hartley 1953, Altmann 1974).

ii) An analysis of the food particles in the frass produced by larvae which had been allowed to feed on a range of plant materials in the choice experiments.

Method (ii) is a direct method evaluating the selection of food. Its main disadvantages were that it was excessively time consuming and it required a considerable degree of experience in identifying food particles within the frass as belonging to a particular plant species. Accordingly two sets of initial studies were made:

1) To demonstrate that methods i) and ii) gave comparable results.

2) Three preliminary experiments were carried out to ensure that in i) consistent methods were used and that:

a) the effect of compacting did not affect the choices made,

b) light did not affect the choices made, and

c) the mobility of the larvae was sufficient to obtain a series of independent results from the same group of larvae.

4.3.1. Method for the choice experiments

4.3.1.1. Larval availability and collection

Restraints were imposed by larval availability of *T. subnodicornis*, and these were caused by the population 'crash' in 1989 (Coulson, unpublished data) as larvae were relatively uncommon. The site with the highest larval density on Chapel Fell, at about 600m, of 142 larvae per m^2 was inaccessible in the winter months and larvae had to be collected between October and mid-December 1990 and in March and April 1991 when the weather was suitable, to enable the use of fourth instar larvae before pupation in early May. The experiments for *T. confusa* were run after those for *T. subnodicornis*, because of demands on time and the differences in its life-cycle. Larvae of *T. confusa* were collected in May and June 1991, and experiments were carried out on fourth instars which had not become quiescent on entering a pre-pupating diapause. Pupation occurred between mid-July and mid-August.

The fourth instar larvae of *T. confusa* used for the food choice experiments were collected from Waskerley Common (Grid Ref. NY 025455). The larvae were found on sites which had been burnt in the last few years and were dominated by short swards of *Campylopus introflexus*, a moss species which was introduced into Britain in 1941.

To obtain larvae, vegetation and soil samples were collected from the field, and were heat extracted in the laboratory using Berlese funnels (Southwood 1971). The extraction of larvae took 7 to 14 days to complete.

4.3.1.2. Justification of the experimental methods

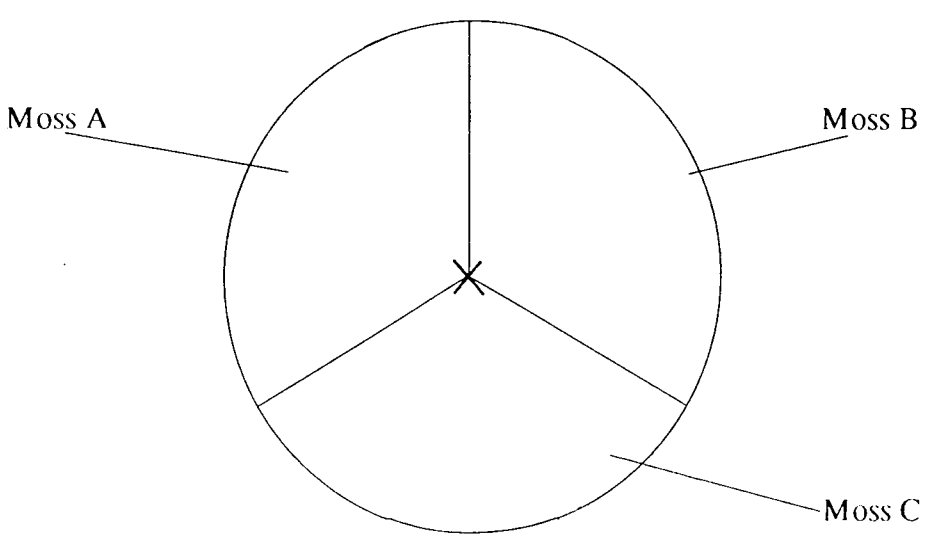
The food choice experiments were set up in a similar manner to the feeding experiments carried out on slugs and snails and other invertebrates by other researchers: Broadhead (1958); Grime *et al.*, (1968); Pallant (1969); Cates and Orians (1975); Molgaard (1986); Davidson and Longton (1987) and Davidson *et al.* (1990).

The food preference experiments were undertaken using a simultaneous choice

of three mosses for a single larva and replicated in each of ten petri-dishes per experiment, (rather than the more usual choice between pairs of moss species), and observing the position of the fourth instar larvae through time. As fourth instar larvae develop rapidly towards pupation, the time available for such studies was limited to less than two months. To increase the information obtained in the time available, it was decided to carry out the choice experiments using three plant species at a time rather than the more traditional choice of two plant species. This maintained an interface between each of the three moss species offered in each experiment. A choice of more than three mosses would not allow such interfaces between each pair of moss species.

4.3.1.3. Experimental details.

In the experiments moss species and larvae were kept in 9cm diameter petri-dishes, each containing a single sheet of Whatman 9 cm filter paper, which was kept damp to prevent desiccation of the mosses and the experimental animal. The three moss species to be used in the experiment were added to each petri-dish in equal proportions, and, as far as possible, with equal compaction. The mosses were arranged so that each edge abutted the other two mosses. Only one larva was used in each dish. A diagrammatic representation of the arrangement is shown in Fig. 4.1.



The larvae were kept at 10°C from the date of extraction from the Berlese funnels, and in all experiments. The animal was placed in the centre of the three mosses (at the point X on Fig. 4.1) and left without disturbance for one day. Observations were made twice daily to give the larvae ample time to make a new selection after the previous spot observation. This procedure was continued for eleven days for each experiment in order to obtain an total of approximately 220 observations.

4.3.1.4. Method for the calculation of the results

The percentage of observations on each bryophyte species in each experiment was determined. For each bryophyte species, a mean percentage of observations was then calculated. A mean percentage of observations of 33% on a moss species meant there was no selection for that species. Likewise, a mean percentage significantly above 33% indicated there was a preference for that moss species and a mean percentage significantly below 33% demonstrated that species was avoided. For each species of *Tipula*, t-tests were carried out to determine which moss species were significantly preferred or avoided. Further, a hierarchical preference for the use of the ten bryophyte species used as food plants was determined for both *T subnodicornis* and *T. confusa*.

4.3.1.5. Food choice experiment between *Eriophorum vaginatum*, *Campylopus paradoxus* and *Sphagnum papillosum*

The same procedure as described above was used for the experiments involving *Campylopus paradoxus*, *Sphagnum papillosum* and the aerial part *Eriophorum vaginatum*. Only *T. subnodicornis* was used in this experiment as it was to test the preference of this species for potential food plant species found on the field site supporting the highest densities of *T. subnodicornis*. The experiment was carried out between November 1990 and January 1991. Three aspects of food choice preferences

were investigated:

i) which plant species was preferred overall,

ii) to determine if the time of year, and hence the developmental stage of the larvae, influenced any preference shown, considering the larvae collectively and individually, when the experiment was divided into three time periods, and

iii) were all larvae homogeneous in the proportion of time they spend on each food plant.

4.3.2. Method for analysis of the frass, to compare with the spot observation method

The ten larvae from six of the choice experiments were removed after 5 days and placed separately on damp tissue paper for a minimum of 24 hours. The *T. subnodicornis* larvae were from experiments 2, 4, 9, 10, 11 and 14 and the *T. confusa* larvae were from experiments 6, 7, 8, 9, 10, and 19. The frass produced from each larva was spread on slides and, from the cell structure, the particles in the frass were identified and the numbers, and hence the proportions, of the particles of each food plant were determined. At intervals throughout each experiment further samples of the frass were collected and examined. The total number and proportion of particles of each moss species in the frass, for each of the experiments investigated, was also determined. The mean percentage of particles (± standard error) for each moss species found in the frass of the ten larvae was calculated.

An investigation was made to justify the use of the observational method to determine preferences. To achieve this, the spot observation method and the frass analysis method were compared.

For each moss species, the mean percentage of particles found in the frass was compared with the mean percentage of observations on the moss species throughout the whole experiment.

In the comparison of the two methods, it was found that the results obtained from measures of the time spent on individual moss species agreed closely with the results obtained from the frass analysis, (see Section 4.4.3.) Accordingly, this second method was employed more extensively.

4.3.2.1. Experiments using choices of single or mixed food plants

As a check on the possibility that some larvae are attracted to certain plant species because of a thigmotaxic response, a further set of experiments was designed and carried out using *T. subnodicornis* and three of its potential food plants: *Eriophorum vaginatum*, *Campylopus paradoxus* and *Sphagnum papillosum*. These experiments were carried out to determine any differences in the proportions of spot observations on food plants when they were offered, in petri-dishes, either singly or mixed in equal quantities with another food plant or, as in one experiment, when two mixtures of food plants were offered. Details are given in Table 4.10, and each experiment comprised ten replicates and were carried out in December 1990 and January 1991. The numbers of observations on either single or mixed food plants were determined, by the method described in Section 4.3.1. The methods of frass collection and examination have been described in Section 4.3.2 and the number of particles of the food plants in the frass were determined.

4.3.3. Methods for the three preliminary experiments

4.3.3.1 Compaction experiment

The aim of this investigation was to determine if the compaction of the mosses used in the experiments had an influence on the choice made by a larva. The experiment used *T. subnodicornis* and *Sphagnum papillosum*. This moss species was a suitable

substrate for this study as it could readily be presented in three levels of compacting which were i) 'normal' moss - *i.e.* the form in which the moss is found in the field, ii) 'loose' moss - i.e. in a less compact form than that found in the field and iii) moss which had been compacted to about twice its natural density. One larva was placed in each of ten petri-dishes, each with *Sphagnum papillosum* of the three different compactions occupying one third of the petri-dish. The experiment was carried out using the same procedures as in the other food choice experiments described in Section 4.3.1.

4.3.3.2. Effect of illumination and darkness on food choices

The choices made by larvae in the light and in the dark were investigated, using *T. subnodicornis* and three of its known food plants: *Eriophorum vaginatum, Campylopus paradoxus* and *Sphagnum papillosum.* The preferences of the larvae for the food plants, in the 12h light and in the 12h dark regime, were determined by placing ten larvae in separate petri-dishes, with equal quantities of the three food plants. The position of each larva were checked four times in every 24 hour period, twice when the petri-dishes were illuminated and twice when the petri-dishes were in darkness, for 20 days.

4.3.3.3. Mobility of the larvae in multiple food choice petri-dishes

To test if larvae were making repeated choices through a period of time, and not remaining on one food plant, the following experiment was carried out. One larva of *T. subnodicornis* was placed in each of ten petri-dishes, containing equal quantities of *Sphagnum papillosum* and *Campylopus paradoxus.* The position of each larva was checked at two-hourly intervals for a period of 24 hours, and the mean number of movements across the interface between the two food plants by each of the ten larvae was determined, over both a 12 hour and a 24 hour period.

4.4. Results

4.4.1. Results of the preliminary experiments

4.4.1.1. Compaction experiment

The results for the *Sphagnum papillosum* compaction experiment, using *T. subnodicornis* are presented in Table 4.1. A total of 254 observations were made. There was a significant difference in the preference shown by the larvae between the three densities of the food plant offered (X^2=46.1 df=2 $p<0.001$) but this was mainly caused by an avoidance of the 'low density' option and there was no significant difference between the proportions found on the compacted moss and the normal moss, (X^2=2.4 df=1 NS). Additional studies with other mosses led to the same conclusion, namely that the normal compaction of the moss was the most appropriate form of the plant to use in the choice studies.

4.4.1.2. Effect of illumination and darkness on food choices

A total of 391 and 397 spot observations were made in the light and dark respectively when kept under a diurnal light regime. It is evident from Table 4.2 that the food preference of the larvae of *T. subnodicornis* did not differ significantly during the part of the day that the containers were illuminated or were in the dark (X^2=1.3 df=2, NS). When the food preferences were considered separately in the light and in the dark, there were no significant differences found throughout the illuminated or dark periods. Accordingly, illumination was not considered to be an important variable.

4.4.1.3. Mobility of the larvae in multiple food choice petri-dishes

The results of the mobility experiments on *T. subnodicornis* larvae between *Campylopus paradoxus* and *Sphagnum papillosum* are presented in Table 4.3. A mean

Table 4.1. Distribution of larvae of *Tipula subnodicornis* when given a choice of equal quantities of *Sphagnum papillosum* of three different compactions.

Numbers of observations of larvae.		
Compact	Normal	Loose
120 (47%)	97 (38%)	37 (14%)

$X^2=46.1$ df=2

$p<0.001$

Table 4.2. Distribution of larvae of *Tipula subnodicornis* in the light and in the dark, when given a choice of equal quantities of three food plants.

	Numbers of observations on each food plant		
	Eriophorum vaginatum	*Campylopus paradoxus*	*Sphagnum papillosum*
Light	159 (41%)	113 (29%)	119 (30%)
Dark	146 (37%)	125 (31%)	126 (32%)

$X^2=1.3$ df=2

NS

Table 4.3. The number of movements made between two food plants, *Campylopus paradoxus* and *Sphagnum papillosum*, by *Tipula subnodicornis* over a period of 12 hours and 24 hours.

Larva	Number of movements made between the two food plants	
	in 12 hours	in 24 hours
1	3	5
2	1	1
3	1	1
4	3	3
5	3	5
6	1	3
7	3	4
8	3	5
9	2	3
10	3	4
	x=2.30 ±0.30 (S.E.)	x=3.40 ±0.48 (S.E.)

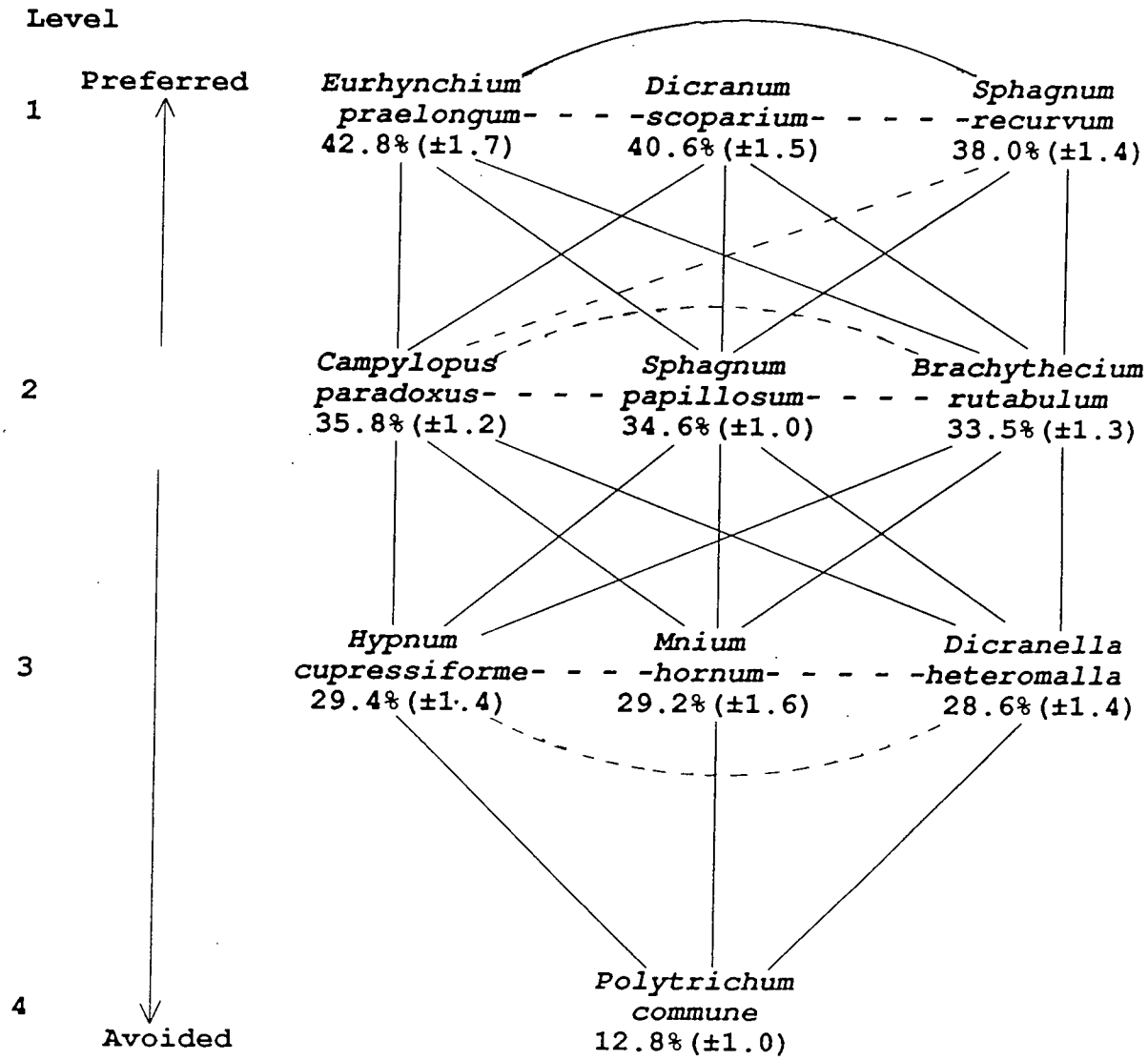
of 2.30±0.30 (S.E.) movements between the two moss species were recorded in a 12h period, from a total of 60 observations, and a mean of 3.40±0.48 (S.E.) movements were recorded in a 24h period from a total of 120 observations. Thus twice-daily observations are likely to reflect the selection and re-selection of a food plant and the larvae are not remaining throughout the 12h or 24h period in the same sector of the petri-dish. Accordingly, it was considered appropriate to consider each observation on a larvae at 12 hour intervals to be independent for statistical analysis.

4.4.2. Results of food choice experiments

The hierarchical preferences are presented in Figs. 4.2 and 4.3 for *T. subnodicornis* and *T. confusa*, respectively, with significant deviations from 33% indicating preference or avoidance. In *T. confusa* seven levels of preference/avoidance and in *T. subnodicornis* four levels of preference/avoidance were detected. Each level of preference/avoidance was determined by those moss species having mean percentage of observations which were not significantly different from each other, but were significantly different from the mean percentage of observations on moss species in the levels immediately above and below. Using the range of mean percentage of observations on the moss species as a measure of variation in preference, it is evident that it is much greater in *T. confusa* (ranging from 70% to 4%), than in *T. subnodicornis* (ranging from 43% to 13%).

In *T. subnodicornis*, the greatest preference was for *Eurhynchium praelongum*, (42.8±1.7% (S.E.) of observations), but this was not significantly higher than that for *Dicranum scoparium*, (40.6±1.5% (S.E.)) which, in turn, was not significantly higher than *Sphagnum recurvum* (38.0±1.4% (S.E.)). However the mean percentage of observations on *Eurhynchium praelongum* was significantly higher than that on *Sphagnum recurvum*. For *T. confusa*, *Dicranella heteromalla* was significantly the most preferred moss species (70.0±1.4% (S.E.) of observations), *Brachythecium*

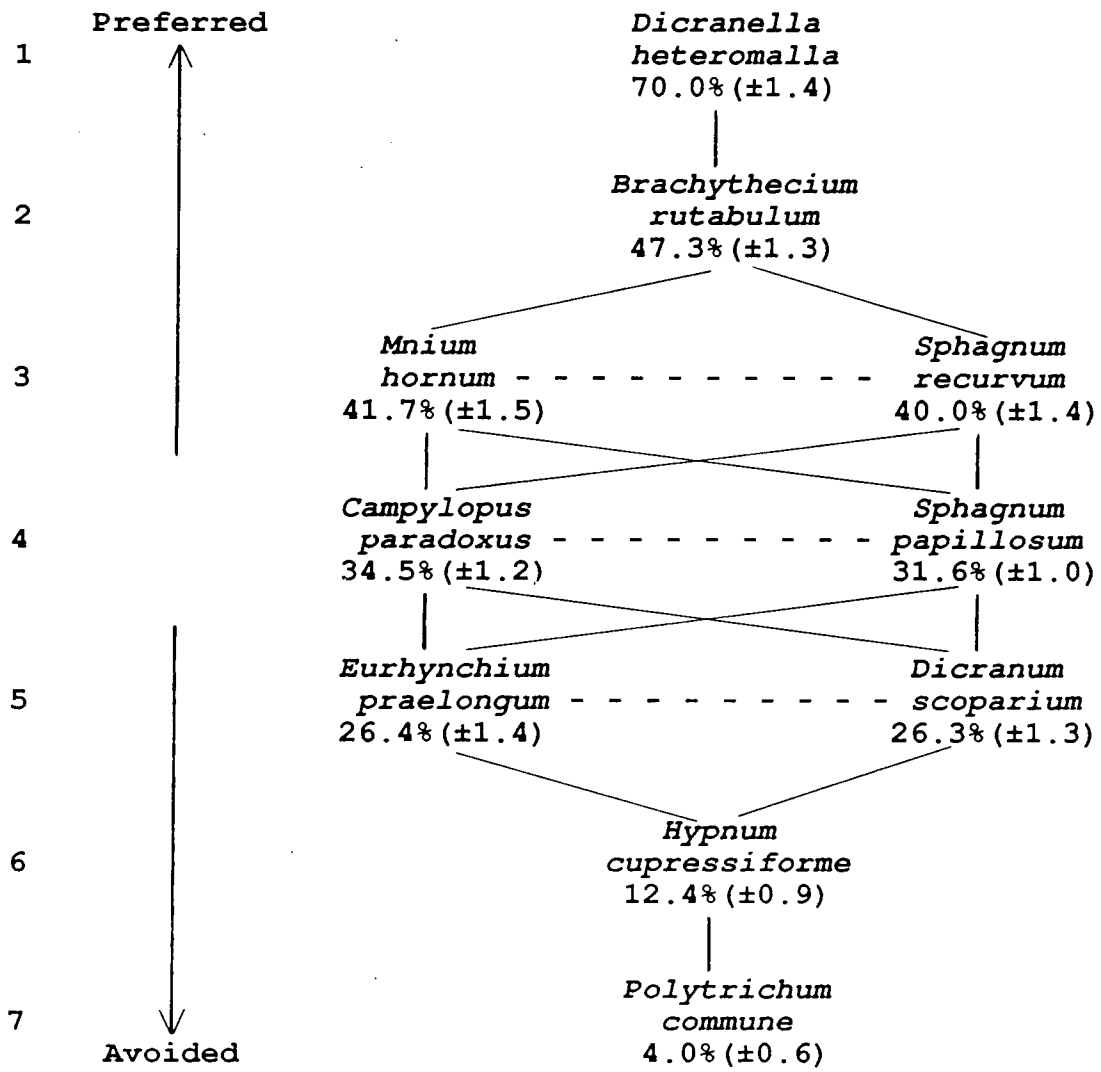
Fig 4.2. *Tipula subnodicornis*. Hierarchical preference of moss species as food plants with the mean percentage of observations (± S.E.).



——— indicates those pairs of moss species in which the mean % observations which are significantly different, using t-tests, at $p<0.05$.

- - - - indicates those pairs of moss species in which the mean % observations are not significantly different.

Fig 4.3. *Tipula confusa*. Hierarchical preference of moss species as food plants with the mean percentage of observations (± S.E.).



———— indicates those pairs of moss species in which the mean % observations which are significantly different, using t-tests, at $p<0.05$.

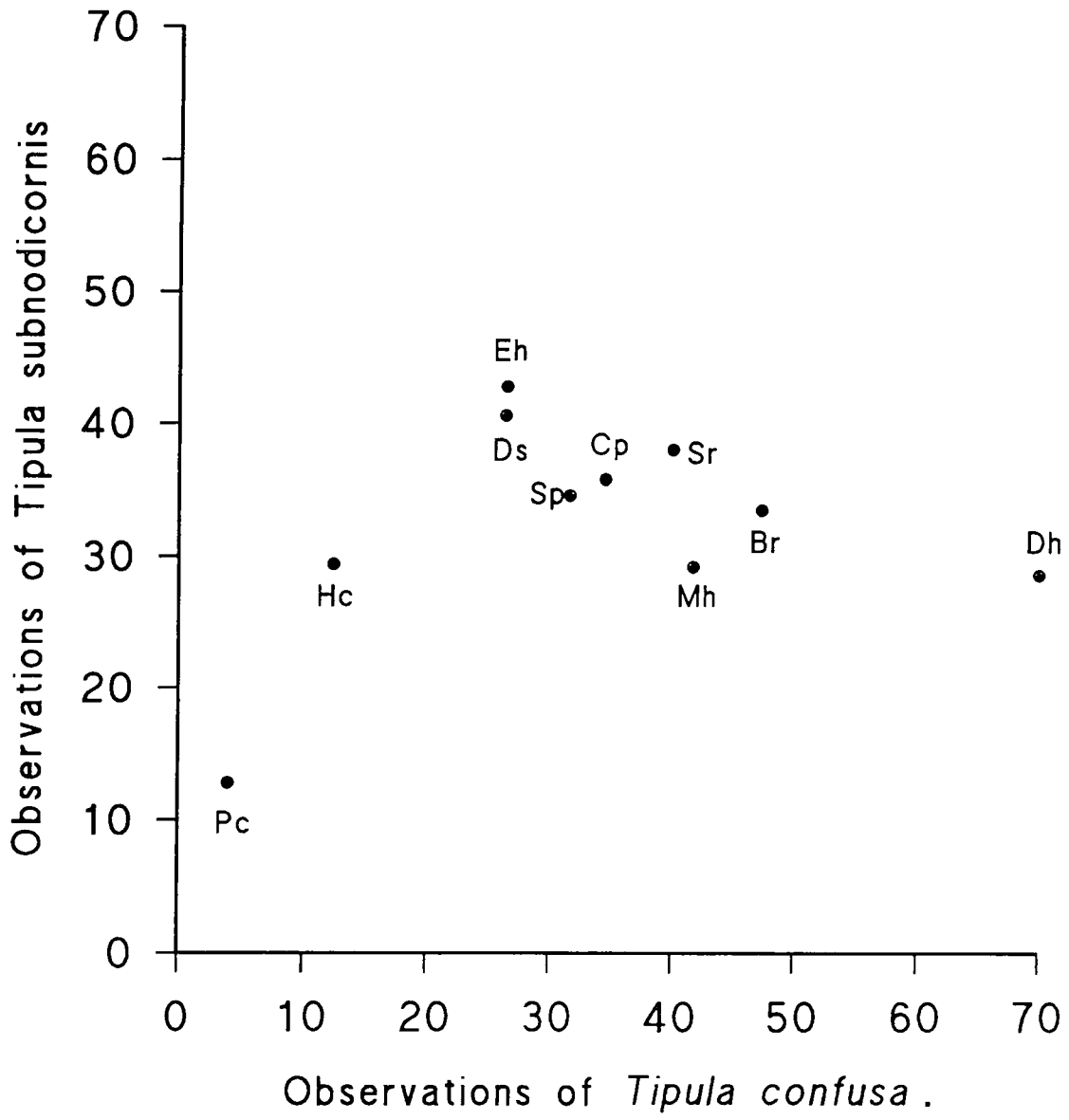
- - - - indicates those pairs of moss species in which the mean % observations are not significantly different.

Fig. 4.4

The relationship between the mean percentage of observations of *Tipula subnodicornis* against those of *Tipula confusa* on each moss species.

The correlation is not significant: $r_8 = 0.28$.

Cp = *Campylopus paradoxus*, Ds = *Dicranum scoparium*, Sp = *Sphagnum papillosum*, Sr = *Sphagnum recurvum*, Hc = *Hypnum cupressiforme*, Pc = *Polytrichum commune*, Br = *Brachythecium rutabulum*, Dh = *Dicranella heteromalla*, Mh = *Mnium hornum* and Eh = *Eurhynchium praelongum*.



rutabulum, another woodland species, was second (47.3±1.3% (S.E.)), and both a woodland species, *Mnium hornum* (41.7±1.5% (S.E.)), and a moorland species, *Sphagnum recurvum* (40.0±1.4% (S.E.)) were third.

In *T. subnodicornis*, levels 2 and 3 have three moss species in each, with the mean percentage observations not significantly different within each level but are significantly different from those in the levels above and below, (apart from *C. paradoxus*, in level 2). Again, in *T. confusa* the mean percentage of observations on moss species in levels 4, 5 and 6 are not significantly different within the levels but are between levels. In both *Tipula* species, *Polytrichum commune* is the least preferred moss species, and is significantly less preferred than any other moss species studied.

Fig. 4.4 presents a correlation of the mean percentage of observations of *T. subnodicornis* against those of *T. confusa* on each moss species used in the choice experiments. There is no overall relationship between the moss preferences of *T. subnodicornis* and those of *T. confusa* (r^2=0.08 NS). *Polytrichum commune* is the only moss species which is avoided by both species of *Tipula* and the highest preference shown is for *Dicranella heteromalla* by *T. confusa*. The horizontal axis (showing the preferences of *T. confusa*), is much more extended than the vertical axis (showing the preferences of *T. subnodicornis*), indicating again that the range of preferences is much greater in *T. confusa* than in *T. subnodicornis*.

Table 4.4 presents the results of a strict comparison between choice experiments carried out on *T. subnodicornis* and *T. confusa*. For both *Tipula* species the preferences shown by the larvae for the moss species are significantly different in each experiment, apart from in Experiment 9.

In *T. confusa* the moss species preference agrees with the overall hierarchical order in Experiments 2, 4 and 11 whereas in *T. subnodicornis*, in only Experiments 1 and 3 does the moss species preference agree with the overall hierarchical order. In each experiment, variations occur in the preferences for the moss species between the two *Tipula* species. The X^2 values are given for comparisons between the number of observations of *T. confusa* and *T. subnodicornis* on each moss species in each

Table 4.4. The numbers of observations of *Tipula confusa* and *Tipula subnodicornis* on moss species in identical food choice experiments. (i) X^2 has been used to test whether each tipulid species in each experiment showed a significant preference or avoidance between the three moss species offered. In addition, (ii) X^2 has been made comparing the proportons of the three mosses taken by the two *Tipula* species.

Experiment	Species	Moss species used in experiments with number of observations			n	X^2 (i) df=2 for preference	X^2 (ii) df=18 for homogeneity
1		Sp	Pc	Br			
	T. confusa	101 (46%)	16 (7%)	100 (46%)	217	65.8 ***	9.4 **
	T. subnodicornis	92 (41%)	38 (17%)	95 (42%)	225	27.4 ***	
2		Pc	Hc	Mh			
	T. confusa	13 (6%)	44 (21%)	158 (74%)	215	162.6 ***	32.2 ***
	T. subnodicornis	41 (18%)	76 (34%)	108 (48%)	225	29.9 ***	

\contd.

Table 4.4. (Contd.)

Experiment	Species	Moss species used in experiments with number of observations			n	X^2 (i) df=2 for preference	X^2 (ii) df=18 for homogeneity
3		Sp	Sr	Ep			
	T. confusa	66 (31%)	59 (27%)	91 (42%)	216	7.9 *	3.1 NS
	T. subnodicornis	51 (23%)	67 (30%)	103 (47%)	221	19.2 **	
4		Hc	Br	Dh			
	T. confusa	19 (10%)	64 (32%)	117 (58%)	200	72.2 ***	10.0 **
	T. subnodicornis	41 (18%)	81 (36%)	101 (45%)	223	25.1 ***	

\contd.

Table 4.4. (Cont.)

