

"Control of chironomid flies breeding in sewage filters"

by

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Summary

The literature concerning the identification, habits and control methods of common sewage filter flies has been reviewed with special reference to the chironomid Metriocnemus hygropetricus (Kieff.) which was the nuisance species breeding on the filters at Tamworth. References have been given for the identification of larval and adult stages of filter flies, the habits of flies in relation to varying filter operation methods and basic physical variables such as light and temperature, and the various chemical and operational methods of fly control used in the past. Other literature concerning general filter ecology and relations between members of filter fauna has been reviewed.

The methods used for sampling filters for film, microfauna and macrofauna, together with the various methods of fly trapping and toxicity testing have been described. Methods have been developed for culturing certain filter insects.

The results from this project can be summarised as follows. The insecticide (Actellic M20) has been applied to filters and has controlled M.hygropetricus. The success of this method is limited when factors such as timing of dose, financing a sampling programme and resistance are considered. The insecticide has been found to effect other common filter fauna at various concentrations. Resistance to the insecticide by M.hygropetricus after 3 years of treatment has been suggested.

Certain operational modifications applied to filters have controlled M.hygropetricus and it is considered that these methods are preferable to insecticidal methods on an ecological and financial basis.

Using laboratory scale filters the specific habits of M.hygropetricus and Psychoda alternata Say have been identified and competition between these species has been proved which has important implications when considering the control mechanisms of the operational modifications.

Miscellaneous findings have included the following. Diurnal emergence patterns have been found with M.hygropetricus, and Psychoda severini Tonn., also it has been found possible to culture M.hygropetricus, P.severini and P.alternata from eggs to adults allowing investigation of the various life cycle stages.

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Introduction

With the development of sewerage systems and the consequent removal of wastes away from dwellings the rivers were the initial site of sewage deposition. The onset of the industrial revolution and the sharp increase in population of the industrialised nations caused the rivers to rapidly decline in quality and some thoughts were given to possible methods of reducing the polluting characteristics of sewage before it was discharged to the rivers. The poor state of the rivers was highlighted by the first Royal Commission on river pollution (1868) and this suggested certain methods of sewage treatment. Initially land treatment of sewage was utilised however ever increasing flows and land scarcity lead to the development of the sewage filter and the first large scale stone filters were constructed at Salford by Josephe Corbett in 1893. The design of sewage filters has not radically altered since that time and the filter has been the most common type of secondary treatment stage on sewage treatment works for over 30 years.

The sewage filter consists of a circular or rectangular container filled with "media" which provides a large surface area for microbial growth. Settled sewage is sprayed on the surface media and it is oxidized and nitrified as it percolates through in contact with the microbial film. The effluent then escapes through perforated tiles in the filter floor. The sewage filter is a good example of a self regulating entity. The microbial film is induced to grow by the constant feeding of sewage however it is prevented from rising to excess levels by the action of certain invertebrates which feed on the film; the grazers. These invertebrates are very limited in diversity and consist of individuals which have been found in mud flats (Lloyd 1944b) and organically polluted rivers. The invertebrates found are limited to a few species of mites, spiders, worms and insects.

Problems have been found in the past concerning adult insect emergences from

filters. A characteristic and common ecological pattern of cyclical population levels of insects are found in filters whereby at any one time in a filter there is a certain stage of the life cycle of one particular insect which is in greater abundance than other life cycle stages. Problems arise when the adults are in greatest abundance and there are large numbers of adults leaving the filter. Some species perform dances prior to mating in which large swarms of males congregate near angular objects e.g. corners of buildings. One of these species is M.hygroptericus, the particular nuisance species found at Tamworth Water Reclamation Works. When these swarms are formed near private dwellings complaints from the public are often received which can cause great public alarm and attract media coverage as was the case at Tamworth. When this situation arises it is often found that the blame for the nuisance is levelled against many people, most often against operational management at the works concerned who cannot be expected to realise the intricacies of ecological life cycle patterns and the habits of filter insects. When all of the facts are considered it appears that many people are to blame including the engineers who designed the works, the biologists for not communicating fully with the engineers and the operations staff, and the planners for allowing private dwellings and sewage works to be built in close proximity.

Certain factors seem to tip the ecological balance in filter beds with the result of increasing fly populations and these have mainly occurred when certain operational practices have been introduced to increase filter efficiency. For example most filters were initially fed by static distributors which simply sprayed sewage continuously onto a filter. Such filters often suffered from excessive film build up causing ponding problems and such systems were gradually replaced by systems where sewage was applied to the filter at intervals usually by means of a travelling distributor. The advantages of this change were to decrease film levels and increase efficiency however it is not difficult to imagine how a build up in fly populations would be favoured by this change.

The first periodically dosed sewage filters were generally circular and had rotating distributors consisting of 2 or 4 arms radiating from a central column, each arm issuing sewage onto the filter. The method of rotating the arms was initially by simply letting the sewage issue out the same side of each arm thus propelling the distributor round by hydrostatic forces. With such a system certain problems were apparent. Often high solids levels and a consequent decrease in efficiency was experienced particularly in the winter. This was probably a direct cause of the period between doses being insufficient to cause significant negative growth effects of the bacterial film.

A method of overcoming this problem was to simply increase the length of the period between doses of sewage i.e. to decrease the dosing frequency. This was achieved by many methods including dragging heavy objects e.g. chains from the distributor arms, using sewage flow powered paddle wheel drive mechanisms or by motorising the distributor mechanisms. These measures produced the desired decrease in winter film levels however an unfortunate side effect of these measures was that often population levels of chironomid flies, particularly the nuisance species M.hydropetricus and Orthocladius minimus (Mg.) were increased. This was caused by the fact that the larvae of these flies are particularly adapted to live and thrive in the low film and high scouring action conditions associated with low dosing frequency.

As the size of sewage treatment works increased a trend towards installing rectangular filters was apparent. This was obviously preferable on a cost basis as there would be a greater filtration area per unit area of land available. Such rectangular filter systems often use large cable hauled distributors running on rails forwards and backwards along the filter's length. Due to the physical size of these systems and limitations of the machinery the distributors tend to travel quite slowly giving a low dosing frequency, thus again favouring nuisance fly species. The particular works studied in this project had filters of this type and coupled with the

close proximity of housing it is not surprising that a chironomid fly nuisance problem was apparent.

When the problems of fly nuisance were first appreciated various methods were used to solve them. These mainly involved drastic measures such as filter flooding and the addition of chemicals such as gas liquor (a trade waste), these methods obviously had a detrimental effect on filter efficiency. Insecticides in various forms were used after this and are still in use at the present time. Many types have been used and throughout the years the trend has been to choose more complex target specific chemicals rather than those which affect the whole community in a filter. This trend is desirable as it is clear that the removal of non-target organisms in a filter is detrimental to efficiency.

Other methods of controlling fly nuisance have been used involving operational changes on the filters however the use of these forms of control seems not so common as the use of insecticide control measures probably because the latter produces almost instant results. Another reason why operational methods of control were not so common in the past could be that the ecology of the filter environment and the habits of the species found within filters were not fully understood.

Given the chironomid fly problem at Tamworth Water Reclamation Works a research project was planned with the following objectives.

Firstly a programme of insecticide treatment was followed using an organophosphate insecticide; Actellic M20 (active ingredient pirimphos-methyl). This insecticide had only recently been used for this purpose and the consequences of its use were not fully known. A sampling programme was carried out over 3 years investigating filter solids levels, macrofauna population levels and adult fly emergence levels. The reasons for this was to clarify if Actellic M20 treatment caused changes in filter

solids levels (and indirectly filter efficiency); to find out if it is effective against the target species and to find out how non target species are affected by regular usage of the insecticide. At the end of the project work was carried out testing various concentrations of the insecticide against the target species and various non target species to give a greater understanding of its effects.

Secondly certain operational methods of control were investigated over 3 years in a similar manner to the insecticide treatment experiment. Filter solids, macrofauna and adult fly emergence levels were monitored to show the full effects of the modifications. As the theoretical mechanisms of control offered by some of the operational modifications involved increases in filter film levels a special experiment was devised to check their effects on filter efficiency whereby 12 pilot filters were constructed using some of the modifications tested on the main filters. The purification efficiency of these filters was closely monitored and the fact that flies were caught from them gave a cross check on the effectiveness of the modifications for fly control.

Thirdly an experiment was planned to give a better understanding of the ecosystems operating in filters and the particular habits of the members of the filter community. It was thought that this information would be useful in predicting the effects of operational modifications on common filter fauna. The opportunity was provided for these investigations by the construction of 18 laboratory scale filters operating in a uniform environment, various operational parameters could therefore be studied without extraneous interference.

Finally the general objectives of this research project were to investigate various methods of fly control and to evaluate the long term effectiveness and feasibility of these methods. In order to achieve such objectives it was necessary to fully understand the mechanisms operating in filters and the habits of filter inhabitants. It

was hoped that the work carried out would lead to a better understanding of the above points and possibly stimulate future work on allied subjects as in most research projects it is often the case that the uncovering and clarification of certain points tends to pose more questions on the subject concerned.

Review of the Literature

Sewage filters have been in operation since the late 19th century and it is possible to say that almost every filter has contained or will contain at some time a population of one or more species of fly which may cause a nuisance.

The main species found belong to the order Diptera and lists of these species have been produced by Lloyd et al. (1940), Solbē et al. (1967) and Learner (1975). Learner did a survey of 48 sewage works in the United Kingdom and found 28 different species from 11 dipteran families occurring frequently in filters, of these the most common families found were Anisopodidae, Chironomidae, Psychodidae and Sphaeroceridae.

Identification of the adults of the common species is fairly simple, a useful but very basic description of the common filter flies was given by Tomlinson (1946). Lloyd (1945) described and gave diagrams of the common nuisance species of that time including the 3 common species of Psychoda (which are easy to distinguish as the diagnostic features are as simple as antennal segmentation and wing markings), Metriocnemus longitarsus^{Goet.} and Anisopus cinctus^{Fab.} More detailed keys concerning the families have been produced. Del Rosario (1936) described the American species of Psychoda whilst Tonnoir (1940) described the British species. Other less common filter flies such as the Trichoceridae, Ptychopteridae and the Anisopodidae have been described by Freeman (1950) and the Sphaeroceridae by Richards (1930). The Chironomidae are perhaps the most difficult family to identify and a comprehensive key was produced by Coe (1950) dealing with the adults. Generally different species can be identified by external features such as wing venation but in some cases the male hypopygial structure has to be studied. Coe pointed out that it is very difficult if not impossible to distinguish between the females of closely related species. Identification of chironomid larvae is not easy, however two useful keys were produced by Bryce (1960) and Bryce and Hobart (1972). It was suggested by Bryce and

Hobart (1972) that often identification is only possible to a species group within a genus but if difficulty is encountered they should be reared to the adult stage and identified using the key of Coe (1950). If further information regarding other families is required, Kerrich, Meikle and Tebble (1967) should be consulted.

The Anisopodidae, Chironomidae and Psychodidae have regularly been associated with problems and nuisance in past years. The occurrence of flies on works either from filters or sludge drying beds is by no means confined to the United Kingdom. Flies have been reported to occur in such areas as North America (Usinger and Kellen 1955 and Rawn 1949), South Africa (Murray 1939 and Klipfontein Organic Products 1949) and the Netherlands (Otter 1966).

Actual reported nuisances of flies whether to works employees or to the public have been quoted by several workers. In the case of sewage sludge a problem with Musca domestica^{L.} from drying beds was described by Klipfontein Organic Products (1949) in Johannesburg and Rawn (1949) in Los Angeles, also sepsid problems were described by Olroyd (1946) and Green (1970). In the case of biological filters many problems have been reported. By far the most common problem species in the past has been Sylvicola fenestralis^(Scop.) which was reported to be a nuisance by Khalsa (1948) and Hudson and Phillips (1975) at Knostrop, Leeds, and Jenkins, Baines and Hawkes (1949) and Hawkes (1951a) at Minworth, Birmingham. Anisopus cinctus was reported to be a problem by Lloyd (1945) in the Aire Valley, Yorkshire. Problems have also been found with Psychoda. Psychoda alternata nuisances have been reported by Lloyd (1937) at Knostrop, Leeds, Reynoldson (1941) at Huddersfield, Murray (1939) in South Africa and Lloyd (1945), also problems with both Psychoda alternata and Psychoda severini have been reported by Otter (1966) in the Netherlands. Problems with chironomids have been reported by Lloyd (1945) concerning Orthocladius perennis^{Mg.} and Chironomus dorsalis, other problems have been reported by Watson and Fishburn (1964) who found a seasonal problem at Keighley. A similar problem was described by Woods, Williams

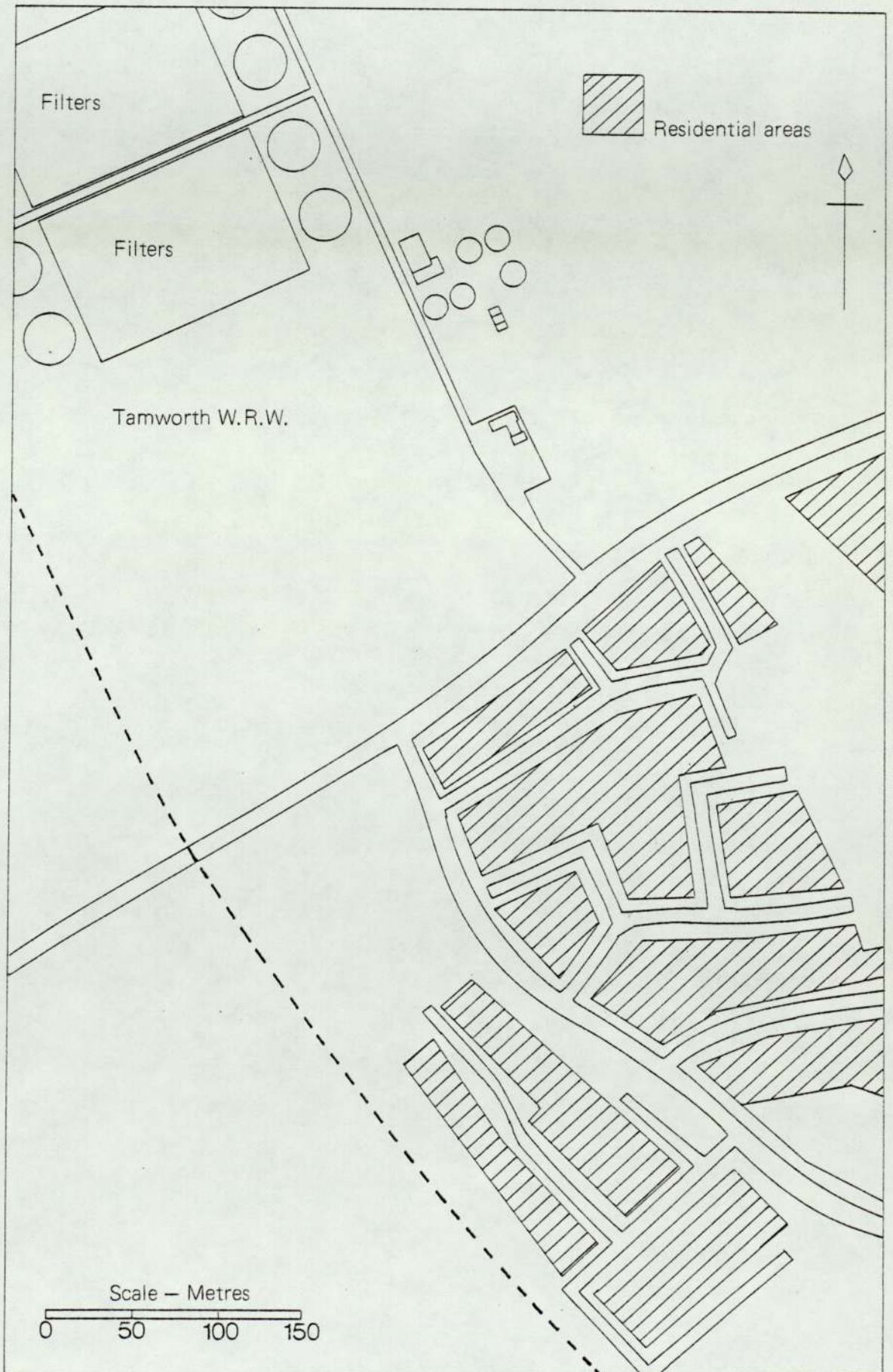
and Croyden (1978) at Tamworth whose work led to the development of this project.

To put the occurrences of fly nuisances into perspective it can be seen from Woods et al. (1978) that from a survey of 700 works using biological filtration, a fly problem was only significant in 20 of these and there were only 12 instances of public complaints, it was considered that the problem was significant at 3 of the works. The remedial taken in these cases was the use of insecticides. No operational methods of control were employed as it was considered that no correlation could be found between specific operational methods and the fly nuisance at the works studied.

In many cases of reported fly problems the siting of the works in relation to housing development has had much to do with the extent of the problem. Lloyd (1943c) showed that Psychoda caused a considerable problem when works and housing development were in close proximity, also Scheltinga (1977) described the Netherlands Public Nuisance Act and said that to avoid nuisance new installations should be sited away from residential areas. He also recommended the minimum distances for the siting of developments from different sized works.

In some cases long standing works sites are encroached upon by housing development as was the case at Tamworth (Woods et al. 1978) where a small works was built in 1907 and after modifications and extensions, work was started on a completely new works on the old site in 1969, to take into account the town's expansion. However in 1970 permission was given by the local authority for a large area of land immediately adjacent to the works to be developed for housing. The position of this development can be seen in Fig.1, it should be noted that the nearest dwelling is only 266 m. distant from 288,000 m² of filter area also the majority of the development lies between a S.E. and S. direction from the filters so N.W. winds would tend to aggravate the problem as the flies would be blown towards the housing estate.

Fig. 1. Map of housing development in relation to Tamworth Water Reclamation Works



Studies on the range of flight of filter flies has been carried out in the case of Psychoda by Headlee (1919), Simpson (1933), Holtje (1943) and Satchell (1947). All of these authors came to the same conclusion that the maximum range of flight of Psychoda was 1.6 Km, however Satchell (1947) found that the total range of Psychoda may be extended if there were intermediate breeding grounds within the 1.6 Km distance. Since chironomids are more sturdy, larger flies it would not be unreasonable to assume that their range is well in excess of that for Psychoda.

An intensive study was carried out by Gibson (1945) into the habits of certain chironomids and it was found that most of them would produce "mating" swarms if certain conditions were favourable. The conditions concerned were wind direction in relation to physical objects, wind velocity, light intensity and temperature. It is well known that swarm formation and the development of a complex mating dance occurs as a prelude to mating in the Tipulidae, Culicidae and Chironomidae however it has not been observed in the Psychodidae. The reason for swarm formation is not fully understood. It is known that swarm formation is needed for successful reproduction in the case of Orthocladus minimus and Metriocnemus hirticollis^{Staeg.} (Lloyd 1937), however M.hygroptericus which can mate successfully in confined spaces still produces swarms when conditions are favourable. Attempts have been made to breed O.minimus in captivity with no success. Lloyd et al. (1940) managed to get adults to pair but out of many attempts only one effective mating was obtained. Similarly Gibson (1945) managed to get good pairing in a bell jar, but out of 74 females taken from the jar only 47% oviposited and then only two laid fertile eggs. It was stated that these fertile eggs may have been obtained from females that had been fecundated prior to the experiment as adult flies were taken from the filters for the experiment, obviously it would have been more conclusive to use pupae which could then have hatched out in the bell jar without any possibility of being fecundated.

The effect of certain parameters has a great bearing on swarm formation and

consequently fly nuisance. It should be noted that in the case of chironomids fly complaints concern not individual flies but swarms round houses and settling of pairing couples on vehicles and washing etc. Gibson (1945) described how swarm formation is a male phenomenon, the females lie beneath the swarm and periodically flies upwards to meet a male, here they pair and drop to the ground, all within a fraction of a second. The length of mating is then very variable, with O.minimus (Gibson 1945) it has varied from almost instantaneous parting to 10 minutes. The main parameters that affect swarm formation and positioning are wind direction and velocity in relation to fixed objects e.g. buildings. It is obvious that the problem will be aggravated if any development is in the direction of the prevailing wind from the works, this was found by Satchell (1947) with Psychoda. Hawkes (1961a) working with S.fenestralis found optimum temperatures for fly emergences and it was found that these temperatures were affected by the wind velocity. For example the optimum emergence temperature with a wind velocity of 3.2-6.4 kmhr⁻¹ was 21.1-23.8°C this fell to 18.3-21.1°C with a wind velocity of 6.4-9.6 kmhr⁻¹ and 15.5-18.3°C with a wind velocity of 9.6-12.8 kmhr⁻¹. Gibson (1945) made a comprehensive study of the effects of wind velocity on swarm formation in which O.minimus individuals were released into a mating cage which had been adapted as a wind tunnel. These were then subjected to known wind velocities and the sizes of the swarms were noted. It is known that wind is not essential for swarming as swarms have been induced in the laboratory. Gibson (1945) found that up to 25.4 cm⁻¹ no effect was noticeable on the swarms except that there was a general alignment of the flies to face the wind, above this level the swarms were reduced in size up to 50.8 cm⁻¹ when swarming ceased, however the flies could be induced to take to flight by tapping the substratum but they soon settled again. Above 101.6 cm⁻¹ it was not possible to induce flight of any kind. Experiments similar to this were carried out in the field and results were fairly similar but generally it seemed that the flies could withstand higher wind velocities in the field than in the laboratory, the explanation given for this was that in the field the wind was gusty with periods of lower velocity allowing recovery, whereas in the

laboratory it was constant. Some work was carried out with Metriocnemus and it was found that reduction of the swarm began at the same wind velocity as with O.minimus but swarms were still observed with velocities as high as 142.3 cms^{-1} , this was to be expected as Metriocnemus are more sturdy and larger than O.minimus.

Many workers have noticed orientation of swarms in relation to raised objects (Howard, Dyer and Knab 1912, Edwards 1929, Rao and Russell 1938, Marshall 1938 and Gibson 1945) and one aspect of the nuisance is that Metriocnemus tend to swarm round the eaves of dwellings in the lee of the wind. Gruhl (1924) found that in the absence of wind, swarms would form round the highest object, this was also found by Gibson (1945) with Metriocnemus. Gibson also found that the orientation of swarms in relation to raised objects was due to a combination of the effects of light intensity and shelter from the wind.

The effect of light intensity on swarm formation was also studied by Gibson (1945). He found that Metriocnemus and O.minimus had diurnal habits and would swarm long before dawn when light intensity was very low. The size of the swarms increased with increasing light intensity throughout the day. These results were backed up by laboratory experiments. The effect of light intensity on S.fenestralis was studied by Hawkes (1951a) using tray fly traps with and without windows of various sizes. It was generally found that S.fenestralis was attracted by shady areas and sought shelter under the trays from the light, also it was found that S.fenestralis tended to emerge during the evening and continued throughout the hours of darkness until dawn when the emergence fell to a lower level.

The effect of temperature on fly emergence has been studied by Williams (1940), Gibson (1945) and Hawkes (1951a and 1961a). Williams (1940) found that the catch of insects in a light trap was doubled for a 2.8°C increase in minimum temperature or a 3.9°C increase in a maximum temperature. Gibson (1945) reported that there seemed

to be no upper limit (of temperatures recorded in this country) for swarming of O.minimus and M.longitarsus. Both were observed swarming in the sun when the shade temperature was 21.7–23.9°C. However he found the existence of a definite lower limit, with O.minimus no swarms were noted below 7.8°C and during a 79 day period mating was only observed twice at 8.9°C and twice at 10°C. With Metriocnemus longitarsus the limits were lower, swarming was still found to occur at 6.1°C and 5°C and mating was observed once at 8.3°C.

Hawkes (1951a and 1961a) studied the effect of temperature on the emergence of S.fenestralis from filters. He found a close correlation between temperature and fly emergence but this only held true in the colder periods of the year, seasonal effects come into being for other parts of the year and these will be discussed later.

The influence of seasonality on the emergence of flies from filters has been noted by many authors. A comprehensive study of five common flies namely P.alternata, P.severini, M.longitarsus, M.hirticollis and O.minimus was carried out by Lloyd (1937). Similar work was carried out on P.alternata, P.severini and O.minimus by Lloyd et al. (1940) also work on S.fenestralis has been reported by Tomlinson (1943), Hawkes (1951a, 1961a) and Hawkes and Jenkins (1958), on P.alternata by Reynoldson (1941), Lloyd (1943b) and Solbē et al. (1967) and on O.minimus by Lloyd (1941). The observed effects of seasonality can be mainly attributed to temperature (Uvarov 1931) and to a lesser degree by day length (Hawkes 1961a). The concept of insect development times being attributable to temperature was stated by Uvarov (1931) in his "Progress temperature" law which briefly states that for each life cycle stage of an insect there is a temperature threshold below which no development can take place and a thermal constant for development which is the total amount of energy required to carry on development through a particular stage of the life cycle, thus at different temperatures the time required to assimilate this fixed amount of energy will vary. This relationship was expressed using the following formula.

$$D = \frac{C}{T-K}$$

- Where D = Development period (days)
 T = Temperature ($^{\circ}\text{C}$)
 C = Thermal constant for particular species (day degrees)
 K = Minimum threshold temperature for particular species ($^{\circ}\text{C}$)

There is also an upper temperature limit of development above which development ceases and if protracted causes death, in a similar manner this is constant for the particular stage of the life cycle and the species concerned. Work carried out by Lloyd (1937), Lloyd et al. (1940), Khalsa (1948) and Tomlinson (Unpublished) has resulted in a comprehensive table of data concerning insect development quoting thresholds for development and thermal requirements for different life cycle stages for most of the common species of filter fly (See table 1).

TABLE 1. PUBLISHED DATA ABOUT FLY DEVELOPMENT (Learner 1975)

Species	Egg incubation		Larva		Pupa		Maturation of female		Total for life-cycle		Temperature range at which there is no detectable temp. mortality ($^{\circ}\text{C}$)	Mating bar ($^{\circ}\text{C}$)
	Threshold temp. ($^{\circ}\text{C}$)	Thermal constant (day degrees)	$^{\circ}\text{C}$	Day degrees	$^{\circ}\text{C}$	Day degrees	$^{\circ}\text{C}$	Day degrees	$^{\circ}\text{C}$	Day degrees		
<i>Hydrobaenus minimus</i>	4.3	38	3.5	383	5.5	37	0.0	63	4.5	450	9-20	10
<i>Metriocnemus hygroetricus</i> ..	1.0	63	1.7	440	2.5	32	—	—	1.0	540	5-18	very low
<i>M. hirticollis</i>	4.5	46	3.0	660	3.7	26	—	—	4.5	610	10-20	10
<i>Psychoda alternata</i>	5.9	20	5.5	244	7.7	31	2.0	46	6.0 5.0**	315 240**	15-28	6
<i>P. severini</i>	2.2	48	0.6	353	1.5	77	0.2	35	1.7 1.0**	480 480**	8-18	partheno-genetic
<i>P. cinerea</i>	—	—	—	—	—	—	—	—	3.5**	350**	—	—
<i>Sylvicola fenestralis</i>	—	—	—	—	—	—	—	—	2.1* 3.5**	814* 680**	<9-18**	very low

All data from Lloyd (1937) and Lloyd et al. (1940), except* Khalsa (1948) and** T. G. Tomlinson (unpubl).

Thus from these data it is possible to predict the time required for an insect to complete its life cycle or one of its stages assuming the filter temperature is known, this information is invaluable as it is then possible to predict either when the bulk of a fly population is going to be in the adult stage or the optimum time for use of insecticide control measures (Woods et al. 1978).

At this stage a distinction should be made between the actual seasonal rhythm of flies i.e. certain species being prevalent at certain times of the year and peaks of development whereby a series of peaks of emergence of the adult insect is observed throughout its period of prevalence during the year. The phenomenon of peaks in adult fly emergence was explained by Lloyd (1941) and is brought about by a sudden rise in temperature in spring tending to cluster the offspring of parents whose emergence may have been widely separated in time. Therefore after the first peak it follows that the progeny should mainly develop at the same rates as they would be subjected to the same temperature influences giving rise to a series of peaks throughout the insects' season. Lloyd also explained the production of "daughter" peaks due to temperature changes near the beginning or end of the life cycle as follows. For example if a fall in temperature occurs during a period in the life cycle when most of the population consists of fourth instar larvae and newly developed pupae (it must be appreciated that there would be some overlap) the temperature change would affect the larvae differently to the pupae. The larvae would probably stay in that stage longer than the pupae would stay in their stage, thus causing in the future two emergences, one due to the pupae developing and the other later due to the larvae pupating and emerging. Thus a fall in temperature could cause an extra peak to appear when only one was expected. The periods during the year when specific filter flies are in greatest abundance can be attributed to their specific thermal requirements and to competition with other species (Lloyd et al. 1940 and Lloyd 1943b). Various workers have shown when the specific periods of abundance are for different species, these are summarised in Table 2.

Table 2 Periods of maximum abundance for certain insects

Species studied	Author					
	Lloyd 1937	Lloyd et al. 1940	Lloyd 1941	Lloyd 1943	Hawkes 1951/61	Solbe et al. 1967
<u>S.fenestralis</u>	-	-	-	-	May-Jul. Sep-Oct.	-
<u>O.minimus</u>	Aug-Oct.	Jul-Oct.	May-Dec.	-	-	-
<u>P.alternata</u>	May-Aug.	Apr-Aug.	-	May-Aug.	-	May-Sept
<u>P.severini</u>	Mar-Jun.	Feb-Jul.	-	-	-	Mar-Jun.
<u>M.hygropetricus</u>	Oct-Feb. Apr-Jun.	-	-	-	-	Jul-Nov.
<u>M.hirticollis</u>	Nov-Feb. May-Jul.	-	-	-	-	-

Thus the explanation for P.severini peaking before P.alternata is due to their different threshold for development temperatures, P.severini has a lower value and therefore can develop earlier in the year than P.alternata (Woods et al. 1978).

Competition was tentatively suggested by Lloyd (1937) to explain the late arrival of O.minimus peaks at Barnsley, it was suggested that large numbers of P.alternata were causing this and these large numbers were explained by periodic resting of the filters giving extremes of temperature which only P.alternata could withstand. Competition will be fully discussed later. Some work has been carried out on the seasonality of less common species of flies found in filters. Solbē et al. (1967) showed that Sphaerocerid flies (mainly genus Leptocera) had a period of prevalence from May to September whilst Terry (1952) suggested that ephydriids and sphaerocerids mainly emerge from July to September.

Work concerning the public health problems due to the nuisance fly species has been quite sporadic. Problems have been found due to M.domestica from sludge drying

beds at Johannesburg (Klipfontein Organic Products 1949) obviously this was due to the connection between the habits of the fly on sludge and on food. The rest of the work concerned flies from filters. Psychoda was considered to be a problem to works operatives as being weak fliers they never migrated very far from the vicinity of the works and could easily be inhaled (Lloyd 1945). It was reported by Johnson (1914) that the hairs of Psychoda could cause skin irritation on humans also Ordman (1946) working in South Africa reported 13 cases of bronchial asthma in works operatives which he attributed to Psychoda. He found that the problem was intensified in the summer when there were large numbers of P.alternata present around the filters. It was thought that the workers were producing an allergic reaction to dust resulting from the disintegration of fly bodies. This was backed up by tests in which it was shown that the workers gave a negative reaction against common allergens e.g. feathers, dust etc. but a positive reaction against P.alternata adult extract.

Other problems with Psychoda have been found by Steinhaus and Brinley (1957) who found certain bacteria present in the hairs and body of Psychoda. Another species which has been studied from this viewpoint is S.fenestralis. Hawkes (1951a) said that they were attracted to food and tended to deposit their egg masses in moist shady places such as on tooth brushes or on food. Thus S.fenestralis must be considered a public health hazard because of its close association with sewage and food, however it is yet to be proved that any disease has been caused by the fly. Hawkes also mentioned the sheer aerial density of the fly during an emergence making work difficult. The main problem with flies seems to be their physical presence causing psychological distress. Woods et al. (1978) also mentioned the psychological hazards of M.hydropetricus to the public. Work was carried out by the above authors testing for the presence of pathogens in settled sewage, filter film and the adult flies. Whilst pathogens were present in the settled sewage and film none was isolated from the flies. This seems to back up earlier views that the problem is mainly physical and psychological.

Various methods of control of filter flies have been attempted in the past. Some of the early methods of control are interesting in being quite drastic and yet authors claim that they had no effects on filter efficiency. A very early method of controlling Psychoda in America was reported by Headlee (1919). He controlled them by flooding the filter for 24 hours every 10 days, it was stated that this method was widely practised in America. Also in America Kessler and Noorgaard (1942) who encountered fly problems with high rate filters controlled the flies using a combination of flooding and chlorination.

Other early methods are fairly standard in employing such chemicals as sodium chloride, creosote, paraffin, calcium chloride and chlorine gas. It is interesting to note that rather obnoxious mixture consisting of 909 l (200 galls.) creosote emulsified with 6.6 kg (14 lbs.) of grease and 90.9 l (20 galls.) of soda ash was used by Scouller and Goldthorpe (1932) to control Psychoda. It was reported by the above authors to have no effect on filter efficiency!

Much of the early work on fly control was carried out in South Africa. Murray (1939) described successful methods used to control P.alternata including the use of calcium hypochlorite and chlorine gas dosed into the sewage prior to filtration. More successful methods included the use of creosote oil, organic substances dispersed in oil e.g. paraffin, and calcium hypochlorite applied direct to the filter media. Murray (1939) also carried out some elementary laboratory work to test the effects of some of these substances on Psychoda adults. He placed the adults in a solution containing a monomolecular layer of paraffin on the surface and the flies which usually floated on the surface sank quickly and died, this was obviously due to the surface tension effects. He also tried placing flies in tubes containing calcium hypochlorite and found that the flies become very agitated due to the chlorine gas. Other reagents which gave rise to agitation included hydrogen sulphide and ammonia.

Wilson (quoted in Murray 1939) discussed the use of bleaching powder as a control method but it has a disadvantage in being expensive, other cheaper methods quoted have been the use of common coarse freezing salt sprinkled on the surface of a resting bed and dissolved with tank effluent causing the larvae to rise to the surface and die, also the use of crude gas liquor diluted with water was reported to be effective in controlling both Psychoda larvae and adults. Another South African worker namely Allison (1940) used a combination of unusual methods to control Psychoda during wartime when supplies of calcium hypochlorite were low. It consisted of using four bags of sodium chloride per filter weekly, the filter was then rested and dusted with one drum of calcium hypochlorite when the larvae had been forced to the surface. Other methods used in combination with this included painting filter walls with old machine oil and physically burning the flies utilising sludge gas. Early methods utilised in the United Kingdom have been described by Reynoldson (1941), Tomlinson and Jenkins (1947) and Lloyd (1945). Reynoldson (1941) at Huddersfield used creosote to control P.alternata but later hinted that control may be achieved using a carefully balanced flow on a double filtration system. Tomlinson and Jenkins (1947) described some early methods of controlling S.fenestralis which included the use of bleaching powder, creosote, sodium chloride and gas liquor. The results of the specific treatments could be summarised as follows. Gas liquor treatment was shown to be doubtful as a 25% dilution took 30 minutes to have any observable effect on S.fenestralis larvae. Sodium chloride was not much better, it was noticed that an application of 2055 kg ha^{-1} (5 tons acre⁻¹) resulted in a larval kill of only 15-20%. Bleaching powder had some success however it was found that it had to be applied at a rate of 1.63 kg m^{-2} (31b yd⁻²) in order to control S.fenestralis effectively and that such a treatment was only effective for 5 weeks. Certain disadvantages reported with this operation was that bleaching powder was non-selective in its action and the spreading of it was a disagreeable operation. Similarly treatment with creosote met with a limited success. It was found that 9.1 l (2 galls.) of 2% creosote per 0.84 m^2 (1 yd²) was needed to control S.fenestralis for 40 days and

that again this treatment was non-selective and killed non-target species such as Hypogastura ^(Tullberg) viatica and Lumbricillus ^(Mull.) lineatus. This had the effect of delaying the spring unloading of film until populations of grazers had built up in the summer. The various costs of these methods were reviewed by the author.

Lloyd (1945) gave a comprehensive review of early control methods and looked at other types of control. He reported operational methods for the control of Psychoda by altering the filter environment to suit Metriocnemus. Few workers previous to this stated the effects of sometimes very drastic treatment methods on other non-target members of the filter fauna. Treatment using some of the substances stated would be out of the question today considering the regulations concerning discharges to watercourses.

Following these early methods of control chemical methods using specific insecticides come into use and are still in use today. Insecticides can generally be classified into 3 main groups according to their structure. These are inorganic, organo-metallic and organic insecticides. The main group involved in the successful control of fly larvae in filters are the organochlorines of which D.D.T. and B.H.C. are members and the organophosphates which contain parathion, malathion and pirimiphos methyl to name but a few. Recently compounds other than these such as the new non-phosphorous insecticide "Dimilin" 1-(4-Chlorophenyl) -3-(2,6-difluorobenzoyl) urea have been used. The structures and formulae of the commonly used insecticides are given in Table 3.

The mode of action of organochlorines has been suggested by Reay (1969) to be as follows. D.D.T. is thought to stimulate the nervous system to produce large amounts of toxins which are normally present in very small amounts, this causes a general disorganisation in nervous co-ordination. Hyperactivity of the peripheral sensory apparatus is characteristic of D.D.T. poisoning which then leads to fatal paralysis.

Table 3. Structure and formulae of commonly used insecticides

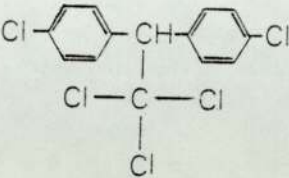
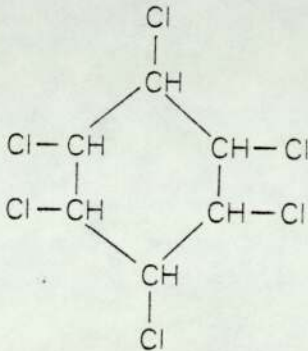
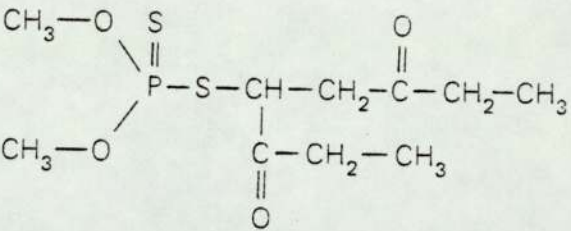
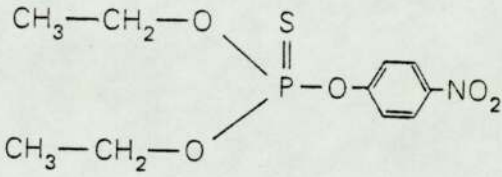
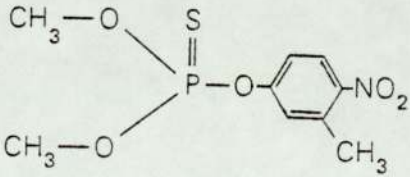
Common and chemical names	Structural formulae
D.D.T. Dichlorodiphenyltrichloroethane	
γ B.H.C. (Gammexane) Gamma-1,2,3,4,5,6- Hexachlorocyclohexane	
Malathion O,O - dimethyl phosphoro - dithioate ester of diethyl mercaptosuccinate	
Parathion O,O - diethyl - O - P - nitrophenyl phosphorothioate	
Fenitrothion O,O - diethyl O - (3 - methyl - 4 - nitrophenyl) phosphorothioate	

Table 3. continued

Common and chemical names

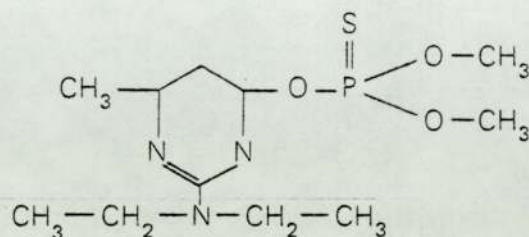
Pirimiphos - methyl (Actellic)

2 - Diethylamino - 6 -

methylpyrimidin - 4 - yl

dimethyl phosphorothionate

Structural formula

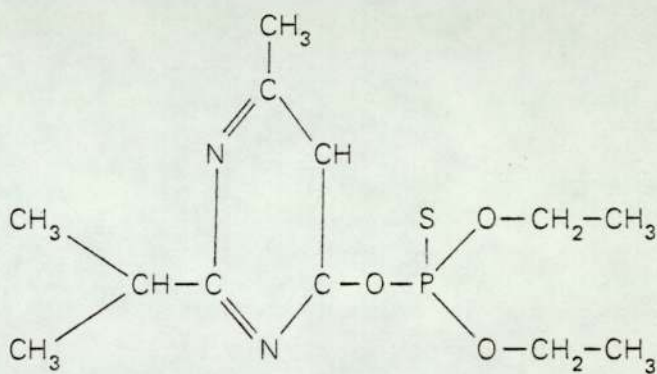


Diazinon

O,O - Diethyl - O - (2 - isopropyl -

6 - methyl - 5 - pyrimidinyl)

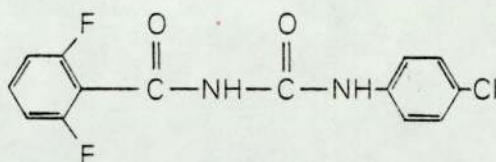
phosphorothioate



Dimilin (TH - 6040)

1 - (4 - chlorophenyl) - 3 - (2,6-

difluorobenzoyl) urea



Acetyl-choline is known to increase but this is not thought to be due to acetylcholinesterase inhibition as is the case with organophosphates. According to Reay (1969) other organochlorines such as B.H.C. have a slightly different mode of action in acting centrally as opposed to peripherally as is the case of D.D.T. Reay also suggested that B.H.C. itself can act as an antimetabolite since it has a similar structure to meso-inositol (a vitamin of the B group). The presence of this substance can offset the symptoms of B.H.C. poisoning but it is not sure how important this vitamin is to the vital processes of insects.