Experiment	Species	Moss species used in experiments with number of observations			n	X^2 (i) df=2 for preference	X^2 (ii) df=18 for homogeneity
9		Cp	Ds	Sp			
	T. confusa	70 (31%)	75 (33%)	81 (36%)	226	0.8 NS	0.2 NS
	T. subnodicornis	63 (33%)	60 (31%)	68 (36%)	191	0.5 NS	
11		Dh	Mh	Ep			
	T. confusa	189 (86%)	20 (9%)	11 (5%)	220	274.3 ***	113.5 ***
	T. subnodicornis	75 (36%)	52 (25%)	78 (38%)	205	6.0 *	

Footnote:

Cp = *Campylopus paradoxus*
Ds = *Dicranum scoparium*
Sp = *Sphagnum papillosum*
Sr = *Sphagnum recurvum*
Pc = *Polytrichum commune*
Hc = *Hypnum cupressiforme*
Br = *Brachythecium rutabulum*
Dh = *Dicranella heteromalla*
Mh = *Mnium hornum*
Ep = *Eurhynchium praelongum*

NS $p>0.05$ * $p<0.05$ ** $p<0.01$ *** $p<0.001$

experiment. In only Experiments 3 and 9 are the proportions of the observations of the two *Tipula* species not significantly different.

4.4.2.1. Food choice experiment between *Eriophorum vaginatum*, *Campylopus paradoxus* and *Sphagnum papillosum*

The results of this experiment are presented on Tables 5, 6, and 7. Considering the preferences for the food plants over the whole experiment which compares *Eriophorum vaginatum*, *Campylopus paradoxus* and *Sphagnum papillosum*, *T. subnodicornis* preferred the sedge *Eriophorum vaginatum*, to the moss species *Campylopus paradoxus* and *Sphagnum papillosum*, X^2=10.3 df=2 p<0.01, (see Table 4.5). When the results are divided into the three time periods, shown on Table 4.6, the preferences of *T. subnodicornis* for the moss species altered over time. For the first two periods (*i.e.* from 12-24 November and 25 November to 9 December 1990 respectively) the preferences follow the overall pattern *i.e. Eriophorum vaginatum* was the preferred plant species. In the third period; from 10 December 1990 to 9 January 1991, this preference changed significantly, and *Sphagnum papillosum* became the preferred moss species (X^2=7.5 df=2 p<0.05) and there was no significant difference between the preference for *Eriophorum vaginatum* and *Campylopus paradoxus* (X^2=0.85 df=2 NS).

The individual preferences of the ten larvae throughout the experiment (Table 4.7) were heterogeneous (X^2=36.6 df=18 p<0.01).

4.4.3. Analysis of moss particles in frass and the comparison between the spot observation and frass analysis methods

Table 4.8 gives the number and proportions of food particles in the frass of *T. subnodicornis* larvae from food choice experiments 2, 4, 9, 10, 11 and 14, and likewise, Table 4.9 gives the total number and proportions of food particles in the frass

Table 4.5. Distribution of larvae of *Tipula subnodicornis* when given choice of equal quantities of *Eriophorum vaginatum*, *Campylopus paradoxus* and *Sphagnum papillosum*

Numbers of observations of larvae on each food plant

Eriophorum vaginatum	*Campylopus paradoxus*	*Sphagnum papillosum*
305 (39%)	238 (30%)	245 (31%)

$X^2=10.3$ df=2

$p<0.01$

Table 4.6. Distribution of larvae of *Tipula subnodicornis*, using the spot observation method, when given a choice of equal quantities of three food plants: comparing proportions of the number of records by testing, in each time period, for homogeneity and the assumption that the proportions of observations on the three food plants are 33%.

	Food plant				
	Eriophorum vaginatum	*Campylopus paradoxus*	*Sphagnum papillosum*	n	x^2
Period					
1	113 (43%)	81 (31%)	70 (26%)	264	11.3**
2	113 (47%)	66 (28%)	59 (25%)	238	21.8**
3	79 (27%)	91 (32%)	116 (41%)	286	7.5 *
			x^2=28.8 ***	df=4	

Footnote: Period 1 = 12 November 1990 to 24 November 1990
Period 2 = 25 November 1990 to 9 December 1990
Period 3 = 10 December 1990 to 9 January 1991

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table 4.7. Distribution of 10 individual larvae of *Tipula subnodicornis*, with n number of observations, when given a choice of three food plants.

	Food plant				
Larvae	*Eriophorum vaginatum*	*Campylopus paradoxus*	*Sphagnum papillosum*	n	X^2 df=2
1	36	22	22	80	1.4 NS
2	26	23	33	82	3.4 NS
3	29	30	21	80	2.2 NS
4	43	14	22	79	9.0 **
5	39	25	18	82	3.7 NS
6	28	33	19	80	5.0 NS
7	31	27	25	83	0.2 NS
8	24	28	25	77	2.3 NS
9	24	12	28	64	6.1 *
10	25	24	32	81	3.3 NS

X^2=36.6 ** df=18

NS p>0.05 *p<0.05 **p<0.01 ***p<0.001

Table 4.8. The numbers of particles of each moss species, used as food plants, in the frass of fourth instar *Tipula subnodicornis* obtained from six food choice experiments.

Experiment	Number of particles of moss species in the frass (% given in bold)			n	X^2 df=2 for preference
2	Pc	Hc	Mh		
	17	63	30	110	30.6 ***
	(15%)	(57%)	(27%)		
4	Hc	Br	Dh		
	39	33	44	116	1.6 NS
	(34%)	(28%)	(37%)		
9	Cp	Ds	Sp		
	46	116	29	191	66.8 ***
	(24%)	(61%)	(15%)		
10	Pc	Ds	Dh		
	2	105	46	153	104.7 ***
	(1%)	(69%)	(30%)		
11	Ep	Mh	Dh		
	158	100	85	343	26.0 ***
	(46%)	(29%)	(25%)		
14	Ds	Sr	Br		
	80	58	20	158	35.0 ***
	(50%)	(37%)	(13%)		

Footnote:

Cp = *Campylopus paradoxus*
Ds = *Dicranum scoparium*
Sp = *Sphagnum papillosum*
Sr = *Sphagnum recurvum*
Pc = *Polytrichum commune*
Hc = *Hypnum cupressiforme*
Br = *Brachythecium rutabulum*
Dh = *Dicranella heteromalla*
Mh = *Mnium hornum*
Ep = *Eurhynchium praelongum*

NS $p>0.5$ *** $p<0.001$

Table 4.9. The numbers of particles of each moss species, used as food plants, in the frass of fourth instar *Tipula confusa*, obtained from six food choice experiments.

Experiment	Number of particles of moss species in the frass (% given in bold)			n	X^2 df=2 for preference
6	Mh	Sp	Sr		
	81	14	11	106	88.7 ***
	(76%)	(13%)	(10%)		
7	Ds	Mh	Hc		
	16	98	34	148	75.3 ***
	(11%)	(66%)	(23%)		
8	Ep	Pc	Br		
	34	1	155	190	207.8 ***
	(18%)	(0.5%)	(81%)		
9	Hc	Sp	Sr		
	14	24	8	46	8.5 *
	(30%)	(52%)	(17%)		
10	Pc	Ds	Dh		
	1	2	41	44	70.8 ***
	(2%)	(4%)	(93%)		
19	Dh	Sp	Cp		
	351	52	4	370	451.6 ***
	(85%)	(14%)	(1%)		

Footnote:

Cp = *Campylopus paradoxus*
Ds = *Dicranum scoparium*
Sp = *Sphagnum papillosum*
Sr = *Sphagnum recurvum*
Pc = *Polytrichum commune*
Hc = *Hypnum cupressiforme*
Br = *Brachythecium rutabulum*
Dh = *Dicranella heteromalla*
Mh = *Mnium hornum*
Ep = *Eurhynchium praelongum*

* $p<0.5$ *** $p<0.001$

of *T. confusa* larvae from food choice experiments 6, 7, 8, 9, 10 and 19. The preferred moss species of the two *Tipula* species, ascertained by this method in each experiment, has been determined by the X^2 value calculated from the preferences given in Tables 4.8 and 4.9.

The graphs of the mean percentage of particles of each moss species against the mean percentage of observations on the moss species throughout the experiment are presented on Figs. 4.5 and 4.6 for *T. subnodicornis* and *T. confusa* respectively.

For both *Tipula* species, 83% of the variation is explained by the regression equations in Fig.4.5 and 4.6. The slopes of the regression line are not significantly different from 1.0 in either Fig. 4.5 ($t=0.92$ $df=16$) or Fig. 4.6 ($t=0.93$ $df=16$). Thus in *T. subnodicornis* and *T. confusa* the mean percentage of spot observations on a particular moss species can be used as a reliable estimator of the percentage of particles of each moss species consumed by the larvae.

4.4.4. Experiments using choices of single or mixed food plants

The results of these experiments to determine preferences for food plants offered singly or as a mixture and compare spot observations with particles in frass are given in Tables 4.10 and 4.11. Table 4.10 gives the numbers of observations, for the four experiments, using single or mixed food plants. Table 4.11 gives the total number of particles of the food plant species found in the frass in each experiment. The expected values for each moss species for the X^2 tests in this table are calculated from the proportion in which they are present in the petri-dishes.

In Experiment 1, where a food plant (*Eriophorum vaginatum*) is offered singly or mixed with *Sphagnum papillosum*, the numbers of observations for the mixed food plants are significantly higher, ($X^2=18.6$ $df=1$ $p<0.001$), than for the single food plant (Table 4.10). Also, considering the particles in the frass (Table 4.11), there is a significantly greater number of *Eriophorum vaginatum* particles ($X^2=5.3$ $df=1$ $p<0.05$).

Fig. 4.5

The relationship between the mean percentage of moss particles in the frass and the mean percentage of observations on the moss species in *Tipula subnodicornis*. $y = 1.11x + 0.01$, $r_{16} = 0.91$, $p<0.001$.

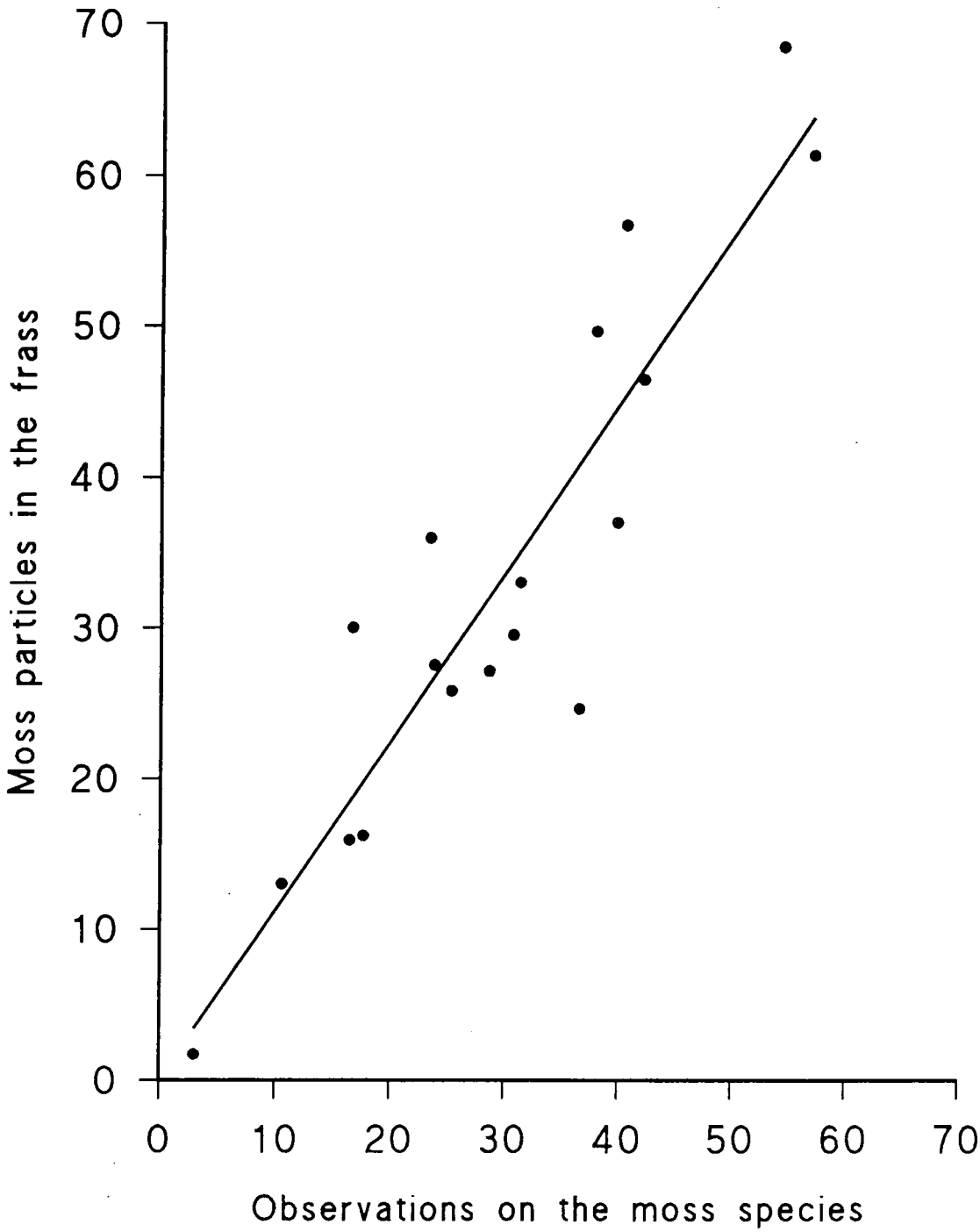


Fig. 4.6

The relationship between the mean percentage of moss particles in the frass and the mean percentage of observations on the moss species in *Tipula confusa*. $y = 1.13x - 3.78$, $r_{16} = 0.90$, $p<0.001$.

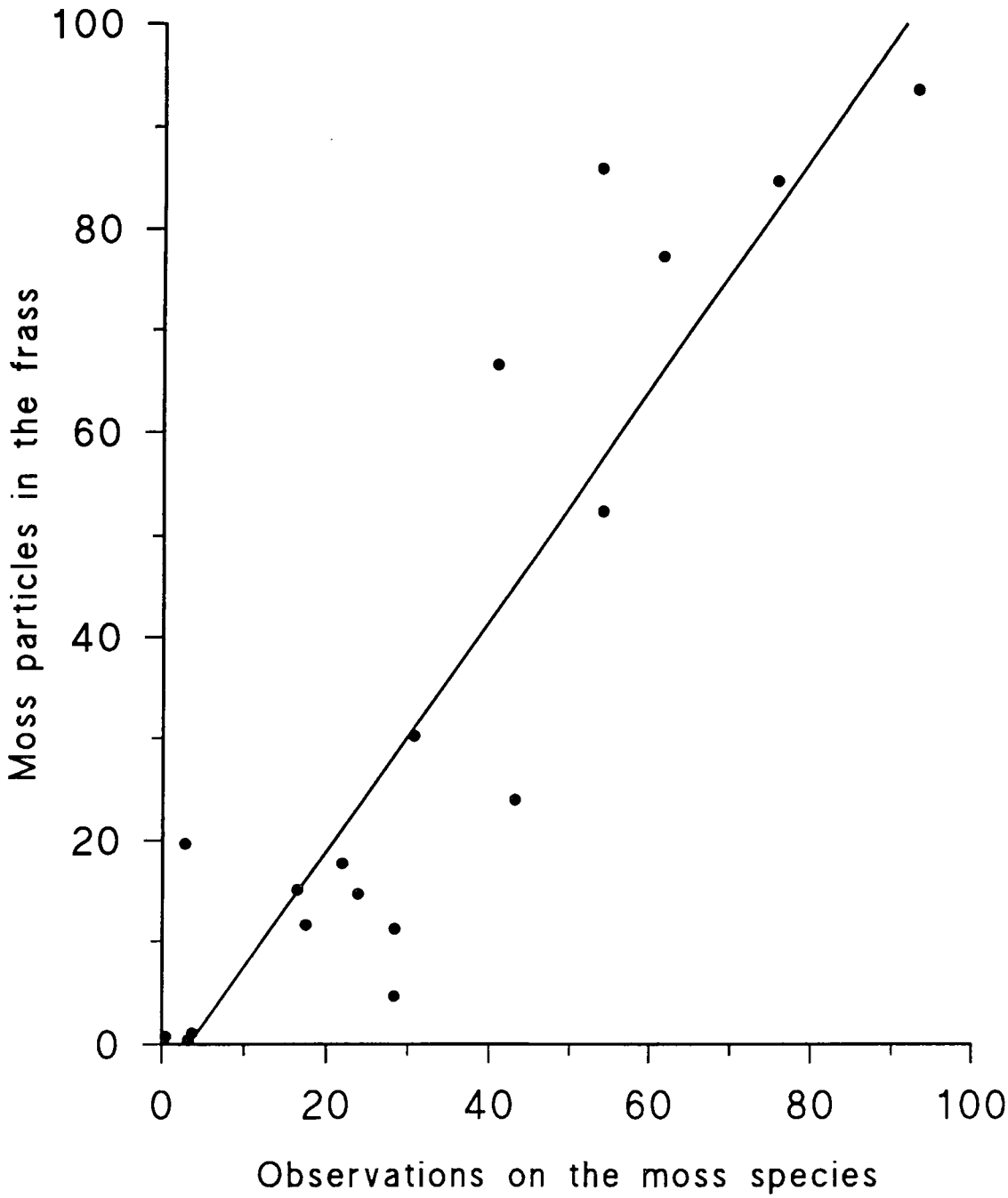


Table 4.10. The numbers of observations of fourth instar larvae of *Tipula subnodicornis* in a series of choice experiments on food plants offered singly, or as a mixture.

Experiment	Moss species used with numbers of observations (% given in bold)		n	X^2 df=1 for preference
1	*Eriophorum vaginatum* 67 (35%)	*Eriophorum vaginatum* and *Sphagnum papillosum* 127 (65%)	194	18.60 ***
2	*Sphagnum papillosum* 97 (33%)	*Sphagnum papillosum* and *Eriophorum vaginatum* 197 (67%)	294	34.01 ***
3	*Sphagnum papillosum* 66 (38%)	*Sphagnum papillosum* and *Campylopus paradoxus* 110 (62%)	176	11.00 ***
4	*Sphagnum papillosum* and *Campylopus paradoxus* 132 (46%)	*Sphagnum papillosum* and *Eriophorum vaginatum* 153 (54%)	285	1.54 NS

NS $p>0.05$ *** $p<0.001$

Table 4.11. The numbers of particles of moss species in the frass of fourth instar larvae of *Tipula subnodicornis* in a series of choice experiments on food plants offered singly, or as a mixture; the numbers given being the total of particles per 10 larvae in each experiment. The expected values for each moss species for the X^2 tests are calculated from the proportion in which they are present in the petri-dishes.

Experiment	Moss species used with numbers of particles in frass (% given in bold)			n	X^2 for preference
1	*Sphagnum papillosum*	*Eriophorum vaginatum*			
	50 (33%)	101 (67%)		151	5.3 * df=1
2	*Sphagnum papillosum*	*Eriophorum vaginatum*			
	147 (24%)	473 (76%)		620	869.8 *** df=1
3	*Sphagnum papillosum*	*Campylopus paradoxus*			
	154 (26%)	430 (76%)		584	736.6 *** df=1
4	*Sphagnum papillosum*	*Campylopus paradoxus*	*Eriophorum vaginatum*		
	181 (17%)	481 (43%)	444 (40%)	1106	502.9 *** df=2

*p<0.05 *** p<0.001

In Experiments 2 and 3, where *Sphagnum papillosum* is offered singly, or as a mixture with either *Eriophorum vaginatum* or *Campylopus paradoxus* respectively, significantly more observations were recorded on the mixtures (Table 4.10). Considering the number of particles in the frass, significantly greater numbers of *Eriophorum vaginatum* and *Campylopus paradoxus* particles were found in Experiments 2 and 3 respectively, (Table 4.11).

In Experiment 4, where there was a choice between either a mixture of *Sphagnum papillosum* and *Campylopus paradoxus* or *Sphagnum papillosum* and *Eriophorum vaginatum*, there was no significant difference between the number of observations on either of these food plant mixtures (Table 4.10). Considering the particles in the frass in Experiment 4 (Table 4.11), there is a highly significant difference between the three food plants represented: *Sphagnum papillosum*, *Campylopus paradoxus* or *Eriophorum vaginatum*, this being the result of a greater number of *Campylopus paradoxus* and *Eriophorum vaginatum* particles in the frass than *Sphagnum papillosum* particles, the number of *Campylopus paradoxus* and *Eriophorum vaginatum* particles not being significantly different (X^2= 1.48 NS).

The results indicate that thigmotaxic responses are occurring with *Sphagnum papillosum*, in that when a favoured food plant (*Eriophorum vaginatum*) is offered in a mixture with *Sphagnum papillosum*, the larvae of *T. subnodicornis* choose this as opposed to *Eriophorum vaginatum* offered singly. The larvae are feeding significantly more on *Eriophorum vaginatum* as verified by the frass analysis.

4.5. Discussion

The hierarchical patterns are very different in the two *Tipula* species studied. *T. subnodicornis*, overall, showed less extensive hierarchical preferences than did *T. confusa*, *i.e.* there is less range of preference/avoidance in *T. subnodicornis* than in

T. confusa. *T. confusa* tended to prefer mosses from a woodland habitat whereas *T. subnodicornis* does not show an overall preference for either moorland or woodland moss species. This could be indicative that *T. confusa* is only secondarily a moorland species and is primarily a species of lower and drier habitats.

Polytrichum commune was the least favoured moss species when offered to both *T. confusa* and *T. subnodicornis*. The midrib in the leaves of this moss species often broadens distally to occupy most of the width of the leaf and the centre of the stem can also thicken - a process which involves polyphenols and encrusting compounds (Longton 1990). These factors probably constitute a strong feeding deterrent on the two *Tipula* species studied here.

In *T. subnodicornis* and *T. confusa*, differences occurred in their preferences for moss species as food plants in the overall hierarchical order. The presence of biflavonoids in cell walls of mosses may function to resist fungal invasion and deter browsing by insects (Geiger 1990), and in their slug study, Davidson *et al.* (1989) suggest that cell wall resistance due to wall bound phenolic compounds may be an important defence against herbivory. It is possible that the different concentrations of biflavonoids and phenolic compounds in the cell walls of bryophytes could deter herbivorous invertebrate species to different extents, and this could account for the differences in preferences found in *T. subnodicornis* and *T. confusa*. This aspect has not been investigated further in this study.

Although the hierarchical pattern gives an overall preference order for the moss species, individual food choice experiments do show differences from the overall preference order, and individual larvae often show differences from the overall preference shown for the food plants in each experiment.

Gerson (1982) in his review of evidence on bryophyte consumption by insects reports some feed exclusively on mosses whilst others feed on mosses and other plants, but considers the latter to be uncommon. In this study, *T. subnodicornis* was found to feed on, and indeed favour, the sedge *Eriophorum vaginatum*, as well as two bryophytes, *Campylopus paradoxus* and *Sphagnum papillosum*. This preference

changed though through time - towards pupation (which, in this experiment in the laboratory, occurred between mid- February and early April) and it is possible that the changing preference in favour of *Sphagnum papillosum* was a thigmotaxic response associated with the selection of a pupation site. In the experiments which gave *T. subnodicornis* a choice of single or mixed food items (carried out in December 1990 and January 1991), there was a response to *Sphagnum papillosum* as a thigmotaxic medium, but *T. subnodicornis* will more readily feed on other food plants which are mixed with this moss species.

There are few references in the literature of the chemical and/or nutrient feeding deterrents present in the bryophyte species studied here. Without analysing each moss species for chemical and nutrient levels, it is difficult to ascertain which bryophyte species are, potentially, the most palatable to insect species, in this case dipterous larvae. From the choices made by the two species of *Tipula* studied, predictions about the palatability of these moss species to other insect herbivores can be made.

Chapter 5. The effects of heavy metal loads in moss species on the choices made by *Tipula subnodicornis* larvae for food plants

5.1. Introduction

Mosses are known to have the ability to accumulate heavy metals from water and the atmosphere, and to build them up to remarkably high concentrations. It is well known that bryophyte tissue is a powerful ion exchanger, (*e.g.* Anschutz and Gessner 1954), and the lack of a protective cuticle, simple organisation of tissues and large surface area to weight ratio, (Tyler 1990), facilitates the uptake of heavy metal ions into moss species. There is usually a linear relationship between the logarithm of the concentrations of heavy metals in plants and the logarithm of the concentration of heavy metals in the water in contact with the mosses, as found for Zn^{2+} ions by Say and Whitton (1983) and Whitton (pers. comm.).

The heavy metal retention efficiency over a wide range of concentrations for the moss, *Hylocomium splendens*, is in descending order, Cu^{2+}=Pb^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+}=Mn^{2+}, Ruhling and Tyler (1970).

This study was carried out to determine if heavy metal loads, (*i.e.* Pb^{2+} and Zn^{2+} ions), introduced into mosses deterred cranefly larvae from choosing those mosses as food items *i.e.* can cranefly larvae detect and avoid the presence of heavy metal loads in moss species, avoidance of moss species being used as a means of showing detection.

Some naturally occurring concentrations of lead and zinc, from Allen *et al.* (1989) and Grimshaw *et al.* (1989), are given below:

	Pb^{2+}	Zn^{2+}
Soils	2-20μgg^{-1}	1-40 μgg^{-1}
Plant material	0.05-3 μgg^{-1}	15-100μgg^{-1}
Animal material	0.1 -3 μgg^{-1}	100-300μgg^{-1}
Freshwater	1-20μgg^{-1}	5-50 μgg^{-1}

Tyler (1990), reviewing the results of experiments from many workers, concluded that the relative toxicity to the following heavy metal ions in moss species, at equal concentrations, decreased in the order $Hg^{2+} > Cu^{2+}=Cd^{2+} > Pb^{2+} > Zn^{2+}$.

5.2. Methods

5.2.1. Experimental procedure for the determination of preferred food plant

In this study, heavy metals were introduced into mosses by soaking the moss in known concentrations of solutions of a salt of the heavy metal involved. Brown and Bates (1972) fed lead to the moss *Grimmia doniana* in a similar manner, by adding Pb $(NO_3)_2$ solution to quantities of the moss, shaking for one hour, filtering and thoroughly rinsing. In this study, zinc and lead were used on the mosses *Campylopus paradoxus* and *Sphagnum papillosum*. Fourth instar larvae of *Tipula subnodicornis* were used to determine whether or not they showed a preference or avoidance of mosses containing heavy metal loads. Details of the collection of *C. paradoxus* and *S. papillosum* are given in Chapter 4, Section 4.2., and of *T. subnodicornis* in Chapter 4, Section 4.3.1.1.

A total of 17 experiments were carried out - each involving both *C. paradoxus* and *S. papillosum* - one moss having been soaked in a heavy metal solution (the experimental moss species) and the other soaked only in de-ionised water (the control moss species). To be comparable to the food choice experiments described in

Chapter 4, where *C. paradoxus* and *S. papillosum* were presented as a choice to *T. subnodicornis*, these two moss species were used here in each experimental run. Details of each experiment are given in Table 5.1, which divides the experiments into eight sets (except Experiment 1); each set containing a pair of experiments.

The overall experimental procedure for each set of experiments using the same heavy metal and concentration is given below.

A one litre beaker which had been cleaned and rinsed in distilled water, was filled with distilled water and left for three hours. This water was then discarded, the beaker rinsed once more, and filled with one litre of fresh distilled water. The required amount of the heavy metal salt was weighed out and this was added to the beaker and stirred with a clean glass rod until it had dissolved. The solution was then divided between two clean 500ml beakers: to one was added a quantity of the moss *Campylopus paradoxus*, and to the other an equal quantity of *Sphagnum papillosum*, and these were left soaking for three days, to ensure uptake of the heavy metal ions. These moss species were used as the experimental mosses.

This procedure was repeated for a second 1l beaker, except that no heavy metal salt was added to the water. The two moss species were left to soak in de-ionised water only, for three days, to be absolutely comparable to the above procedure. These moss species were used as the control mosses in each petri-dish. This procedure was used also for Experiment 1, the control experiment.

After three days soaking in either the heavy metal solution or de-ionised water, all the mosses were thoroughly rinsed in de-ionised water for ten minutes and thereafter drained. This procedure was repeated for the range of soaking solutions listed in Table 5.1.

Two sets of ten petri-dishes were set up (only one set for Experiment 1) - each with a piece of damp filter paper in the base. To the first set of petri-dishes: equal quantities of *C. paradoxus*, as the experimental moss species, and *S. papillosum*, as the control moss species, were added to each petri-dish. To the second set of petri-dishes: equal quantities of *S. papillosum*, as the experimental moss species, and

Table 5.1. Moss species and concentrations of the heavy metal salt solutions used in the choice experiments with fourth instar *Tipula subnodicornis*.

Set	Experiment	Moss soaked in heavy metal solution	Heavy metal Salt	Concentration of soaking solution (ppm)
	1	*Campylopus paradoxus* *Sphagnum papillosum*	-	-
1	2	*Campylopus paradoxus*	$ZnSO_4$	1.0
	3	*Sphagnum papillosum*	$ZnSO_4$	1.0
2	4	*Campylopus paradoxus*	$Pb(NO_3)_2$	3.0
	5	*Sphagnum papillosum*	$Pb(NO_3)_2$	3.0
3	6	*Campylopus paradoxus*	$ZnSO_4$	4.5
	7	*Sphagnum papillosum*	$ZnSO_4$	4.5
4	8	*Campylopus paradoxus*	$Pb(NO_3)_2$	12.5
	9	*Sphagnum papillosum*	$Pb(NO_3)_2$	12.5
5	10	*Campylopus paradoxus*	$ZnSO_4$	22.8
	11	*Sphagnum papillosum*	$ZnSO_4$	22.8
6	12	*Campylopus paradoxus*	$Pb(NO_3)_2$	62.5
	13	*Sphagnum papillosum*	$Pb(NO_3)_2$	62.5
7	14	*Campylopus paradoxus*	$ZnSO_4$	44.5
	15	*Sphagnum papillosum*	$ZnSO_4$	44.5
8	16	*Campylopus paradoxus*	$Pb(NO_3)_2$	125.0
	17	*Sphagnum papillosum*	$Pb(NO_3)_2$	125.0

C. paradoxus, as the control moss species, were added to each petri-dish. In Experiment 1 equal quantities of *C. paradoxus* and *S. papillosum* with no heavy metal enhancement were added to each of ten petri-dishes. In all the experiments the two moss species in each petri-dish were arranged so each one occupied half the available space.

One fourth instar larva of *Tipula subnodicornis* was added to the centre of each petri-dish. The experimental chambers were left for at least one day without disturbance. Thereafter the position of each animal in the petri-dish was checked twice daily, as in the food choice experiments, described in Chapter 4. The larvae, from the time of their collection, had been kept at 10°C, and all experiments were run at this temperature.

To determine if the choices made by *T. subnodicornis* in each experiment varied over time, each experiment was divided into ten equal time periods of between one and two weeks and the proportion of time spent on the two moss species within each time period was compared to the overall proportions found in the experiment as a whole.

5.2.2. Methods for the 24 hour experiments

These were carried out to determine the movements of the larvae from one moss species to the other in each experiment, over a 24h period, using 2 hourly checks. They were also used to determine how long larvae spent on each moss species, and whether this was influenced by the heavy metal loads of the moss species involved.

The investigation involved each experiment listed in Table 5.1. The experiments began with the first observations at 0800h, with the positions of the animals checked every two hours until 0800h the following day.

5.2.3. Atomic spectrometer analysis to determine the actual concentrations of heavy metal ions in the moss species

The procedure followed for both the preparation of the samples for analysis, and the actual analysis was adapted from the methods described by Allen *et al.* (1989) for lead and by Grimshaw *et al.* (1989) for zinc.

A sample of both the experimental moss and the control moss, from every experiment carried out, had been collected at the time of the experiment, oven dried at 40°C for 24 hours, and stored dried until required for analysis. This resulted in 17 samples of both *Campylopus paradoxus* and *Sphagnum papillosum.*

5.2.3.1. Preparation of samples

The samples were prepared for analysis using the mixed acid procedure. Each sample was ground with a pestle and mortar, using liquid nitrogen as a freezing agent to facilitate the grinding. Each sample was weighed accurately, to the nearest 0.0001g, placed in a boiling tube and the following added: 1.5ml 95% nitric acid, 0.1ml 96% sulphuric acid and 0.2ml 60% perchloric acid. Two blanks were also prepared, containing only the acid mixture. All boiling tubes and contents were incubated at 40°C for two hours. The heat was increased to 110°C for one hour, and then, after the appearance of white fumes, the contents were allowed to digest for one hour at 140°C. The vessels were then cooled, and 5ml of de-ionised water was added to each. The contents of each boiling tube were placed in a 25ml graduated flask, and made up to 25ml with de-ionised water, thoroughly rinsing the boiling tube. The samples were then ready for analyses.

5.2.3.2. Analysis of samples

Samples with enhanced concentrations of Pb^{2+} ions were analysed for actual

concentrations of Pb^{2+} ions, and samples with enhanced concentrations of Zn^{2+} ions, were analysed for actual concentrations of Zn^{2+} ions. The samples containing the overall control mosses, were analysed for both heavy metal ions.

Stock solutions of both 100 ppm Pb^{2+} ions and 50 ppm Zn^{2+} ions were prepared, using $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$, respectively and from these working standards were prepared in 1% nitric acid solutions. For lead the working standards were 0 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm; and for zinc the working standards were 0 ppm, 0.2 ppm, 0,4 ppm, 0.6 ppm, 0.8 ppm and 1.0 ppm. The samples were analysed in an Instrumentation Laboratories Video II atomic absorption spectrophotometer. The methods used were similar for both metal ions analysed.

The wavelength selected on the machine for Pb^{2+} ion analysis was 283.3nm with a narrow slit. Other adjustments on the machine required for the analyses were carried out as directed by the instructions for the machine. A calibration curve for Pb^{2+} ions, was calculated, using the working standards, after setting the 0 ppm standard to zero and the top standard to a suitable read out value. Each intermediate standard was then aspirated to produce a calibration curve for Pb^{2+} ions. The two blank samples were aspirated through the machine to produce base line readings.

Each of the 18 moss sample solutions to be analysed for Pb^{2+} ions were aspirated in turn, the two blank samples being aspirated after every four samples, to check that the readings were consistent. The readings obtained from the standards were also checked frequently, using the 0 ppm, 10 ppm and an intermediate standard. The calibration curve produced was used to obtain the concentration of Pb^{2+} ions in the sample solutions in ppm.

This procedure was repeated using the zinc standards and the 18 moss sample solutions that were to be analysed for Zn^{2+} ions. This time a wavelength of 213.8nm was used.

The mean reading determined from the blanks in each set of analysis was subtracted from the concentrations of either Pb^{2+} or Zn^{2+} ions in ppm to give a corrected reading for the concentrations.

The equation given below was then used to calculate the concentration of either Pb^{2+} or Zn^{2+} ions in $\mu g g^{-1}$.

$$Pb/Zn\ \mu g g^{-1} = \frac{C_{(ppm)} \times \text{solution volume (ml)}}{\text{sample weight (g)}}$$

where C = corrected concentration of soaking solution and the solution volume is 25ml.

Appendix 1 gives the weights of each sample of moss which was analysed to determine the actual concentrations of the heavy metal ions in the moss species. These data were used to calculate the actual concentrations of the heavy metal ions in $\mu g g^{1}$.

5.2.4. Method for analysis of the frass to compare with the spot observation method

This comparison was made to determine if it was feasible to use the observational method to determine the preferences of *T. subnodicornis* larvae for moss species as food plants. Frass was collected from the ten larvae in six of the experiments carried out, and was prepared and analysed as described in Chapter 4, Section 4.3.2.

The six experiments investigated in this way were Experiments 2, 3, 4, 5, 6 and 8, listed in Table 5.1.

5.3. Results

5.3.1. Actual concentrations in the mosses

The same weight of lead nitrate and zinc sulphate was used for each set of concentrations of heavy metal ions. As a consequence, the concentrations in the soaking solutions of Zn^{2+} ions, were lower than the concentrations of Pb^{2+} ions, for the same weight of heavy metal salt used.

Table 5.2 gives the concentrations of the lead nitrate solution and the zinc sulphate solution in which the moss species were soaked, and the actual concentrations of Pb^{2+} and Zn^{2+} ions resulting in both moss species, *C. paradoxus* and *S. papillosum*, in $\mu g g^{1}$, respectively, as determined by the atomic absorption spectrophotometer.

It is immediately apparent that with Pb^{2+}, (see Table 5.2A), *S. papillosum* has the ability to concentrate the heavy metal ions to a much greater degree than *Campylopus paradoxus*, at soaking concentrations above 3 ppm. At 3 ppm Pb^{2+} in the soaking solution, the concentration in *S. papillosum*, at 565 $\mu g g^{-1}$, is 0.8 times the concentration in *C. paradoxus*. However at the higher soaking solution concentrations, *i.e.* at 12.5 ppm, 62.5 ppm and 125.0 ppm, the concentrations found in *S. papillosum* are greater than those found in *C. paradoxus*, by factors of 2.8, 2.7 and 2.1, respectively.

With Zn^{2+} ions the situation is similar, (see Table 5.2B), with *S. papillosum* concentrating the heavy metal ions to a greater degree, at soaking solution concentrations of 1 ppm and above. At soaking solution concentrations of 1 ppm, 4.5 ppm, 22.8 ppm and 45.5 ppm, the concentrations found in *S. papillosum* are greater than those found in *C. paradoxus*, by factors of 2.6, 3.8, 4.1 and 2.3, respectively.

When the uptakes of Pb^{2+} and Zn^{2+} ions are compared in *S. papillosum*, Zn^{2+} ions are concentrated to a greater extent than Pb^{2+} ions: when, overall, the soaking solution concentration is increased by a factor of 45.5, for Zn^{2+} ions and 41.7 for Pb^{2+} ions, the concentration of Zn^{2+} ions and Pb^{2+} ions in the moss increases by

Table 5.2. Concentrations of heavy metal ions in the soaking solutions, and the resulting concentrations in the moss species determined from atomic absorption spectrometry.

A. Moss species soaked in lead nitrate solution.

Concentration in soaking solution (ppm)	Concentration of Pb^{2+} ions in *C. paradoxus* $\mu g g^{-1}$	Concentration of Pb^{2+} ions in *S. papillosum* $\mu g g^{-1}$
0	365	265
3.0	660	565
12.5	1220	3360
62.5	1470	3985
125.0	2130	4570

B. Moss species soaked in zinc sulphate solution.

Concentration in soaking solution (ppm)	Concentration of Zn^{2+} ions in *C. paradoxus* $\mu g g^{-1}$	Concentration of Zn^{2+} ions in *S. papillosum* $\mu g g^{-1}$
0	120	170
1.0	180	470
4.5	640	2420
22.8	1040	2465
44.5	2905	6615

factors of 14.1 and 8.1, respectively. The same is true when the uptake of the two heavy metal ions are compared in *C paradoxus*. In this case the concentration of Zn^{2+} ions and Pb^{2+} ions in the moss increased by factors of 16.1 and 3.2, respectively.

In each moss species with either heavy metal loads of Pb^{2+} ions or Zn^{2+} ions, there is a linear relationship between the logarithm of the concentration in the moss and the logarithm of the concentration of the soaking solutions, (see Figs. 5.1-5.4).

5.3.2. Comparison between the frass analysis method and the spot observation method

The regression of the mean percentage of particles of each moss species in the frass of *T. subnodicornis* (y) against the mean percentage of observations of *T. subnodicornis* on the moss species (x) throughout the experiment is presented on Fig. 5.5. For *T. subnodicornis*, 66% of the variation is explained by the regression equation,

$$y = 1.79(\pm 0.44\ (S.E.))x - 48.64(\pm 22.30\ (S.E.))$$

and the slope of the line is not significantly different from 1.0 ($t=1.80$ $df=10$ NS). Thus, as found with *T. subnodicornis* in the food choice experiments, the mean percentage of spot observations can be used as an alternative method to estimate which moss species is being used as the preferred food plant in each experiment.

Fig. 5.1

The relationship between the logarithm of the concentration of Pb^{2+} ions in *Campylopus paradoxus* and the logarithm of the concentration of Pb^{2+} in the soaking solution. $y = 0.34x + 2.61$ $r_3 = 0.98$ $p<0.01$

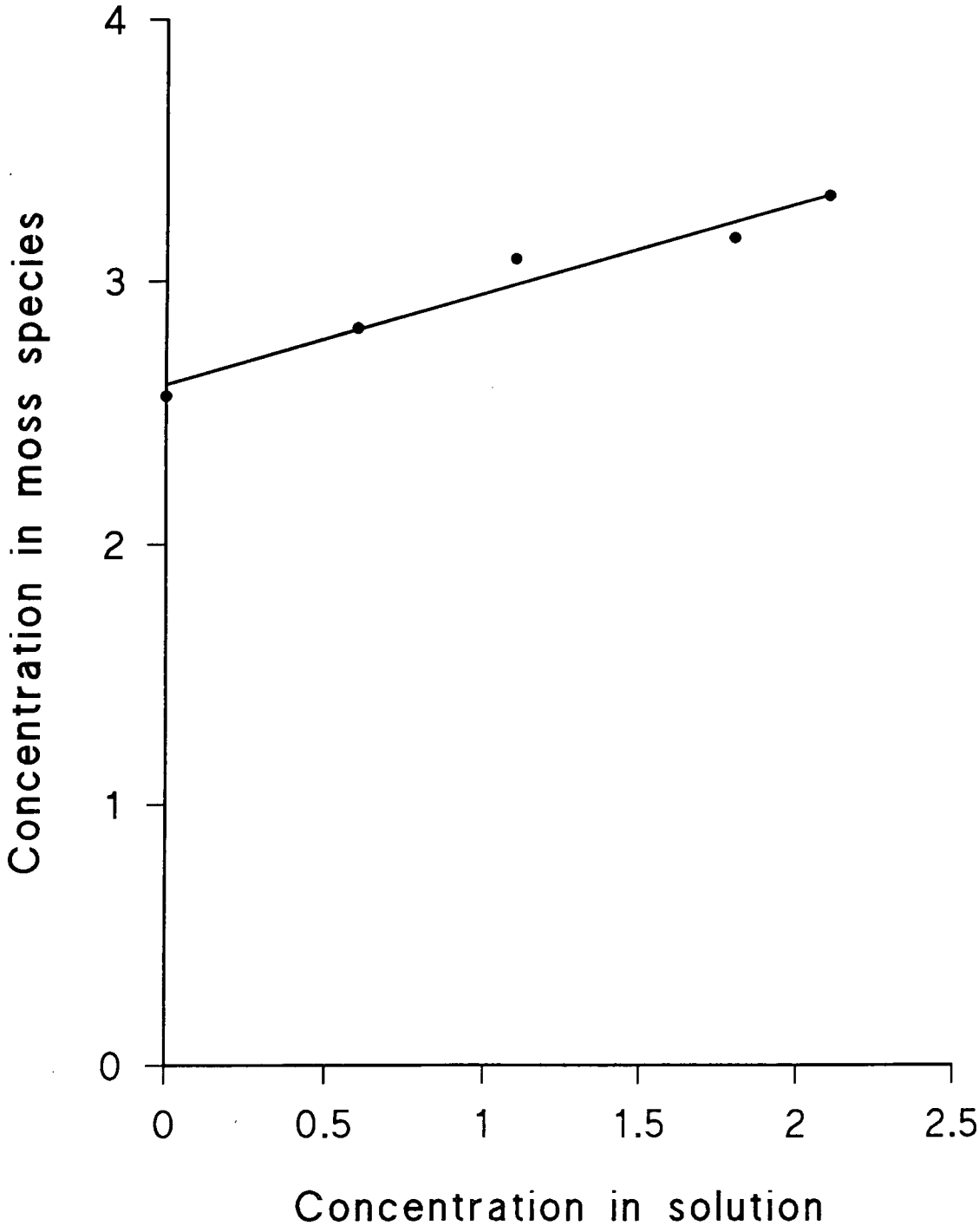


Fig. 5.2

The relationship between the logarithm of the concentration of Zn^{2+} ions in *Campylopus paradoxus* and the logarithm of the concentration of Zn^{2+} in the soaking solution. $y = 0.78x + 2.08$ $r_3 = 0.98$ $p<0.01$

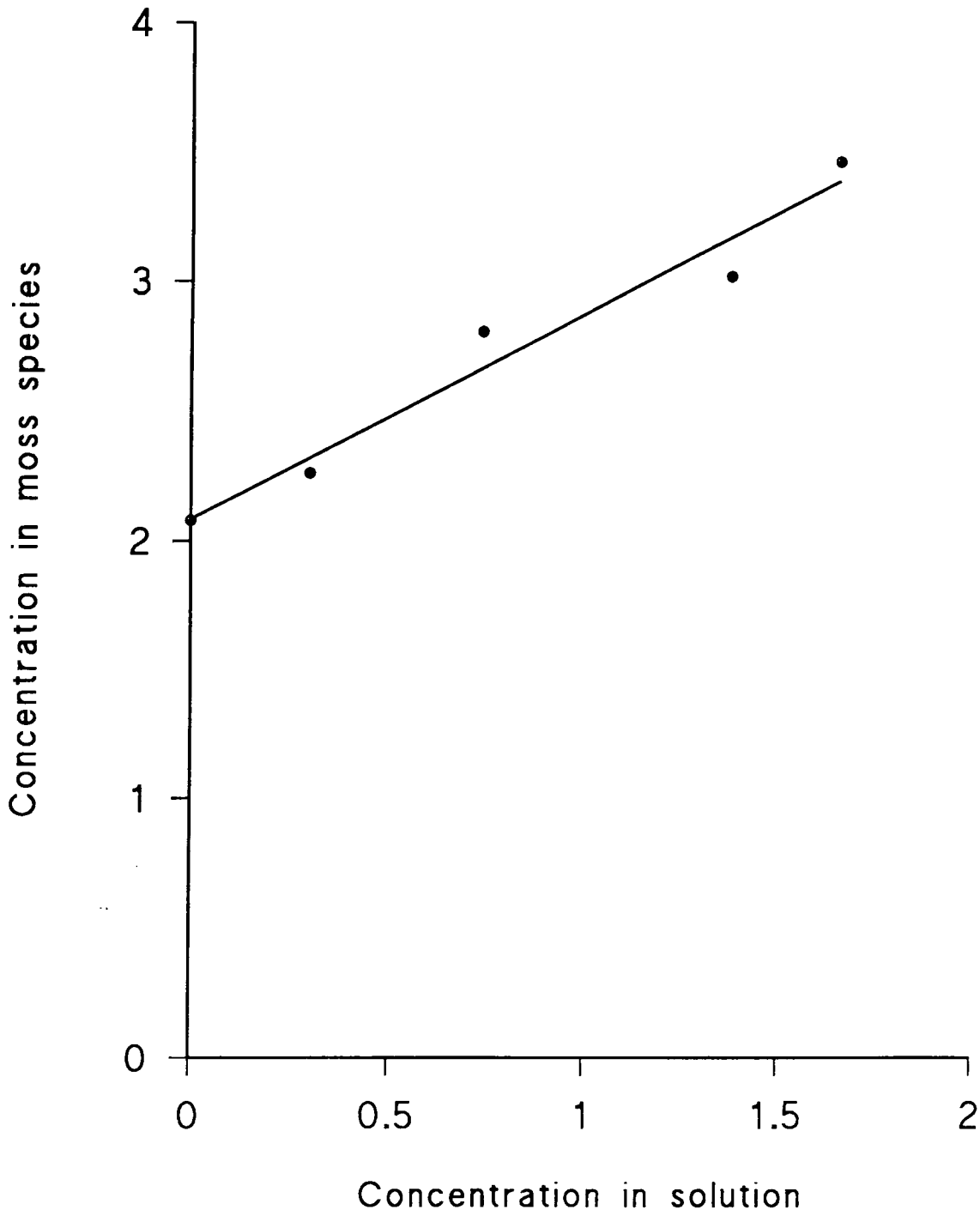


Fig. 5.3

The relationship between the logarithm of the concentration of Pb^{2+} ions in *Sphagnum papillosum* and the logarithm of the concentration of Pb^{2+} in the soaking solution. $y = 0.62x + 2.50$ $r_3 = 0.94$ $p<0.05$.

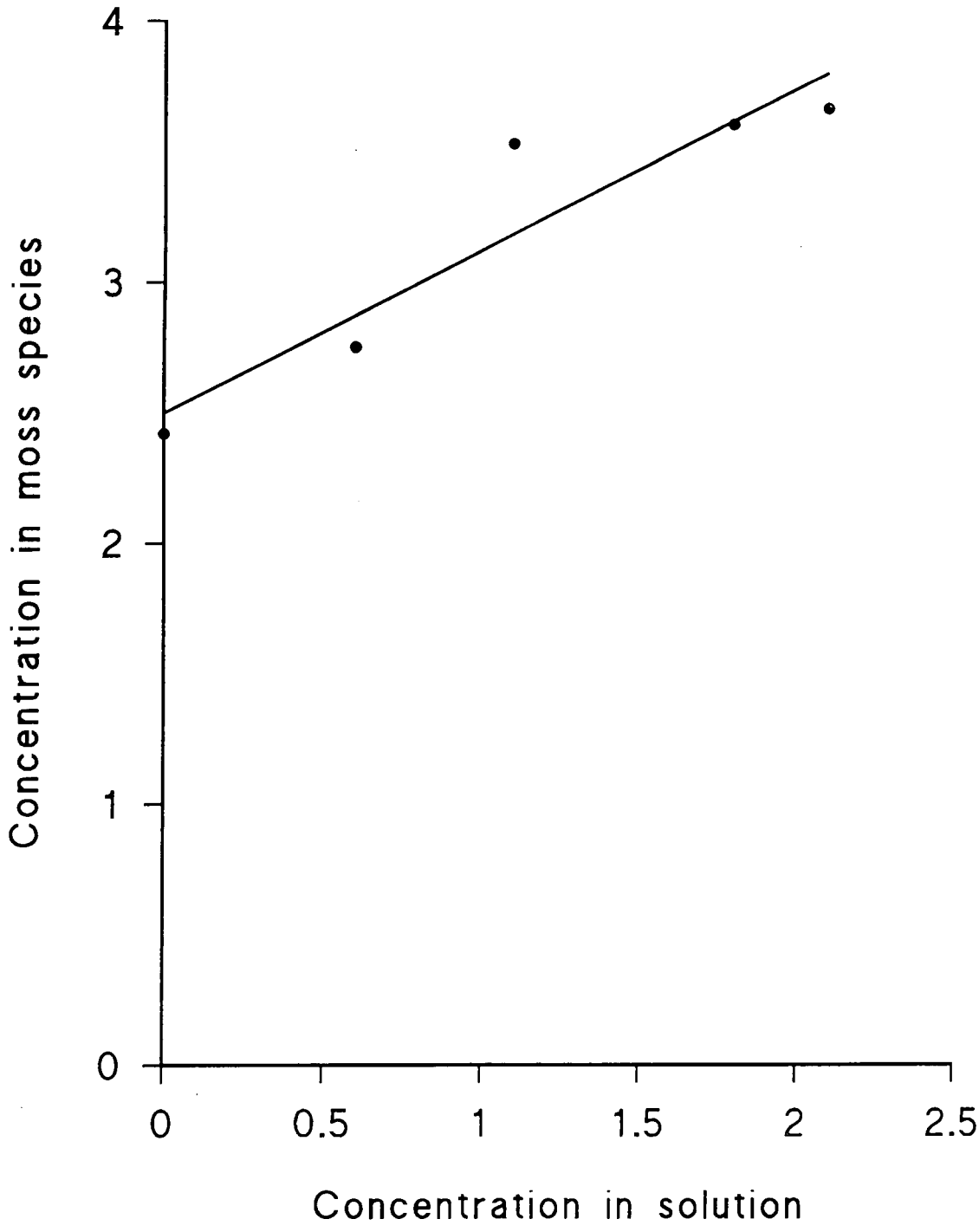


Fig. 5.4

The relationship between the logarithm of the concentration of Zn^{2+} ions in *Sphagnum papillosum* and the logarithm of the concentration of Zn^{2+} in the soaking solution. $y = 0.92x + 2.40$ $r_3 = 0.96$ $p<0.01$.

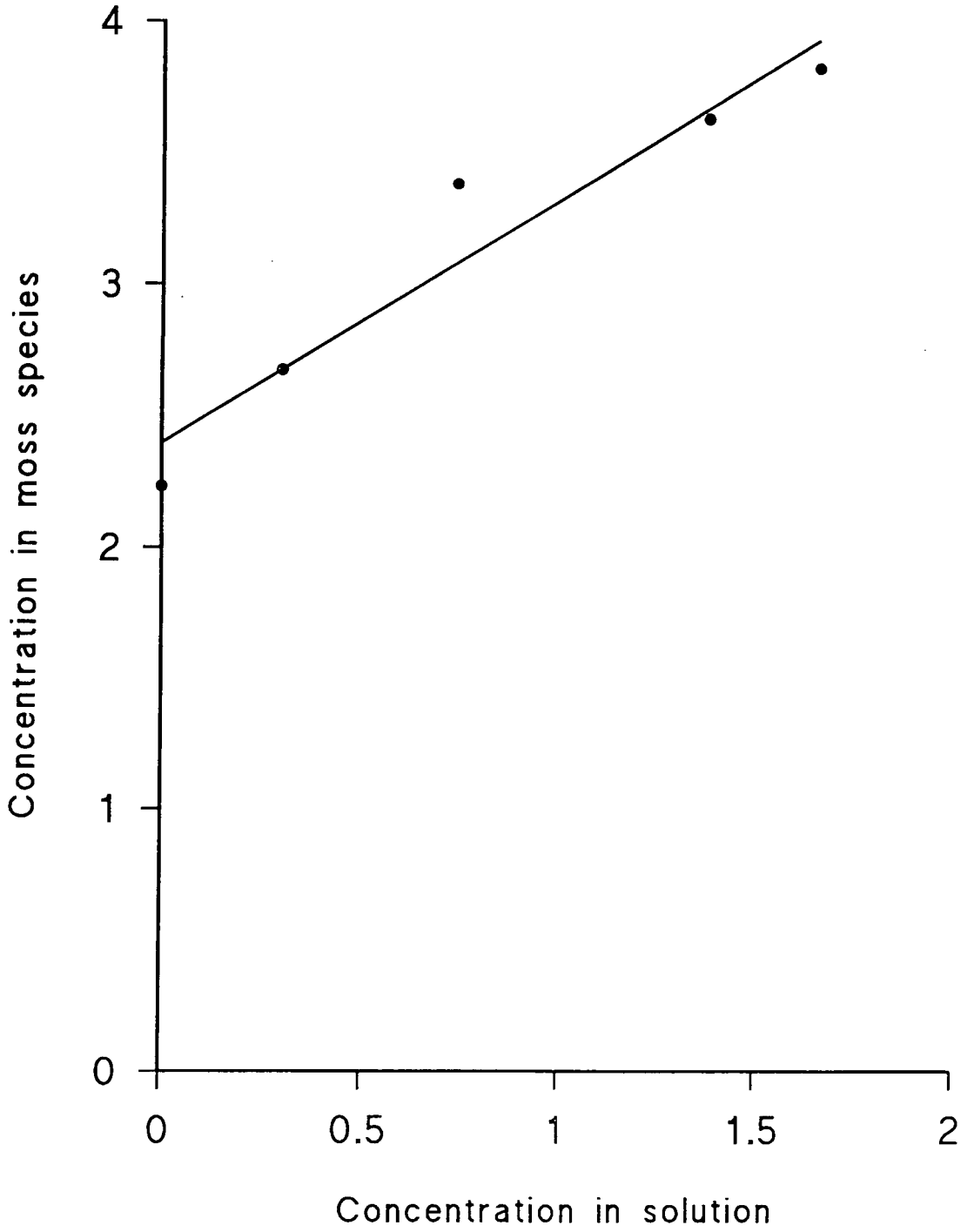


Fig. 5.5.

The relationship between the mean percentage of moss particles in the frass and the mean percentage of observations on the moss species. $y = 1.97x - 48.64$, $r_{10} = 0.81$, $p<0.01$.

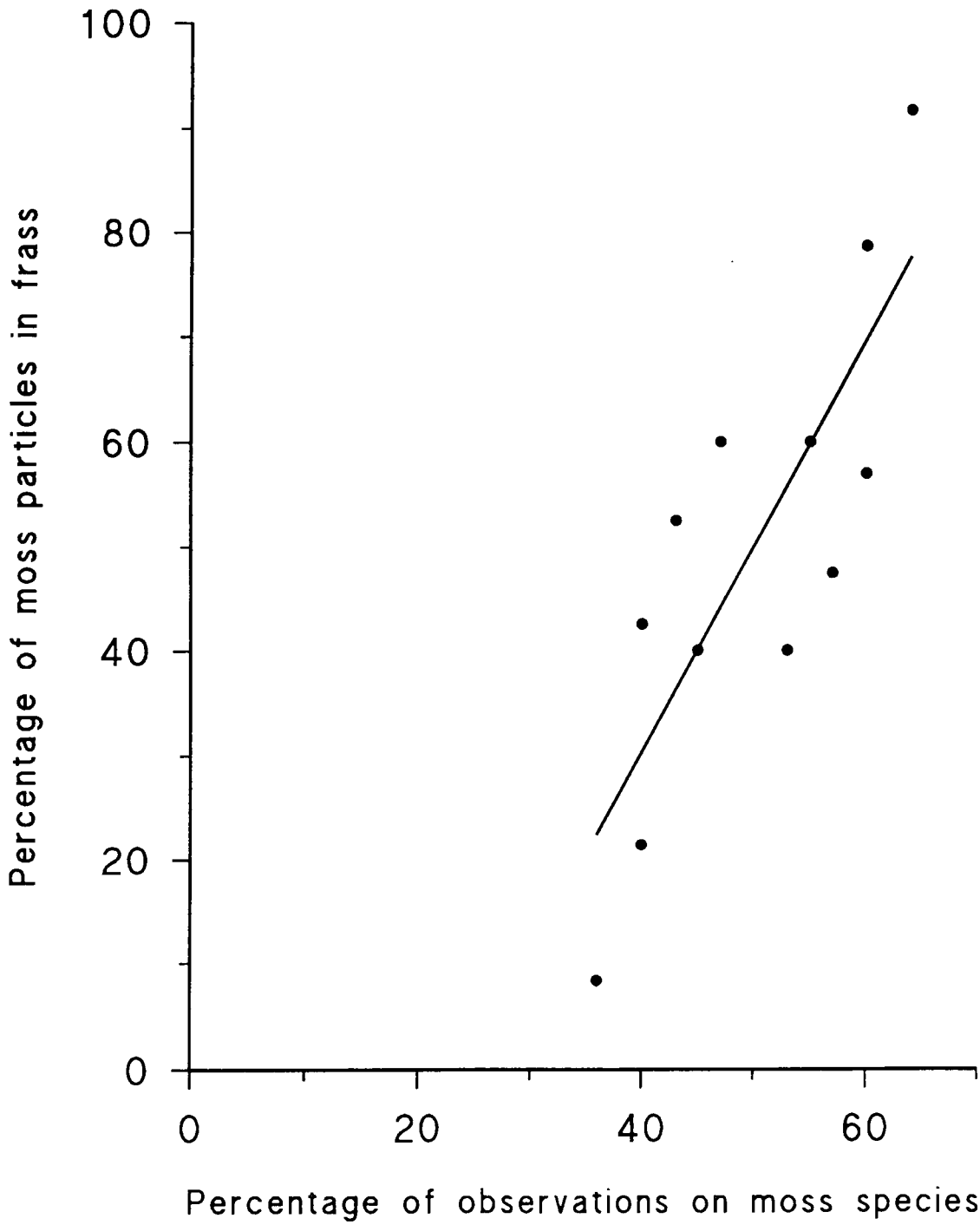


Table 5.3. Comparison of the numbers of observations of fourth instar *Tipula subnodicornis* on *Campylopus paradoxus* or *Sphagnum papillosum* when one moss species was soaked in water containing concentrations of a heavy metal.

A. *Campylopus paradoxus* treated with Pb^{2+} ions.

Experiment	Concentration of Pb^{2+} in *C. paradoxus* ($\mu g g^{-1}$)	n	Numbers of observations on *C. paradoxus* (%)	Comparison with control experiment X^2
Control	365	683	403 (59%)	-
i	660	523	314 (60%)	0.1 NS
ii	1220	471	201 (43%)	50.8 ***
iii	1470	361	148 (41%)	48.9 ***
iv	2130	341	123 (36%)	73.7 ***

NS $p>0.05$ *** $p<0.001$

Mean concentration of Pb^{2+} ions in control moss, *S. papillosum* = 351.0 (±47.4) $\mu g g^{-1}$

\cont'd.

Table 5.3. (cont'd)

B. *Campylopus paradoxus* treated with Zn^{2+} ions.

Experiment	Concentration of Zn^{2+} in *C. paradoxus* ($\mu g g^{-1}$)	n	Numbers of observations on *C. paradoxus* (%)	Comparison with control experiment X^2
Control	120	683	403 (59%)	-
i	180	537	347 (64%)	6.9 **
ii	640	463	198 (43%)	50.2 ***
iii	1040	383	167 (44%)	31.5 ***
iv	2905	336	151 (44%)	27.2 ***

** $p<0.01$ *** $p<0.001$

Mean concentration of Zn^{2+} ions in control moss, *S. papillosum* = 263.0 ±56.0$\mu g g^{-1}$

\cont'd.

Table 5.3. (cont'd)

C. *Sphagnum papillosum* treated with Pb^{2+} ions.

Experiment	Concentration of Pb^{2+} in *S. papillosum* ($\mu g g^{-1}$)	n	Numbers of observations on *S. papillosum* (%)	Comparison with control experiment X^2
Control	265	683	280 (41%)	-
i	565	412	185 (45%)	2.6 NS
ii	3360	380	194 (52%)	18.3 **
iii	3985	332	162 (49%)	8.4 **
iv	4570	336	162 (52%)	16.8 ***

NS $p>0.05$ $p<0.05$ ** $p<0.01$ *** $p<0.001$

Mean concentration of Pb^{2+} ions in control moss, *C.paradoxus* = 496.6±53.2 $\mu g g^{-1}$

\cont'd.

Table 5.3. (cont'd)

D. *Sphagnum papillosum* treated with Zn^{2+} ions.

Experiment	Concentration of Zn^{2+} in *S. papillosum* ($\mu g g^{-1}$)		Numbers of observations on *S. papillosum* (%)	Comparison with control experiment X^2
Control	170	683	280 (41%)	-
i	470	346	162 (47%)	4.8 *
ii	2420	407	168 (41%)	0.01 NS
iii	4265	338	204 (60%)	52.3 ***
iv	6615	343	152 (44%)	1.2 NS

NS $p>0.05$ $p<0.05$ *** $p<0.001$

Mean concentration of Zn^{2+} ions in control moss, *C. paradoxus* = 165.0 ±43.7$\mu g g^{-1}$

\cont'd.