The mode of action of organophosphates is a simple reaction involving inhibition of normal synaptic functioning resulting in death. In insects it has been found that acetylcholine is a transmitter substance in the central nervous system which is a chain of small ganglia (Smallman and Mansingh 1969).

The chemistry of synaptic transmission was described by Eto (1974). To summarise, a nerve impulse, when arriving at the pre-synaptic membrane causes a release of acetylcholine which then travels across the synapse and causes excitation of the post-synaptic membrane, thus the nerve impulse is carried over the synapse from one nerve to another. The acetylcholine released as a result of excitation is then rapidly hydrolysed into non-reactive acetic acid and choline by acetylcholinesterase produced on the post synaptic membrane. The hydrolysis of acetylcholine is important as in order for the post-synaptic membrane to revert to its original non-excited state all of the acetylcholine must be broken down. This is necessary in order to allow the post-synaptic membrane to be sensitive to a second nerve impulse whenever one arrives. Thus any inhibition of acetylcholinesterase results in a disturbance of nervous function which leads to severe if not fatal damage to the organism. Several organophosphates inhibit this action. Reay (1969) suggested that such compounds as parathion and malathion are not themselves acetylcholinesterases but are converted to metabolites within the insects gut. Parathion is converted to paroxon and

malathion to maloxon (an oxidation process from thiophosphate to phosphate, P=S to P=O) both of which interfere with acetylcholinesterase activity.

Recently new non-phosphorus insecticides such as "Dimilin" have been used against Psychoda (quoted by Buckland, private communication) with mixed success. This insecticide is interesting as it acts not by synaptic inhibition but by inhibiting chitin production (Ishaya and Casida 1974). This has the effect of causing moulting larvae to burst, also it has ovicidal properties.

The past use of insecticides to control nuisance fly species has been widespread. Frye et al. (1931) described the use of common insecticides to control P.alternata on filters. The insecticides used included such substances as chlorine, borax, carbon disulphide, copper sulphate, arsenates and derivatives of naturally occurring compounds e.g. nicotine sulphate. Later uses of insecticides mainly concerned the use of organochlorines such as D.D.T. and B.H.C. (gammexane). Present day uses are usually confined to organophosphates such as malathion, parathion and pirimiphos-methyl due to their generally low mammalian toxicity (Reay 1969 and Eto 1974) and low persistency in the environment (Woods et al. 1978).

Early work with insecticides was reported by Brothers (1946) using D.D.T. to control P.alternata in high rate filters in Texas, he found that 3 mg l^{-1} applied for 24 hours was sufficient to control the fly for 20-30 days however he recommended a dose of only 1 mg l^{-1} . Work carried out in South Africa (Klipfontein Organic Products 1949) showed that B.H.C. dust controlled a population of M.domestica found breeding in sludge drying beds. The level of the film in filters at the time of treatment was found to be an important factor in the success of that treatment by Tomlinson and Muirden (1948) and Tomlinson et al. (1949). They used D.D.T. and gammexane against Psychoda and S.fenestralis and found that treatment was only effective when film conditions were fairly low indicating some adsorption of the insecticide by the film.

These effects were also described by Hawkes (1951a) when it was found that a better control of S.fenestralis could be effected when film levels were low.

Target selectivity of D.D.T. and gammexane treatment was shown by Tomlinson and Jenkins (1947). They controlled P.alternata and S.fenestralis successfully with the above insecticides and gave the opinion that they had no effect on two other important grazers, L.lineatus and H.viatica when used in the specific concentrations required to kill the target species. Obviously if higher concentrations are used some problems may be encountered. Effective control of S.fenestralis was achieved using D.D.T. and gammexane by many workers. Jenkins, Baines and Hawkes (1949) used both insecticides and found them both effective however it was stated that gammexane was preferable on a cost basis. Hawkes (1951a, b, and 1957) and Hawkes and Jenkins (1951) also described the effective control of S.fenestralis using gammexane, it was also found by Hawkes (1951a) that the method of application of the insecticide was important. An emulsion of B.H.C. gave an 18.5% reduction in S.fenestralis output whilst when used as a water dispersable powder this reduction was increased to 70%. Another success in controlling S.fenestralis was achieved by Watson and Fishburn (1964) using the organophosphate malathion. This is one of the first reported uses of organophosphates in the United Kingdom to control filter flies.

The important concept of the timing of an insecticide dose in relation to the stage of the life cycle in which the bulk of the target species are in has been mentioned by a few workers. Hawkes (1951a) and Hawkes and Jenkins (1951) found that gammexane was more effective against S.fenestralis larvae than against other life cycle stages, also Woods et al. (1978) suggested that pirimiphos methyl may be most effective against larval stages. These authors also quoted measures taken against other stages of the life cycle of target species such as the use of aerial sprays or fogging machines or by applying insecticide to the filter walls and surrounding areas but all of these techniques met with limited success. Obviously once a fly has emerged and is mobile

it is difficult to control, therefore it must be preferable to attack the target species when it is in a fairly non-mobile state such as a larva, in such a stage it is confined to a specific habitat, being the filter, onto which it is easy to apply the insecticide. No one author has discussed why insecticides should be most effective on larval stages but this is probably due to the fact that larvae have a high metabolic rate and are more active than for example pupae and as most insecticides are contact effective they would be absorbed more quickly by the larvae. Another reason for this, most notably in the case of chironomids is that the pupae are enveloped in a mucus sheath which may act as a physical barrier to the absorption of the insecticide.

Control of chironomids by insecticide treatment has not been so well documented as it has for other species, this may be due to the number of works on which there are a nuisance being limited compared to the numbers of works on which other species are a nuisance. It was stated by Jackson (1943) that chironomid larvae were controlled in activated sludge tanks by applying pyrethrum powder (0.7 – 0.9% pyrethrins), he suggested that a safe dose to use was 1 kg to 20043 l (1 lb to 2000 gall) of tank capacity. Woods et al. (1978) used a range of insecticides in attempts to control the chironomids M.hydropetricus and O.minimus at Tamworth. Initially malathion was used but because of its high toxicity to fish the filters had to be treated in stages, one small area followed by another, this was to ensure that the amount of insecticide reaching the watercourse was limited, with this method the whole treatment took 8 days and the results were not successful. B.H.C. was then investigated, because of its high fish toxicity the dosing method had to be extended to 16 days and the results were that only 60% of the target larvae were killed. The explanation of the low kill using B.H.C. and malathion was that during the long period needed for dosing there was a chance of reinfestation of the treated areas from the untreated areas however considering the persistence of these compounds (Edwards 1975) it is postulated that low success rates may have been due to some other factor such as low susceptibility of these species to malathion and B.H.C.

Woods et al. (1978) also used pirimiphos-methyl against chironomid larvae with great success. A concentration of 5 mg l^{-1} in the settled sewage was found to be an effective larvicide and it was found that insecticide was readily degradable by a variety of factors including sunlight, hydrolysis, volatilization and bacterial degradation. Thus it was not persistent in the filters, it did not reach the receiving watercourse in appreciable quantities and treatment could be effected in 2 hours without interrupting works operation.

Other observations noted with this insecticide was that it seemed to have no detrimental effect on purification efficiency and therefore the film, however studies are continuing on this matter, also it seemed that pirimiphos-methyl at 5 mg l^{-1} was not effective against P.severini which were found at Tamworth in small numbers. The explanations the authors gave for this is that either P.severini may be less susceptible to the insecticide than chironomids or that the insecticide may be absorbed by the film in the upper levels of the filter and never reach P.severini in sufficient quantities to kill. It should be appreciated that P.severini is prevalent in a specific sub-surface level in filters (Fair 1934, Tomlinson and Hall 1950, Solbē et al. 1967 and Hawkes and Shephard 1972).

Considering the large scale use of organochlorine insecticides in previous years it seems that little thought was given regarding their breakdown or accumulation in the environment. One of the first reports came from Tomlinson and Jenkins (1947) who noted the toxic effects of gammexane and D.D.T. on trout and had reservations about discharging effluent to watercourses after insecticide treatment. Fleming et al. (1962) and Busvine (1971) stated that organochlorines are so toxic to freshwater invertebrates that these invertebrates have actually been used to bioassay insecticides for several years. Edwards (1975) described fully the effects of insecticides on the environment and from his work it seems that most problems were encountered using organochlorine insecticides. The accumulation of insecticides

through trophic levels in the aquatic environment has been shown by Bridges et al. (1963), Kallman et al. (1962), Keith (1966) and Keith and Hunt (1966). Also Edwards (1975) compiled lists of organochlorine insecticide residues found in freshwater invertebrates and fish. From these lists it is obvious that D.D.T. persists and is accumulated to a much greater extent in the environment than B.H.C., also generally higher residues were found in America than in the United Kingdom. Examples of levels of these insecticides found in certain aquatic organisms can be seen in Table 4.

Table 4 Insecticide residues in certain organisms

Location	Organism	Insecticide	Mean residue in organism MgKg ⁻¹	Author
U.S.	Plankton	D.D.T.	0.005	Hannon et al.1970
U.S.	"	B.H.C.	0.0002	" " "
"	Crayfish	D.D.T.	0.002	" " "
"	"	B.H.C.	0.001	" " "
"	Whirlygig Beetles (Gyrinidae)	D.D.T.	0.919	" " "
"	" "	B.H.C.	0.001	" " "
"	Clam (<u>Gaudea</u> sp)	D.D.T.	8.2	Godsil & Johnson 1968
"	Scud (Amphipoda)	D.D.T.	13	Peterle 1965
G.B.	Perch	D.D.T.	0.14	Holden 1966
U.S.	"	D.D.T.	0.71	Reinke et al.1972
G.B.	Salmon Parr	D.D.T.	0.03	Holden 1966
Sweden	Salmon	D.D.T.	3.4	Jensen et al.1969
Canada	"	D.D.T.	15.33	Duffy & O'Connell 1968

Obviously from these results it can be seen that the organochlorines concerned are very persistent. This persistency was noted in early work by Tomlinson et al. (1949) who found as much as 90% of applied B.H.C. was adsorbed on to the film and this persisted for as long as 3 weeks. The fate of another organochlorine larvicide

Methoxychlor, which was used against blackfly (Diptera: Simuliidae) was studied by Wallace et al. (1976) who found appreciable levels in non-target life forms in the watercourse concerned. Methoxychlor was used extensively in Canada to control mosquitoes and blackflies, a review of its effects on fish including sub-lethal concentrations was given by Gardner and Bailey (1976).

Organophosphorus insecticides are more easily degraded and this probably explains their increase in use in recent years. Woods et al. (1978) have stated a number of ways in which pirimiphos methyl degrades when applied to filters apart from which it is adsorbed by the film. Woods, Williams and Croyden (unpublished) also stated the half life of pirimiphos methyl in works final effluent to be as low as 6 hours. Similarly experiments were carried out to investigate the fate of pirimiphos methyl and one hour after "spiking" humus sludge with a 5 mg l^{-1} solution it was found that only 28% of the original concentration was recovered only 2% of this being in the "top" liquor. After 3 hours only 7% was recovered all of this being in the solids. It was postulated that this accounted for the disappearance of pirimiphos methyl after dosing. Khan, (1972) explained these mechanisms of adsorption on humic substances and studied the effects of certain factors such as temperature and pH on the adsorption mechanism. The fate of another organophosphate, Malathion, has been documented. Wolfe et al. (1977) found that malathion was susceptible to alkaline degradation, similarly pirimiphos methyl is thought to be. However the U.S. Environmental Research Agency (1974) state that when malathion breaks down in water at 1.7°C it forms malathion acids which may possess some of the toxic qualities of malathion and that these acids persist longer in the environment than malathion.

Although organophosphates seem fairly labile it should not be assumed that they do not reach the environment in sufficient quantities to affect fish. Thirugnanam and Forgash (1977) found evidence of acetylcholinesterase inhibition in fish which were

predators of mosquito larvae in salt marshes. The mosquito larvae were subject to a programme of treatment using the organophosphate chloropyrifos (Dursban). It was found that after dosing the larvae 4 times at 14 day intervals acetylcholinesterase inhibition in the fish increased by 56 — 100%, so the effects seem cumulative. Even 69 days after the final application there was still some evidence of acetylcholinesterase inhibition, therefore it was concluded that the use of this larvicide in salt marshes where a specific predator - prey relationship has evolved created an environmental hazard which endangered the fish population.

Other instances where acetylcholinesterase inhibition has been found in aquatic life have been shown by Coppage and Matthews (1974) who found that inhibition of the enzyme was caused by organophosphates in estuarine fishes and pink shrimps. This inhibition seems to be not the only effect of organophosphates on fish. Bull and McInerny (1974) found that sub-lethal concentrations of the organophosphate fenitrothion suppressed feeding behaviour in salmon. This behaviour was found to be suppressed at 1 mg l^{-1} and it was concluded that this reaction could be adapted to be a sensitive indicator of low levels of organophosphate pollution. The promising new non-phosphorus insecticide Dimilin described earlier is known to be very toxic to fish and Daphnia (Buckland private communication). Schaeffer and Dupras (1976) studied the stability of this insecticide in water and found that it persisted long enough to control mosquito larvae but did not have long term stability. It seemed to be affected by photolysis and adsorption on to organic matter and it was found to be least stable when the water temperature and pH were both high. Reports on the effects of insecticides on fish are countless and it is not intended to describe these any further apart from mentioning the work of Toobey et al. (1975) who tested the acute toxicity of 102 pesticides and miscellaneous substances to harlequin fish, the substances were listed in increasing toxicity based on a 24 hr. L.C.50 values for that specific fish.

The use of insecticides on filters is strictly controlled by the government in the scheme described in Woods et al. (1978). The Department of the Environment Health and Safety Executive control the use of insecticides and have to clear them for use in accordance with the pesticides safety precautions scheme. This scheme prevents the insecticide manufacturers marketing products containing any new chemical, or introducing a new use for an existing chemical or introducing a new formulation of a chemical without the specific government department stating recommendations for safe use. Edwards (1975) reviewed the limitations on the use of specific insecticides internationally, as follows. The use of D.D.T. in America has been controlled state by state for several years and was then banned in July 1969. This was a probable reaction to a Department of Health, Education and Welfare investigation into D.D.T. levels in man in April 1969, the alarming result was that the average American had D.D.T. levels in the body of 12 mg l^{-1} . In the United Kingdom the Advisory Committee on Pesticides and other toxic chemicals reported to the Department of Education and Science (Wilson report) in 1970 that there was insufficient evidence for a complete ban on organochlorines and that they should continue to be used freely except for certain restrictions on specific chemicals.

Internationally the uses of D.D.T. are generally restricted or banned altogether. Countries which have banned the use of D.D.T. include Bulgaria, West Germany, Greece, Hungary, Italy, Sweden, Switzerland and the United States, whilst countries allowing restricted use include Austria, Belgium, Canada, Denmark, France, Netherlands, Portugal, Poland, Norway, Spain, Turkey, United Kingdom and the U.S.S.R.

Other reasons for the banning of D.D.T. by many countries is the drastic increase in resistance shown by target species not specifically to D.D.T. but to a whole range of insecticides. This is by no means a recent problem. Quarterman and Schoof (1958) produced a comprehensive report listing confirmed and some unconfirmed cases of

insecticide resistance appearing in insects of public health importance, they listed 14 confirmed species showing resistance in the United States alone whilst in Europe the figure was only 5, obviously this must have had something to do with the early widespread use of organochlorines in the United States and since the mid 1950's organophosphates have had to be used in that country. Quarterman and Schoof (1958) identified the problem of resistance as of major importance becoming more critical annually in different ways. Firstly there may be an increase in the number of resistant species, secondly the area in which a given species is resistant may increase and thirdly the types of insecticide to which a given species is resistant may increase. The above authors showed that from 1946-1956 the number of insect species that were confirmed physiologically resistant to insecticides increased from 2 to 25. Quarterman and Schoof also recognised 2 forms of resistance, firstly a physiological type where the toxicant that has entered the body is tolerated physiologically, this is the type most commonly encountered, and secondly a behavioral type where the target species avoids contact with the toxicant by means of altered behavioral responses, this has been shown in the field and in laboratory tests by Trapido (1952 and 1954). According to Quarterman and Schoof (1958) large scale resistance built up in 1948 when M.domestica became resistant to D.D.T. in most of the United States, since 1952 it became resistant to organochlorines in general and since 1956 organophosphates were brought into general use and apart from isolated incidents no general resistance to these compounds has been reported. Isolated reports where resistance has built up have been quoted by Gjullin and Isaak (1957) who demonstrated that the larvae and adults of the mosquito Culex tarsalis^{Coq.} were resistant to malathion but susceptible to parathion.

Problems have been reported with Chrysomia putoria (a species found breeding in dung) in South Africa by Bervoets et al. (1957 unpublished)*. It was found that after initial success with B.H.C. a resistance built up. Then the organophosphate diazinon was used with success until resistance built up to this in the second year of

* Source - Quarterman and Schoof (1958)

treatment. The nature of this resistance was investigated and it was found that 363 mg l^{-1} was needed to kill larvae from a treated zone compared to 65 mg l^{-1} needed to kill larvae from an untreated zone. In addition the more serious implications of cross resistance were shown. The treated and untreated larvae were tested against a range of insecticides and it was found that specific doses of chlorothion, malathion and parathion were needed to give a 100% kill of untreated larvae, however the same doses gave a 42.5, 1.9 and 9.0% survival rate over 16 hours when tested against the diazinon resistant strain.

In Europe a similar pattern seems to be appearing. The first reported resistances of M.domestica to D.D.T. were from Missiroli (1947) and Saccā (1947), later Wichmand (1953) reported the failure of D.D.T. and related compounds for fly control in Denmark. Quarterman and Schoof (1958) reported similar problems with diazinon in Europe to those reported in Africa.

Resistance in the sewage works venue is not so well documented. Bruce and Decker (1950) working in the United States reported that P.alternata was found to be resistant to organochlorine insecticides similarly Watson and Fishburn (1964) at Keighly found that after treating S.fenestralis for 10 years with B.H.C. it was apparent that the control was failing and they assumed that S.fenestralis was becoming immune to the treatment. They then tried the organophosphate malathion with success against S.fenestralis but they then reported sporadic emergences of chironomids. However it is not sure whether resistance was operating in this case as Jenkins (in discussion of Watson and Fishburn 1964) pointed out that successful control of S.fenestralis had been achieved at Minworth Birmingham using B.H.C. for over 10 years, so other factors may be operating here. One of these factors may have been an increase in film levels which has been known to cause a failure in insecticide treatment (Tomlinson and Muirden 1948 and Tomlinson et al. 1949).

An interesting sideline is the resistance shown by oligochaetes to insecticide treatment. Some of these are important grazers in filters and it was stated by Tomlinson and Jenkins (1947) that the enchytraeid worm L.lineatus seemed to be unaffected by the concentrations of D.D.T. and gammexane needed to control S.fenestralis and P.alternata. A similar resistance in lumbricid worms was explained by Laverack (1963) and it is not unreasonable to assume that the mechanisms may be similar in enchytraeids. It was suggested that the tolerance of low concentrations of insecticide was due to a large amount of acetylcholinesterase found in the body wall, thus an unusually large amount of the inhibiting insecticide would be needed to tie up the extra acetylcholinesterase, thus the worms can survive insecticide treatment.