5.3.3. Comparison of numbers of observations on the two differently treated moss species

In the overall control experiment, with a choice for the *T. subnodicornis* larvae between *C. paradoxus* and *S. papillosum* and with no introduced heavy metal concentration in either moss species, there were significantly more observations on the *C. paradoxus* (59%) than on the *S. papillosum* (41%), X^2=22.2 df=1 p<0.001, (Table 5.3).

Also individual differences existed in the choices made by the ten larvae that were involved in each experiment. From the total of 16 experiments carried out using different concentrations of the two heavy metal ions in the two moss species, in ten experiments significant variations existed in the choices made by the ten larvae at the p<0.05 level. Examples of these analyses are given in Table 5.4 below:

Table 5.4.

Experiment 8: *C. paradoxus* with 1220µgg^{-1} Pb^{2+} ions.

Larva	Number of observations with larvae on:			
	Campylopus paradoxus		*Sphagnum papillosum*	
	Observed	Expected	Observed	Expected
1	19	20.1	28	26.9
2	16	19.6	30	26.4
3	17	19.2	28	25.8
4	17	19.6	29	26.4
5	23	20.9	26	28.1
6	24	20.1	23	26.9
7	28	21.3	17	28.7
8	20	20.5	28	27.5
9	20	20.5	28	27.5
10	16	19.2	33	25.8

X^2=19.9 df=9 p<0.05

Table 5.4 (cont'd)

Experiment 11. *S. papillosum* with 4265µgg^{-1} Zn^{2+} ions.

	Number of observations on with larvae on:			
Larva	*Sphagnum papillosum*		*Campylopus paradoxus*	
	Observed	Expected	Observed	Expected
1	28	21.2	7	13.8
2	24	21.2	11	13.8
3	13	20.0	20	13.0
4	20	21.2	15	13.8
5	24	21.2	11	13.8
6	10	19.9	23	13.0
7	28	19.9	5	13.0
8	22	20.5	12	13.4
9	9	19.4	23	12.6
10	26	19.4	6	12.6

X^2=54.6 df=9 p<0.001

5.3.3.1. Choice experiments between *Campylopus paradoxus* with introduced Pb^{2+} ions, and *Sphagnum papillosum* as a control

In the three experiments with concentrations above 660 µgg^{1} Pb^{2+} in *C. paradoxus*, (*i.e.* experiments ii), iii) and iv)), there are significantly more observations of *Tipula subnodicornis* on *S. papillosum*, than on *C. paradoxus*, compared to the control experiment (Table 5.3A). There was no significant difference in the proportions of observations on the two moss species in these three experiments (X^2= 3.71 df=2 NS). In experiment i) the proportion on the two moss species did not differ significantly from the control experiment. Overall there was a

mean of 19±3% fewer observations on *C. paradoxus* than on *S. papillosum*.

5.3.3.2. Choice experiments between *Campylopus paradoxus* with introduced Zn^{2+} ions and *Sphagnum papillosum* as a control

A similar pattern of results was found here. In the three experiments with concentrations of $ZnSO_4$ above 180 $\mu g g^{-1}$ in *C. paradoxus*, (*i.e.* experiments ii), iii) and iv)), a significantly greater number of observations of *T. subnodicornis* was found on *S. papillosum* than on *C. paradoxus*, compared to the proportions found on the mosses in the control experiment (Table 5.3B). No significant difference in the proportions were found on the two moss species in these three experiments (X^2=0.38 df=2 NS). In experiment i) significantly more observations of *T. subnodicornis* were found on *C. paradoxus*, than on *S. papillosum*, compared to the control experiment. Overall a mean of 15±0.3% fewer observations were made on *C. paradoxus* than on *S. papillosum*.

The larvae appear to avoid *C. paradoxus* soaked in Zn^{2+} ions, at a lower concentration than *C. paradoxus* soaked in Pb^{2+} ions. This is surprising since lead is usually considered the more potently toxic heavy metal.

5.3.3.3. Choice experiments between *Sphagnum papillosum* with introduced Pb^{2+} ions, and *Campylopus paradoxus* as a control

Here, significantly more observations of *T. subnodicornis* were found on *S. papillosum* in the three experiments in which this moss species contained concentrations above 565 $\mu g g^{-1}$ Zn^{2+} ions, (*i.e.* experiments ii), iii) and iv)), compared to proportions found on the control mosses, (Table 5.3C). Again the proportions overall found on the mosses in the three experiments were not significantly different (X^2=0.75 df=2 $p<0.05$). The proportions of observations found on *S. papillosum*, containing 565 $\mu g g^{-1}$ Zn^{2+} ions, and *C. paradoxus* were not

significantly different to the proportions found on the control mosses. Overall there was a mean of 8±2% more observations on *S. papillosum* than on *C. paradoxus*.

5.3.3.4. Choice experiments between *Sphagnum papillosum* with introduced Zn^{2+} ions and *Campylopus paradoxus* as a control

In experiment i), with a concentration of 470 $\mu g g^{-1}$ Zn^{2+} ions in *S. papillosum*, compared with the proportions of observations found on the mosses in the control experiment, significantly more observations were on the *S. papillosum* (Table 5.3D). In experiments ii) and iv), with 2420 $\mu g g^{-1}$ and 6615 $\mu g g^{-1}$ Zn^{2+} ions in *S. papillosum* respectively, there was no significant difference in proportion of observations on the two mosses compared with control experiment. In experiment iii) however, with a concentration of 4265 $\mu g g^{-1}$ Zn^{2+} ions, there was a significantly greater proportion of observations on the *S. papillosum* compared to the control experiment. Overall a mean of 7±4% more observations were made on *S. papillosum* than on *C. paradoxus*. There is an anomaly here. *S. papillosum* concentrates Pb^{2+} and Zn^{2+} ions to much higher concentrations than *C. paradoxus* and yet the *T. subnodicornis* larvae do not appear to be affected or influenced by these concentrations in the *Sphagnum*.

For all sets of experiments, the proportions of observations on the two moss species were divided into ten equal time periods in each experiment, and were compared to the overall proportions. For each experiment no significant differences were found, indicating that the proportions of observations did not alter during the course of the experiments. See Appendix 2 for examples of these analyses.

5.3.4. 24 hour experiments

These experiments were carried out to determine if there was a difference in the frequency of movements per larva, (from the total potential movements per larva),

when on moss species containing heavy metal loads. The results are presented on Table 5.5.

A comparison of the frequency of movements between *C. paradoxus* with enhanced concentrations of Pb^{2+} ions, and the controls showed no significant differences at the two lowest concentrations. However, at the two highest concentrations, *i.e.* at 1470 and 2130μgg^{1} , there was a significantly higher mean percentage of movements from the experimental moss (*C. paradoxus*) than from *S. papillosum* (see Table 5.5A).

For *C. paradoxus*, with concentrations of Zn^{2+} ions, the pattern was similar, with no significant differences between the percentage of movements from the experimental and control mosses at the two lower concentrations of Zn^{2+} ions. However there were a higher percentage of movements from the experimental moss species than in the control moss species (*S. papillosum)* at the two higher concentrations of Zn^{2+} ions, *i.e.* at 1040 and 2905μgg^{1} , (see Table 5.5B).

For *S. papillosum* with concentrations of Pb^{2+} ions, there was no significant difference in the mean percentage of movements from the experimental and control moss species except for the highest introduced concentration of Zn^{2+} ions (4570$\mu g\bar{g}$ ^{1}), where there was a significantly higher mean percentage of movements from the experimental moss (see Table 5.5C).

In the case of *S. papillosum* with increased concentrations of Zn^{2+} ions, the results showed a similar pattern, in that there was no significant difference in the mean percentage of movements from the experimental and control moss species in the experiments at the three lowest concentrations of Zn^{2+} ions. In the experiment in which *S. papillosum* has an introduced Zn^{2+} ion concentration of 6615$\mu g\bar{g}^{1}$, there were significantly more movements from the *Sphagnum* than from the *Campylopus* (see Table 5.5D).

Throughout the experiments there were no significant differences in the movements between any set of controls. In all four sets of experiments, the frequency of movements from the experimental mosses increased as the heavy metal

Table 5.5. Comparison between the mean percentage of movements per larva, as a percentage of the total potential number of movements per larva, from moss species containing heavy metal loads, and controls, with n as the number of larvae.

A. *Campylopus paradoxus* treated with Pb^{2+} ions, *Sphagnum papillosum* as control.

Concentration of Pb^{2+} ions in $\mu g g^{-1}$ in *C. paradoxus*	n	Mean percentage of movements per larva from: *C. paradoxus* (±S.E.)	*S. papillosum* (±S.E.)	t-value df=18
i 660	9	30.4(±3.7)	25.5(±5.2)	0.77 NS
ii 1220	10	36.8(±5.3)	23.5(±5.2)	1.79 NS
iii 1470	10	39.0(±4.2)	28.0(±3.1)	2.11 *
iv 2130	10	42.0(±4.1)	26.1(±4.5)	2.61 *

t-value between: i and iv, t=2.10 * df=17

B. *Campylopus paradoxus* treated with Zn^{2+} ions, *Sphagnum papillosum* as control.

Concentration of Zn^{2+} ions in $\mu g g^{-1}$ in *C. paradoxus*	n	Mean percentage of movements per larva from: *C. paradoxus* (±S.E.)	*S. papillosum* (±S.E.)	t-value df=18
i 180	10	25.0(±3.2)	18.3(±3.4)	1.43 NS
i 640	10	28.2(±4.1)	18.1(±4.2)	1.72 NS
iii 1040	10	34.8(±3.5)	16.0(±3.4)	2.85 *
iv 2905	10	41.8(±5.1)	18.1(±7.4)	2.64 *

t-values between: i and iii, t=2.06 * df=18
i and iv, t=2.70 ** df=18
ii and iv, t=2.07 * df=18

\contd.

Table 5.5. (cont'd)

C. *Sphagnum papillosum* treated with Pb^{2+} ions *Campylopus paradoxus* as control.

Concentration of Pb^{2+} ions in $\mu g g^{-1}$ in *S. papillosum*	n	Mean percentage of movements per larva from: *S. papillosum* (±S.E.)	*C. paradoxus* (±S.E.)	t-value df=18
i 565	9	19.8(±4.6)	14.8(±3.5)	0.86[+] NS
ii 3360	10	23.3(±2.8)	20.5(±1.7)	0.85 NS
iii 3985	10	32.1(±3.7)	27.1(±4.9)	0.81 NS
iv 4570	10	38.0(±3.3)	24.9(±4.7)	2.28 *

t-value between: i and iii, t=2.08 * df=17
i and iv, t=3.20 ** df=17
ii and iv, t=3.40 ** df=18

D. *Sphagnum papillosum* treated with Zn^{2+} ions *Campylopus paradoxus* as control.

Concentration of Zn^{2+} ions in $\mu g g^{-1}$ in *S. papillosum*	n	Mean percentage of movements per larva from: *S. papillosum* (±S.E.)	*C. paradoxus* (±S.E.)	t-value df=18
i 470	10	23.4(±1.0)	20.0(±2.3)	1.36 NS
ii 2420	10	27.8(±2.0)	25.4(±6.6)	0.35 NS
iii 4265	10	34.0(±5.1)	25.0(±6.6)	1.08 NS
iv 6615	10	42.6(±7.9)	21.7(±4.8)	2.26 *

t-value between: i and iii, t=2.04 * df=18
i and iv, t=2.41 * df=18

[+]df=16 NS=not significant *p<0.05 **p<0.01

concentration increased. These differences in frequency of movements from the experimental mosses with the highest and lowest heavy metal concentrations were significant in all the sets (Table 5.5). Other significant differences in the frequency of movements from experimental mosses with different heavy metal concentrations in each experimental set are given in Table 5.5.

5.3.5. Comparison of amount of time spent on the mosses

This was carried out during the 24 hour experiments. In each experiment, the mean length of time spent per larva, in the 24 hour period, on the moss species with introduced concentrations of heavy metals, and on the control moss species, was calculated. These results are presented on Table 5.6. These means were compared and the results of these comparisons are also presented on Table 5.6.

Considering firstly the set of experiments in which *Campylopus paradoxus* contains different introduced concentrations of Pb^{2+} ions, (Table 5.6A). The mean time spent per larva on *C. paradoxus* with the two highest concentrations of Pb^{2+} ions, *i.e.* 1470 and 2130 $\mu g\bar{g}^{1}$, was significantly less than the mean time spent per larva on *C. paradoxus* in the control experiment by 6.6h and 7.0h respectively.

Considering the set of experiments in which contains different introduced concentrations of Zn^{2+} ions, (Table 5.6B). These experiments show a similar pattern to the set of experiments described above. At the two highest concentrations, *i.e.* 1040 and 2904 $\mu g\bar{g}^{1}$, again the mean time spent per larva on *C. paradoxus* is significantly less than the mean time spent per larva on *C. paradoxus* in the control experiment by 6.8h and 5.7h respectively.

In the two sets of experiments in which *S. papillosum* contained increasing concentrations of Pb^{2+} and Zn^{2+} ions, (see Table 5.6C and 5.6D respectively) there was no significant difference between the time spent per larva on the *S. papillosum* containing introduced concentrations of the heavy metal ions and that spent on

Table 5.6. Comparison of the amount of time spent by fourth instar of *Tipula subnodicornis* on *Campylopus paradoxus* or *Sphagnum papillosum* when one moss species was soaked in water containing concentrations of a heavy metal.

A. *Campylopus paradoxus* treated with Pb^{2+} ions.

	Concentration of Pb^{2+} ions in *C. paradoxus* ($\mu g g^{-1}$)	Number of larvae	Time (hours) Total	Mean per larva on *C. paradoxus* (±S.E.)	Comparison with control experiment t-value (df=18)
Control	365	10	240	15.0 (±1.7)	-
i	660	9	211	12.6 (±2.7)	0.3 NS[+]
ii	1220	10	240	12.7 (±3.1)	0.7 NS
iii	1470	10	240	8.4 (±1.4)	3.0 **
iv	2130	10	236	8.0 (±0.6)	3.9 ***

B. *Campylopus paradoxus* treated with Zn^{2+} ions.

	Concentration of Zn^{+} ions in *C. paradoxus* ($\mu g g^{-1}$)	Number of larvae	Time (hours) Total	Mean per larva on *C. paradoxus* (±S.E.)	Comparison with control experiment t-value (df=18)
Control	120	10	240	15.0 (±1.8)	-
i	180	10	226	15.5 (±2.4)	0.2 NS
ii	640	10	240	10.7 (±2.3)	1.5 NS
iii	1040	10	228	8.2 (±2.1)	2.5 *
iv	2905	10	226	9.3 (±1.1)	2.9 **

\cont'd.

Table 5.6. (cont'd)

C. *Sphagnum papillosum* treated with Pb^{2+} ions.

	Concentration of Pb^{2+} ions in *S. papillosum* ($\mu g g^{-1}$)	Number of larvae	Time (hours) Total	Mean per larvae on *S. papillosum* (±S.E.)	Comparison with control experiment t-value (df=18)
Control	265	10	240	9.0 (±1.7)	-
i	565	9	216	11.2 (±2.5)	0.7 NS[+]
ii	3360	10	236	12.6 (±1.4)	1.6 NS
iii	3985	10	236	13.6 (±1.8)	1.9 NS
iv	4570	10	234	11.7 (±1.6)	1.2 NS

D. *Sphagnum papillosum* treated with Zn^{2+} ions.

	Concentration of Zn^{+} ions in *S. papillosum*	Number of larvae	Time (hours) Total	Mean per larva on *S. papillosum* (±S.E.)	Comparison with control experiment t-value (df=18)
Control	170	10	240	9.0 (±1.7)	-
i	470	10	240	8.8 (±1.1)	0.1 NS
ii	2420	10	212	13.0 (±2.0)	1.5 NS
iii	4265	10	240	11.4 (±1.4)	1.1 NS
iv	6615	10	238	12.0 (±2.1)	1.1 NS

[+] df=16

NS $p>0.05$ *$p<0.05$ ** $p<0.01$ ** $p<0.01$ *** $p<0.002$

S. papillosum in the control experiment, in each set.

5.4. Discussion

Mosses have been found to accumulate the heavy metal ions investigated to very high concentrations; Zn^{2+} ions being accumulated to a greater extent than Pb^{2+} ions, in both *C. paradoxus* and *S. papillosum*. The uptake of Zn^{2+} ions in the moss *Fontinalis antipyretica* has been suggested to occur in three stages (Pickering and Puia 1969). Firstly the movement of the Zn^{2+} ions into the free space in the cell wall, then the penetration by the Zn^{2+} ions of the protoplast and finally, the accumulation of Zn^{2+} ions in the cell vacuole.

With lead uptake, however, there is a disagreement as to how the ions are taken up in moss species. Brown and Bates (1972), state that the uptake of lead from $Pb(NO_3)_2$ in *Grimmia doniana* is by a passive physical process with no lead passing through the cell wall, and the uptake in live and dead material is identical. However, lead-containing particles have been found in the nucleus of some cells of *Rhytiadelphus squarrosus* (Skaar *et al.*, 1973; Gullvag *et al.*, 1974), but the cell wall of another species *Hylocomium splendens* was found to be a barrier to lead penetration.

These varying methods of uptake of Pb^{2+} and Zn^{2+} ions which have been proposed could be the cause of the differential concentrating of the heavy metal ions. The moss species *S. papillosum* concentrated both heavy metal ions to a greater extent than did *C. paradoxus*. Concentrations of both heavy metal ions in *S. papillosum* was approximately twice that in *C. paradoxus*. This could be due to the high percentage of uronic acid in the tissues in *Sphagnum* species, as the cation exchange capacity in these species is closely related to the tissue concentration of these pectic substances (Knight *et al.*, 1961).

Overall the concentrations of heavy metals in the mosses did not appear to deter *Tipula subnodicornis*, to the extent that it was initially expected. The number of spot observations of a larva on moss species gives a sound estimate of the period of time spent on the mosses. A strong positive correlation was found between the percentage of moss particles in the frass of a larva and the percentage of spot observations on that moss species *i.e.* the amount of time a larva spends on a moss species the more likely it is to be feeding on that moss species.

In the majority of cases, there were significantly more spot observations than expected from the control experiments on *S. papillosum* containing the higher concentrations of the heavy metals. This could be due, in part, to a small proportion of the larvae nearing pupation, and, as discussed in Chapter 4, moving onto *S. papillosum* under a thigmotaxic response to a pupation site, rather than for feeding.

In *C. paradoxus* significantly fewer observations were found on the moss containing the higher concentrations of the heavy metals.

Heavy metal concentrations in *S. papillosum* do not appear to have as great an affect as in *C. paradoxus* on the behaviour of *T. subnodicornis* larvae. The number of spot observations, and hence the amount of feeding occurring on *S. papillosum*, did not decrease with increasing concentrations of heavy metal ions. Also, at all heavy metal concentrations, there was no significant difference between the amount of time spent on the moss, although there were a greater number of movements from the moss species with the highest concentrations of heavy metal ions.

In *C. paradoxus*, however, all aspects of the behaviour of *T. subnodicornis* larvae studied appeared to be affected: less spot observations on; more movements from; and less time spent on *C. paradoxus* as the concentration of heavy metal ions increased in the plant.

Therefore there is some evidence that *T. subnodicornis* larvae are deterred from feeding on mosses containing concentrations of heavy metal ions, but to differing degrees with the two moss species studied.

Further studies on this subject which could be carried out include the determination of

the levels of heavy metals actually present in the larvae themselves; to follow closely the affects of the heavy metals have on the larvae as regards survival and fitness and to determine the nutrient levels in *C. paradoxus* and *S. papillosum.*

Campylopus introflexus, *Calluna vulgaris* and bare peat: a typical habitat of *Tipula montana* larvae at Waskerley Common, Co. Durham

Chapter 6. Aspects of the biology of the moss-feeding cranefly, *Tipula montana* at Waskerley Common

6.1. Introduction

Tipula montana and the closely related species *Tipula excisa* are the commonest craneflies in the boreal and alpine areas of the Western Palearctic Region (Theowald and Mannheims 1962). In the past there has been confusion about the presence of *Tipula montana* and *Tipula excisa* in Britain and elsewhere in Europe. Theowald and Mannheims (1962) critically examined museum collections and report that only *Tipula montana* is found in Great Britain. This species is also found in Fennoscandia and in the south and middle European alpine areas whilst *Tipula excisa* is distributed only in Fennoscandia and across the northern part of the former U.S.S.R. References in the literature to *Tipula excisa* in the British Isles refer to *Tipula montana*.

Tipula montana is generally found in montane habitats, particularly on areas with little or no vegetation cover. Coe *et al.*, (1950) state that *Tipula excisa (=montana)* is found only above 2000ft (610m) a.s.l. in the British Isles. However, in May 1990, fourth instar larvae were found during this study on a moorland habitat - Waskerley Common, Co. Durham (Grid Ref.NZ025455), at 400m a.s.l. A small number of adults of this cranefly species had been found in 1976 and 1978 at Waskerley Common, Grid Ref. NZ016447, at 380m a.s.l., (Coulson and Butterfield 1980). At Waskerley, the larvae feed on live and dead *Campylopus introflexus*, (a moss species introduced into Britain in 1941 from the Southern Hemisphere (Watson 1968)), and other mosses growing on recently burnt *Calluna* heath. Details on the feeding of *Tipula montana* are given in Chapters 2 and 3.

Few studies on the biology of *Tipula montana* have been made, although *Tipula excisa* has been studied in Norway (Hofsvang 1972, 1973, 1974). He concludes that in his study area at Finse (1200m a.s.l) all *Tipula excisa* there had a two year life-cycle. In the Cairngorms, with a mean annual temperature of 2.3°C at 4000` at the summit of Cairn Gorm, (Green 1981), it is suggested that *Tipula montana* also has a two year life-cycle, (Galbraith *et al.* 1993). Here adult *Tipula montana* are considered an important food item for dotterel chicks, and their emergence coincides with the hatching of the chicks in June (Galbraith *et al.* 1993). It is of interest to determine the life-cycle of *Tipula montana* at Waskerley because of the lower altitudes and higher temperatures which are present there, (Waskerley Common has a mean annual temperature of 6.2°C (Jennings 1982)), and also because the main emergence takes place a month and a half later than in the Cairngorms.

6.2. Field sites

Waskerley Common is a northern heath, ranging in altitude from 350m to 550m. Thirteen areas within Waskerley Common in which the heather *Calluna vulgaris* had been burnt within the last ten years were chosen as sites and these bare peat areas were partially colonised by *Campylopus introflexus* to varying degrees. Table 6.1 gives a description of these field sites. As Sites W1 (at 440m, Grid Reference NZ014454) and W2 (at 465m, Grid Reference NZ009450) yielded the greatest number of *Tipula montana* these were accordingly investigated intensely.

In addition, seven sites with 100% cover of *Calluna vulgaris* were also investigated. Details of these are given on Table 6.2.

Table 6.1. Description of field sites at Waskerley in which *Campylopus introflexus* is the dominant vegetation.

Fieldsite with Grid Ref.	Altitude (m)	Approximate year of last burning *	Cover of *Campylopus introflexus*
W1 (NZ014454)	440	1984	40%
W2 (NZ009450)	465	1987	65%
WA (NZ045456)	350	1983	90%
WB (NZ040454)	370	1985	75%
WC (NZ035455)	390	1981	70%
WD (NZ031455)	395	1986	50%
WE (NZ026454)	400	1982	60%
WF (NZ021453)	420	1982	70%
WG (NZ042458)	340	1983	60%
WH (NZ012456)	430	1990	<10%
WI (NZ014453)	455	1990	<10%
WJ (NZ025457)	400	1990	<10%
WL (NZ038455)	390	1990	<10%

* Information from D. Dodds (pers. comm.)

Table 6.2. Description of field sites at Waskerley with 100% cover of *Calluna vulgaris*.

Fieldsite with Grid Ref.	Altitude (m)	Height of *Calluna vulgaris* (cm)
H1 (NZ015456)	430	3-6
H2 (NZ016455)	440	10
H3 (NZ015457)	410	40-50
H4 (NZ048456)	360	5-10
H5 (NZ047455)	360	20-30
H6 (NZ044454)	370	50
H7 (NZ024457)	400	50

6.3. Field collection of *Tipula montana*

Collections of *T. montana* larvae, pupae and adults from the field were made between May 1991 and May 1992. Second, third and fourth instars were found in the field together with a few pupae and adults. Two methods were employed in the collections:

i) Collection of soil samples from the sites and heat extraction of larvae (and pupae) from these in Berlese funnels (Southwood 1971).

ii) Using searching techniques: 30 minute time searches for larvae and pupae; 10 minute time searches for adults, or thorough searching over a defined area.

Additional pupae and adults were obtained from larvae which were kept in the laboratory (see Section 6.5.1.).

Table 6.3 shows the total number of each stage of *T. montana* collected at each site investigated. First instar larvae were obtained from eggs kept in the laboratory.

In addition the development period to hatching of eggs kept at different constant temperatures was determined (see Section 6.7).

6.4. Determination of the number of larval instars in *Tipula montana*

Members of the Tipulinae have four instar stages (Alexander 1920, Coulson 1956, Brindle 1960). These instars can readily be separated using the diameter of the spiracular disc at the posterior end of the animal (Coulson 1956, Hemmingsen 1965, Hadley 1971). Separation of the instars using other linear measurements of the larvae have not proved reliable. Hemmingsen (1965) states that the presence of head capsule size groups (when measured at the maximum width) is no proof of corresponding instars, and this probably applies to the mandible length also.

Table 6.3. Numbers of *Tipula montana* found at each fieldsite with unit effort on Waskerley Common 1991/92.

Site	Total number of *Tipula montana* found					
	Larval instars				Pupae	Adults
	2nd	3rd	4th	Total		
W1	21	69	83	173	7	29
W2	0	19	22	41	0	24
WA	0	1	11	12	0	18
WB	2	0	3	5	1	20
WC	0	4	3	7	0	8
WD	4	2	4	10	1	34
WE	0	12	10	22	1	18
WF	0	0	2	2	0	21
WG	0	0	6	6	0	5
WH	0	0	5	5	0	-
WI	0	2	7	9	0	-
WJ	0	0	0	0	0	-
WK	0	0	0	0	0	-
H1	0	0	0	0	0	-
H2	0	0	0	0	0	-
H3	0	0	0	0	0	-
H4	0	0	0	0	0	-
H5	0	0	0	0	0	-
H6	0	0	0	0	0	-
H7	0	0	0	0	0	-

Footnote: i) 1st instar larvae were not found on the field sites.
ii) For Sites WH-Wk and Sites H1-H7 there was only one search for larvae and they were not searched for adults.

The head capsule grows and expands within an instar and grows differently in different areas and so is not a reliable measurement for separating the instar stages (Coulson 1956, Hemmingsen 1965). For example, there is less growth in the anterior, fully sclerotised part of the head capsule than in the parts which are imperfectly sclerotised and embedded in the body of the larva. It had been suggested that body length increased in 'leaps' between instars (Sellke 1936) *i.e.* the larvae soon reach a certain body weight after a moult which they retain until the next moult. However Hemmingsen (1965) found that there was growth in body length throughout an instar and Byers (1961) states that body size (length) in *Dolicopeza* increases rapidly immediately after moulting to the next instar and then increases more slowly. Thus the growth within each instar causes overlapping in the distribution of body lengths between the instars.

It soon became evident that the spiracular disc diameter was the most reliable indicator of instar. The diameter of the left spiracular disc was measured at the same magnification for all the instars *i.e.* x50. The length of the left mandible and the body length of each larva examined were also measured to determine the distributions within each instar.

Fig. 6.1 shows a histogram of the distribution of spiracular disc diameters and indicates the presence of the four larval instars. Each instar has a discrete range of diameters, with no overlapping between the instars. Therefore larvae of *T. montana* can be confidently ascribed to one of the four instars by the spiracular disc diameter. The mean spiracular disc diameter and standard deviation for each instar is given in Table 6.4.

Fig. 6.2 show the logarithm of the mean spiracular disc diameter plotted against the provisional instar number and indicates that no intermediate instar has been missed. The first instar was confirmed by larvae hatched from eggs and the final instar has been confirmed by measurements just prior to pupation. Information was also obtained on the change in spiracular disc diameter for individual larvae which moulted whilst in culture. These changes were entirely consistent with those in Fig. 6.1.

Fig. 6.1.

Frequency histogram of the spiracular disc diameters of *Tipula montana* larvae from Waskerley Common, 1991/1992.

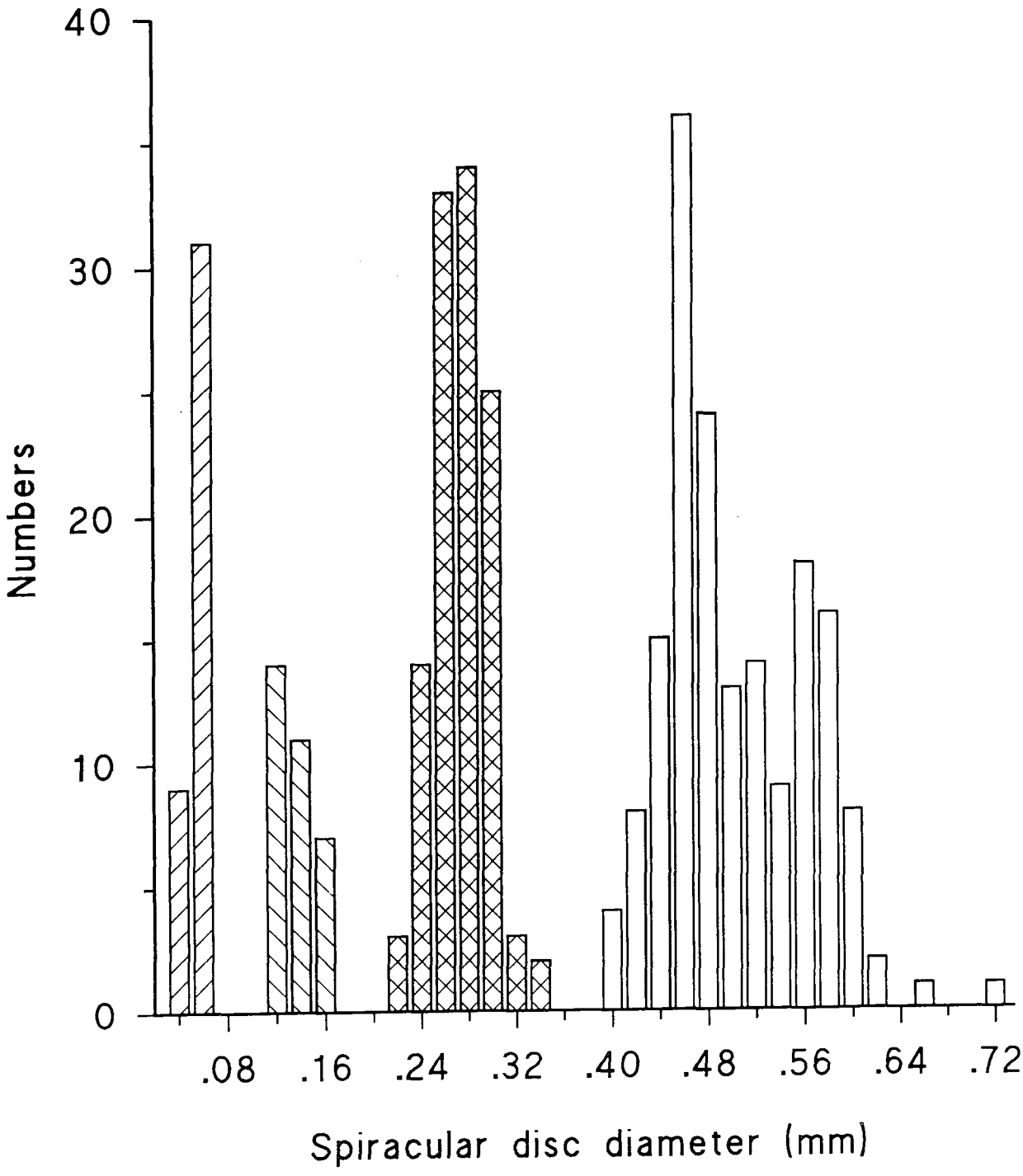


Fig. 6.2.