Obviously insecticide control measures are not ideal for long term control of flies in sewage filters and it is apparent that resistance is building up in other environments. Quarterman and Schoof (1958) stated that there was little evidence that organophosphates were losing their general effectiveness however the initial reports of resistance may have only been a forerunner of future extensive difficulties. Similarly Edwards (1975) stressed that other forms of control should be looked at in controlling pests generally including cultural and ecological methods. He stated that insecticides should only be used sporadically to obtain the maximum effect with a minimum dose, too often large doses of insecticides were used just as an insurance. Finally he stated that biological control must always be preferable.

Many forms of operational control have been noticed to have an effect on filter fauna. The first one to be discussed is the hydraulic loading. As early as 1943 Edmonson and Goodrich working on a high rate filter noticed that Psychoda larvae were present in the filter but the high rate of application of the sewage prevented them from emerging, however they found that if the distributor was stopped the flies soon emerged. The conclusion from this work was that the high rate of application did not stop Psychoda breeding but did prevent them from rising to nuisance

proportions. This was backed up by Wheatley and Williams (1976) who studied high rate filters on loadings up to $2.4 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$ and found Psychoda along with enchytraeids to be the dominant grazers some 2 years after commissioning. Similar results to this were expressed by Bruce and Merckens (1970) who found P.alternata present in high rate filters on loadings up to $6 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$ but never observed them to emerge. The explanation for these observations is probably that the near continuous application of sewage encountered on these loadings prevents the adults from gaining access to the filter surface and emerging. It is known that P.alternata can mate in the confines of the filter so mating probably takes place in some sheltered crevice of the media. An intensive study of the effects of hydraulic loading on both filter fauna and film throughout the depth of filters was carried out by Tomlinson and Hall (1950). They studied a whole range of loadings from $0.5 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$ to $5.7 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$ and some of the results can be seen in a graphical form in Learner (1975). The results show increases in the springtail H.viatica, Psychoda and L.lineatus up to respective peaks after which the levels fell rapidly. The explanation for this could be that initially the film which was the food for the grazers was limiting and as the loading increased the amount of film increased (Tomlinson and Hall 1950) and this allowed an increase in the number of grazers. In a similar manner an increase in Psychoda levels corresponding to an increase in film was shown by Tomlinson and Stride (1945). The reason for the numbers of grazers falling after certain hydraulic loadings is most likely due to the increased scouring action of the sewage limiting film levels (Holtje 1943 and Bridge Cooke 1959) which in turn caused a decrease in grazers. This was borne out by Tomlinson and Hall (1950) whose results show an increase in the mean numbers of grazers throughout the depth of filters as the hydraulic load increased but after a certain level the numbers were found to decrease.

Another factor found to affect film levels and therefore the fly output from filters was the organic loading or the strength of the sewage quite apart from the physical volume applied. One of the first authors to mention the effects of this was Lloyd

(1945) who commented on the differences in fauna in 2 works receiving different quality sewages. He found that at Leeds where the sewage was well balanced and of medium strength a full range of flies was found whereas at Huddersfield where the sewage was of a strong industrial nature the only flies to be found were 3 species of Psychoda and their predator Spathiophora hydromyzina Fall.

An intensive study into the effects of organic loading on film and fauna was carried out by Tomlinson and Stride (1945). As expected they found a direct relationship between the amount of film and the organic loading however some interesting results were obtained regarding the relative abundance of different fly species at different loadings. They found that the abundance of Psychoda increased with increased loading and related this to the increase in film and therefore food, however a drop in the numbers was noted on the highest loadings of $0.336 \text{ kg BOD m}^{-2} \text{ day}^{-1}$ ($3000 \text{ lb BOD acre}^{-1} \text{ day}^{-1}$), it was not stated whether ponding conditions occurred in the filters but it was noticed by Otter (1959) that on severely overloaded filters Psychoda disappeared altogether. It was also found that generally there was an inverse relationship between chironomids and organic loading. O.minimus did not occur in any filters above a loading of $0.224 \text{ mg BOD m}^{-2} \text{ day}^{-1}$ ($2000 \text{ lb BOD acre}^{-1} \text{ day}^{-1}$). Metriocnemus was found to have similar habits and it was concluded that these species preferred clean lightly loaded filters. No specific relationship between fly trapping and organic loading was noticed in the case of S.fenestralis or O.perennis, both seemed to appear in appreciable numbers from both lightly and heavily loaded filters. However work done by Hawkes (1951b) does in fact indicate some relationship between S.fenestralis and loading. It is important at this stage to distinguish which stage of the insect was being used in the studies to determine any relationship. Tomlinson and Stride (1945) based observations on the incidence of S.fenestralis adults however Hawkes (1951b) compared numbers of larvae pupae and adults at 3 loadings and found that a certain correlation in the larvae was repeated in the pupae but not so in the adults, in fact the highest loading of $0.325 \text{ kg BOD m}^{-2} \text{ day}^{-1}$

(2900 lb BOD acre⁻¹ day⁻¹) which produced the maximum number of larvae and pupae produced the minimum number of adults. It should be noted here that work done by Hawkes (1951a) showed that the method of trapping used by Tomlinson and Stride (1945) which was tray trapping on the surface of the filter actually attracted S.fenestralis from underneath the tray and from other areas of the filter therefore this gave inaccurate information as to the numbers of S.fenestralis that emerged from the filter in normal conditions. However even though Hawkes' (1951b) results show a direct correlation between S.fenestralis numbers and increasing organic loading the author suggested that no such simple loading relationship occurred and that it was possible that in filters having a mixed fauna interspecific competition had a more important effect on S.fenestralis abundance.

Terry (1956) studied the effects of loading in relation to media size on specific fauna, the concept of media size will be discussed later but his general conclusions specifically concerning loadings were similar to Tomlinson and Stride's (1945) views that Metriocnemus prefer clean lightly loaded filters and that P.alternata flourished in wet badly aerated conditions as would appear on a heavily loaded filter. Similar relations between Psychoda and heavily loaded filters have been described by Reidmuller (1957) in Germany and Otter (1966) in the Netherlands. Otter (1966) described a method of controlling Psychoda by means of recirculation which had the effect of slightly increasing organic loading but drastically increasing the hydraulic loading which had a scouring effect on the film described by Holtje (1943) and Bridge-Cooke (1959), thus the bulk of the film which was Psychoda's food supply was washed out of the filter.

The general relationship between different species and organic loadings was explained by Learner (1975). He stated that the main reason Psychoda was dominant in high film conditions whereas chironomids were hardly found at all was that Psychoda larvae possess posterior respiratory siphons by means of which they can take in

atmospheric oxygen whilst completely enveloped in film. However chironomids have a closed tracheal system with no such adaptation so have to rely on the simple diffusion of oxygen through the body surface so this explains their restriction to generally clean low loaded filters.

Therefore it is obvious that film levels have a great bearing on the type of fauna present in filters. These film levels per unit volume of filter media were shown to depend on the organic loading and the media size (Terry 1956). He said that the two factors operate in combination giving a specific film level which determines the presence or not of certain species. Terry showed this interaction by proving that P.severini and O.minimus seemed to be found on small media fed with weak sewage and large media fed with strong sewage (both giving rise to low film levels per unit volume), however they were not found on small media fed with strong sewage (which gave rise to high film levels) in which case P.alternata was found in abundance. In this case no relationship was found between M.hygroperiticus and media size except that they generally preferred low film levels. Terry (1956) also found that S.fenestralis did not develop successfully on small grade media, a similar opinion was held by Watson and Fishburn (1964) however all workers do not agree on this as Hawkes and Jenkins (1955) stated that along with film levels and numbers of Psychoda, numbers of S.fenestralis larvae increased with decreasing particle size. It should be noted that the smallest media referred to in the case of Hawkes and Jenkins (1955) was 3.18 cm (1.25 in) cracked granite whereas in the case of Terry's (1956) work the smallest media was 1.9 cm (0.75 in) hard coal, thus there may be a critical size of media (assuming identical film levels per unit volume) below which S.fenestralis does not survive successfully.

Concerning the effects of media on fly emergence one of the earliest studies was carried out by Dyson and Lloyd (1933) who stated that a medium consisting of smooth pebbles was preferable to prevent excessive fly emergence. Other workers have

noticed that the addition of a topping of small media can have a significant effect on fly emergence (Wilson and Johnson quoted in Fair 1934, Lloyd 1945, and Tomlinson and Stride 1945). Wilson and Johnson supposed that a 150-300 mm (6-12 in) depth of small rounded pebbles 25mm (1 in) diameter would inhibit Psychoda emergence, however no mention here is made of any problems that may have been encountered with excessive film build up. Lloyd (1945) from a comprehensive study of filters at various works quoted specific conditions which seem to favour either Metriocnemus or Psychoda. He said that the most favourable conditions for Metriocnemus was a topping media of 51-76mm (2-3 in) in which case there was a high density of L.lineatus but very few Psychoda whereas the most favourable conditions for Psychoda was with a topping media of 25-51mm (1-2 in) grade in which case Metriocnemus was much sparser and L.lineatus and Psychoda were far denser. In both of these cases the operating conditions and loadings were similar. A more detailed investigation into the effects of small media topping was carried out by Tomlinson and Stride (1945). They excavated a portion of the filter surface and filled areas to a 76mm (3 in) and 230mm (9 in) depth with 19mm (0.75 in) diameter media. They found a large reduction in the numbers of S.fenestralis emerging in both cases however numbers of Psychoda were not greatly affected, also they found that generally less flies emerged from the 230mm depth than the 76mm depth of media. The authors in this case did appreciate that problems may arise from excessive film build up. They hinted that the fine medium is more liable than coarse medium to become choked with solids most especially during the winter and that if this form of control was used this would have to be taken into account. However they noticed that numbers of the springtail H.viatica were higher on the small media filters than the coarse media and this insect has been proved to be an active scourer (Parkinson and Bell 1919) so film build up may not have been so drastic as expected. The subject of small media topping was also brought up by Watson (in discussion of Watson and Fishburn 1964) who suggested that small media "topping up" of filters may have had to be turned to as an alternative form of control of S.fenestralis if insecticide treatments failed.

It must be appreciated that with small media the size of the interstices between the individual media particles is much reduced and that it does not take much film build up to cause them to block. Both Hawkes and Jenkins (1951) and Learner (1975) suggested that the reduction in fly emergence from a small media area may be the physical effect of a barrier caused by the blocking of these small interstices, however Learner (1975) suggested that the reduction may be partly physical due to the above reasons and partly physiological due to the small media and film reducing ventilation and thus reducing the amount of oxygen available to the larvae and so limiting their development.

Other studies on the effects of small media topping have been made by Otter (1966) in the Netherlands. He found that a 60-70% reduction in Psychoda numbers could be achieved using a 15 cm thick layer of smooth surface media of 2 grades, 1-4 cm and 10-18 cm grade media. Otter also suggested that his experience did not support the general assumption that small media became quickly clogged with solids.

It has already been mentioned that S.fenestralis was easily controlled by 19 mm media (Tomlinson and Stride 1945), it is easy to imagine how difficult it would be for a fly the size of S.fenestralis to emerge in such small interstices, however Hawkes and Jenkins (1951) studying even smaller media found that Psychoda which are very small compared to S.fenestralis can be controlled using media less than 16 mm (0.625 in) diameter however no mention here was made of any film accumulation problems as it has been shown that excessive film accumulation can adversely affect filter efficiency (Hawkes 1957). It was also noticed that Psychoda was more common on small media in the summer than in the winter, the reason for this was thought to be due to competition effects which will be discussed later.

Hawkes and Jenkins (1955 and 1958) did extensive studies into the effects of 4 grades of media from 32- 64 mm (1.25 - 2.5 in) on purification, film accumulation and fauna

on filters on single filtration (1955) and alternating double filtration (1958). It was found that film accumulation and mean numbers of Psychoda and S.fenestralis increased with decreasing particle size and the largest medium supported a community with Lumbricillus as the dominant grazer. Also Hawkes and Jenkins (1955) found that the effluent quality was related to the size of the medium as expected the area with the smallest media produced the best effluent as the smallest media would have the greatest surface area to volume ratio, however this held true only if ventilation was adequate. During winter the small medium was subject to surface ponding however the standard of the effluent produced did not fall in this case, it was suggested that the lower depths of the filter received sufficient aeration to retain efficiency. The general conclusion given was that the optimum size of media for any circumstance would be the smallest grade which could be used without impeding aeration by excessive solids accumulation in the interstices. The study carried out by Hawkes and Jenkins (1958) into the same 4 grades of medium working on alternating double filtration pointed to generally the same conclusions except that it was stressed that it was not just the physical characteristics of the media alone that determined efficiency, it depended on a whole range of operational factors such as the system of filtration and the application method, so it was not possible to suggest an ideal media for one and all filters. One important result found from this study was that although small media on single filtration gave seasonal problems with film accumulation (Hawkes and Jenkins 1955), these problems were not so marked when operating on alternating double filtration.

The specific efficiencies of different media have been tested by many workers including Balakrishnan (1969) and Novelli (1975) who looked at this along with other operational variables. Much work has been done in recent years on the development of plastic media in both random and modular form (Bruce and Merkens 1970, Duddles, Richardson and Barth 1974, and Wheatley and Williams 1976). The main reason for its development has been its use in high rate filters where film accumulation would be

excessively high using mineral media, whereas the plastic media offers a very high voidage allowing film to build up yet still allowing adequate ventilation. The subject of plastic media is not going to be reviewed here as there have not been many reports of fly nuisance specific to plastic media.

The effect of the application method of the sewage onto filters has a profound effect on the types of grazers and distribution of the film. A few studies have been made on this subject and by far the most comprehensive work was that of Hawkes (1959). This involved studying the horizontal distribution of film and fauna under 6 types of applicators ranging from fish tails and splash plates to straight jets spaced at different intervals along the distributor arm. Hawkes stated that the efficient running of a filter had to be a compromise between using the maximum area possible on the surface of the filter (by using splash plates giving a thoroughly wetted surface) and the maintenance of a balanced grazing fauna consisting of a variety of species which are in competition with each other (by allowing the sewage to fall onto the bed in discrete jets giving "wet" and "dry" areas). The fish tails and splash plates gave the most even distribution of film however with the fish tails the scouring action was much reduced giving high film accumulations which were later shown to affect efficiency detrimentally (Hawkes 1957). The splash plates and straight through jets (150 mm spacing) both gave a fairly good distribution, the former was slightly better however it was considered that since 150 mm spaced jets gave a more balanced community it was preferable. H.viatica was found in the "dry" areas between the jets and S.fenestralis was found in the "wet" areas under the jets. The general conclusion was that it was preferable to have conditions where mixed populations are present as is the case of the 150 mm spaced jets rather than have a fauna dominated by one species.

A similar study involving a different species was described by Otter (1966) who found that in order to control Psychoda it was necessary to give complete "sprinkling" of

the sewage on to the filter so that no "dry" areas appeared. This was achieved by boring extra holes in the distributor arms allowing the whole of the bed to be wetted. It was the author's view that Psychoda were hampered in their effort to reach the surface and emerge. The fact that flies need relatively dry or sheltered areas to emerge was stated by Hawkes (1951b) who found that S.fenestralis larvae migrated from a subjet to an interjet position on filters just prior to pupating, the advantages of emerging from a "dry" area are obvious.

The effects of the dosing frequency of the sewage onto filters has been very well documented both from fly control and the purification efficiency points of view. One of the earliest investigations was carried out by Lumb and Barnes (1948) on circular filters at Halifax. It was found that the most efficient system was a frequency of 4-9 minutes using a 4 armed distributor however no mention was made of any effects on the fauna. Tomlinson and Hall (1951 and 1955) did detailed studies into the effects of dosing frequency at Minworth, Birmingham (1951) and Minworth and Finham, Coventry (1955). They agreed with Lumb and Barnes (1948) in finding a 4-8 minute period (using a 4 armed distributor) the most efficient and on further investigation they found the 8 minute period more efficient than the 4 minute period. Film accumulation is affected by the application method described previously and in addition it is affected by the dosing frequency. Tomlinson and Hall (1955) showed that very high dosing frequencies in the order of 0.25 -2 mins. gave high film levels causing ponding. Problems of film accumulation and its control by low frequency dosing have been quoted by Hawkes (1955a, b, and 1957) and Hawkes and Shephard (1972). An important result of lowering the frequency of dosing is the increase in instantaneous dose (the force of the sewage hitting the bed in unit time), the effect of this was described by Hawkes (1955a and 1961b). These high instantaneous dose rates found on low frequency dosed filters were shown by Hawkes (1961b) to alter the community of filters from a fly dominated population to a worm dominated one. The explanation given for this was that the high instantaneous dose rates produced a

scouring action which the fly larvae (in this case P.alternata and S.fenestralis) could not withstand, however the prehensile enchytraeid L.lineatus can withstand these flows and survived in the absence of other species. The low film levels on the surface found with low frequency dosed filters according to Hawkes (1961b) were not so much a result of scouring action, which was the view of American workers including Holtje (1943) and Bridge-Cooke (1959) but more a result of the long periods between doses giving rise to a negative growth of the film, this was fully described by Hawkes (1961b). The consequences of high instantaneous dose rates have been appreciated by many workers. Both Lumb and Barnes (1948) and Peach (1957) found that a high instantaneous rate significantly reduced the frequency of ponding in filters. Staynes (quoted in the discussion of Goldthorpe 1948) stated that filters with high instantaneous dose rates were very efficient and in these filters solids were washed down giving no problems with film accumulation on the surface. Barritt (1939) gave the impression that high rates of flow result in the monopoly of filters by one or two species which agree with results given by Hawkes (1961b) concerning worm domination of low frequency dosed filters.

A specific control of Psychoda and S.fenestralis was quoted by Hawkes (1955b) using a dosing frequency as low as 42-55 minutes on a 4 armed distributor (a true frequency of 10.5 – 13.75mm). In cases such as these the benefits of fly control by this method have to be weighed against any loss in purification efficiency. Some authors have expressed that purification efficiency may drop if dosing frequency is reduced markedly. Work done by Water Pollution Research (1956) has shown that this can occur, but Stanbridge (1956) and Water Pollution Research (1956) have both stated that the mean retention time and therefore the purification efficiency can be increased by decreasing dosing frequency within reason. The theories of purification in low frequency dosed filters has been comprehensively described in Hawkes (1961b).

Recent work on dosing frequency has been quoted by Learner (1975) who related the

effects of dosing frequency in the light of different film levels. He suggested that a low dosing frequency caused a low film accumulation allowing the insect fauna to have an increased diversity which is to be preferred if a well balanced fauna is desired. Also Cook and Crame (1976) in the United States studied different dosing programmes including continuous dosing and come to the conclusions that continuous dosing gave the best effluent which does not agree with previous work described, however this was the result after comparing continuous dosing with dosing periods of 7.5, 5, and 2.5 minutes in 10 minute programmes. The authors did state that periodic dosing was an aid in preventing solids accumulation and it was also found that a 5 minute dose and 5 minute rest programme was the most efficient in controlling flies but no explanations were given for this. It has also been shown that a low dosing frequency, in addition to its observable effects on surface film and fauna has a profound effect on the vertical distribution of these within the filter (Hawkes and Shephard 1972). It was found that generally a low dosing frequency caused a peak in the solids in the low levels of filters which would have been found at the surface on a filter with a higher frequency of dosing. It was suggested that this peak was caused by the scouring action of the sewage washing down humus material thereby causing an accumulation at a lower level.

Various operational methods have been used in previous years to control film levels and indirectly fly populations, these have included the use of double filtration, recirculation and alternating double filtration. The main effect of these systems is to decrease film levels and the theories behind these practices were explained by Hawkes (1961b).

The main reason for using double filtration systems was to improve the performance of existing plant and these systems initially were not introduced to reduce fly output. Reynoldson (1941) controlled P.alternata by means of a double filtration system. He found that the weak feed to the secondary filters caused near starvation conditions

therefore reducing the fauna due to lack of food, he also suggested that P.alternata populations on the primary filter may have been controlled by carefully balancing the flow to keep the film levels down. Later at the same works Reynoldson (1942) found P.alternata output to be less from the primary than the secondary filter due to excessive build up of film causing ponding problems. The reason for this was probably the heavy load received during wartime conditions.