The relationship between the logarithm of the mean spiracular disc diameter and instar in *Tipula montana* from Waskerley Common.

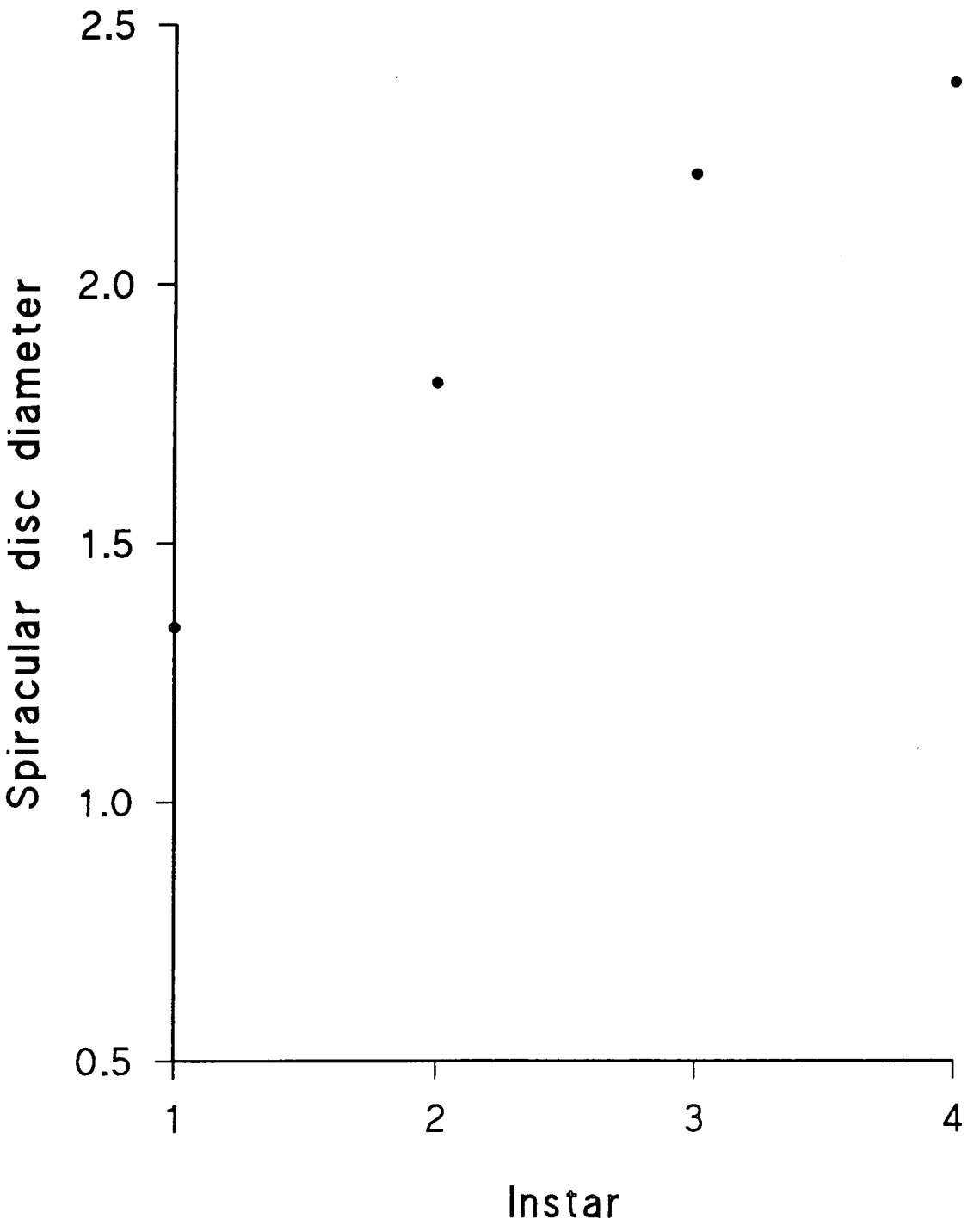


Table 6.4. Biometric measurements of each instar of *Tipula montana*, with n being the total number of individuals involved in the analysis.

Biometric measurement	Instar			
	I	II	III	IV
Spiracular disc diameter				
n	40	27	109	156
Mean	0.046	0.136	0.274	0.507
SD	0.009	0.016	0.027	0.057
Increase	x3.0	x2.0	x1.8	
Mandible length				
n	20	28	51	133
Mean	0.113	0.167	0.263	0.410
SD	0.015	0.015	0.049	0.075
Increase	x1.5	x1.6	x1.6	
Body length				
n	20	22	51	133
Mean	2.366	5.157	12.520	16.835
SD	0.509	0.703	1.385	2.993
Increase	x2.2	x2.4	x1.3	

Fig. 6.3.

Frequency histogram of the mandible lengths of *Tipula montana* larvae from Waskerley Common, 1991/1992.

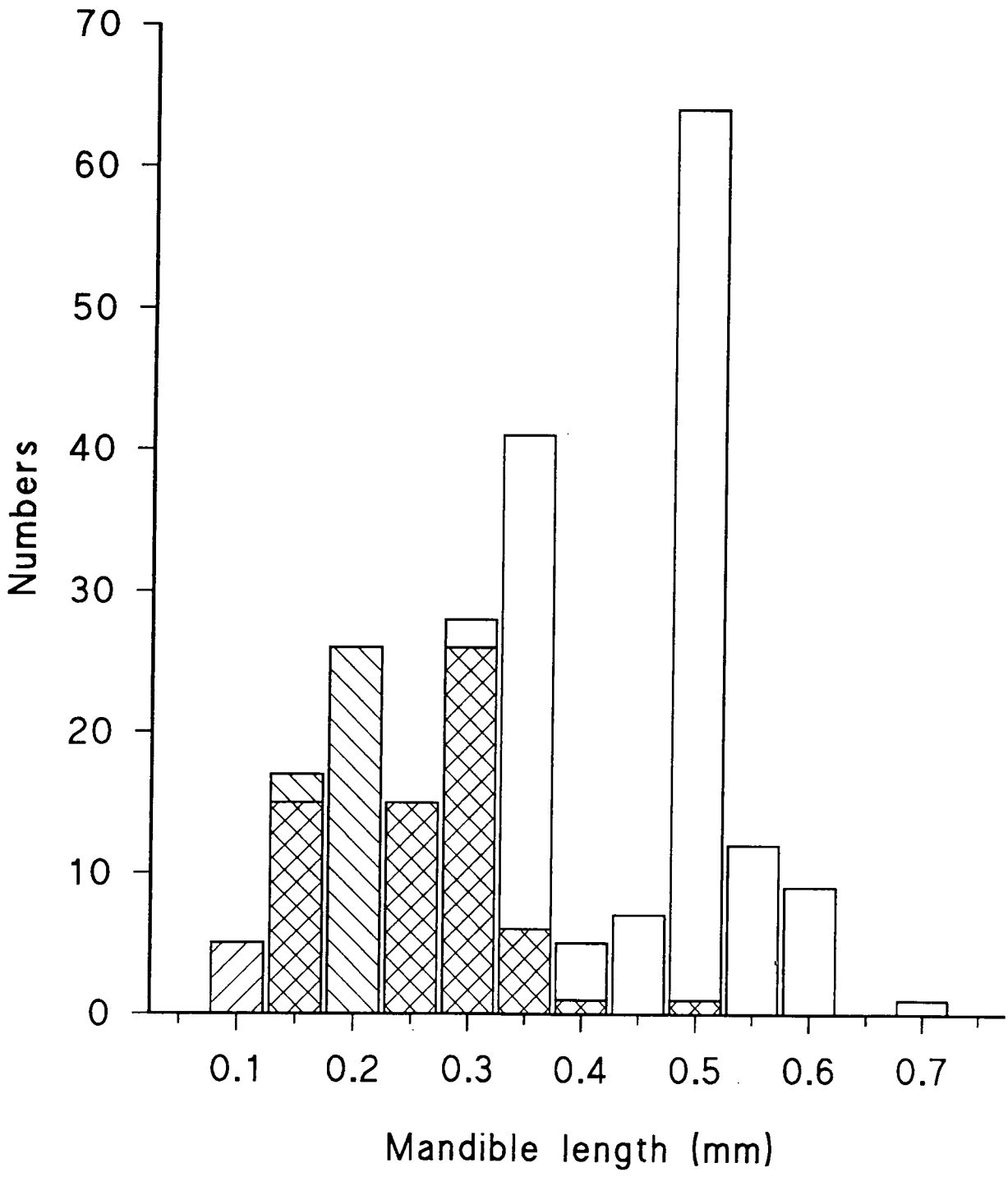
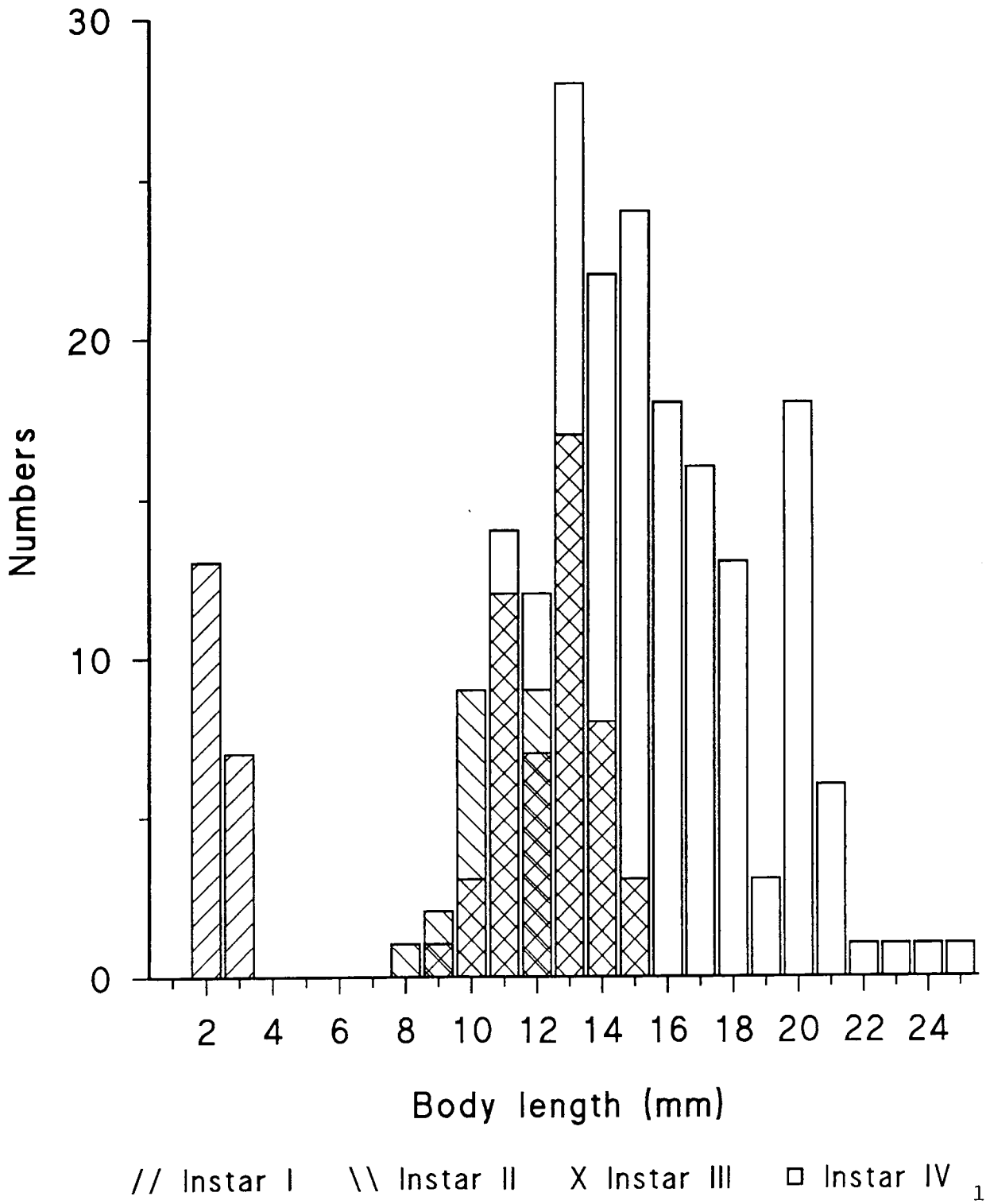


Fig. 6.4.

Frequency histogram of the body lengths *Tipula montana* larvae from Waskerley Common, 1991/1992.

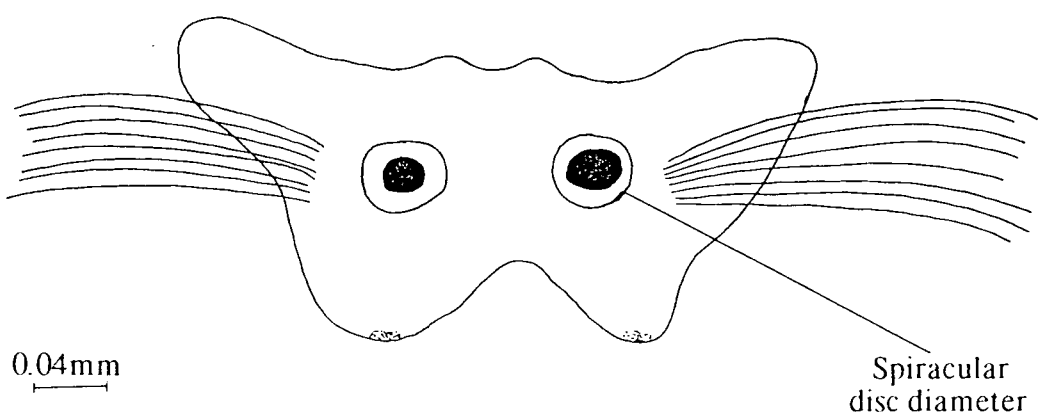


The histogram of the distribution of mandible lengths is shown in Fig. 6.3. As overlapping occurs in the mandible length this does not show the discrete separation corresponding to the instars. The mean mandible length for each instar (determined by the spiracular disc diameter) is given in Table 6.4.

The distribution of body length in relation to the instar is shown in Fig. 6.4. It clearly shows that there is considerable growth within each instar and that this parameter is of little value in separating instars. The mean body length of each instar is given in Table 6.4.

Larvae in instar I are different from the other instars in having a smaller spiracular disc diameter; by having a group of up to eight long hairs protruding at the posterior end of the larva from either side of the spiracular field (the area which contains the spiracular discs); the dorsal lobes are very much reduced and are not sclerotised and the sclerotisation on the ventral lobes is lighter in colour. Instars II, III, and IV are similar to each other, except for the differences in size *e.g.* spiracular disc diameter. Drawings of the posterior end of instar I and instar IV are given below in Fig. 6.5.

Instar I



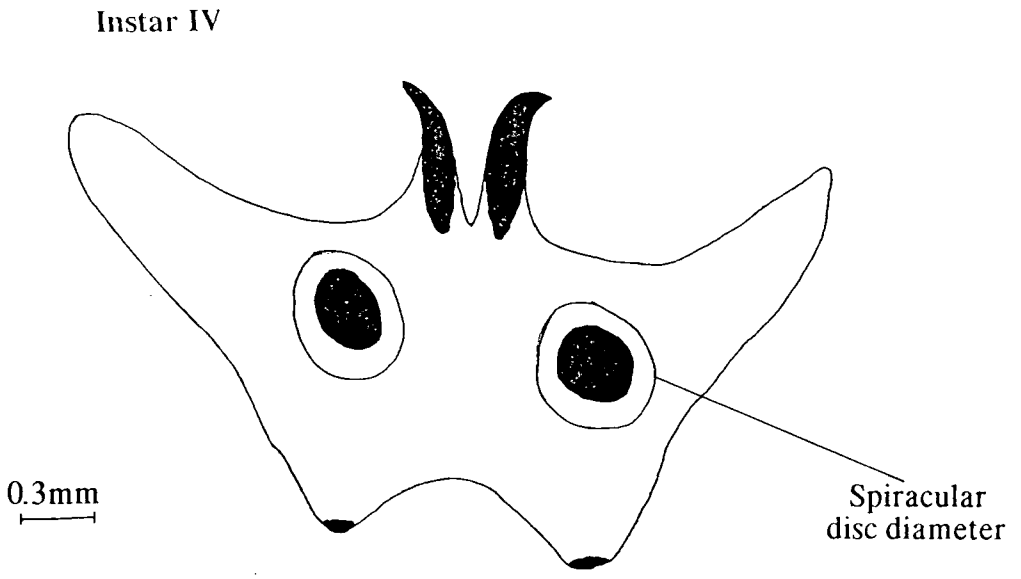


Fig. 6.5

6.4.1. Use of Dyar's Law

Dyar's Law (Dyar 1890) states that biometric measurements augment by a constant multiplier from one instar to the next in many insect larvae and has been applied to tipulids by Hemmingsen (1959) and Byers (1961). In *T. montana*, mandible length follows Dyar's Law, with increases of a factor of 1.5-1.6 between instars; see Table 6.4. The spiracular disc diameters and the body lengths do not appear to follow Dyar's Law closely - see Table 6.4.

Increases in the spiracular disc diameters between instars II and III and instars III and IV are similar and follow Dyar's Law, but not between instars I and II. It is possible that the deviation resulted from the first instars being measured when first hatched and so there would not be the range of measurements on individuals throughout the instar, as in instars II, III and IV.

Dyar's Law is not followed by body length throughout all the instars. This could be due to the posture of the animal when measured in the fourth instar stage as

the larvae have the ability to expand and contract, as it does appear to hold between instars I and II and between instars II and III.

Dyar's Law does not appear to be held for weights and the reasons for this are discussed in Section 6.6.1.

6.5 Bimodality of biometric measurements in fourth instar larvae

Freeman (1964) reported a bimodal variation in the head capsule length in fourth instar larvae of *Tipula luna* and suggested that this reflected a size difference between the sexes. In this study, bimodality was found in the spiracular disc diameters of fourth instar larvae and there were clear differences between larvae which eventually became male or female pupae, (see Figs. 6.1 and 6.6). Those fourth instar larvae with a spiracular disc diameter below 0.51mm were males and those with a spiracular disc diameter above 0.50mm were females, with complete separation between the two sexes, (Fig. 6.6).

The mean spiracular disc diameters in all male fourth instars is 0.476±0.003mm (S.E.), and that for all female fourth instars is 0.565±0.004mm (S.E.), a significant difference ($t=19.6$, $df=166$, $p<0.001$). Bimodality was not found in the mandible lengths (see Fig. 6.7).

Fourth instar larvae were divided into males and females using the spiracular disc diameters and the body length was measured (Fig. 6.8). There are two peaks, which can be attributed to male and female larvae. There is much overlap between the sexes, but only females are present in the highest categories, and only males present in the lowest categories. This overlapping could be in part due to the posture of the animal when measured as mentioned in Section 6.4.1.

Fig. 6.6.

Frequency histogram of the spiracular disc diameters of fourth instar *Tipula montana* larvae which pupated, from Waskerley Common, 1991/1992.

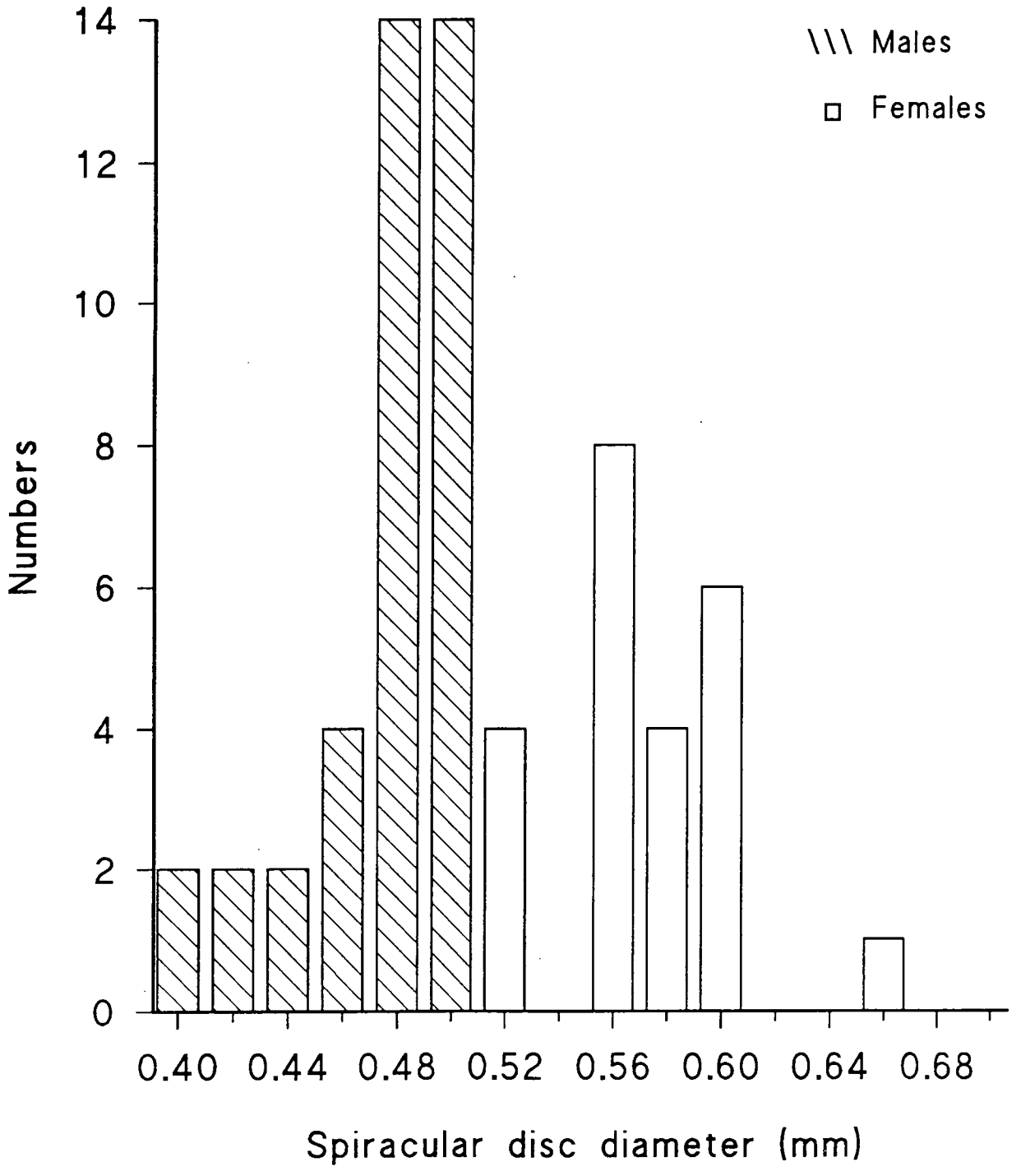


Fig. 6.7.

Frequency histogram of the mandible lengths of fourth instar *Tipula montana* larvae which pupated, from Waskerley Common, 1991/1992.

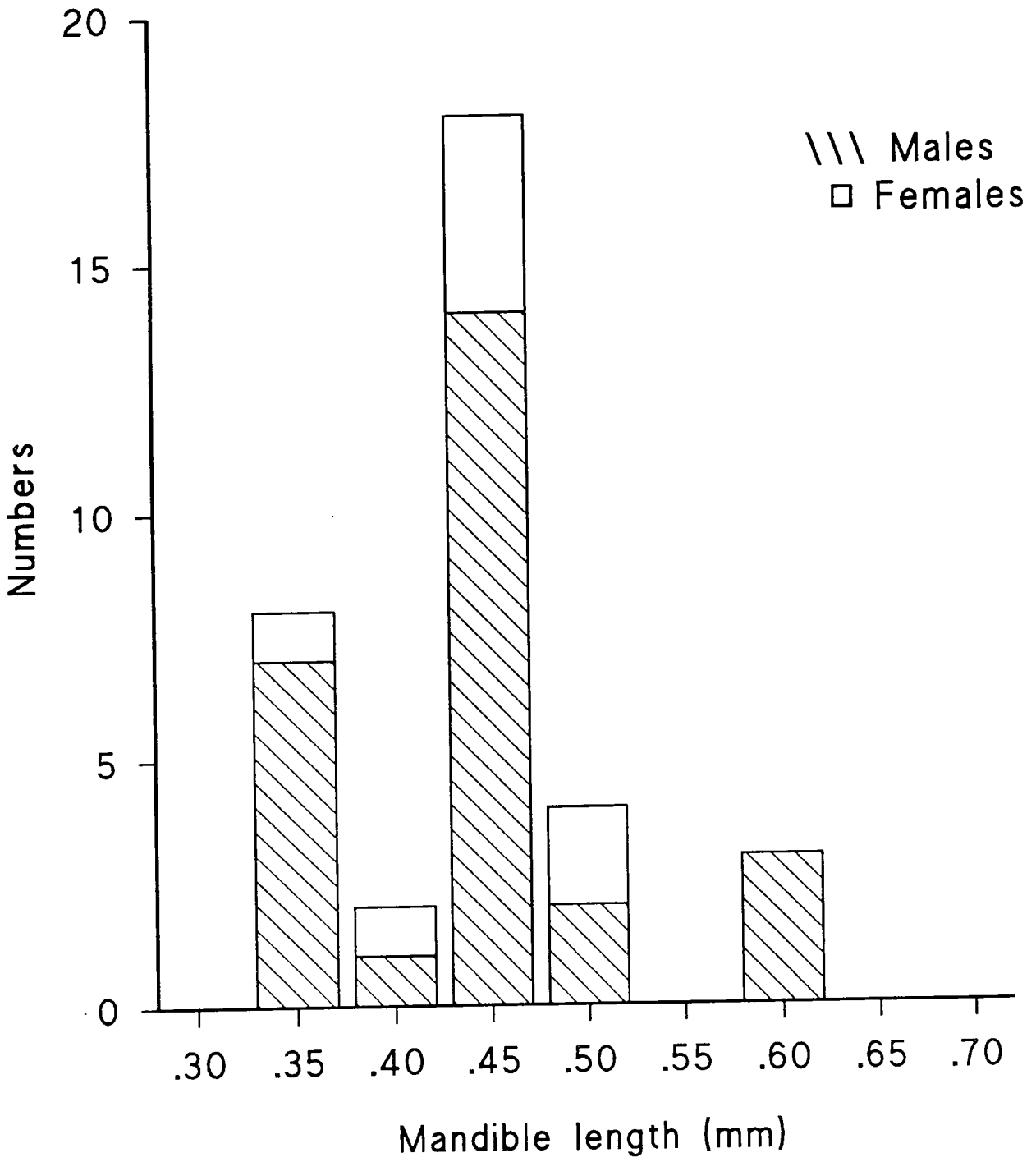
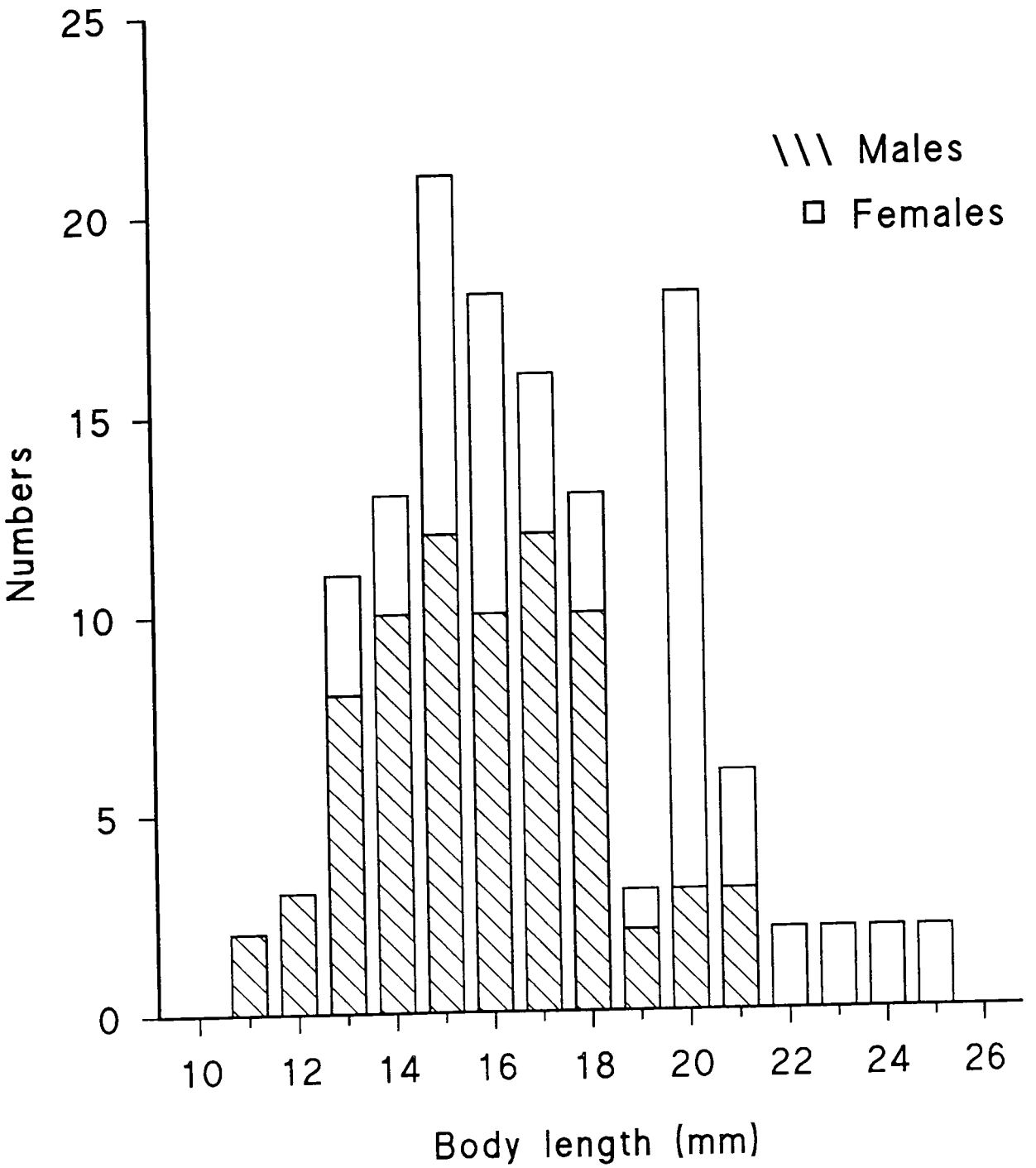


Fig. 6.8.

Frequency histogram of the body lengths of fourth instar *Tipula montana* larvae which pupated, from Waskerley Common, 1991/1992.



6.6. Laboratory studies on *Tipula montana*

Each larva collected in the field or extracted from the Berlese funnels was placed separately in a 9cm petri-dish with damp filter paper and an adequate supply of food: *Campylopus introflexus* from the field site. In this way, the progress of an each individual through the instars could be monitored and weight changes towards and at pupation could be determined. All larvae were kept at 10°C.

6.6.1. Determination of larval weights

Each larva and pupa collected in the field or extracted from the Berlese funnels was weighed before being placed in its individual petri-dish. The instar of each larvae had been determined using the spiracular disc diameters, and therefore these weights were used to determine the mean weight of larvae in each instar (Table 6.5). Dyar's Law does not appear to be followed for weights. This could be due to similar reasons as given for the spiracular disc diameters in Section 6.4.1., *i.e.* that a range of weights was not obtained throughout the first instar stage, but only from newly hatched individuals. The increases in mean weights between second and third instars, and between third and fourth instars are similar (x3.5 and x2.7 respectively) compared to the increase between first and second instars which is an order of magnitude greater (x36.0).

The weight of each individual fourth instar larva was monitored up to and at pupation. The maximum live weight reached by each fourth instar male and female larva of *T. montana* was determined. The sex of the individual was identified when pupation occurred, using the shape of the tip of the abdomen, where the ovipositor is obvious in the female.

There were two clear size classes in the maximum weights recorded in each of

Table 6.5. Mean weights of each instar of *Tipula montana*.

Instar	Number measured	Mean weights in mg (±S.D.)	Increase
1	80	0.36 ± 0.04 (at hatching)	x36.0
2	30	13.00 ± 2.74	x 3.5
3	131	46.00 ±11.44	x 2.7
4	174	124.00 ±52.76	

the fourth instar larvae. Larvae in the smaller size class became male pupae and those in the larger size class became female pupae (Tables 6.6 and 6.7). The mean maximum weight for female larvae, 227.6±8.9mg (S.E.) is significantly higher than the mean maximum weight for male larvae, 132.4±4.0mg (S.E.), t=9.8 df=35 p<0.001. The mean weight of male larvae is 58% of the mean weight of female larvae.

Fig. 6.9 gives the distribution of weights of fourth instar male and female larvae. Female larval weights are distributed throughout the range, and male larval weights range from 0.04mg to 0.17mg. The weights presented here are from larvae throughout the fourth instar stage and no male larvae exceeds 0.17mg in weight - all larvae of this weight and above being females.

6.6.2. Determination of pupal weights

Individual pupae collected from the field and those obtained in the laboratory were used in the calculation of the mean weight of male and female pupae. There was no significant difference between the weights in the two sources :

Mean weight of male pupae (±S.E.):

All individuals (n=48) = 88.4±2.6mg (±18.44mg S.D.)

Field individuals (n=32) = 90.2±3.1mg

t=0.7 df=46ns

Laboratory individuals (n=16) = 85.7±5.3mg

Mean weight of female pupae (±S.E.):

All individuals (n=28) = 129.0±6.0mg (±31.89mg S.D.)

Field individuals (n=16) = 138.1±9.1mg

t=1.9 df=26ns

Laboratory individuals (n=12) = 117.7±5.9mg

The mean weight of male pupae is 68% of the mean weight of female pupae.

Table 6.6. Maximum weights of female instar larvae of *Tipula montana*.

Larva	Weight (mg)	Days before pupation	Pupal weight (mg)	% loss in weight
86	211.8	15	103.5	51.1
94	206.2	24	108.1	47.6
96	231.0	13	128.3	44.4
98	168.0	15	84.7	49.6
99	246.0	13	131.0	46.7
158	241.7	20	129.7	46.3
169	216.0	10	134.9	37.5
171	267.5	16	167.1	37.5
173	246.5	10	155.8	36.8
175	264.3	10	184.1	32.6
459	205.0	5	70.2	65.6
Mean	227.6 ±8.9(S.E.)	13.7 ±1.5(S.E.)		

Table 6.7. Maximum weights of male fourth instar larvae of *Tipula montana*.

Larva	Weight (mg)	Days before pupation	Pupal weight (mg)	% loss in weight
67	105.7	12	61.1	42.2
76	101.4	4	58.5	42.3
93	121.2	10	81.0	33.2
95	135.1	16	82.2	39.2
97	107.0	16	60.5	43.4
101	134.0	12	90.7	32.3
103	104.8	3	76.8	26.7
154	148.0	10	104.4	29.4
157	134.0	12	82.5	38.4
159	168.2	10	112.7	33.0
161	125.1	16	88.2	29.5
162	125.5	13	94.9	24.4
163	131.0	20	87.1	33.5
164	177.6	13	-	-

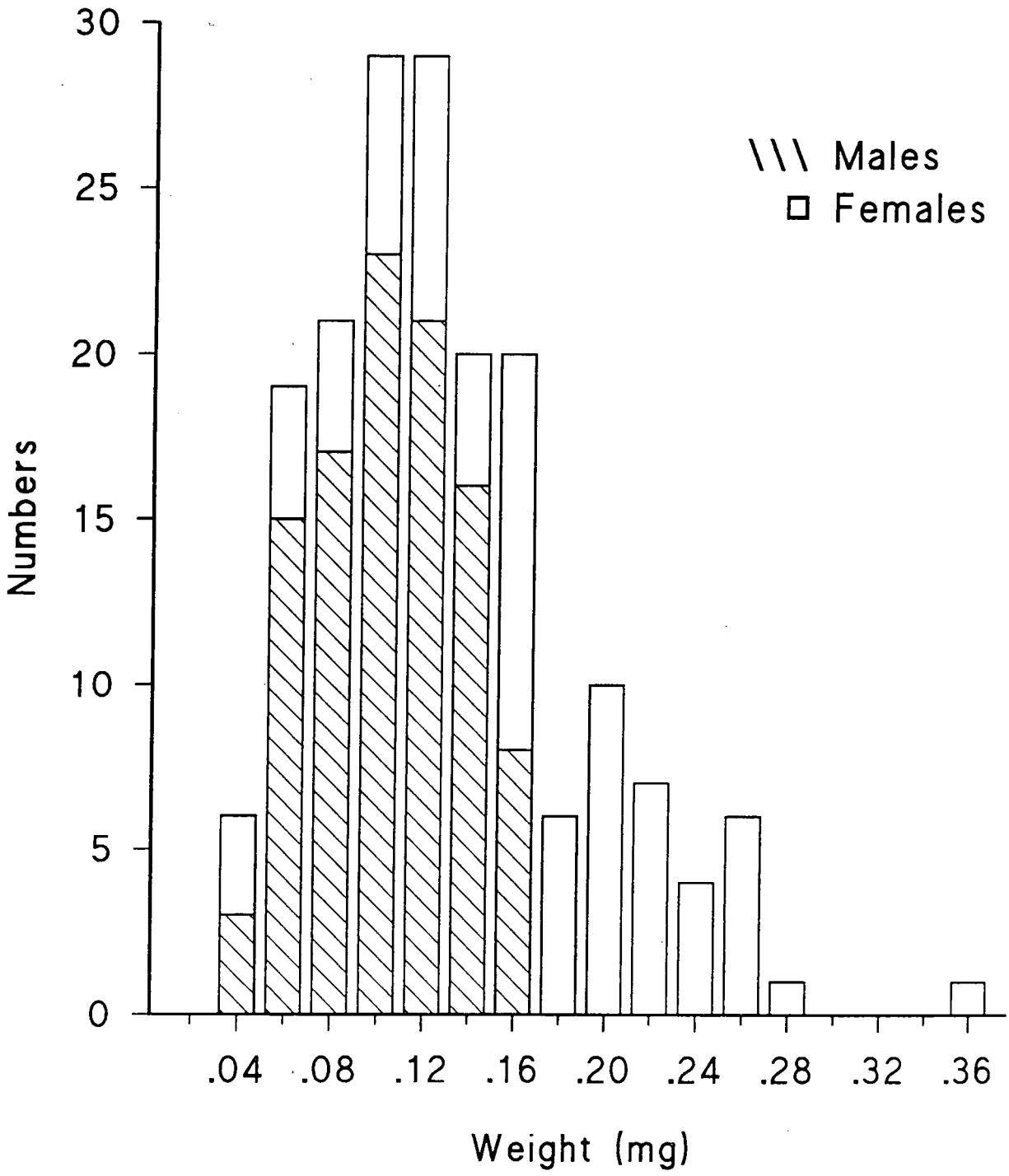
cont'd\

Table 6.7 (cont'd).

Larva	Weight (mg)	Days before pupation	Pupal weight (mg)	% loss in weight
165	122.0	19	87.0	28.7
166	140.1	16	94.4	32.6
167	139.2	13	99.7	28.4
168	163.2	16	-	-
170	141.0	19	92.2	34.6
176	142.3	28	83.0	41.7
177	138.2	10	98.0	29.1
179	166.3	10	122.0	26.6
260	152.0	6	121.2	20.3
261	114.1	6	75.3	34.0
540	125.1	10	-	-
541	89.5	7	54.8	38.8
543	125.7	4	81.0	35.6
544	123.6	7	78.1	36.8
Mean	132.4 ±4.0(S.E.)	13.2 ±1.0(S.E.)		

Fig. 6.9.

Frequency histogram of the weights of fourth instar *Tipula montana* larvae which pupated, from Waskerley Common, 1991/1992.



The weight of final instar larvae in the genus *Tipula* decreases just before pupation *e.g. T. oleracea* lost 40% to 50% of its live weight in the week prior to pupation (Laughlin 1960), and in *Tipula subnodicornis* the weight loss was found to be 38% (Coulson 1962).

Male pupae of *T. montana* lose 33.4±1.2% of their live weight in changing from a larva (using the maximum recorded weight) to a pupa. For female pupae this figure is 45.1±2.7% thus there is a significantly greater loss in weight at pupation in females, than in males ($t=3.9$ $df=34$ $p<0.01$). These losses in weight are probably mainly due to the expenditure of reserves involved in the formation of the pupa (and eggs in the female), but also to the emptying of the larval gut, water loss prior to pupation and the cast larval skin (Laughlin 1960). The reasons for the differential weight losses in male and female larvae were not investigated further in this study.

Growth curves for fourth instar larvae are presented as a percentage of pupal weights in Fig. 6.10 for five females and in Fig. 6.11 for eight males. These show that there is a general increase in larval weights up to approximately 15 days before pupation, after which time the weight decreases up to the time of pupation. For female larvae (Fig. 6.10) 69% of the variation is explained by the regression equation for larval weights 15 or more days before pupation. For male larvae (Fig. 6.11) this figure is lower, at 50%. The mean minimum number of days before pupation when weight had increased in the fourth instar larvae is 14.2±0.6 (S.E.) days for females and 15.7±1.5 (S.E.) days for males, with no significant difference between them. In *T. oleracea*, the peak weight is reached one week before pupation occurs (Laughlin 1960).

6.7 Adult emergences in the laboratory and in the field

There were two distinct emergences, in early June and August 1991, of

Fig. 6.10

Weights of fourth instar female larvae of *Tipula montana* as a percentage of the pupal weight. The regression equation for larval weights 15 days or more before pupation (●) is:

$y = -1.37x + 220.31$ $r_{11} = 0.83$ $p<0.001$.

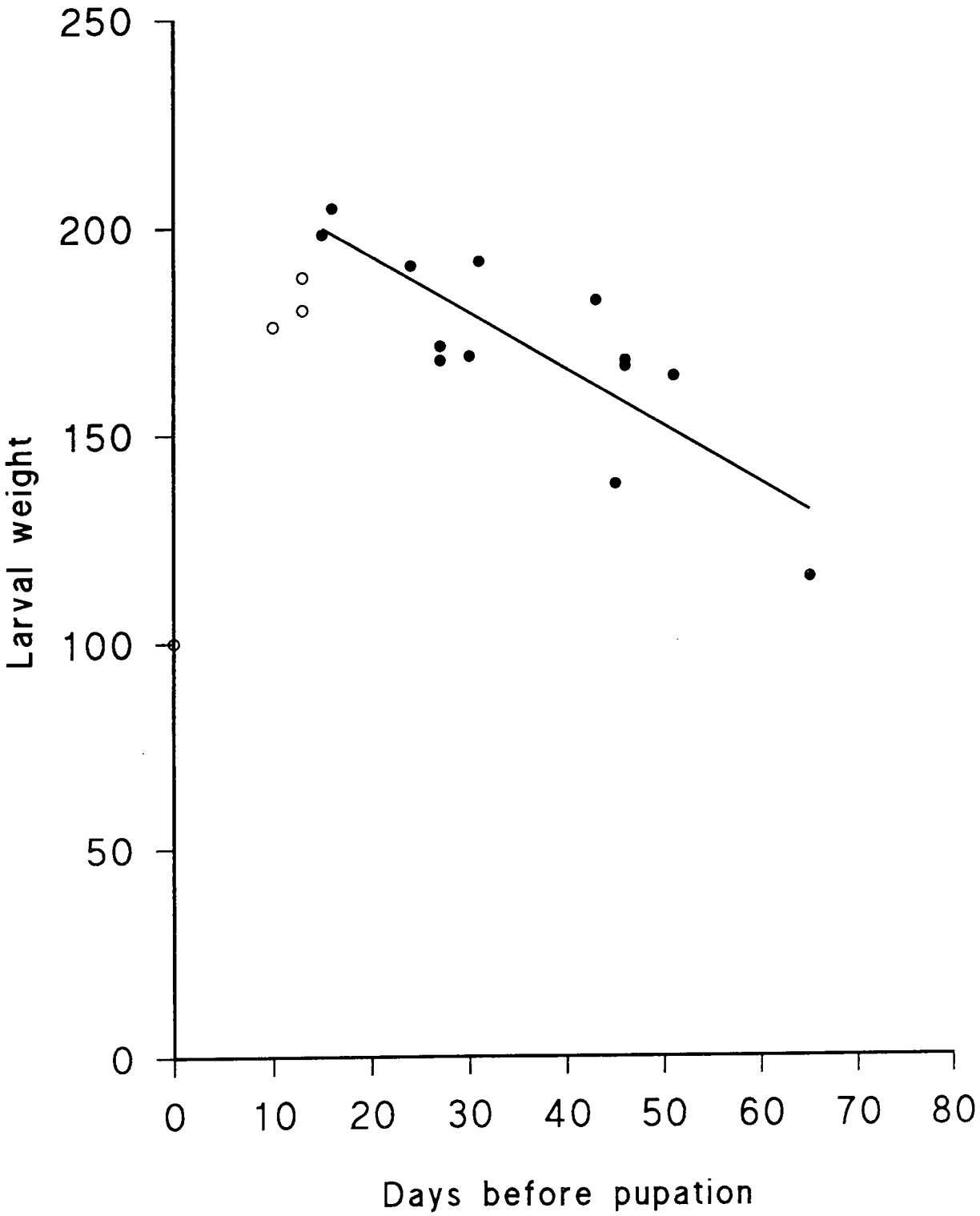
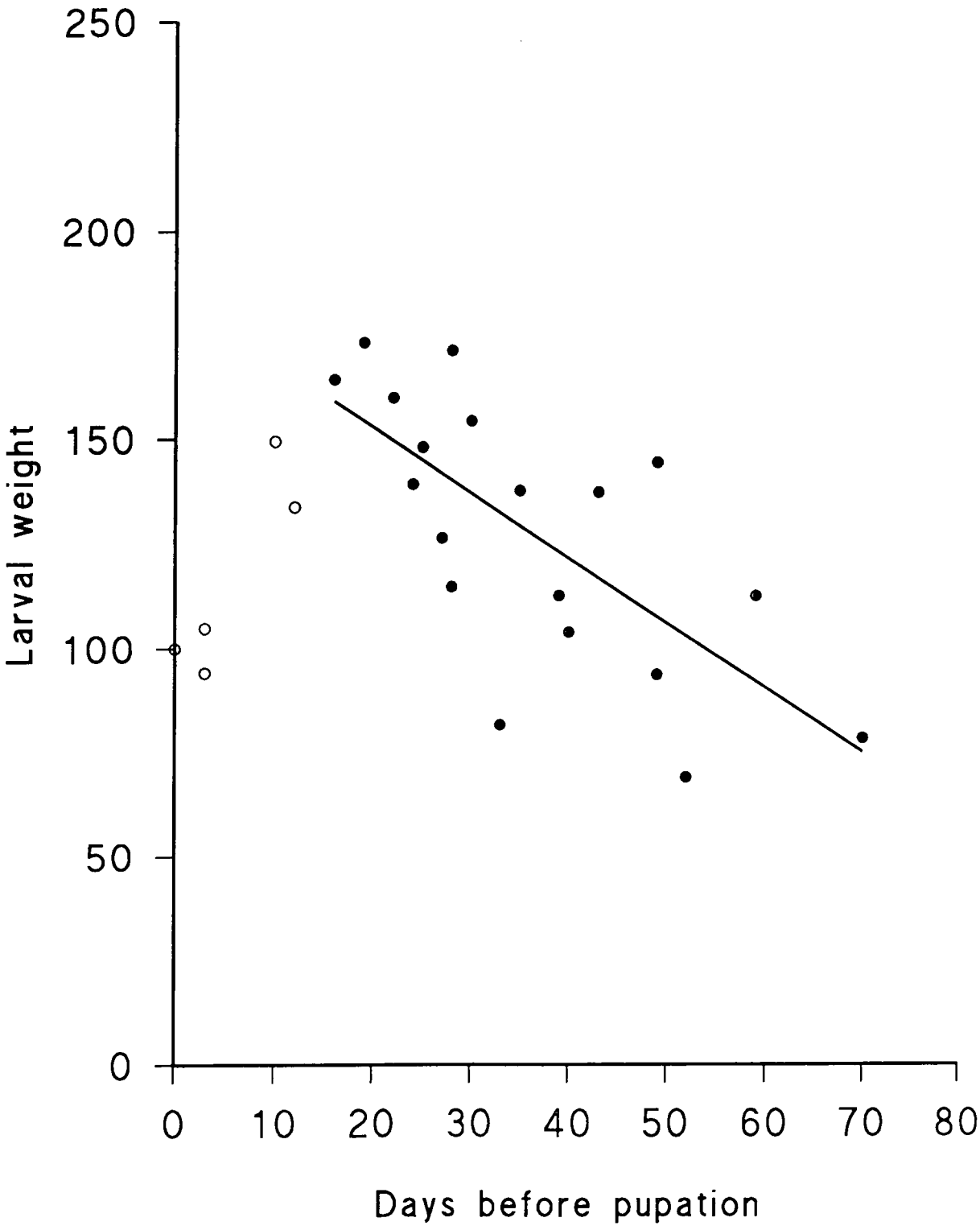


Fig. 6.11

Weights of fourth instar male larvae of *Tipula montana* as a percentage of the pupal weight. The regression equation for larval weights 15 days or more before pupation (●) is: $y = -1.56x + 184.08$ $r_{17} = -0.70$ $p<0.01$.



T. montana adults in the laboratory from larvae collected in May from Waskerley Common (the larvae and pupae of which had been kept at 10°C). Only one emergence was observed in the field, from mid-June to mid-July 1991. In late April, four pupae (two male and two female) developed in the laboratory at 10°C, and adults emerged from these in early June. Thereafter no further adult emerged until early August (see Fig. 6.12 for details of the laboratory emergences). This suggests that there is possibly two emergences of *T. montana* per year - the earlier one being very much smaller than the later one. As a result of this, intensive searches for adults in the field began in mid-June, and continued through the emergence period, at four day intervals. Emergences before mid-June would have been missed.

The first adults were observed in the field on 17 July 1991. From then and until mid-August (the end of the emergence period) a total of 186 adults were collected: 163 males and 23 females; see Fig. 6.13. In 1992, fourteen adult *T. montana* were found in pitfall traps at Waskerley Common (Grid Ref. NZ014454) between mid-May and early June (Coulson pers. comm.), verifying the existence of the smaller spring emergence in the field suggested by the laboratory emergence in 1991. The summer emergence began on 27 July 1992; seven adult males and two adult females being observed (pers. obs.).

There was no diurnal pattern observed in the time of copulation; when females emerged and the time when males were searching for females - these activities occurred at any time during daylight hours. Observations on activities at night were not carried out in this study.

From the number of pupae obtained in the laboratory, there is a ratio of males:females of approximately 2:1. From the numbers of adults found in the field, this ratio was 7:1 (males:females). This is probably due to behavioural differences in the sexes in that the males were found to be much more mobile than the females: females were found on the peat, often camouflaged and in copulation, whereas the majority of males were found on the wing or walking across the surface of the peat. Adults in both sexes lived only for a maximum of six days in the laboratory with no

Fig. 6.12.

Frequency histogram of the number of adult *Tipula montana* emerging in the laboratory in 1991.

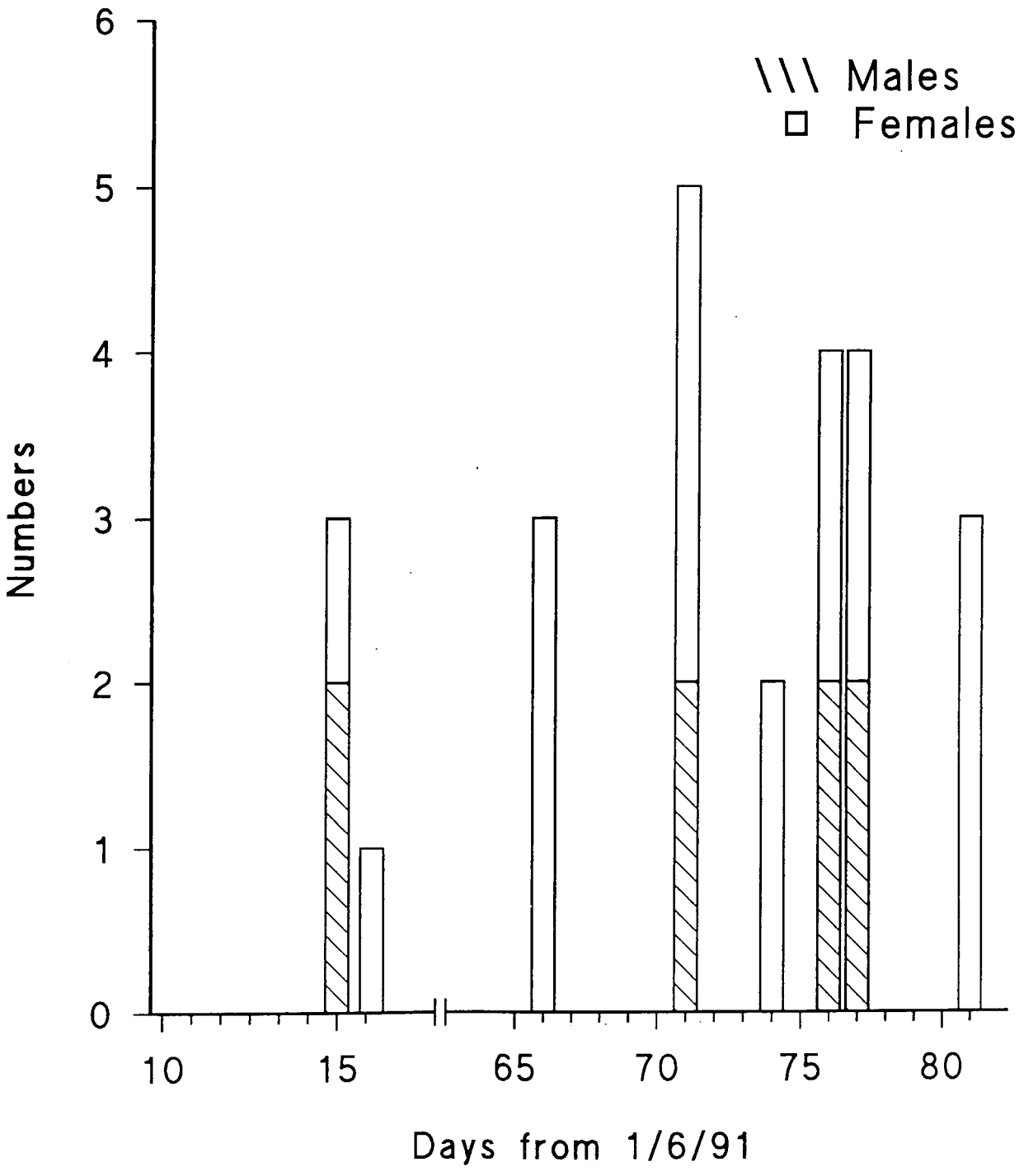
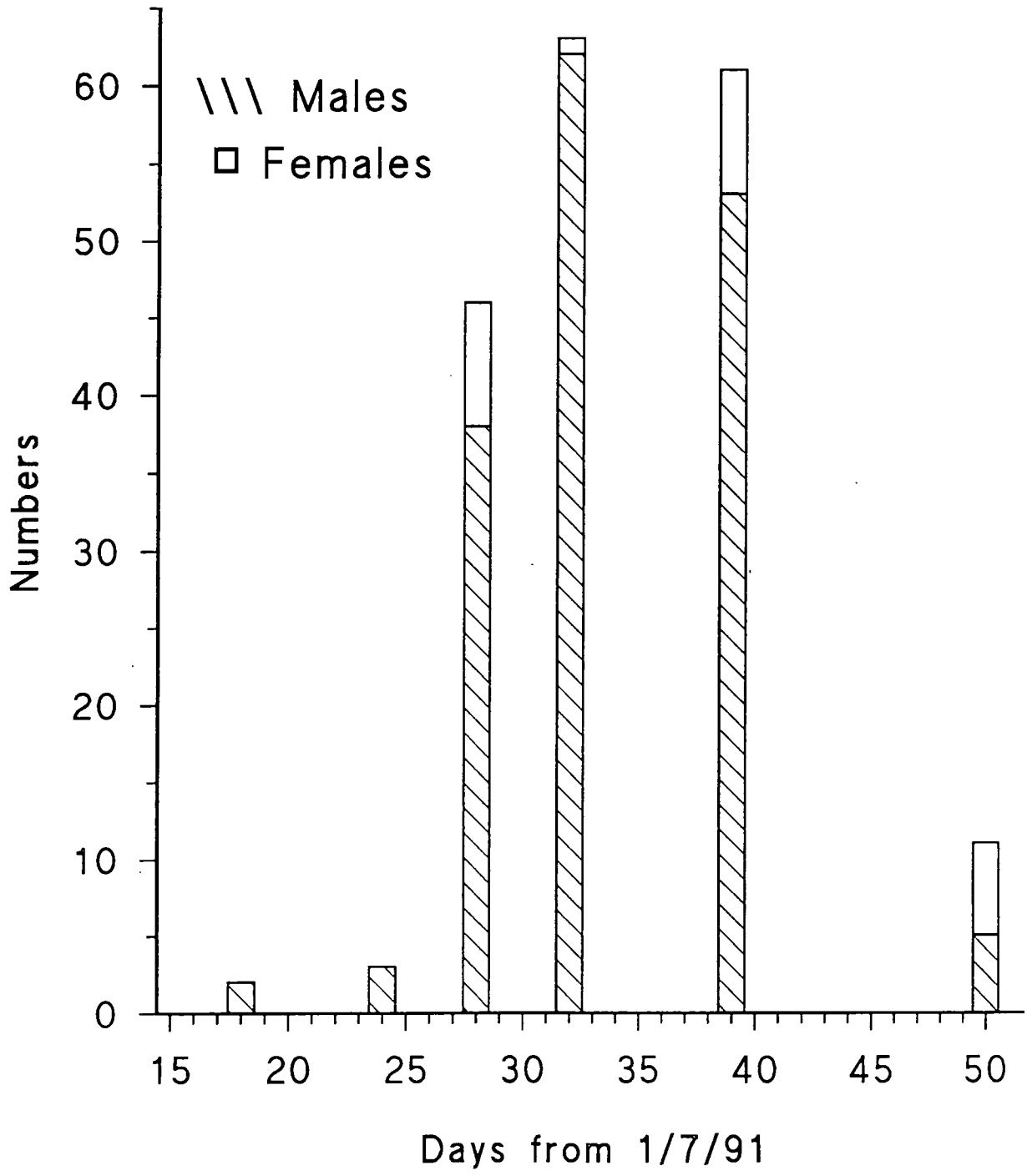


Fig. 6.13.

Frequency histogram of the number of adult *Tipula montana* collected at Waskerley Common in 1991, with unit effort on each date.



difference between the sexes.

6.7.1. Development of eggs in the laboratory

A number of the adult females which had been collected in the field or had emerged in the laboratory were placed separately in large plastic pots with damp tissue paper and two or three adult males. The eggs obtained were placed in 9cm petri-dishes containing damp filter paper, and kept at a range of different constant temperatures and a long photo-period (18h light).

Table 6.8 gives the time to hatching of *T. montana* eggs kept at the different temperatures. Fig. 6.14 gives the rate of development at constant temperatures of eggs of *T. montana*. This appears to be more similar to, although higher than, the development rate of *T. subnodicornis* eggs than to the development rate of *T. pagana* eggs (Butterfield and Coulson 1988).