The relative efficiencies of different operational processes have been reviewed by Gast (1974) and Peach (1957). Peach (1957) found the double filtration system to be most efficient, however a recent study into a double filtration system by Bruce, Merkens and Haynes (1975) gave opposing results, the authors agreed that this was against normal views and in their opinion the poor results may have been due to high temperature differentials which are common in small pilot scale plants as it was observed that there were large seasonal fluctuations in nitrification.

Concerning recirculation the only report of control of flies by this method was by Otter (1966) who reported a control of Psychoda achieved by washing out its food supply (film).

Work on alternating double filtration systems has been well documented with special reference to fly nuisance. As early as 1931 O'Shaughnessy conceived the theory of alternating double filtration in order to control excessive film accumulation. The process was first studied by Tomlinson (1941) who tested different periods of alternation. He found that alternating double filtration, apart from controlling film in the winter gave a reduced fly output. Similarly Tomlinson (1943) found that S.fenestralis could be controlled by alternating double filtration at high rates of application with short periods of alternation. It was also stated by Tomlinson and Stride (1945) that fewer flies generally emerged from alternating double filtration than from single filtration in proportion to the amount of sewage treated. He added

that the advantages of alternating double filtration was that it gave a more even distribution of film on the surface thus allowing no dry areas to persist. The reason for this was probably that higher hydraulic loads could be handled with alternating double filtration than on other systems giving rise to better distribution. Hawkes and Jenkins (1958) repeated a study on 4 grades of medium operating on alternating double filtration in a similar fashion to the original study using the same media on single filtration (Hawkes and Jenkins 1955). Generally it was found that film levels were more stable throughout the year on alternating double filtration however some problems were found with the smallest medium (32 mm) regarding film accumulation but these may have been due to the severe winter conditions of 1956/57.

Another interesting method of fly control which has been utilised is the physical closing over of filters so preventing the flies escaping. This seemed to be a quite common practice in South Africa at one time. Murray (1939) reported that many works in South Africa had these modifications fitted however in the author's opinion the enclosing led to high uniform temperatures which in turn led to an increase in fly breeding, and the flies managed to escape via the false floor and the air ports of these filters. However Lundie (1940 quoted in discussion of Murray 1939) expressed that success was achieved on 22.9m (75 ft) diameter enclosed filters and that fly escape was prevented by screening the air ports, however in neither case were any results given regarding purification efficiency which may have suffered as a result of impeded ventilation. Recent work has been carried out by Jennings et al. (1976) who described a model for the biological removal of organic matter in a submerged filter.

Many of the operational methods described above achieve their required objectives by means of altering the ecosystem operating in filters. This ecosystem is very complex and has been shown by many workers to involve both interspecific and intraspecific competition. Interspecific competition can be identified as follows. If the conditions of a filter are changed the reason a certain species is excluded or reduced is probably

due to the new conditions favouring a rival competing species which then has the effect of curtailing the other species. Intraspecific competition can be seen to be operating if after filter conditions are changed the numbers of a certain species fall and are not replaced by any other species it can be assumed that certain stresses are being applied to that species which then is regulating its own population level. This type of situation is often found when food is limiting.

Much work has been carried out on the effects of competition and as early as 1914 Johnson suggested that H.viatica was a food competitor with Psychoda as they were never found in large numbers together. A similar view was held by Fair (1934). Also Hawkes (1957) noticed that after removing S.fenestralis by chemical treatment H.viatica populations quickly took their place which may be more evidence of food competition. Competition between insect larvae and worms has been shown by Lloyd (1943a and 1945) and Reynoldson (1948). In the case of Lloyd (1943a) this was suggested when it was found that M.longitarsus and P.severini numbers seemed to be lower than usual after a warm winter. The reason given for this was that the warm winter allowed L.lineatus populations to increase causing competition for food with the insects. It was Lloyd's view that the reason why certain species were found at specific times was due to a combination of the influence of weather directly and competition from other species regulated by the weather. Lloyd (1943a) also found that in filters the strongly carnivorous chironomids competed with each other, the larger M.longitarsus would reduce O.minimus numbers at the surface levels however Psychoda avoided this by inhabiting a lower level of the filter (Fair 1934). The carnivorous habits of Metriocnemus in reducing Psychoda numbers were described by Golightly (1940) and Lloyd (1945). Similarly the smaller chironomid O.minimus was found to be carnivorous and reduced numbers of P.alternata (Lloyd 1941 and 1945). The effect of film levels on competition in relation to food supply was discussed by Lloyd (1945), Lloyd et al. (1940) and Hawkes and Jenkins (1951). It was stressed that chironomids would only attack Psychoda when food was scarce (Lloyd et al. 1940).

Similarly Hawkes and Jenkins (1951) found S.fenestralis and Psychoda to be food competitors by showing that reducing S.fenestralis levels had the effect of increasing Psychoda levels, also competition for food was shown to operate between P.alternata and P.severini (Lloyd 1945).

Other effects of competition have been described such as the effects of competition on the seasonal abundance of flies. Lloyd (1944) and Hawkes and Jenkins (1951) explained the early abundance of P.alternata in small media filters in the light of the exclusion of competitors. Intraspecific competition was shown to have an effect on the seasonal incidence of P.alternata by Lloyd (1943b). He found that P.alternata peaks alternate from small to large and explained that this was a result of the initial generation depleting film levels so that the subsequent generation were subject to food stresses and did not develop in large numbers, the numbers increased in the next generation and the pattern repeated itself. Intraspecific competition was also shown by Golightly (1940) who found that the stresses of temperature and crowding both affected P.alternata and P.severini populations in relation^{to} their individual physical size. The sizes of the flies were reduced in both cases when compared to flies not subject to these stresses. Lloyd et al. (1940) explained some of these competition effects by carrying out a series of predacity tests where different life cycle stages of insects and worms were exposed to certain insect larvae in isolation and their reactions were observed, some useful results were obtained from this. Generally Lloyd (1943a and 1945) summed up competition as a useful force because it resulted in a balanced fauna and prevented any one species rising to nuisance proportions, he also stated that this balanced fauna could be attributed to operating conditions in the works. One interesting exception where the presence of one species caused an increase in another was quoted by Williams and Taylor (1968) who stated that enchytraeid worm populations increased more rapidly in the presence of P.alternata. This was explained by the conditioning of the film by the fly larvae which had a shorter life cycle.

Another possible method of control not yet described is specific biological control. This type of control of insects was described by Reay (1969) to be the type of control involving predators, parasites and pathogens including bacteria, viruses, fungi and protozoa. As far as is known none of these methods has been used in the sewage works venue however they are in use in other areas of insect problems. For example Bacillus thuringiensis^{Berliner} has been used against Lepidopteran larvae and B. popilliae^{Dutky} against certain grubs (Thomson 1973). Also Remaudiere et al. 1973 used this form of control against aphids in orchards. Obviously biological methods are much preferable to chemical ones as has been stated by Edwards (1975), Reay (1969) and Taylor (1955), however shortcomings in specific biological methods were outlined in Reay (1969) in being very slow to operate. Taylor (1955) suggested that the restricted success of specific biological methods of insect control were due to such examples as introducing predators into incompletely understood conditions and not knowing fully the requirements of the predators, it was the author's view that situations like this led to chemical control being more effective.

Both Edwards (1975) and Reay (1969) suggested that the future means of insect control should be a combination of the advantages of chemical, biological and ecological controls: an integrated control using insecticides only sporadically to get the maximum effect from the minimum dose. It was stated by Reay (1969) that in order to use this type of control system much investigation had to be carried out into the habits of the target species. For example he stated that the timing of a treatment could be arranged so that other non-target species were not around, or insecticides could be coated in a protective covering that only the target species digestive system could remove, thus it would not act against any other species or as a contact insecticide. It is not unreasonable to assume that this type of control could not be extended to the filter bed habitat as certain advantages are present here in that the fauna is very restricted in diversity and much is known about their habits.

Methods

Full scale filter experiments

I Description of plant and its method of operation

The filter beds at Tamworth Water Reclamation Works are rectangular and are fed by cable hauled distributors which siphon settled sewage from central troughs each feeding two filters. They consist of eight filters, each 137.2 x 22.9 m with a depth of 1.8 m split into two blocks, one block consists of filters 1A, 1C, 2A and 2C and the other consists of filters 3A, 3C, 4A and 4C (see Fig. 3). The filters were designed to be used as a single filtration system however during the project not all of the filters were operated in this way as the flow was not sufficient to allow this. The reason for this was that when the filters were designed extra capacity was allowed for any future town expansion.

Prior to the start of the project filters 3A, 3C, 4A and 4C were used on single filtration with a variable amount of recirculation, the other filters were not used, however in October 1975 when the project commenced the flow was switched to filters 1A, 1C, 2A and 2C and the other filters were left idle. In February 1976 and for the rest of the duration of the project a system of double filtration was used whereby filters 3A, 3C, 4A and 4C were used as the primary filters and filters 1A, 1C, 2A and 2C were used as the secondary filters. In this system of double filtration the primary and secondary filters did not receive equal hydraulic loads, the flow to the secondary filters was pumped and was a constant $9720 \text{ m}^3 \text{ day}^{-1}$ limited by the capacity of the pumps. This was independent of the flow to the primary filters assuming the flow to the primary filters was equal or in excess of this flow.

The loadings on these filters for the duration of the project can be seen in table 5.

Table 5. Loadings (organic and hydraulic) imposed on main filters during project duration

	Primary filters		Secondary filters	
	Organic Kg BOD $\text{m}^{-3} \text{ day}^{-1}$	Hydraulic $\text{m}^3 \text{ m}^{-3} \text{ day}^{-1}$	Organic Kg BOD $\text{m}^{-3} \text{ day}^{-1}$	Hydraulic $\text{m}^3 \text{ m}^{-3} \text{ day}^{-1}$
Oct. 1975	0-20	0-65	—	—
Nov. 1975	0-12	0-62	—	—
Dec. 1975	0-17	0-64	—	—
Jan. 1976	0-14	0-66	—	—
Feb. 1976	0-20	0-68	0-02	0-42
Mar. 1976	0-18	0-78	0-03	0-42
Apr. 1976	0-17	0-65	0-01	0-42
May 1976	0-14	0-66	—	0-42
Jun. 1976	0-13	0-69	—	0-42
Jul. 1976	0-15	0-68	—	0-42
Aug. 1976	0-13	0-59	—	0-42
Sept. 1976	0-16	0-73	—	0-42
Oct. 1976	0-16	0-91	—	0-42
Nov. 1976	0-12	0-69	0-02	0-42
Dec. 1976	0-14	0-77	—	—
Jan. 1977	0-18	1-09	—	—
Feb. 1977	0-15	1-28	—	—
Mar. 1977	0-14	0-82	—	—
Apr. 1977	0-11	0-66	—	—
May 1977	0-18	0-87	—	—
Jun. 1977	0-19	0-83	—	—
Jul. 1977	0-13	0-59	—	—
Aug. 1977	0-09	0-56	—	—

Table 5. Continued

	Primary filters		Secondary filters	
	Organic Kg BOD $\text{m}^{-3} \text{ day}^{-1}$	Hydraulic $\text{m}^3 \text{m}^{-3} \text{ day}^{-1}$	Organic Kg BOD $\text{m}^{-3} \text{ day}^{-1}$	Hydraulic $\text{m}^3 \text{m}^{-3} \text{ day}^{-1}$
Sept. 1977	0-12	0-52	—	—
Oct. 1977	0-15	0-70	—	—
Nov. 1977	0-12	0-62	—	—
Dec. 1977	0-15	0-76	—	—
Jan. 1978	0-18	0-92	—	—
Feb. 1978	0-16	0-96	—	—
Mar. 1978	0-17	0-79	—	—
Apr. 1978	0-15	0-67	—	—
May 1978	0-15	0-67	—	—
Jun. 1978	0-18	0-58	—	—
Jul. 1978	0-20	0-71	—	—

The works in general has a designed dry weather flow of $27276 \text{ m}^3 \text{ day}^{-1}$, the average daily flows for 1975, 1976 and 1977 were 15,545, 16,367 and $18,434 \text{ m}^3 \text{ day}^{-1}$ respectively, trade effluent constituted approximately 10% of the total flow (1975 figures).

The total filter capacity is $45,840 \text{ m}^3$ and they are filled with blast furnace slag (grade 55-60 mm) as a medium. Each filter bed is split into two as regards the dosing regime, one half being dosed as the distributor travels in one direction and the other half on the return travel. With this system a periodicity of dosing of 15 minutes is obtained. The distributor arms contain straight through jet type nozzles of diameter 25 mm set at a spacing of 155 mm. Each distributor machine contains two arms, one extends the whole width of the filter bed but discharges only on to one half of the filter and the other arm extends only half way across the filter bed and doses the other half of the filter. Changeover between the arms is effected by a system of flaps connected to a swinging boom which is moved by lifting devices set on the trough wall at both ends of the filter. Thus at the end of the machine's travel in either direction flow is switched from one arm to the other.

For the purposes of experimental work certain operational modifications were applied to the filtration system. These modifications involved altering the organic and hydraulic loading, the dosing frequency, the application method and the surface medium size on small portions of the filters. The filters concerned here were one from the primary block (4A) and one from the secondary block (2A). The modifications were as follows:-

(a) Loading

This modification involved redirecting the flow from four nozzles to an area of the filter served by four immediately adjacent nozzles via a system of tubes and a cradle. The four original nozzles were extended by means of rigid polythene pipes to allow

the sewage to fall in the same position as that from the extended nozzles. The arrangement can be seen in Plate 1. Thus this modification had the effect of doubling both the organic and hydraulic loading without the use of pumps on a strip of filter some 620 mm wide for the whole of the filters length. Media sampling and fly trapping was carried out from the centre of this double loaded strip thus keeping as far away from the peripheries as possible to reduce the chance of interference.

(b) Dosing frequency

In a similar manner to above this modification involved redirecting four nozzles by means of tubes and a cradle, however in this case the nozzles chosen for redirection were those on the end of the series on the long arm of the distributor in the centre. There were redirected to impinge on an area of the filter fed normally by the other arm (see Plate 2). Thus a strip of filter some 620 mm wide was fed with sewage both on the outward trip by one set of nozzles and then on the return trip by the set of redirected nozzles. Therefore at a point at the midway of the machines travel the dosing frequency was increased to 7.5 minutes from the original 15 minutes. Sampling and fly trapping were carried out from the centre of the strip as before as close to this midway point as possible as it must be appreciated that true dosing frequency would deviate either side of that point until at the ends of the filter it would be approximately 15 minutes. In order to keep the loadings similar to the rest of the filter all of the eight nozzles utilised in this modification were fitted with smaller apertures.

(c) Application method

The method of application of sewage on to the filter was altered by attaching an aluminium splash plate (see Fig. 2) on to the distributor arm. This had the effect of spreading the sewage flow from the original discrete jets into a continuous sheet thus wetting the whole of the filter area under the apparatus (see Plate 3) leaving no "dry" areas between the jets as are found in the normal situation. The apparatus affected

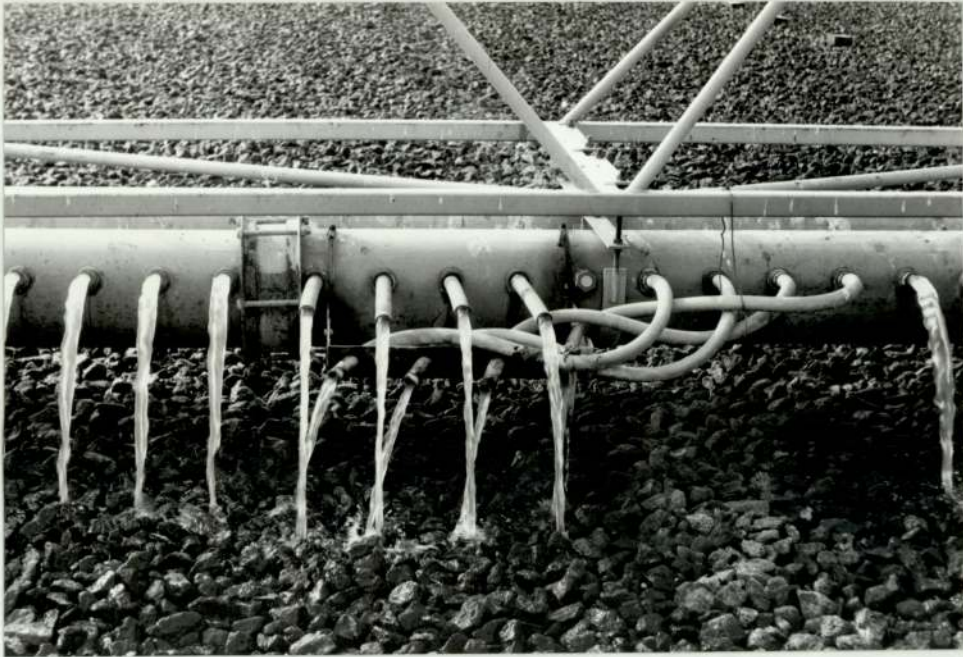


Plate 1. Double loading modification, nozzle arrangement

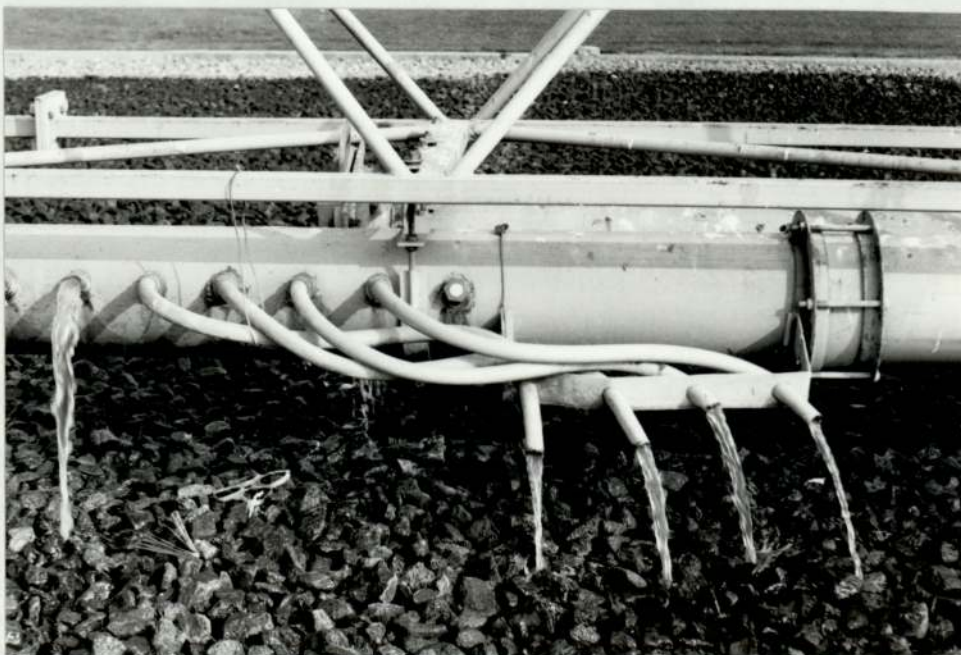
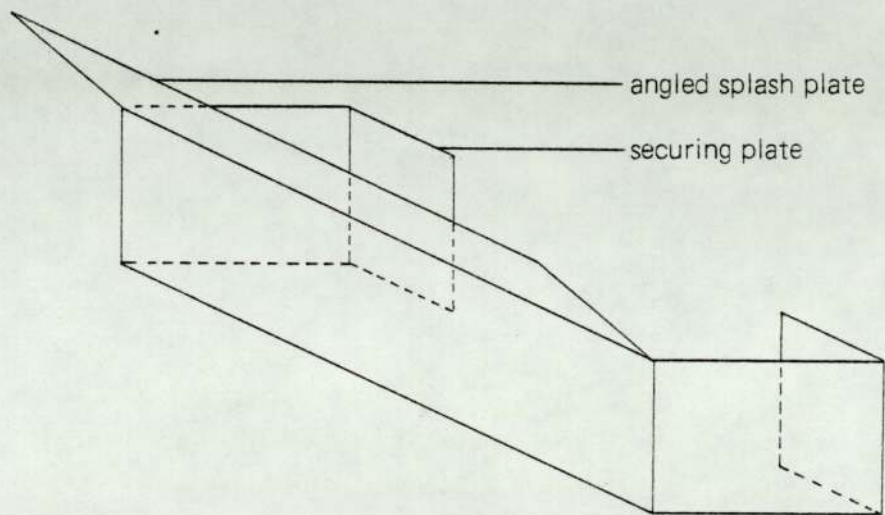


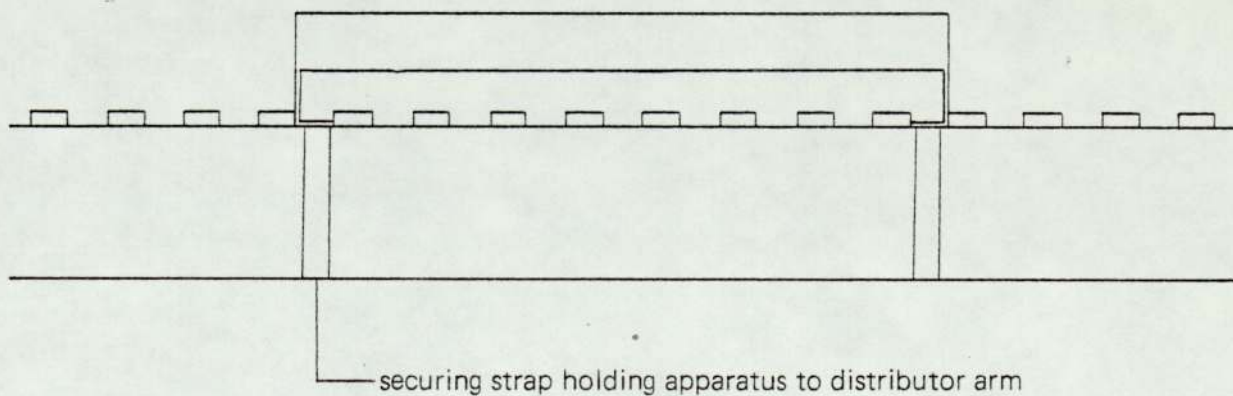
Plate 2. Dosing frequency modification, nozzle arrangement

Fig.2. Splash apparatus

(a) Exploded view of apparatus



(b) Top view of apparatus in position on distributor arm



the flow from eight nozzles thus producing a strip of filter 1.24 m wide running the whole of the filters length—subjected to this modification. Mediumsampling and fly trapping was carried out from various positions within this strip.