6.7.2. Ground temperatures at Waskerley Common

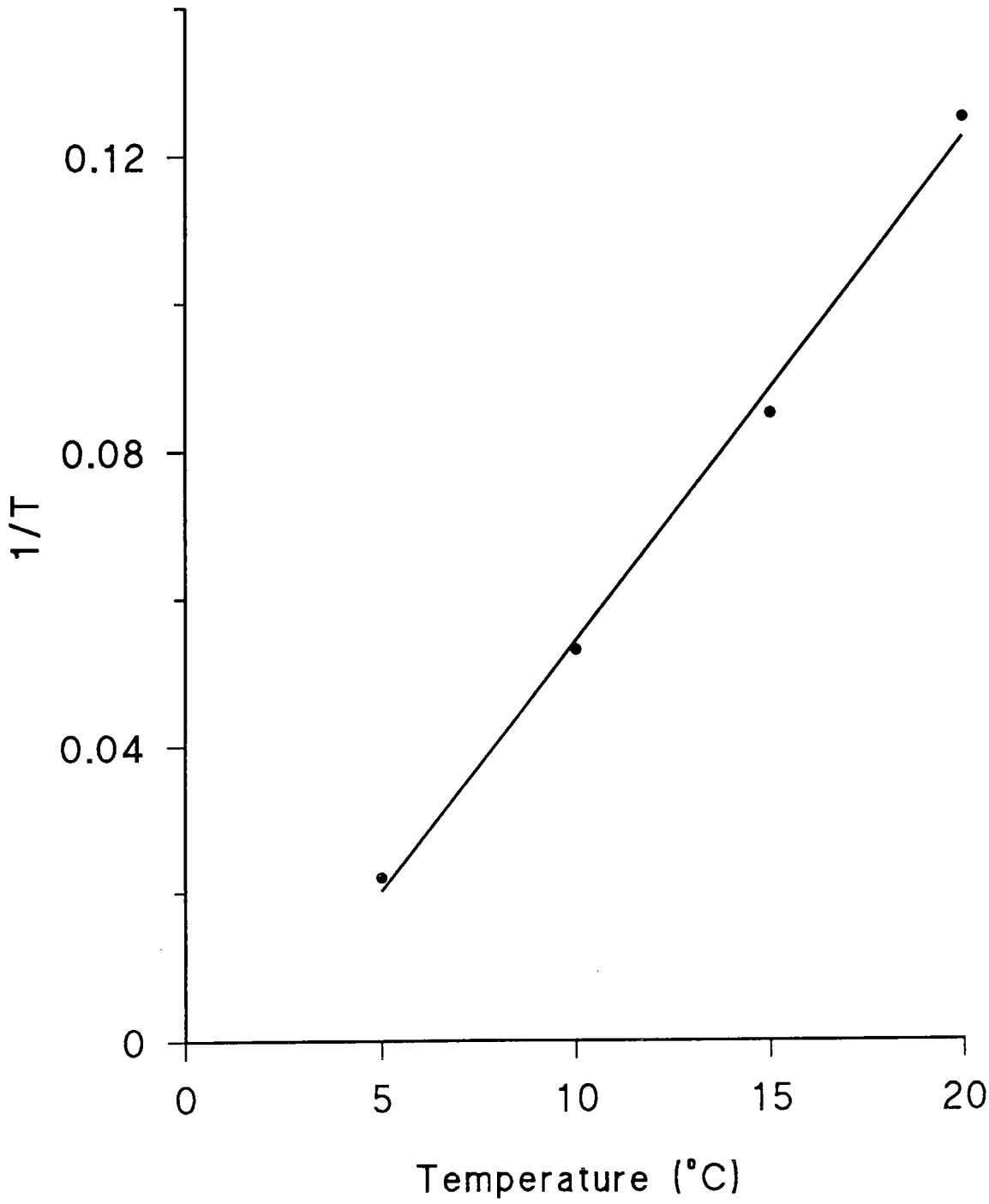
The mean day-time day time ground temperatures of the bare peat/*Campylopus introflexus* sites at a depth of 2cm in July, is 10.4°C (±0.2°C (S.E.)), which is probably a similar temperature to that experienced by the eggs in the field, an assumption used also by Coulson (1962). The mean temperature of the sites with 100% *Calluna vulgaris* cover is lower, at 9.0 (±0.3°C (S.E.)) than that of the bare peat/*Campylopus introflexus* sites and significantly so: $t=3.9$ $df=13$ $p<0.01$. These differences in temperature are probably due to diurnal variation: the temperature of the heather covered areas would not be as high as the bare peat area in the day time, and conversely, would not be as low at night.

Table 6.8. The egg development period of eggs of *Tipula montana* at different temperatures.

Date laid	Temperature (°C)	Number of eggs	Minimum development time (days)
12/8/91	10	52	17
15/8/91	10	58	21
29/7/91	15	50	12
29/7/91	15	50	12
29/7/91	15	30	12
31/7/91	15	8	11
29/7/91	20	50	8
29/7/91	20	30	8
31/7/91	20	8	8

Fig. 6.14.

The relationship between the reciprocal of the mean hatching time (T) and temperature in *Tipula montana* eggs from Waskerley Common. $y = 0.007x - 0.020$, $r_2 = 0.99$, $p<0.01$.



6.8. Life cycle of *Tipula montana*

Table 6.9 shows the numbers of each stage of *T. montana* from Waskerley which were present throughout the year.

Figs. 6.15-6.20 show histograms of the weights of the larval stages found in January, March, May, June, July and August. From January to July there is a general decrease in the numbers of second instars and then in the numbers of third instars until only fourth instars are present in July. In April there is a sudden decrease in the numbers of both third and fourth instars and then a sharp increase in the numbers of the two instars in May. The numbers of third instars decrease again in June and have disappeared by July. Second instars then appear again, with the fourth instars in August. All instars are present in November but first instars have disappeared by January. Pupae and adults were found in the field in July and August.

It would be impossible for the adults from the emergence which began on 17 July to produce the second instars found on 8 August because of the development time of the eggs and the first instar larvae. From Table 6.8, the minimum number of days to hatching at 10°C is between 17 and 21 days. From this study, at 10°C, first instar larvae remained as such from 26 August to 24 September, giving the duration of first instars a minimum of 19 days. This makes an absolute minimum of 36 days for a newly laid egg to develop into a second instar larva *i.e.* second instars from the July emergence could not be present on the field sites until the end of August, at the earliest.

Individuals of *T. montana* at Waskerley Common do not all have a two-year life-cycle, unlike that of *T. excisa*, (Hofsvang 1974). In *T. excisa* from Finse in Norway, two stages are present with one stage between them being absent throughout the year. This is not found in *T. montana* at Waskerley.

The *T. montana* life-cycle is not a simple one-year life-cycle either, as in *T. subnodicornis* and *T. paludosa* (Coulson 1962), as more than two stages are often present at any given time. One alternative is that *T. montana* at Waskerley has two

Table 6.9. Stages of *Tipula montana* present at Waskerley.

Month	Number in each stage present					
	Instar 1	Instar 2	Instar 3	Instar 4	Pupae	Adult
January		6	39	9		
February		*2*	*4*	*5*		
March		16	48	21		
April		*9*	*25*	*1*		
May			47	51		
June			12	51		
July				34	9	50
August		10		2	1	136
September		-	-	-		
October		-	-	-		
November	*2*	*14*	*24*	*1*		
December		-	-	-		

Data courtesy of J.C.Coulson in *italics*

- indicates month not sampled

Fig. 6.15.

Frequency histogram of the weights of *Tipula montana* larvae collected from Waskerley Common in January 1991.

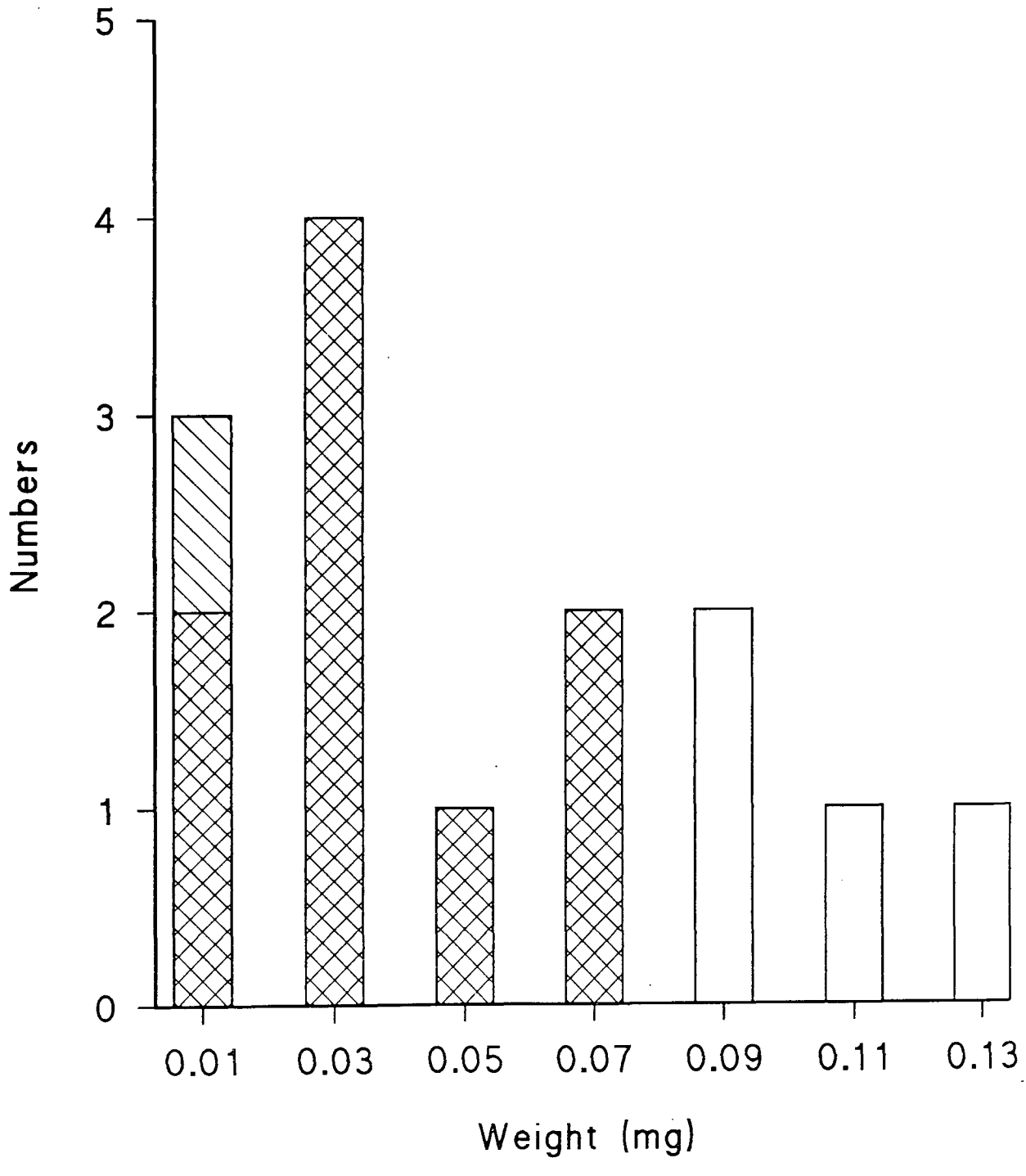


Fig. 6.16.

Frequency histogram of the weights of *Tipula montana* larvae collected from Waskerley Common in March 1991.

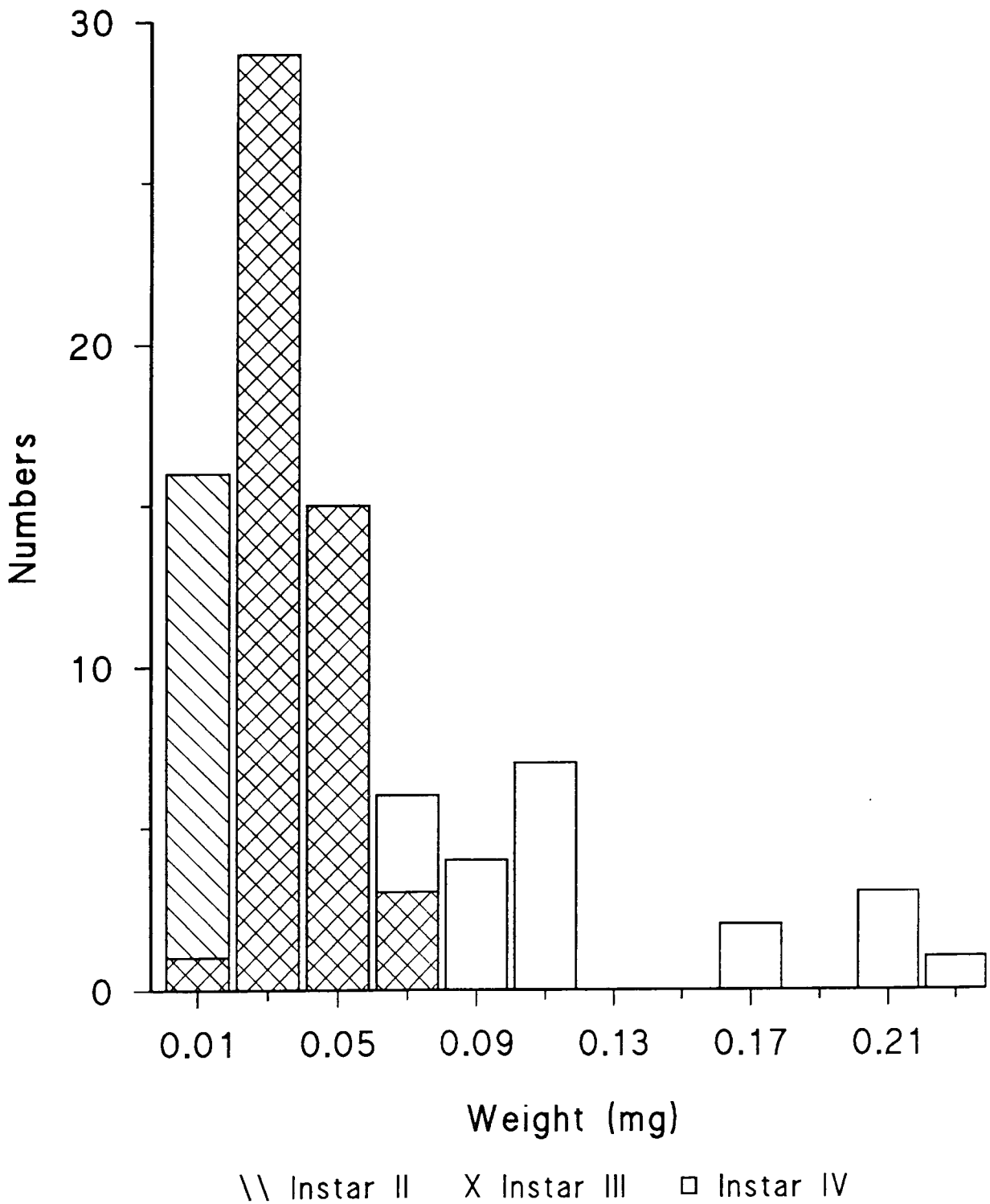


Fig. 6.17.

Frequency histogram of the weights of *Tipula montana* larvae collected from Waskerley Common in May 1991.

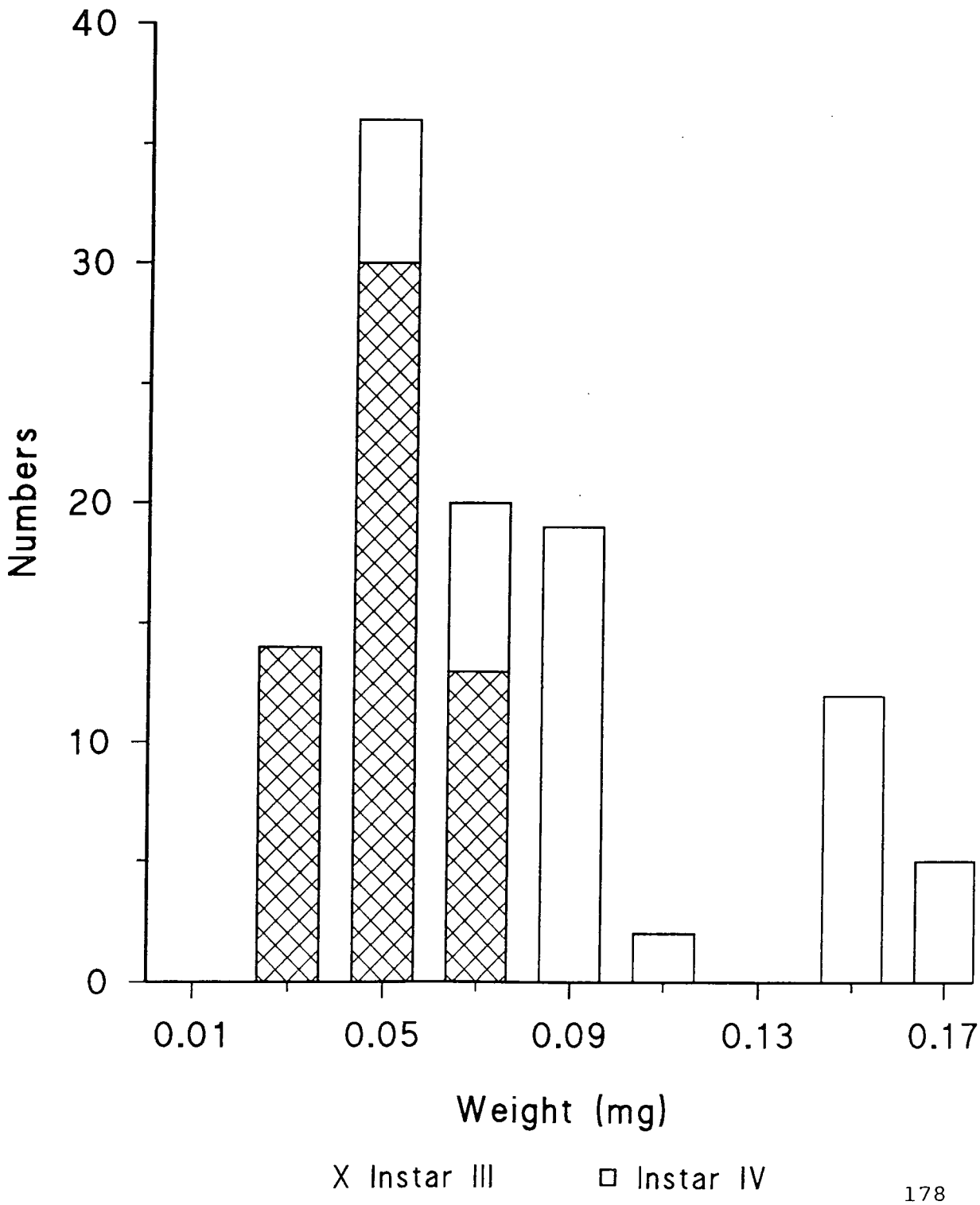


Fig. 6.18.

Frequency histogram of the weights of *Tipula montana* larvae collected from Waskerley Common in June 1991.

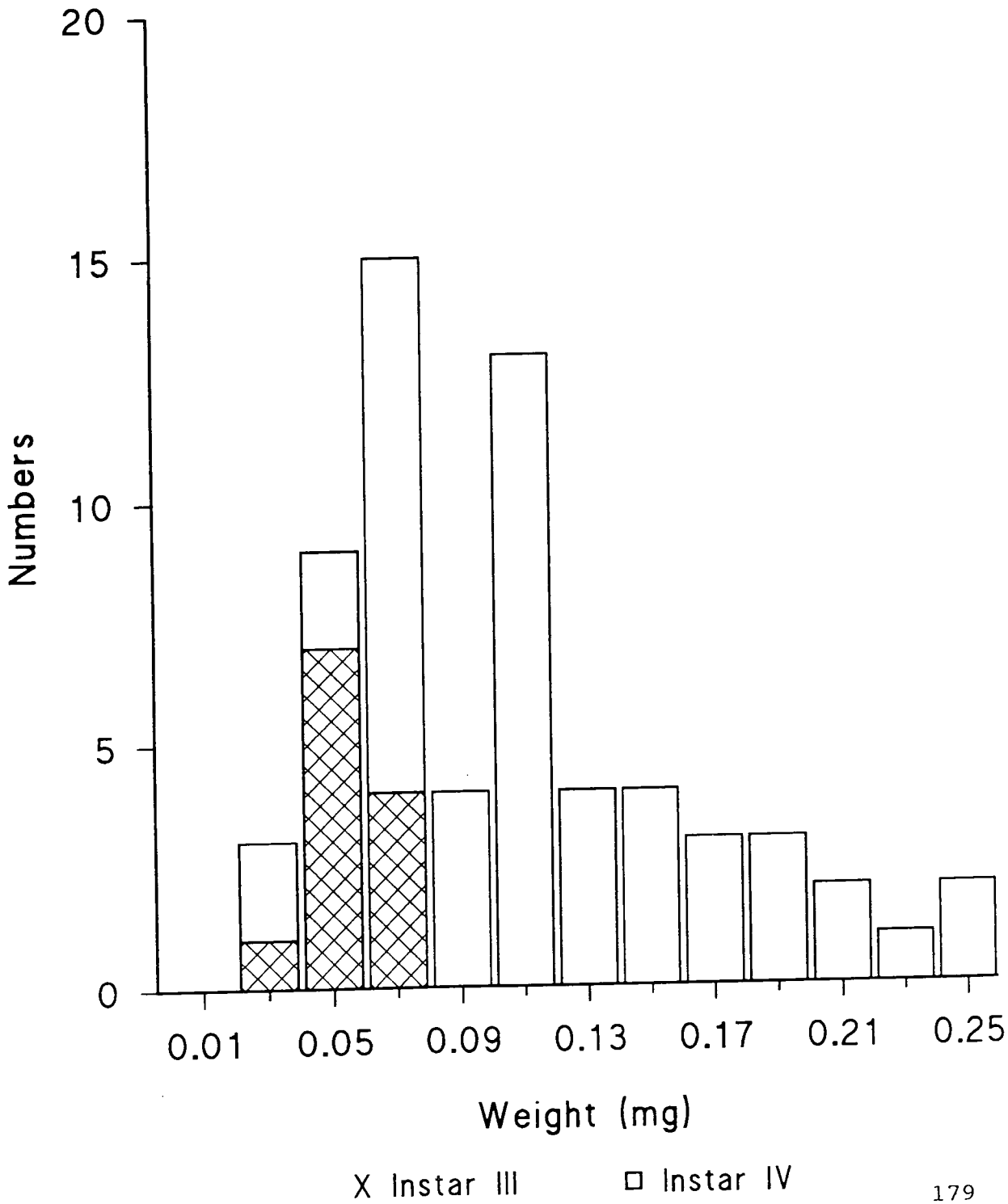


Fig. 6.19.

Frequency histogram of the weights of *Tipula montana* larvae collected from Waskerley Common in July 1991.

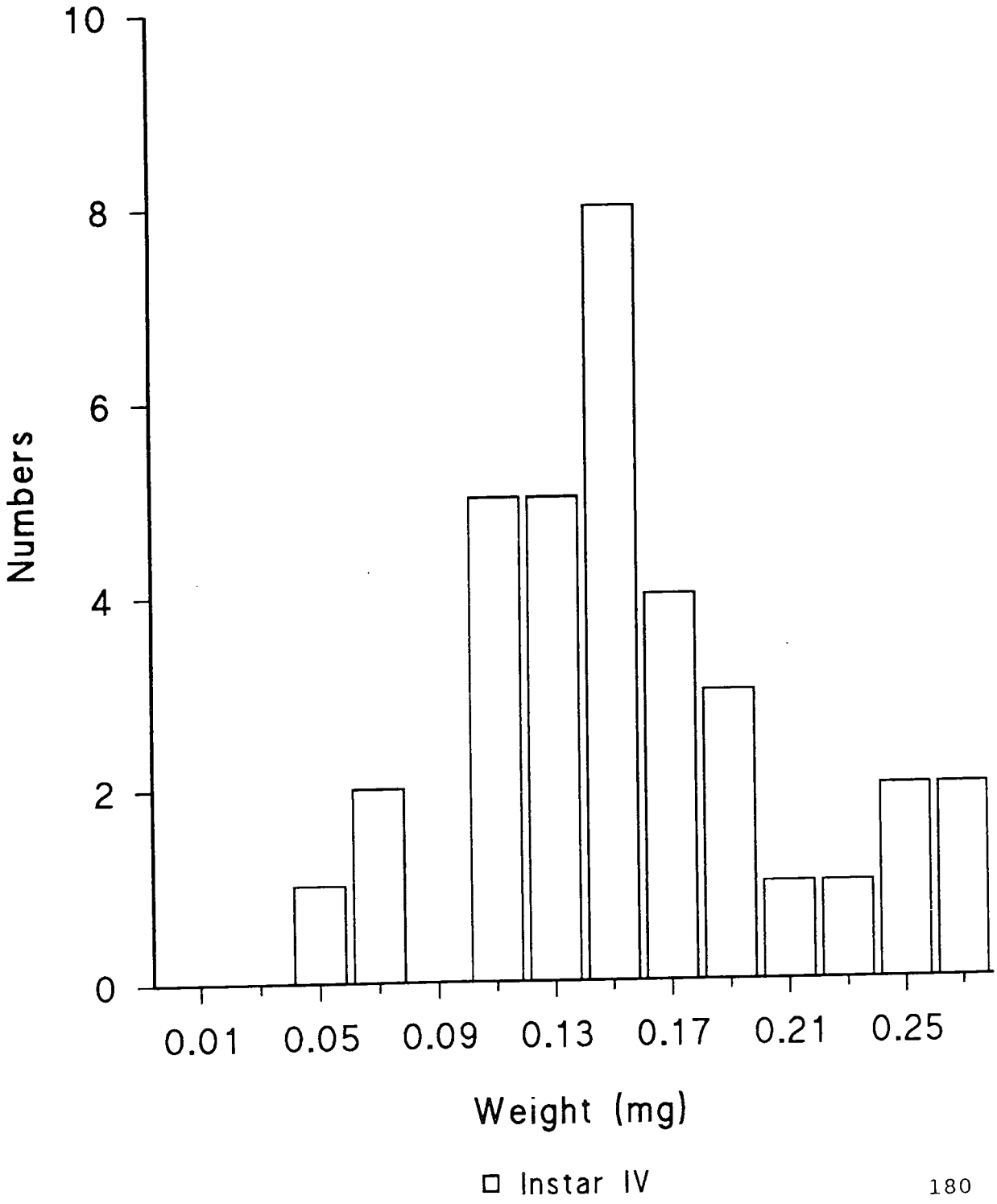
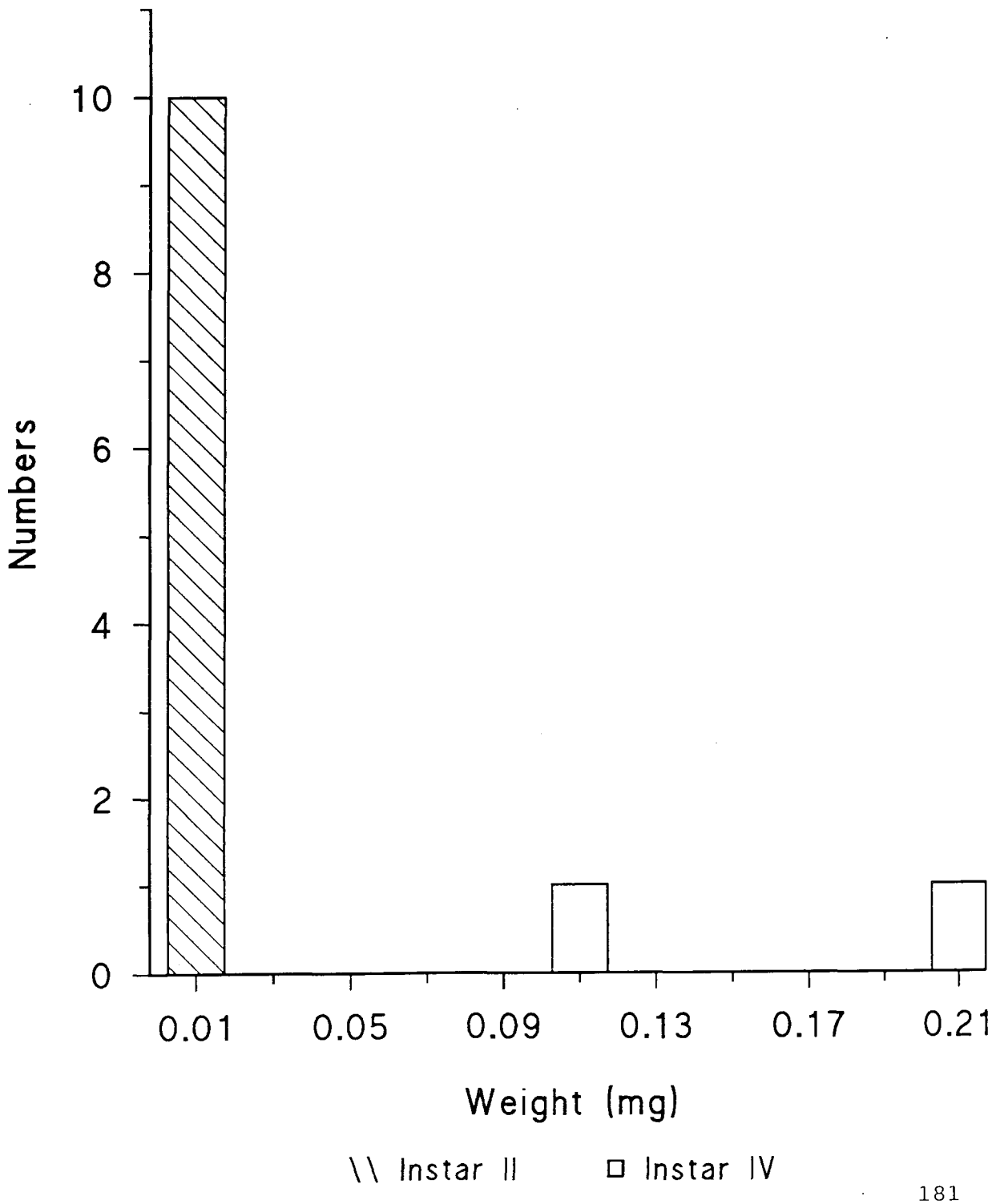


Fig. 6.20.

Frequency histogram of the weights of *Tipula montana* larvae collected from Waskerley Common in August 1991.



asynchronous life-cycles: one producing many more adults than the other. The other alternative is that there is a one-year life-cycle for the majority of individuals, with a few individuals having a two-year life-cycle. These are proposed due to the stages present and the numbers in each stage present in each month (Table 6.9).

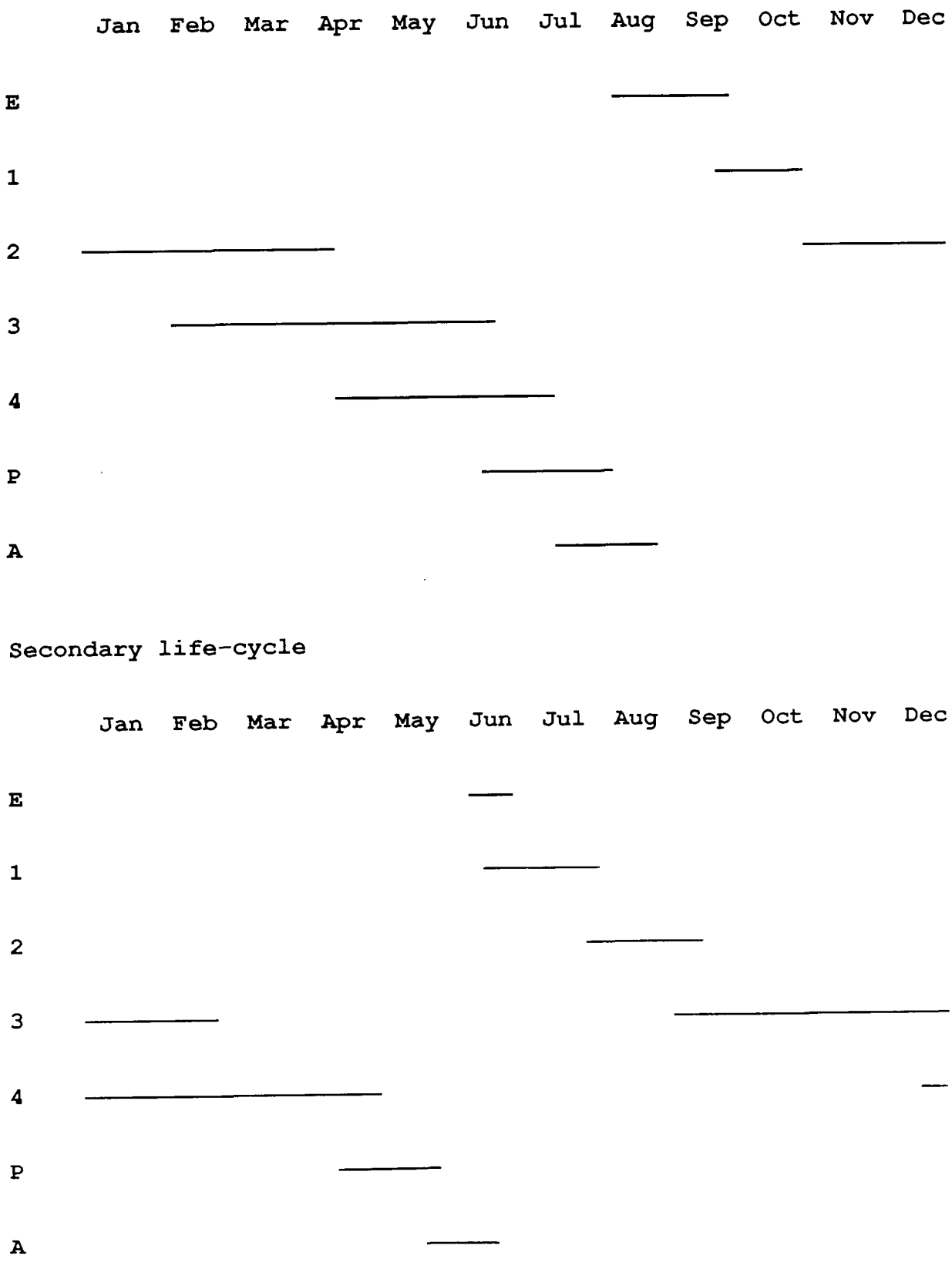
Fig. 6.21 gives the proposed one-year life-cycles of *T. montana* at Waskerley which consists of: i) a primary life-cycle, producing many adults found at Waskerley from mid-July to mid-August, and ii) a secondary life-cycle, with far fewer adults emerging in early June, based on the laboratory emergences.

i) The emergence in July/August would produce the first and second instars which were found in November: that very few first instars were found was probably caused by the difficulty in locating this stage in the field. These overwinter as second instars, and become third instars in March and April. These start to moult to fourth instars in May with the complete change to fourths being completed by early July. Pupae and adults were found in the field in July and August.

ii) If, as in the laboratory, there was a small earlier emergence in the field, which has now been confirmed, *e.g.* in early June, the second instars found in early August would be produced from this emergence. In this secondary life-cycle, the overwintering stage would be mainly third instars (with a few fourth instars) and these change to fourth instars in March. Pupation would occur in May leading to a small emergence in early June. That the third instars have not disappeared by May is thought to be due to the presence of third instars from the primary life-cycle.