In August 1977 a further modification to this strip was introduced by the addition of small medium of diameter 19 mm to the surface of the filter on a 3 m long portion of the strip served by this modified jet. The amount of small medium used was just sufficient to fill the interstices between the larger pieces of medium the filter surface was by no means covered as the large medium was still exposed. Fly trapping was carried out from various positions in this strip.

(d) Media topping

An area of medium (1.5 x 1.5 m) was excavated from the surface of the filters to a depth of 230 mm and was replaced by an equal volume of 19 mm diameter gravel (see Plate 4). The positioning of this modification was such that half of this area fell under the portion of the filter already affected by the application method modification described previously. Thus half of this area was dosed by the normal jet application method whilst the remaining half was dosed through the splash plate jet. The position of the splash plate apparatus when passing over this area can be seen in Plate 5. Mediumsampling and fly trapping were carried out from both halves of the small medium topped area.

II Micrograzer studies

It should be noted that the investigation into micrograzers was not planned to be a specific part of the project, however since "clean" filters were commissioned at the commencement of the project it was considered that an investigation into the order of colonisation and succession of micrograzers on newly commissioned filters would be useful.

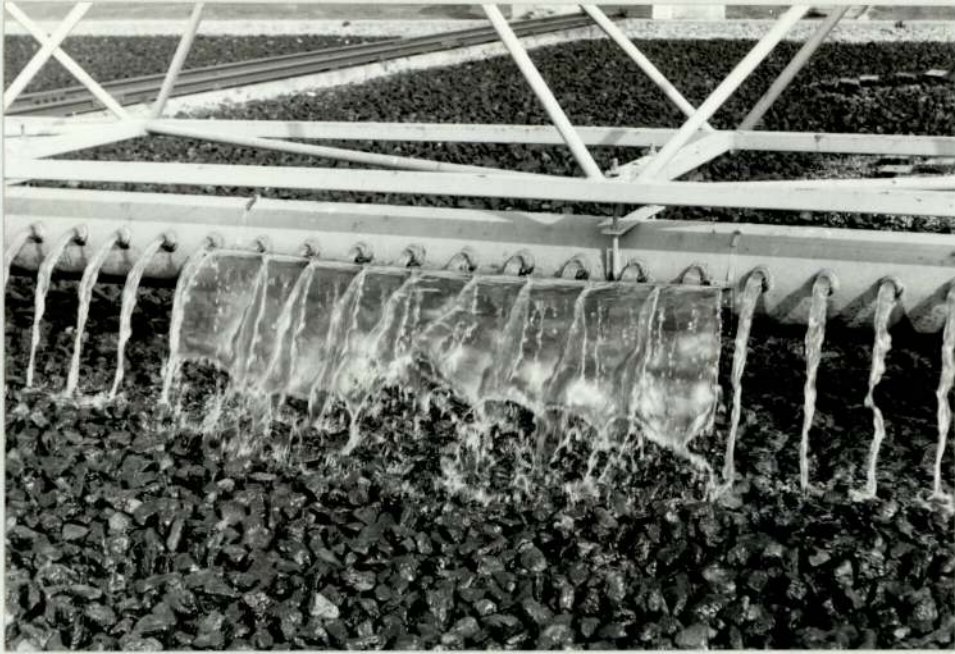


Plate 3. Splash plate modification



Plate 4. Small medium area

In studying micrograzers the following method was used.

- (a) A 200 x 200 x 200 mm basket representing the top 200 mm of the filter containing 0.008 m^3 of medium was removed from the filter and each piece of medium was individually scrubbed with a nail brush in a bucket containing 5 l of water.
- (b) The 5 l of medium washings was then agitated thoroughly to break up the film and 10 ml samples were taken in triplicate from different levels in the bucket.
- (c) These 10 ml samples were then placed in the refrigerator for one hour in order to slow the movement of the micrograzers to facilitate observation.
- (d) After cooling the 10 ml samples were agitated and 1 ml samples were taken from these and placed on graticule haemocytometer slides.
- (e) Once the samples were on the slides 10 random 1 μl squares were scored for micrograzers for each 1 ml sample, then the results were averaged and corrected up to micrograzers per litre.

The frequency of sampling was twice weekly for seven weeks starting 20 days from commissioning of the filter, also on each sampling date samples were taken in duplicate i.e. two baskets of media were pulled.

III Macrograzer and film studies

Intensive sampling of the main filters both primary and secondary was carried out from February 1976 to February 1977. This involved removing sample baskets (see Plate 6) as described previously however in this case two different sizes of baskets

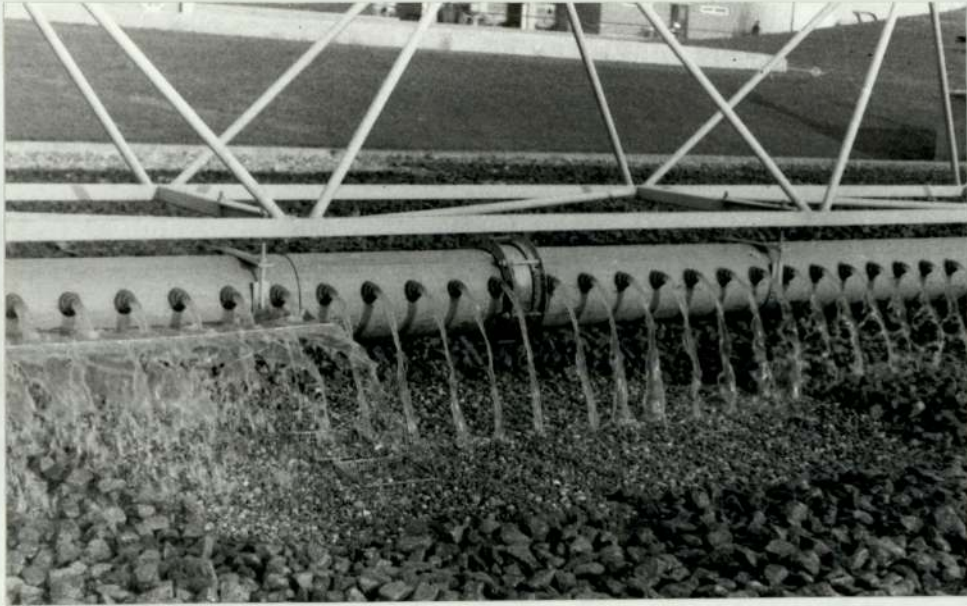


Plate 5. Positioning of splash plate on the distributor arm in relation to the small medium area



Plate 6. Sample basket in position in filter

were used. Baskets of dimensions 160 x 160 x 150 mm and volume 0.0035 m³ were utilised on the control and insecticide treated filters whilst baskets of dimensions 200 x 200 x 200 mm and volume 0.008 m³ were used on the modified operation areas. The methods of treating these samples were virtually identical apart from certain washing water volume changes. In the following description of the method the values given are those for the control and insecticide treated areas, the values for the modified operational areas are given in parentheses.

- (a) 0.0035 m³ (0.008 m³) of medium was removed from a sample basket and each piece was individually scrubbed in a bucket containing 3 l (5 l) of water.
- (b) The washings were then thoroughly agitated and two 1-litre sub-samples were taken from different levels.
- (c) The first 1 litre sub-sample was then immediately passed through a 500 µm sieve to separate the grazers from the liquid and the fine solids. The remaining solids in the sieve were then rinsed with tap water to remove any adherant matter.
- (d) The solids in the sieve were then washed into a sectioned counting tray and were scored for macrofauna, results were expressed as organisms per dm³ (litre) of media.
- (e) The second 1 litre subsample was allowed to settle for 1 hour after which the supernatant liquid was discarded and the solids were transferred to vitreosil dishes.
- (f) The dishes containing the solids were then dried in an oven overnight at 105°C.

- (g) The dishes were then weighed and placed in a preheated furnace at 550°C for 1 hour after which they were allowed to cool and were reweighed.
- (h) The difference in weights allowed calculation of volatile solids levels which were expressed as solids in g per dm³ of media.

It should be appreciated that the presence of large numbers of grazers can give inaccurately high readings as regards volatile solids levels therefore in order to get a true measure of film levels not affected by the numbers of grazers present a correction method described by Shephard (1967) was used.

This involved estimating the weight of members of the grazing fauna and subtracting this from the total weight of the volatile solids obtained. This correction was 0.2 g per 1000 individuals of *Enchytraeidae*, *Psychodidae*, *Chironomidae*, *Arachnida*, *Spathiophora* and *Hypogastura*, and 0.2 g per 100 individuals of *Sylvicola*.

It should also be noted that volatile solids and grazer levels quoted are those found in the top 200 mm of the filter (150 mm in the case of the smaller basket) which only constitutes 11% (8%) of the filter depth. Methods are available for sampling the whole depth of filters.

These have involved using vertical shafts containing baskets of media which are withdrawn from the required depth (Tomlinson 1941, Hawkes 1955, 1965, and Solbē et al. 1967) also horizontal shafts set into the sides of filters at various depths containing removable trays of media (Wheatley and Williams 1976), both of these methods allowed determination of film and grazer levels at specific levels in filters. Another method which has recently become popular is the neutron scatter technique described by Harvey et al. (1963) but this has the disadvantage of only measuring moisture levels and indirectly film levels and not grazer levels. Fortunately the mass

of the grazing fauna and most notably the nuisance species Metriocnemus hygropetricus predominantly inhabit a top band of media, therefore results shown relating to this top 200 mm should give good indications of population levels within the whole filter depth.

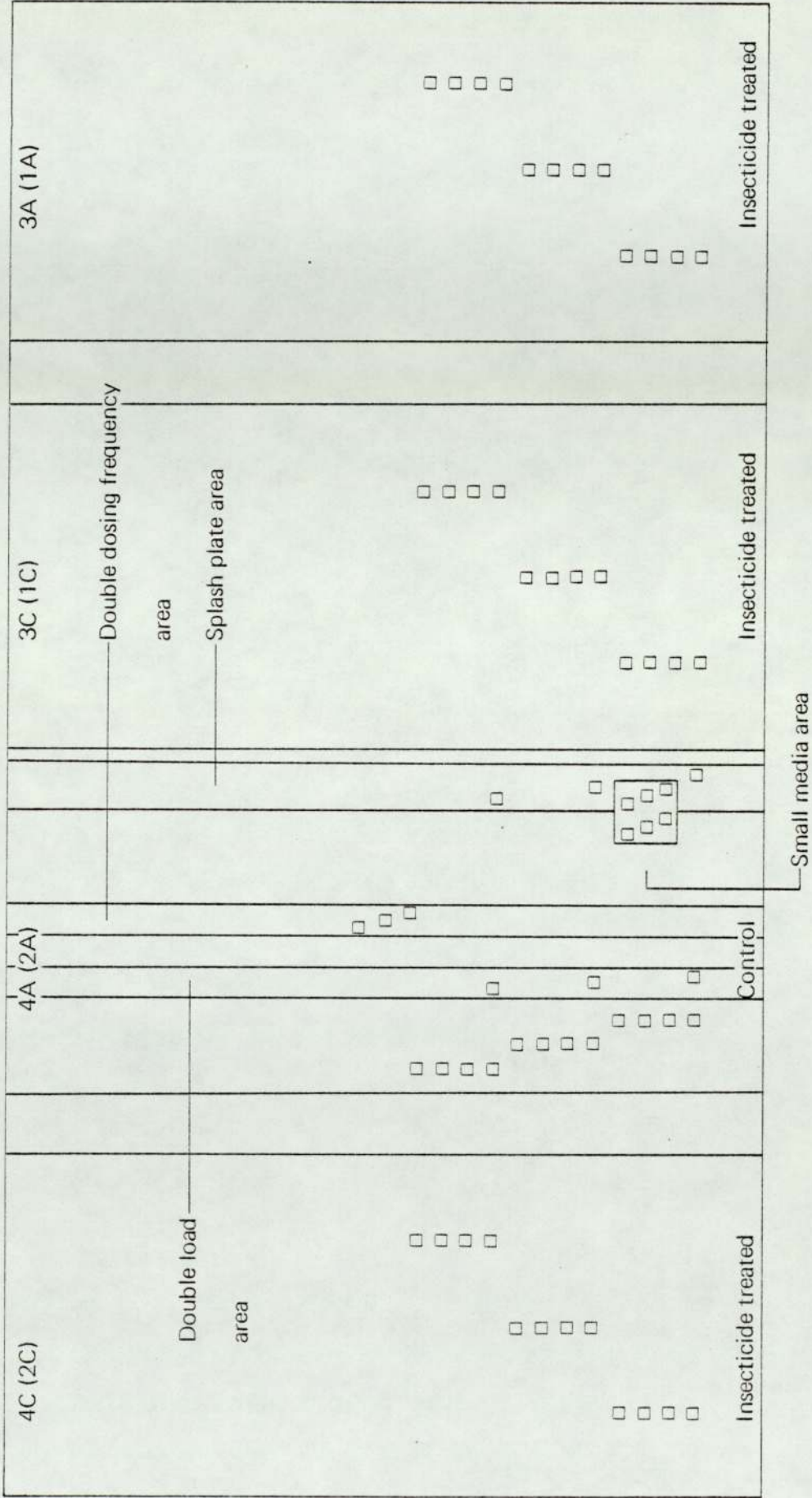
For the first 6 months all media sampling was carried out in triplicate however after this period single sampling was reverted to due to the introduction of other experiments. The frequency of sampling was weekly for both control and insecticide treated areas and monthly for the modified operation areas.

The positioning of the baskets within the filters can be seen in Fig.3. The reason for the use of a number of baskets in each area was to allow sufficient time for conditions to equilibrate in between removing the same basket, also to facilitate equilibration, when the contents of a basket were removed the basket was refilled with media from adjacent areas i.e. media with a good covering of film.

IV Fly trapping methods

Various methods have been used in the past to assess fly emergence from filters. Lloyd (1935) described the use of a shallow wooden tray 305 x 305 mm (1 ft x 1 ft) placed on the surface of a filter catching flies by means of a central aperture covered by a glass jar. Other workers have developed methods (Tomlinson and Stride 1945, Jenkins 1947 and Hawkes 1951 a and b) by using a similar tray set into the filter media but with a removable lid which was replaced on the inverted tray trap after the trapping period and the flies were anaesthetised for counting. This method of trapping was subject to many inaccuracies as quoted by Hawkes (1951a) and as have been described by many workers. Hawkes (1951a) found that the tray trap in fact attracted Sylvicola due to the dark sheltered conditions found under the trap and was thus considered to give an inaccurate indication of fly emergence from the filters. According to Hawkes (1957) one of the most accurate methods of determining the

Fig.3. Positioning of sampling baskets in main filters (Not to scale)



This plan of positioning of baskets applies to both primary and secondary blocks of filters

aerial density of Sylvicola was to use canister traps first used by Tomlinson and Stride (1945) comprising cylindrical tins the bottom of which were replaced by inverted cones, these were lined with insecticide and suspended 1.22 m (4 ft) above the filter surface. Disadvantages with this method was that it was specific to Sylvicola, other species did not seem to enter the traps.

Various other methods have been utilised for the assessment of the aerial density of flies including the disc dropping suction trap designed by Taylor (1951) and described in detail by Hawkes (1961). This trap was generally used when assessing fly emergence per unit time, it could not be used to assess flies per unit area of a filter as it does not relate to any specific area of a filter. This trap was used in this project and the method will be described later.

The methods used in this project have been developments of sticky trap methods first described by Broadbent (1948) in a study of aphid migration. One of the first reported uses of this in a sewage works venue was by Hawkes (1951a) who described the use of 305 mm (1 ft) square sheets of sticky perspex placed over the filters, this was then developed further into a sticky cross trap with 152.5 mm (6 in) square sheets of perspex placed 90° to one another 1.22 m (4 ft) above the ground, the reason for this was that wind direction should not affect the mean numbers of flies caught.

A further development of the sticky trapping method which has been in general use in many studies was the method using the trap described by Solbē et al. (1967). This basically involved the use of a 305 mm (1 ft) square box set into the surface of the media with inclined sticky plates for a lid and provisions for ventilation and dosing of sewage within the trap. These traps were used in this project for the first 4 months after commissioning of the main filters. The method of preparing the sticky lids was as follows.

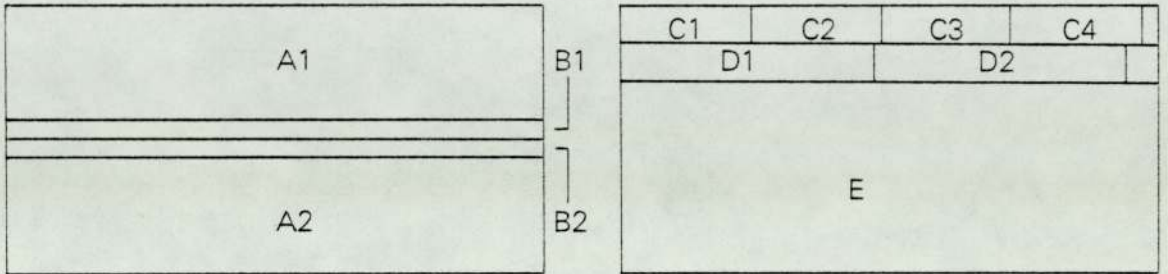
- (a) A container of "Tree grease" (as used on young fruit trees to catch crawling insects) was placed in an oven at 105°C for approximately 2 hours prior to greasing in order to give the grease a soft consistency.
- (b) The perspex plates were placed in the same oven for approximately 2 minutes before removing and greasing by means of an applicator constructed from a large rubber bung mounted sideways on a wire frame to act as a roller which was dipped into the grease and rolled onto the plates.
- (c) The plates were then positioned on the traps.
- (d) After the flies had been caught and the results recorded the plates were degreased using a concentrated emulsion degreasing solution manufactured by Applied Chemicals and then they were rinsed with warm water.

Initially the traps described by Solbē et al. (1967) were used singly on the filters however it was decided that more traps were needed as in order for reasonable accuracy results had to be in triplicate, also a large number of extra traps were needed for the modified operation areas therefore it was decided to design a completely new easily and quickly constructable trap to satisfy these requirements. It was considered that the Solbē trap was too expensive and time consuming to construct when a large number were needed.

The trap designed was in the form of a rectangular perspex box with an open bottom (see Plate 7). The two ends were constructed of nylon mesh for ventilation and the two large sides had guides below the top to accommodate a removable lid which was greased and acted as the fly trap. Advantages of this trap was that it was very simply constructed from two sheets of perspex $140 \times 280 \times 3$ mm (see Fig. 4) and perspex was the only construction material needed apart from the nylon netting, also

Fig.4. Design and construction of fly traps

(a) Perspex sheets cut as marked



A1, A2. - Sides

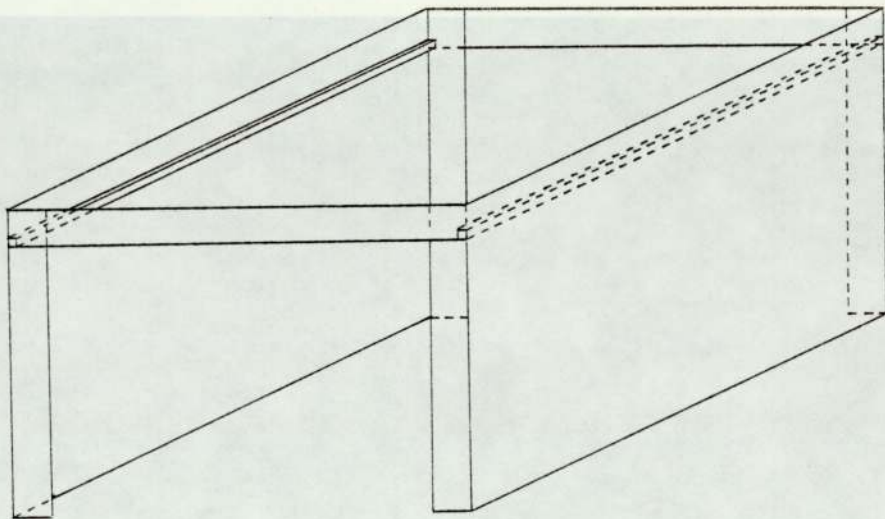
B1, B2. - Ledges for lid

C1, C2, C3, C4. - End pieces

D1, D2. - Cross pieces

E. - Lid

(b) Exploded view of assembled trap



*

Paired 't' test of chironomids from Solbe trap v. new trap.

Trap	Mean weekly emergence per m ²	't' value	'P' value	Significance
Solbe type	16.80 ± 3.75	0.62	>10%	NS
New type	13.61 ± 4.32			

± = Standard error of mean (S.E.M.)

No. observations = 32

the trap was of such dimensions that it could rest in the "dry" area thus obviating the need for an internal distribution system as is found in the Solbē type trap. The placement of the trap in relation to the filter media and the distribution jet nozzles can be seen in Plate 8.

Before constructing the traps in any great number a comparison was made between the numbers of flies caught in a Solbē type trap and a new type trap. Over 32 trappings a mean of 16.80 ± 3.75 chironomids and 392.96 ± 79.56 psychodids per m^2 per week were caught from the Solbē trap compared to 13.61 ± 4.32 chironomids and 214.54 ± 37.52 psychodids per m^2 per week from the new type trap. From this the average of the two chironomid means was calculated as 15.16 ± 1.60 indicating that no significant difference was apparent between the two trappings. Thus chironomid numbers were fairly similar from both types of trap however approximately double the number of psychodids were caught from the Solbē trap compared to the new trap. These results were subjected to further statistical analysis* and no significant difference was found between chironomid numbers from the Solbē and the new type trap. These results may be explained by the distribution system within the Solbē trap which created different conditions to those found on the filters.

The distribution jets within the trap are spaced at 41 mm intervals whereas they are at 155 mm intervals on the main filter distributor arms. Also the scouring effect of the sewage is much diminished as its fall is broken by the trap, therefore this generally gives higher film conditions under the Solbē type trap which may be the reason behind the higher levels of Psychoda found therein. As the chironomid levels were fairly similar from both types of trap it was decided to proceed with the construction of the new type of trap.

This type of trap has been used for all monitoring of fly emergence from the main filters from February 1976. The traps were used in triplicate on both primary and

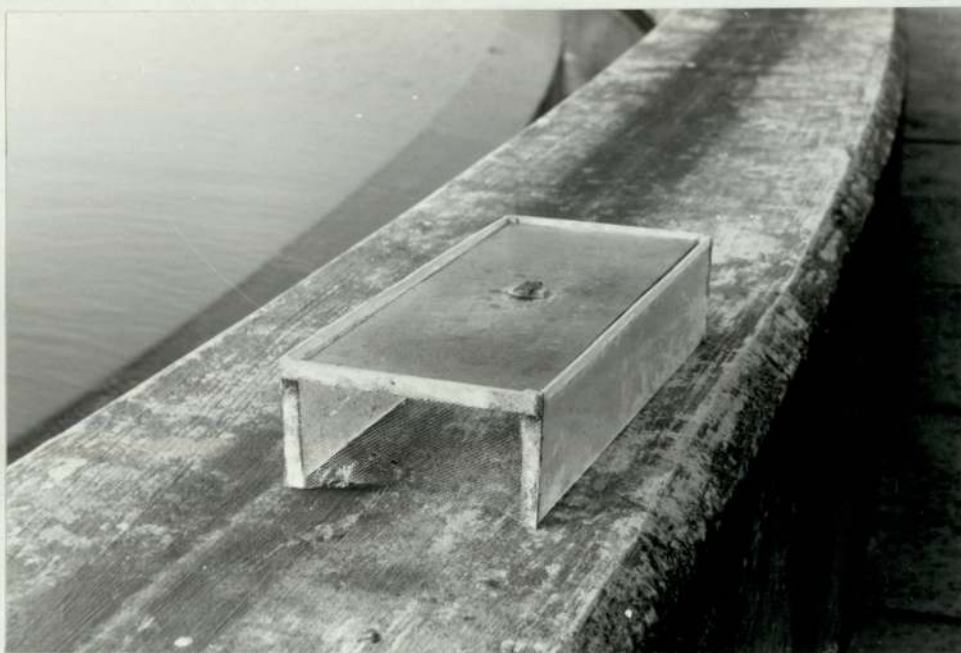


Plate 7. Newly designed fly trap



Plate 8. Newly designed fly trap in position on filter

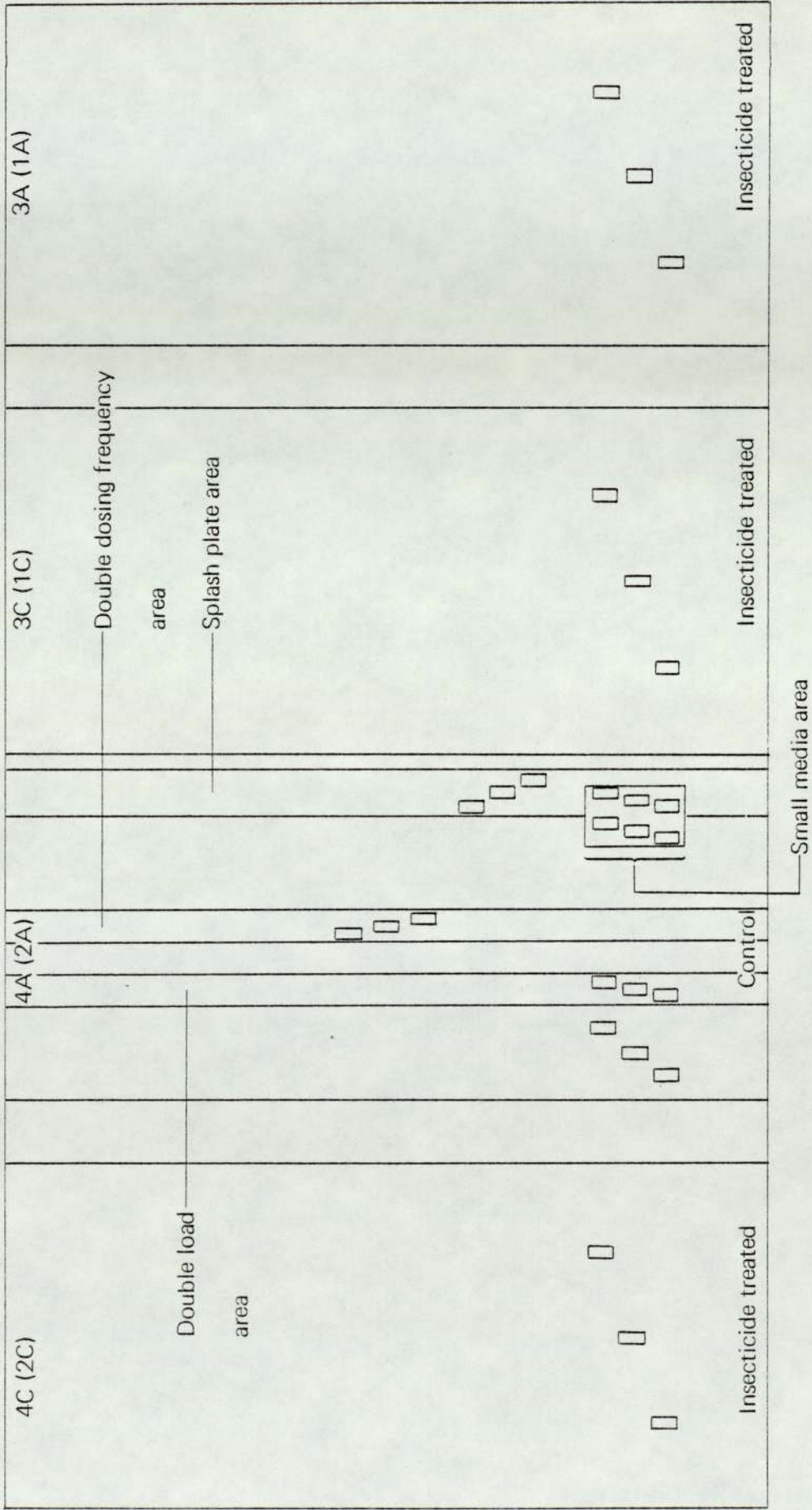
secondary filters on the insecticide treated, modified operation and control areas of these filters up till August 1977 and from that date they were used on the primary filters only and the number was increased to 5 per area. The positioning of these traps on all filter areas can be seen in Fig. 5. The method of preparing the greased lids was identical to that used in the Solbē type trap also the frequency of sampling was weekly. The lids were left on the traps for a maximum of 3 days in 7 so as to allow conditions to equilibrate before replacing the plate. It should be noted that all of the traps were moved to adjacent positions monthly as it was considered that this should reduce the effects of any abnormal conditions that may build up around the trap.

Pilot filter experiments

I Description of plant and operational methods

A series of 12 cylindrical pilot filters each 1.83 m long and 0.25 m in diameter containing media from the main filters were constructed flush to the end wall of one of the main filters on the primary block (4C). A general view of these is shown in Plate 9. These filters (which were constructed of polythene) were stacked in line together in an angle iron framework and were covered with 76 mm (3 in) thick fibre glass lagging and enclosed by 19 mm ($\frac{3}{4}$ in) marine ply board to act as insulation. The positioning of these filters in relation to the 3 m (10 ft) height of the main filter wall was such that provision was made for a feed trough of internal dimensions 2.67 x 0.06 m (145 mm deep) to be placed on the framework directly above the filters. The maximum height of the inner edge of the trough was positioned directly flush with the level of the top of the filter wall. Similarly provision was made for an effluent collecting trough of dimension 2.67 m x 0.127 m (152 mm deep) enclosed in the framework positioned immediately underneath the 12 filters. This was positioned approximately 170 mm above ground level allowing escape of the effluent by gravity via a 12 m long waste pipe to a drain.

Fig.5. Positioning of fly traps on main filters Feb. 1976 – Aug. 1977 (Not to scale)



This plan applies to both primary and secondary filters up till Aug. 1977 when all of the fly traps were removed from the secondary filters and the numbers on the primary filters were increased to 5 per section

The feed system involved the use of the trough already described which was filled with sewage every 15 minutes from the main filter distributor arm via a system of extended nozzles and an inclined corrugated sheet (see Plate 10). This sheet of dimensions 1.82 x 2.72 m was constructed from corrugated P.V.C. sheeting held on an angle iron trestle and framework. It was set at an angle of 4.5° thus when sewage flowed from the 7 extended nozzles on the distributor arm it passed via the corrugated sheet into the feed trough where it flowed out via 12 19 mm ($\frac{3}{4}$ " diameter feed pipes into the filter underneath (see Plate 11). This system can be seen in diagrammatic form in Fig. 6. Thus by using this system many operational conditions present on the main filters can be imitated. For example the filters were fed with the same sewage as the main filters at the same dosing frequency and they contained the same media. The total loading to the filters was optimised to compare with the loading received on the main filters by adjusting the number of extended nozzles on the distributor arm which impinged sewage onto the sheet. The remaining nozzles which were not required to impinge on the ramp were fitted with 90° bends to direct the sewage away from the ramp and onto the main filter.

These filters were used with certain operational modifications in a similar manner to those used on the main filters. These included the use of small medium (19 mm diameter gravel) as a topping in varying depths including 229 mm (9 in.) and 76 mm (3 in.). Other modifications included the use of perforated plastic pots attached to the ends of the feed pipes (see Plate 12) which had the effect of spreading the application of the sewage over the whole of the filter surface leaving no "dry" areas. This modification was also used in conjunction with a 229 mm (9 in) depth of the small medium previously described, also with a surface sprinkling of small medium to fill the interstices between the larger pieces of medium. Another modification used was the covering of the whole of the filter surface with a circular thick rubber disc with a 25 mm (1 in) central aperture through which sewage was applied to the filter. This modification is shown in Plate 13. The desired effect in this case was to restrict the

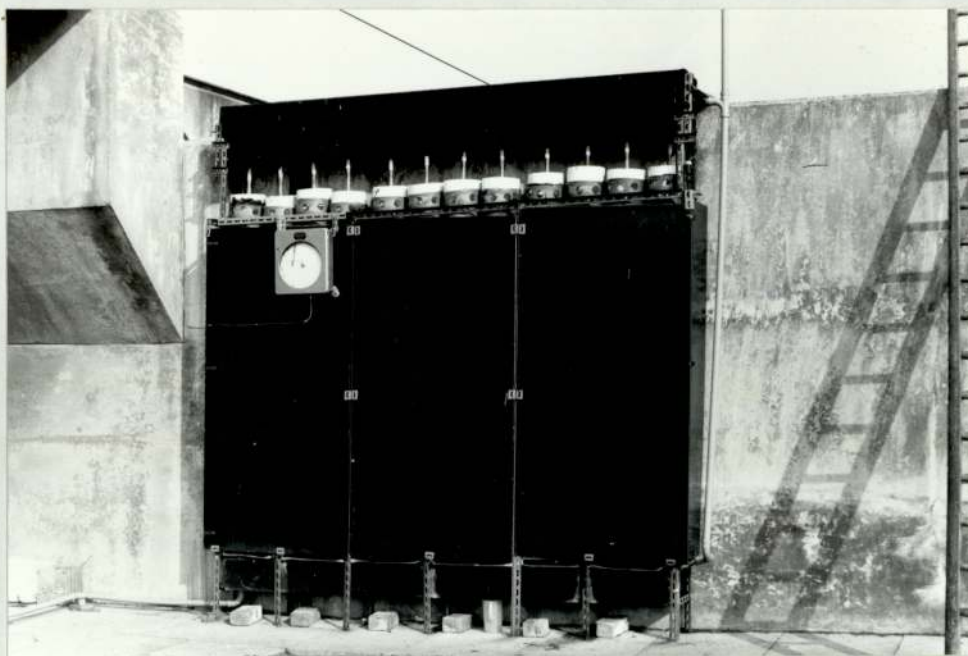


Plate 9. General view of pilot filter system

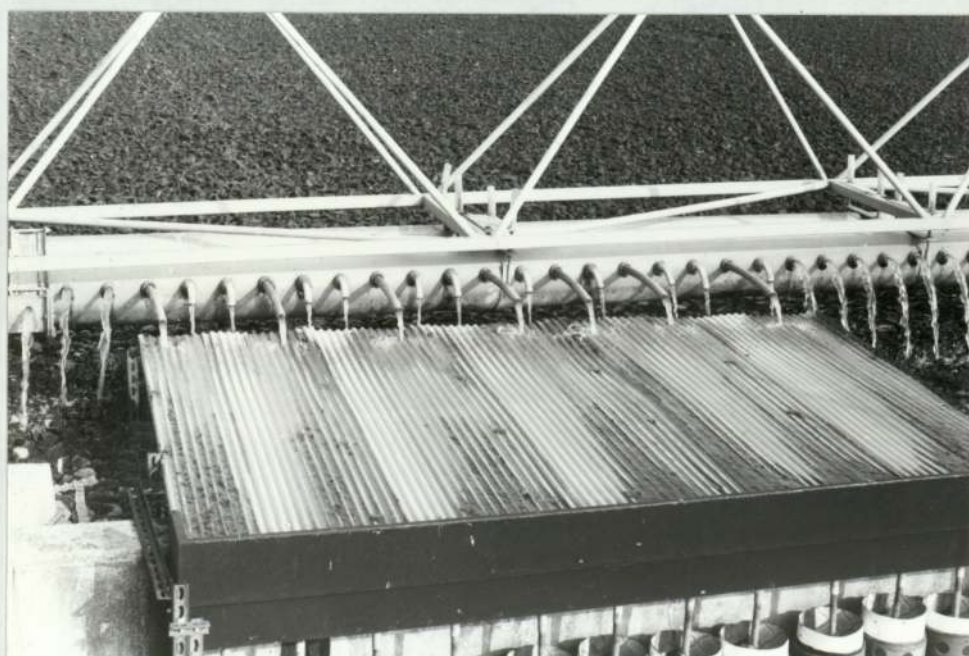
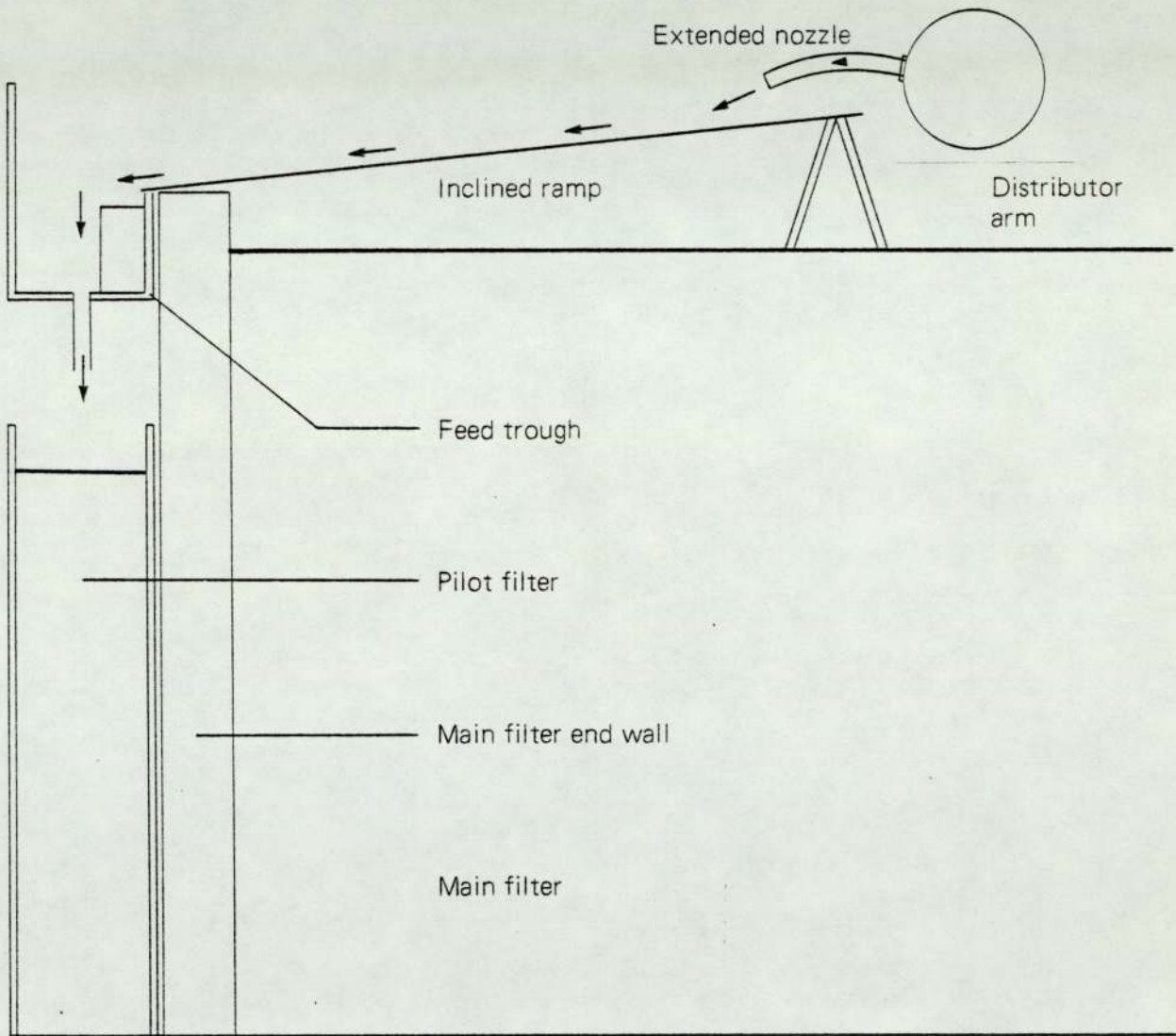