The alternative suggestion for a life-cycle is as follows: the main life-cycle is the primary life-cycle described above - with final instar larvae pupating as they reach the required weight (male or female as appropriate) for pupation. Some of these fourth instars, however, may not attain the required weight to pupate. These larvae would remain as fourth instars, overwintering as such, - therefore explaining the presence of that instar stage then - and pupate under a photo-period stimulus in May (as they would probably reached the weight required for pupation before the winter months) and emerge in early June. These individuals therefore have a two-year life-

Fig. 6.21. Proposed life-cycle of *Tipula montana* at Waskerley.



cycle.

The life-cyles may act together - so the secondary life-cycle (June to June) would occur when the adults emerge from the two-year life-cycle in June. The spring emergence, lasting two weeks, is much more synchronous that the autumn emergence which lasts for approximately a month and a half.

6.9. Discussion

Tipula montana was found to have four instar larval stages; the same number as found in other *Tipula* species. However it is only in the fourth instar that obvious bimodality is found between the sexes in the biometric measurements. Hemmingsen (1965) found that in *Tipula saginata*, spiracular disc diameter and head capsule area not covered by skin obeyed Dyar's Law approximately. In this study, mandible length obeyed Dyar's Law (it is part of the head capsule not covered by skin), but neither spiracular disc diameter or body length obeyed Dyar's Law throughout all the instars.

Hofsvang (1972) states that 'it is possible that *Tipula excisa* found in the mountainous areas of Great Britain have a one-year life-cycle, since the area is very different from Finse with its Arctic temperatures.' He is actually considering here the species which is found in Finse, Norway - but it is *T. montana*, not *T. excisa*, which is found in Great Britain, although the suggestion made may well be valid for *T. montana* in Great Britain. In this study it has been shown that *T. montana* can thrive and complete its life-cycle in Britain at lower altitudes than had previously been thought.

In the Cairngorms the peak emergence is in June and in Waskerley the main emergence is in July/August: at least one and a half months later at the lower altitude, higher temperature site. Coulson (1962) states that the peak emergence of *Tipula paludosa* at Moorhouse is in late July/early August whereas at Rothamsted,

Hertfordshire, the peak emergence is not until September, at least one month later at the lower altitude, higher temperature site (Barnes 1937, Robertson 1939). It is possible that the same pattern could be occurring in *T. montana* as in *T. paludosa*, in that the difference in altitude affects the emergence period. The time of emergence could be related to the annual trend of water content of the soil during the ovipositioning period and possibly at the time of egg-hatching (Coulson 1962).

The proposed two-year life-cycle fits in with the third and fourth instar larvae found at Dun Fell, Cumbria (Grid Ref. NY710323) in October/November (Coulson pers. comm.) and with the emergence time of the adults on the Cairngorms in June. The life-cycle of *T. montana* on Dun Fell (Coulson pers. comm) and on the Cairngorms are suggested to be strictly biannual. At Waskerley, however, the evidence strongly suggests that this is not the case - there is a one-year life-cycle occurring at some point because of the frequency with which the instars change.

The two-year life-cycle would be controlled by the diapause of the fourth instars, which overwinter in the second year, and is broken by a photo-period stimulus in May to produce pupae. In the June to June one-year proposed life-cycle, larvae probably overwinter as fourth instars and this diapause would be broken by a photo-period stimulus in May which initiates pupation. It is more difficult to explain the control of the August to August proposed one-year life-cycles as there is no evidence of a diapause occurring in any stage, and pupation is initiated when the required weight in the fourth instars is reached in June/July.

However, using this model, the life-cycle of *T. montana* at Waskerley can be explained, as a combination of two asynchronous one-year life-cycles and a two-year life-cycle.

Further studies which could be carried out on *Tipula montana* include a comparison of the life-cycle of this species at Waskerley Common, Dun Fell, Cairngorms and northern and southern continental European sites, and the factors which influence the life-cycle at these various sites.

Chapter 7. General Discussion

7.1. Reasons for the low number of moss herbivores

Bryophytes are generally considered unsuitable as food plants for herbivores (Pakarinen and Vitt 1974). Vascular plants are apparently much more suitable since one quarter of the earth's macroscopic animal species are insect herbivores of vascular plants (Strong *et al.* 1984). Bryophytes are an important constituent in the vegetation of many natural and man-made ecosystems (Tyler 1990), but despite their locally high biomass they are seldom freely consumed by herbivores. This poor utilisation of the bryophytes can be explained by: i) their low nutrient value, ii) the cell wall of bryophytes being physically tough or resistant to digestive enzymes, due to lignin-like and other compounds such as polyphenols, and iii) the chemical defences involving solutions of secondary compounds or high concentrations of certain elements.

Mosses have a similar calorific value to higher plants (Pakarinen and Vitt 1974). The protein levels are however lower in bryophytes than in higher plants, the figures being 5% and 15% respectively (Skre *et al.* 1975). Also fat levels in bryophytes, at 2%, are lower than those in higher plants (5%). The percentage of sugars in mosses is between 1% and 5% (Margaris and Kalaitzakis 1974).

Gerson (1982) considered that many bryophyte species possess physical and/or chemical defences against grazing, and few animal species have evolved effective methods to overcome these. Davidson and Longton (1990) suggest that slugs feeding on bryophytes are unable to digest moss leaves completely even though they have enzymes for digesting carbohydrates, including cellulose, as they cannot digest the polyphenols which are also present within the cell wall. Indeed few insect species are known to obtain all, or any of their food energy from bryophytes because

they contain toxic or unpalatable secondary plant substances (Lawrey 1987). In contrast, secondary compounds in grasses are generally at low concentrations, of sporadic occurrence and they can provide all the essential nutrients for insect herbivores (Bernays and Barbehenn 1987).

The polyphenols present in certain moss species can often also have an antibiotic action, and are likely to have a negative effect on grazers either directly or indirectly, by inhibiting the gut micro-organisms. These species include *Bryum, Dicranum, Mnium, Polytrichum* and *Sphagnum* (McCleary *et al.* 1960, Banerjee and Sen 1979). When *Tipula confusa* and *Tipula subnodicornis* larvae were presented with a choice of mosses, *Dicranum scoparium, Mnium hornum* and *Polytrichum commune* were actively avoided by one or both cranefly species. *T. subnodicornis* appeared to be more of a generalist bryophyte feeder than *T. confusa*, as there was less range of preference or avoidance for the moss species investigated in *T. subnodicornis* than *T. confusa*. *T. confusa* showed a significant preference for woodland mosses as opposed to moorland mosses.

It would appear that bryophytes avoid being consumed by lowering their digestive capacity. However, the eight species of *Tipula* in this study which feed exclusively on, and complete their life-cycle on mosses must have some method, as yet unknown, of countering these unpalatable and indigestible substances. Tipulid larvae in this study were presented with mosses containing a range of introduced concentrations of heavy metal ions. They were not deterred from using the mosses as a food source in all cases. This being so, although the effects of secondary plant substances *e.g.* polyphenols, were not investigated here, it is speculated that *Tipula* larvae could cope with these substances in a way similar to that used for the heavy metal concentrations.

7.2. So why are mosses consumed?

Mosses can form a considerable proportion of the diet of some animals *e.g.* Soay sheep, *Ovis aries* (Milner and Gwynne 1974), reindeer, *Rangifer tarandus platyrhynchus* (Ekern and Kildemo 1978), barnacle geese, *Branta leucopsis* (Prop *et al.* 1980), tipulids (Coulson 1962, Pritchard 1983, Richardson 1981, and this study). Chown and Scholtz (1989) state that although bryophyte feeding is a rare occurrence in weevils (Coleoptera: Curculionidae), in two species (*Mesembriorrhinus brevis* and *Dusmoecetes marioni*) from the sub-Antarctic Marion Island, bryophytes are preferred as food plants to angiosperm species. Crafford and Chown (1991) suggest that this is simply because of the absence of angiosperm species in the Pleistocene glacial periods, rather than any nutritional advantage in the bryophytes.

Prins (1981) suggests that, at least in the case of birds and mammals, (*e.g.* geese, caribou (*Rangifer tarandus pearyi*) and reindeer) larger quantities of bryophytes are consumed in cold environments, not because they supply a greater amount of energy, but because the mosses supply polyunsaturated fatty acids (*e.g.* arachidonic acid) which can probably increases cold resistance in these animals. There have been many observations of geese (*e.g.* pinkfoot (*Anser brachyrhychus*), barnacle and brent (*Branta bernicla*) geese) feeding on mosses immediately on arrival at their Arctic breeding grounds. In lemmings, *Lemmus sibericus* from tundra ecosystems, the metabolizable energy intake is eight times lower from mosses than it is from grasses, but they do digest mosses to a limited extent, and a proportion of their diet is made up of mosses (Batzli and Cole 1979), possibly for the reasons given above. However moss leaves have been found to be poorly digested by both reindeer and caribou (Person *et al.* 1980, Thomas and Edmonds 1983). Also, Ekern and Kildemo (1987) showed that there is low digestibility of mosses in reindeer and they are able to gain only a small amount of energy from this food source.

In cold climates, bryophytes are often the only abundant food source, and although the energy and nutrients obtained per unit volume would be low, consuming

large amounts of bryophytes could provide the necessary nutrient and energy requirement of the herbivores feeding on them, albeit in an inefficient way.

In this study the method of digestion in eleven species of *Tipula* has also been found to be very inefficient, so the amount of nutrients and energy obtained per unit volume of bryophytes (and of grasses and algae) would be very low, but this is probably counteracted by feeding on greater quantities. As digestion in these eleven species is so inefficient, as only edge cells are used to obtain the necessary nutrients, maximum number of edge cells need to be damaged per particle by the larvae to maximise the quantity of nutrients obtained from each particle. There are relatively more edge cells on smaller particles than on larger ones, and therefore larger particles are not consistently ingested by the larger larvae, although they do so occasionally, demonstrating that they are physically able to do so.

This results in a higher proportion of the particle passing through the body unused. In effect, these tipulid larvae are bulk feeders - the majority of food passing through the gut unused. These animals need to be bulk feeders in order to obtain sufficient nutrients by their inefficient method of feeding. It has been shown in this study that there is very little increase, compared to the biometric measurements, in the size of vegetation particles which are ingested through each progressive larval instar of each species. This is advantageous to these animals for two reasons: i) that relatively more edge cells can be damaged on smaller particles and ii) it decreases the bulk of food that would pass through the animal unused.

Few studies have considered the size of food particle ingested in invertebrate animals through their developmental stages. Bernays and Janzen (1988) have investigated vegetation particle sizes ingested in saturnid caterpillars, which use a feeding method similar to larvae in the genus *Tipula*. They found that, as also found here, that the only plant tissue readily available for rapid digestion and removal of nutrients is that around the margin of the cells.

Three hypotheses are now put forward in an attempt to explain why *Tipula* larvae should feed on mosses at all.

i) When tipulids evolved, bryophytes were the only available food plant, as in the case of the weevils on Marion Island.

ii) As mosses are a relatively unexploited food source, these species would be able to feed on them with relatively little competition, albeit inefficiently.

iii) Bryophytes are generally less tough than many other food plants, with no waxy cuticle and so are easier to manipulate in the feeding method used by these tipulid species.

These, however, raise the question as to why all tipulid species or many other insects do not feed on bryophytes.

The earliest definitely confirmed bryophyte was from the upper Carboniferous period, approximately 320 million years ago, (Richardson 1981). However grasses only evolved between 26-100 million years ago - there is definite fossil evidence from the mid-Tertiary period and more tenuous fossil evidence from the Cretaceous period, (Ledyard Stebbins 1972). The family Tipulidae, a very ancient family in the Diptera, evolved from ancestors in the Upper Jurassic period, 136 to 165 million years ago, (Alexander and Byers 1981).

It would seem likely that when the Tipulidae first evolved there was, in fact, an absence of angiosperm species although bryophytes would have been well established. Therefore the only available food source was bryophytes. As more plant species, and more herbivorous insect species evolved, competition between insects for food plant species would increase. Tipulids have been shown in this study to be inefficient feeders with no difference in efficiency, in the method of obtaining nutrients, found between moss, grass or algal feeding species. Therefore certain species may have continued as moss feeders - as there was less competition for this food source and the feeding methods used were easier on mosses as is bulk feeding.

Generally it is the larger species *e.g. T. paludosa*, *T. oleracea* and *T. maxima*, which now feed on plants other than bryophytes, (*e.g.* grasses, plant debris or wood) and possibly this is because their larger mandibles are more able to cope with these tougher plant materials, whilst the smaller sized species remained as moss feeders.

Also the moss feeding tipulid species are mainly restricted to the subgenus *Savtshenkia* and members of this subgenus are possibly more adapted to moss feeding.

Animals do not always feed on food of the highest nutritive value. For example, Taylor and Bardner (1968) found that larvae of *Plutella maculipennis* (Lepidoptera: Plutellidae) and *Phaedon cochleariae* (Coleoptera: Chrysomelidae) fed on older leaves with a lower protein content than younger leaves, although they had a significantly higher growth rate when fed exclusively on the younger leaves. This possibly could give credence to the theory that food plants are chosen as such for reasons other than their nutritive value.

When mosses are utilised as food plants by herbivores, a greater volume has to be consumed than when other vegetation types are utilised, as they are low in food quality and nutrients are generally more difficult to extract and, additionally for tipulid species, only a small proportion of the amount ingested is available for digestion and assimilation.

7.3. Directions for further studies

1) Determination of the nutrient and protein levels in each of the moss species investigated, in an attempt to show if the choices made by *T. subnodicornis* and *T. confusa* are reflected in these levels, and to compare these with the levels in grasses and algae.

2) Taylor and Bardner (1968) found that in certain insect species the weight of food consumed depended upon the nutritive value of the food. For example, locusts (*Locusta migratoria*) fed on artificial diets consumed more food when on the least nutritious diet (Dadd 1960). If the actual weights eaten by tipulid larvae of each of the mosses, grasses and algae were determined, the above theory could be tested here.

Also the increased amount of food consumed when fed a nutritionally poor bryophyte diet could be calculated.

3) Extend the study on the effect of introduced concentrations of heavy metal ions in moss species. This could be achieved by using more moss species and measuring the levels of heavy metal concentrations in the larvae feeding on experimental and control mosses.

4) Measurement of the growth rate of the larvae on the different food plants to determine if any differences found in the growth rates were reflected in the nutritive content of the food plant.

5) Investigation of the theory that non-moss feeding *Tipula* larvae are potential moss-feeders, by determining if such larvae can live, and complete their life-cycle, on mosses, *e.g. T. paludosa*, *T. oleracea* and *T. lateralis*.

Summary

1. Bryophytes are little used as a food source by insects and other animals. Larvae of Diptera in the genus *Tipula* are some of the few insect species to feed and complete their life-cycle on bryophytes, despite the ancient origin of insects which matches the peak of abundance of these plants.

2. Mosses have attributes which make them suitable as both a habitat and a food source for insects: they can hold large amounts of water, but can also withstand desiccation; they can provide insulation, and their structurally unprotected leaves can facilitate feeding on them.

3. Eleven species in the genus *Tipula* were investigated. The feeding ecology of eight moss-feeders, *Tipula rufina*, *Tipula confusa*, *Tipula pagana*, *Tipula staegeri*, *Tipula limbata*, *Tipula alpium*, *Tipula subnodicornis* (all from the subgenus *Savtshenkia*) and *Tipula montana* (from the subgenus *Vestiplex*), was compared with the feeding ecology of two grass-feeders, *Tipula paludosa* and *Tipula oleracea* (both from the subgenus *Tipula*) and an algal feeder *Tipula lateralis* (from the subgenus *Yamatotipula*).

4. As particulate feeding only occurs in the larval stages, feeding in each of the four larval instars of the species was investigated.

5. Most damage to the ingested plant material was caused by the action of the mandibles cutting the vegetation particle from the food plant. Frequently, intact cells passed through the gut.

6. Of the species investigated, *Tipula rufina* ingested the smallest vegetation particles in each instar, by at least 25%, and the grass feeders ingested the largest in each instar (except instar I), again by at least 25% .

7. The pattern of difference in mandible lengths was similar, with *Tipula rufina* larvae having consistently shorter mandibles (except instar I) and *Tipula paludosa* consistently longer ones, in each instar.

8. There was a strong positive correlation (r=0.79) between vegetation particle volume ingested and mandible length in the *Tipula* species studied.

9. There was also a strong positive correlation (r=0.96) between the weight of a larva and its mandible length in ten of the *Tipula* species studied, so the smaller individuals consistently have shorter mandibles.

10. In each instar, *Tipula rufina* and *Tipula subnodicornis* had the smallest spiracular disc diameters and *Tipula montana* and *Tipula paludosa* the largest.

11. For each species there was a significant positive correlation between the vegetation particle volume ingested and the spiracular disc diameter.

12. Both moss-feeders and non-moss feeders had a similar range of coefficients of variation for the mean vegetation particle size in the instars of each species. For both groups the relative standard deviation of the vegetation particle volumes ingested by the *Tipula* larvae was 29%.

13. The increases in mandible length, spiracular disc area and weight in the moss-feeders and the non-moss feeders (apart from mandible length in *Tipula oleracea* and *Tipula lateralis*) were greater than the increase in vegetation particle volumes from

instar I to instar IV. In all cases the increases in vegetation particle volume and mandible length were at least an order of magnitude less than the increases in spiracular disc area and weight.

14. It was clear that the majority of plant cell contents passed through the gut of *Tipula* larvae and were not available to the larvae. In effect the larvae are bulk feeders, taking in quantities of food in order to obtain a small proportion of the nutrients.

15. It is not advantageous for animals with this type of feeding system to ingest larger sized food particles as they grow, since the amount absorbed appears to be determined by the proportion of cells damaged by the mandibles.

16. In *Tipula rufina* the percentage of vegetation particle volumes of 19,000μm^3 or less which were ingested decreased with increasing instar, but it always exceeded 50%. Therefore the majority of vegetation particles ingested were in the same size range, irrespective of instar.

17. In the caterpillar of the moth *Hydriomena furcata*, which has a similar type of feeding system to tipulids, the vegetation particle size ingested was closely correlated with the head capsule diameter (r=0.91), and the two measurements increased at approximately the same rate.

18. In the two instars of the locust, *Locusta migratoria*, the vegetation particle volume was closely correlated with the mandible length (r=0.91), and again the two measurements increased at approximately the same rate.

19. There was no significant relationship between mean percentage of damaged cells per particle ingested and the spiracular disc diameter in each *Tipula* species studied,

and so similar proportions of damaged cells were consumed by larvae of the same species through the four instars.

20. The mean percentage of damaged cells in larger food particles was not greater than in smaller particles, in each *Tipula* species studied.

21. Overall, there was no significant difference in the mean percentage of damaged cells per particle either between species or between instars.

22. There was a significant difference in the percentage of damage occurring in the edge cells, between the four instars when all eleven *Tipula* species were considered. More damage to the edge cells occurred in the later instars. This suggests that larger larvae induce a greater amount of damage to edge cells when cutting their food from the plant. Thus larger larvae are probably more efficient in feeding on vegetation particles than smaller larvae.

23. The mean percentage of damage in the edge-cell region was significantly greater than that in the mid-cell region: in instar I by 20±4%, in instar II by 23±4%, in instar III by 31±3% and in instar IV by 31±4%.

24. Food choice experiments were carried out to determine if tipulid larvae showed preferences for particular moss species, and obvious differences were found.

25. In *Tipula confusa* and *Tipula subnodicornis* larvae, the mean percentage of spot observations on a particular moss species could be used as a reliable estimator of the percentage of particles of that moss species consumed by the larvae. There was a significant correlation between the mean percentage of moss particles in the frass of a larva and the mean percentage of spot observations which were on that moss species for both *Tipula subnodicornis* ($r=0.91$) and *Tipula confusa* ($r=0.90$).

26. The percentage of observations of *Tipula confusa* and *Tipula subnodicornis* on each of ten moss species was determined. The range within each *Tipula* species was used as a measure of variation in preference for the moss species. This was much greater in *Tipula confusa* (ranging from 70% on *Dicranella heteromalla* to 4% on *Polytrichum commune*) than in *Tipula subnodicornis* (ranging from 43% on *Eurhynchium praelongum* to 13% on *Polytrichum commune*).

27. There was no close correlation between the preferences made by *Tipula subnodicornis* and those made by *Tipula confusa* (r=0.08).

28. *Tipula confusa* strongly preferred woodland moss species to moorland moss species whereas *Tipula subnodicornis* did not show an overall preference for either moss type.

29. When *Tipula subnodicornis* was given a choice of the sedge *Eriophorum vaginatum* and two moss species, *Campylopus paradoxus* and *Sphagnum papillosum*, it favoured the sedge, although the preference changed to *Sphagnum papillosum* towards the onset of pupation, which suggested that there was a response associated with the selection of a pupation site.

30. *Sphagnum papillosum* concentrated both Zn^{2+} ions and Pb^{2+} ions from standard solutions to a greater extent than did *Campylopus paradoxus*, and Zn^{2+} ions were accumulated to a greater extent than Pb^{2+} ions in both moss species.

31. *Campylopus paradoxus* and *Sphagnum papillosum* with high concentrations of heavy metals were presented to *Tipula subnodicornis* as food items and comparisons were made with 'control' material by means of repeated spot observations..

32. In a choice experiment, a correlation of 0.81 was found between the percentage of moss particles in the frass of a larva and the percentage of spot observations on that moss species. Therefore the mean percentage of spot observations can be used as an alternative method to estimate which moss species was being used as the preferred food plant.

33. In the majority of cases, there were significantly more observations of *Tipula subnodicornis* on *Sphagnum papillosum* containing either 3360µgg-1 of Pb^{2+} ions and above (a mean of 7±4% more observations) or 470µgg-1 of Zn^{2+} and above (a mean of 8±2% more).

34. There were significantly fewer than expected observations of *Tipula subnodicornis* on *Campylopus paradoxus* containing either 1220µgg-1 of Pb^{2+} ions and above (a mean of 19±3% fewer observations) or 180µgg-1 of Zn^{2+} ions (a mean of 15±0.3% fewer observations).

35. Similar heavy metal concentrations in *Sphagnum papillosum* had less effect than the heavy metal concentrations in *Campylopus paradoxus* on the behaviour of the *Tipula subnodicornis* larvae.

36. *Tipula montana* is normally a species of montane habitats above 600m, with a two year life-cycle, feeding on plant debris and dead plant material. In this study it was found on a moorland habitat, Waskerley Common, Co. Durham at 400m, feeding on mosses.

37. The spiracular disc diameters of *Tipula montana* was the most reliable indicator of instar, and showed the presence of four larval instars.

38. Mandible lengths of *Tipula montana* followed Dyar's Law, as they increased by a

factor of 1.5-1.6 between instars.

39. Fourth instar larvae of *Tipula montana* could readily be divided into males and females using the spiracular disc diameter: those with diameters of 0.50mm and below were males and those with diameters of 0.51mm and above were females.

40. Two distinct classes were found in the maximum weight recorded for fourth instar larvae of *Tipula montana*. Those larvae in the larger size class, with a mean weight of 227.6±8.9mm, became female pupae, whilst those in the lower size class, with a mean weight of 132.4±4.0mm, became male pupae.

41. The mean weight of male fourth instar larvae was 58% that of a female larvae of *Tipula montana*, measured at the same time.

42. The mean weight of male pupae of *Tipula montana* was 68% that of female pupae.

43. In fourth instar larvae of *Tipula montana* there was an increase in weight up to approximately 15 days before pupation and thereafter the larval weight decreased.

44. The life-cycle of *Tipula montana* at Waskerley Common is complicated. It is suggested that it is a combination of two asynchronous one-year life-cycles and a two-year life-cycle. The life-cycle is discussed with reference to life-cycles of the species from other localities.

45. Some species of *Tipula* may be moss feeders because when they evolved bryophytes were the only plants available. Also their method of feeding, and being bulk feeders, necessitates an easily manipulated food source such as mosses. The reasons why some species remained as moss-feeders and others did not are discussed.

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Appendix 1. Weights of each moss sample analysed in the atomic absorption spectrophotometer and corrected concentrations of the heavy metal ions found in the moss species.

Heavy metal ion	Concentration of soaking solution (ppm)	Moss* species	Weight (g)	**Corrected concentrations in the moss species (ppm) Pb^{2+}	Zn^{+}
-	0	Cp	0.1156	1.692	0.556
-	0	Sp	0.0639	0.676	0.438
Pb^{2+}	3.0	Cp	0.1224	3.229	
	0	Sp	0.0765	0.070	
	12.5	Cp	0.1396	6.799	
	0	Sp	0.0807	0.319	
	62.5	Cp	0.1202	7.091	
	0	Sp	0.0497	0.172	
	125.0	Cp	0.1184	10.090	
	0	Sp	0.0870	1.897	
Zn^{+}	1.0	Cp	0.1322		0.970
	0	Sp	0.0560		0.390
	4.5	Cp	0.1395		3.580
	0	Sp	0.0544		0.468
	22.8	Cp	0.1124		4.681
	0	Sp	0.0429		0.814
	44.5	Cp	0.1200		13.944
	0	Sp	0.0773		0.870

Appendix 1 (cont'd).

Heavy metal ion	Concentration of soaking solution (ppm)	Moss* species	Sample weight (g)	**Corrected concentrations in the moss species (ppm) Pb^{2+}	Zn^{+}
Pb^{2+}	3.0	Sp	0.0539	1.220	
	0	Cp	0.0822	1.632	
	12.5	Sp	0.0649	8.729	
	0	Cp	0.0544	1.891	
	62.5	Sp	0.0766	12.206	
	0	Cp	0.1186	2.226	
	125.0	Sp	0.0786	14.359	
	0	Cp	0.1208	3.335	
Zn^{+}	1.0	Sp	0.0456		0.858
	0	Cp	0.0752		0.232
	4.5	Sp	0.0807		7.814
	0	Cp	0.1090		0.512
	22.8	Sp	0.0759		12.944
	0	Cp	0.1212		0.900
	44.5	Sp	0.0719		19.024
	0	Cp	0.1281		1.672

* Cp = *Campylopus paradoxus*
Sp = *Sphagnum papillosum*

** Corrected concentration refers to the concentration determined from the calibration curve produced by the spectrometer, with the readings for the blanks subtracted.

Appendix 2. Examples of experiments comparing the numbers of observations on moss species with introduced heavy metal concentrations and on non-treated moss species, when the experiments are divided into ten equal time periods, with the overall number of observations found per experiment.

1. *Campylopus paradoxus* with an introduced concentration of $660\mu g g^{-1}$ of Pb^{2+} ions and *Sphagnum papillosum* as the control.

Time period	Number of observations on *Campylopus paradoxus*	Number of observations on *Sphagnum papillosum*	n	X^2 df=2
1	25	25	50	2.1 NS
2	33	16	49	1.5 NS
3	28	26	54	2.4 NS
4	38	16	54	2.4 NS
5	31	17	48	0.4 NS
6	33	20	53	0.1 NS
7	31	21	52	0.1 NS
8	30	23	53	0.3 NS
9	28	25	53	1.2 NS
10	37	20	57	0.6 NS

$X^2=9.6$ NS df=9

NS $p>0.05$

Appendix 2 (cont'd).

2. *Sphagnum papillosum* with an introduced concentration of 3360$\mu g g^{-1}$ of Pb^{2+} ions and *Campylopus paradoxus* as the control.

Time period	Number of observations on *Sphagnum papillosum*	Number of observations on *Campylopus paradoxus*	n	X^2 df=2
1	22	18	40	3.0 NS
2	18	19	37	0.1 NS
3	23	16	39	0.8 NS
4	18	15	33	0.1 NS
5	25	15	40	1.8 NS
6	21	19	40	0.1 NS
7	22	17	39	0.3 NS
8	19	21	40	0.3 NS
9	16	24	40	2.2 NS
10	13	19	32	1.6 NS

X^2=7.5 NS df=9

NS p>0.05

Appendix 2 (cont'd).

3. *Sphagnum papillosum* with an introduced concentration of 6615µgg^{-1} of Zn^{2+} ions and *Campylopus paradoxus* as the control.

Time period	Number of observations on *Sphagnum papillosum*	*Campylopus paradoxus*	n	X^2 df=2
1	14	20	34	0.1 NS
2	15	20	35	0.1 NS
3	19	16	35	1.4 NS
4	19	16	35	1.4 NS
5	15	19	34	0.1 NS
6	20	15	35	2.3 NS
7	10	23	33	2.6 NS
8	17	18	35	0.3 NS
9	13	22	35	0.7 NS
10	10	22	32	2.2 NS

X^2=11.2 NS df=9

NS p>0.05

Appendix 2 (cont'd).

4. *Campylopus paradoxus* and *Sphagnum papillosum* with no introduced concentration of Pb^{2+} ions or Zn^{2+} ions. (Control experiment).

Time period	Number of observations on *Campylopus paradoxus*	*Sphagnum papillosum*	n	x^2 df=2
1	40	29	69	0.1 NS
2	42	27	69	0.2 NS
3	47	23	70	2.1 NS
4	30	39	69	6.5 *
5	36	33	69	1.2 NS
6	44	25	69	0.8 NS
7	42	24	66	0.6 NS
8	46	24	70	1.3 NS
9	40	24	64	0.4 NS
10	34	35	69	2.6 NS

x^2=15.7 NS df=9

NS p>0.05