Plate 10. Feed system to pilot filters

Fig. 6. Diagrammatic representation of feed system to pilot filters



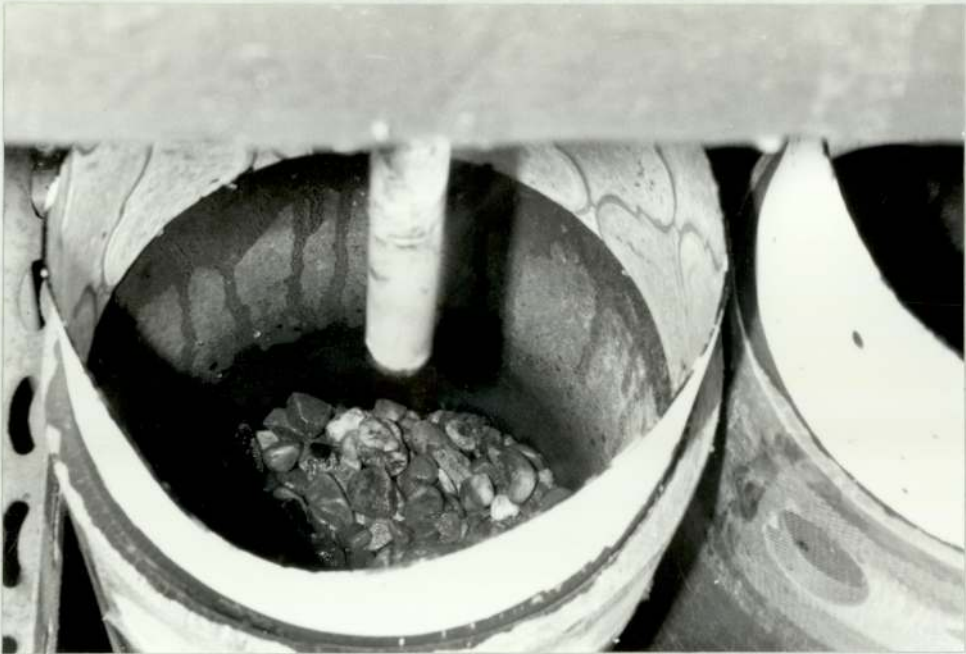


Plate 11. Pilot filter system feed nozzle

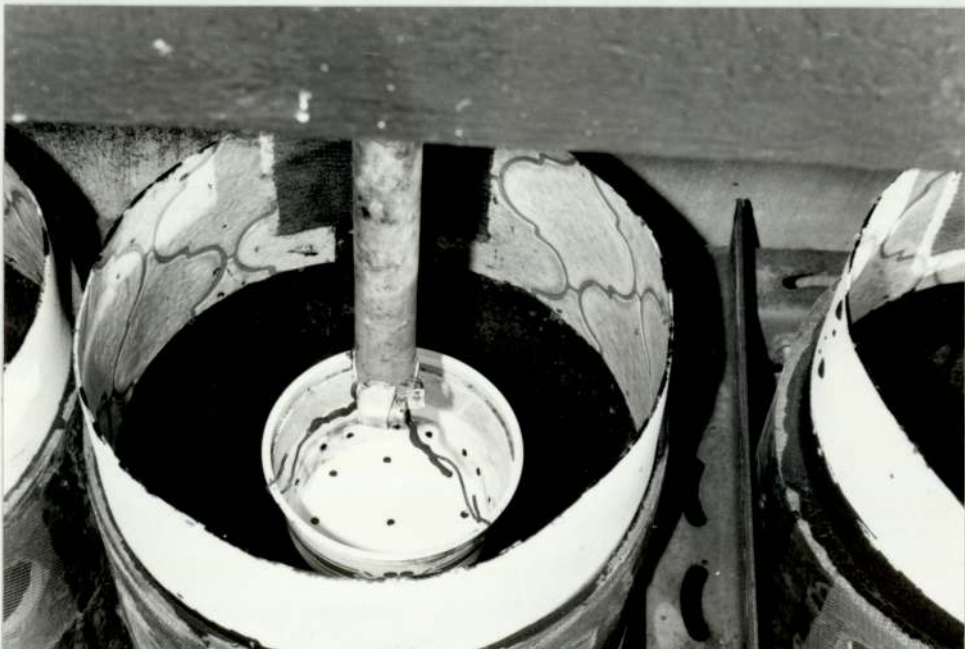


Plate 12. Pilot filter system splash modification

light and the area of possible fly emergence to a minimum. Alterations were carried out to this modification in September 1977 when blackened inverted funnels were placed under the aperture of the disc to cut down the light further.

All of these 6 modifications were investigated in duplicate, therefore 12 filters in all were utilised.

II Flow balancing

When these filters were first commissioned on 18th February 1977 it was apparent that the flow to all of the filters was not sufficiently balanced therefore certain changes were made to the method of operation. One of the first changes was the use of rubber bungs within which small glass tubes (5 mm diameter) were inserted. These slowed the emptying of the trough drastically and evened out the flow through the 12 feed pipes however certain disadvantages were apparent during operation. These were that the small diameter glass tubes often clogged with rag etc. from the sewage also it was considered that the scouring action of the sewage onto the filters was much reduced in comparison to the situation found on the main filters. The bungs and tubes were removed on 26th May 1977 leaving the straight through 19 mm ($\frac{3}{4}$ " diameter jets. At this date the trough was lined with house bricks (80 x 230 x 115 mm) on one side to reduce the effective volume and give a greater head of sewage when filled, this had a similar effect to the bungs and tubes in balancing the flow and the advantage of retaining the scouring action.

Experimental investigations were carried out to compare the flow to all 12 filters in relation to the flow to each individual filter and to the main filter using the following method.

(a) A 2 l beaker was placed under each feed jet of the 12 pilot filters.

- (b) A plastic trough of dimensions 310 x 480 mm was placed on the main filter surface.
- (c) After the distributor arm had passed, the volumes in all of the beakers were measured and compared with the volume collected in the trough on the main filter.

This comparison necessitated relating the area of collection offered by the trough to the area offered by the filter.

$$\text{Area of trough} = 1.568 \text{ m}^2$$

$$\text{Area of filter} = 0.346 \text{ m}^2$$

Therefore if the same relative volumes of sewage were to be collected in both areas the ratio of volumes in the trough to the volumes in the filter would be $1.568 : 0.246$
 $= 4.53 : 1$

This determination was carried out on 3 occasions for 2 hours i.e. 8 doses of sewage at a time, these occasions were 8.30 - 10.30, 10.30 - 12.30 and 13.15 - 15.15 hrs. on separate days. The results of these can be seen in Table 6. Thus it can be seen that the means on separate occasions do vary considerably and this may be due to the result of diurnal variations in the flow pattern to the works, however in all cases readings were taken when no rain had been reported for the previous 24 hours. The means of the flow within one occasion between different filters do compare favourably indicating that flow balancing had been achieved i.e.

Table 6. Flow balancing results

Observation period 08-30 – 10-30 ml. per dose

Modifications												Mean
Control	A	100	900	1950	2050	1250	1550	1550	1100	1250	1300	
Control	B	50	800	1800	2000	1100	1650	1550	1000	1250	1244	
SM.9	A	50	600	1750	1950	850	1250	1500	900	1250	1122	
SM.9	B	100	600	1700	1950	1500	1650	1800	700	1050	1228	
SM.SP.9	A	100	950	1600	1600	1200	1400	1450	1000	1250	1172	
SM.SP.9	B	50	1100	1800	1850	1150	1550	1600	1050	1350	1278	
SM.3	A	50	800	1500	1650	750	1550	1450	800	1200	1083	
SM.3	B	100	850	1500	1750	1050	1650	1600	1000	1250	1194	
SM.SP.CO.V.	A	100	800	1300	2200	1200	1750	1450	950	1200	1217	
SM.SP.CO.V.	B	150	900	1500	2050	1100	1300	1900	1350	1500	1305	
S.CO.V.	A	100	1100	2000	1900	1250	1650	1500	1150	1450	1344	
S.CO.V.	B	150	850	1750	1750	1350	1700	1700	1150	1550	1328	
Mean of separate modification means.											1234	
Main filter flow.		1100	3650	5200	5900	3500	4700	4400	3000	4300	3972	
Ratio of mean main filter flow to mean pilot filter flow											3.22	

Table 6. Continued Flow balancing results

Observation period 10-30 – 12-30 ml. per dose

Modifications										Mean
Control	A	550	400	250	800	1100	850	850	750	694
Control	B	450	300	150	800	1100	800	900	800	663
SM.9	A	450	300	200	850	1200	800	1050	700	694
SM.9	B	700	400	250	1150	1650	1350	750	700	868
SM.SP.9	A	550	300	200	950	1300	950	950	800	681
SM.SP.9	B	550	350	200	950	1100	900	950	800	725
SM.3	A	550	350	150	1100	1400	750	700	650	706
SM.3	B	600	350	150	750	900	900	750	700	637
SM.SP.COV.	A	650	350	350	1350	1200	850	800	700	781
SM.SP.COV.	B	400	300	250	850	1600	1250	800	800	781
S.COV.	A	450	300	250	850	1600	1100	950	850	793
S.COV.	B	350	200	300	650	1500	1250	800	750	725
Mean of seperate modification means										729
Main filter flow		2200	1950	1700	3100	3800	3300	3100	2900	2756
Ratio of mean main filter flow to mean pilot filter flow										3.78

Table 6. Continued Flow balancing results

Observation period 13-15 – 15-15 ml. per dose

Modifications										Mean
Control	A	1300	1250	1000	550	250	300	350	714	
Control	B	1000	1200	900	450	250	200	300	614	
SM.9	A	1350	1350	1050	500	250	250	300	721	
SM.9	B	1600	1200	1000	750	350	350	450	828	
SM.SP.9	A	1300	1250	1000	600	250	250	350	714	
SM.SP.9	B	1550	1400	1200	600	300	300	350	814	
SM.3	A	1400	1400	1050	650	300	250	400	778	
SM.3	B	1650	1350	1050	650	350	300	450	828	
SM.SP.COV.	A	1300	1200	1050	700	400	400	550	800	
SM.SP.COV.	B	1250	1200	900	450	250	200	300	650	
S.COV.	A	1300	1300	900	500	200	200	350	652	
S.COV.	B	1250	1200	850	400	200	200	250	621	
Mean of separate modification means									727	
Main filter flow		4000	4400	3600	3200	3600	2300	2000	3300	
Ratio of mean main filter flow to pilot filter flow									4.54	

* Details of analysis of variance test.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	'F'ratio	'P'value	Significance
(a) Period 8.30-10.30						
Between modifications	11	660283	60025	0.1898	>20%	NS
Within modifications	96	30357305	316221			
Total	107	31017588				
(b) Period 10.30-12.30						
Between modifications	11	360695	32790	0.2220	>20%	NS
Within modifications	84	12405316	147682			
Total	95	12766011				
(c) Period 13.15-15.15						
Between modifications	11	461913	41992	0.1847	>20%	NS
Within modifications	72	16364537	227285			
Total	83	16826450				

<u>Experimental period</u>	<u>Means and standard error</u> <u>of means of flow to separate</u> <u>filters ml per 15 min</u>
8.30 – 10.30	1234 ± 23.0
10.30 – 12.30	729.0 ± 18.9
13.15 – 15.15	727.8 ± 23.3

A one way analysis of variance test was carried out on the results shown in Table 6 with the following results:-^{*}

<u>Experimental period</u>	<u>Variance ratio (F)</u>	<u>Degrees of freedom</u>	<u>P value %</u>
8.30 – 10.30	0.1898	11/96	> 20
10.30 – 12.30	0.2220	11/84	> 20
13.15 – 15.15	0.1847	11/72	> 20

Therefore as all of the values of P were over 20% it is likely that any variations in the flows received by the filters were due to chance.

It should be appreciated that the drastic variations in flow between separate doses on both pilot and main filters may be attributed to the flow to the works varying considerably as the main flow is pumped in short bursts only when the level in a tank in the pumping station has reached a certain level.

Although not all of these results reach the desired 4.53:1 ratio it should be noted that the most comparable set of results was obtained during a medium flow period of 13.15 – 15.15, both morning sets of results showed lower ratios of 3.78 and 3.22:1 indicating that the pilot filters were receiving more sewage per unit volume than the

main filters, however when considering a 24 hour flow pattern ratio, results found during the night when flows are very low must be considered. Although no such results are given it was observed on many occasions during the evening that flow to the pilot filters was much reduced during these low flow periods. Therefore the ratio during the evening and night would probably be much higher compensating for the lower ratios found during peak flow periods.

It was therefore considered that flow to these filters was as comparable to the main filter flow as could practically be achieved using the apparatus described so the filters were then operated on this system to the end of the experiment. When comparing the pilot filter feed volumes to the main filter volumes it should be noted that the experiment did not specifically set out to compare the pilot filters to the main filters, it set out to compare the modifications on the pilot filters to each other therefore slight variations in flow between the pilot filters and the main filters should not be too serious as long as flows between different pilot filters were comparable.

III Fly trapping

Fly trapping was carried out on these filters in a similar manner to that used on the main filters, this is that sticky plates were utilised. Vinyl sheet collars were attached to the inner edges of the tops of the filters to give a level surface on which the plate rested. A central 19 mm ($\frac{3}{4}$ " hole was bored in these plates through which the feed pipe was inserted thus allowing uninterrupted dosing to be carried out whilst the fly plate was in position. A fly plate in position on the top of a filter can be seen in Plate 14. Important differences between this method and the method used on the main filters included the following. The flies caught represent the total output from an area of filter in the pilot filter case whereas in the main filters lateral migration may have some effect on the flies caught under a trap. Also fly trapping could be carried out without interrupting dosing in the case of the pilot filters whereas on the main filters, even though traps were placed in "dry" areas between jets, in cases when



Plate 13. Pilot filter system surface covering modification



Plate 14. Fly plate in position on pilot filters

the wind was fresh lateral shifting of the jets was apparent, thus the traps prevented sewage from reaching the filter underneath in these situations.

The fly trapping plates were greased and cleaned in a similar manner to the method described for the main filter fly traps. The frequency of trapping was weekly and fly plates were usually in position for one day only. The reason for this was that it was considered that isolating the filters by means of having the plate in position for long periods may detrimentally affect free movement of insects from the main filters onto these filters.

IV Effluent sampling

Provision was made in the construction of the pilot filters for easy collection of the effluent from separate filters. A 50 mm (2 in) gap was left between the bottom of the filters and the effluent collecting trough to allow for insertion of sampling trays. The sampling trays were constructed of 254 mm (10 in) diameter polythene piping cut into 25 mm (1 in) cylinders. (This was exactly the same size diameter pipe as used for the actual filters). The cylinders were covered with polythene sheet on the bottom surface and a 12.5 mm ($\frac{1}{2}$ in) square aperture was cut into the cylinders from the bottom edge to allow a 152 x 12.5 x 12.5 mm polythene chute to be inserted (see Plate 15). This tray was then inserted under the filter and lined up exactly with the filter's periphery, effluent collected in the tray then flowed down the chute into a container (see Plate 16). When the sampling period was over the trays were swilled with the effluent collected to collect all of the humus solids that may have accumulated. Certain advantages were apparent with this system, namely that all of the effluent passing through a filter could be collected as the area of the tray was exactly the same as the area of the filter. Also this system allowed isolation and collection of samples of effluent from different stages of the dosing cycle. The effluent collected was subjected to the standard analyses as regards shaken and filtered biochemical oxygen demand (BOD), chemical oxygen demand (COD) and



Plate 15. Pilot filter effluent sampling tray



Plate 16. Effluent sampling tray in position

suspended solids as described in H.M.S.O. (1972) also total oxidized nitrogen (TON) and ammonia determinations were carried out using a chemlab autoanalyser. All of the routine chemical analysis was carried by Severn-Trent Water Authority staff in the divisional laboratories. Sampling was carried out generally alternating either once or twice every week, also at each occasion one of each duplicate pair was sampled, thus on calculation both of the duplicates for each modification were sampled on 3 occasions every month.

V Retention time experiments

The method of effluent collection described above allowed easy determination of the retention time of separate filters. The method followed was basically similar to the method described by McNicholas and Kirkwood (1953) involving the use of a strong solution of sodium chloride added to a filter at a certain time and measuring the concentration of chloride ion in the effluent at different periods from that time. The actual method followed for each filter was as follows.

- (a) A solution of sodium chloride containing 1000 mg l^{-1} of chloride ion was prepared using the method described in H.M.S.O. (1972).
- (b) Standard silver nitrate solution (4.791 mg l^{-1}) was prepared using the method described in H.M.S.O. (1972) and stored in a dark bottle.
- (c) A sampling tray was inserted under the filter to be investigated and effluent was collected over a 30 minute period prior to the start of the experiment.
- (d) 750 ml of $1000 \text{ mg l}^{-1} \text{ Cl}^{-}$ solution was substituted for the normal dose of sewage on the filter under investigation. The effluent was collected from the time of addition of the Cl^{-} tracer (considered time 0).



- (e) Samples of effluent collected between the following time intervals were removed. These were 0-1, 1-2, 2-3, 3-5, 5-10, 10-15 minutes and at each 5 minute interval until 60 minutes had elapsed, further single 5 minute collection samples were then taken at approximately 105 and 150 minutes.
- (f) A sufficient volume of settled sewage feed for 4 doses of 750 ml was collected prior to the start of the experiment a sample of this was retained for analysis and 750 ml of this was fed to the filter at each dosing period i.e. 15, 30, 45 and 60 minutes.
- (g) The samples collected by the above method were measured volumetrically and subjected to analysis for the detection of Cl^- by the volumetric method involving filtration with standard silver nitrate solution with potassium chromate as an indicator as described in H.M.S.O. (1972). The result obtained from the sample of filter effluent collected prior to the start of the experiment was considered to be the basal Cl^- level discharged from the filter, thus results obtained were considered in the light of this, also results obtained from the settled sewage feed sample were expected to be comparable or only slightly higher than the basal filter effluent results, if not the experiment was repeated.
- (h) This procedure was followed 6 times to investigate the 6 operational modifications found on the pilot filters. The results obtained were expressed graphically and the mean retention times were calculated as will be described later.

It is appreciated that this method of determination of retention time is quite crude however when this particular experiment was started time was at a premium and a quick, easy method was required. Similar methods have been described using sodium chloride by Hawkes (1961b) and using aluminium chloride by Tomlinson and Hall

(1950). More complex methods using radioactive tracers have been described by Tariq (1975) and Eden et al. (1964) using Br_{82} , Co_{60} and Co_{58} .

Laboratory small scale filter experiments

I Introduction to the filters and their methods of operation

18 small scale glass filters were set up in a temperature controlled room in order to investigate competition between filter fauna. These filters were in the form of glass cylinders 356 mm long and 63.5 mm in diameter tapered at one end into an outflow. An air vent pipe was provided 335 mm from the top of the filter to allow for ventilation and the filter bodies were covered with black polythene sheeting to prevent light entering. These filters were split into 3 groups of 6, each group was held within a separate angle iron framework with the feed pumps attached to the base of each framework. Thus each framework constituted a unit of 6 filters, pump and the associated pipework. A complete framework with pumps and filters can be seen in Plate 17. The filters were filled with euromatic polythene spheres of diameter 19.5 mm as medium. The tapered bottom end of each filter contained a plastic "practice golf ball" so as to allow easy drainage of the effluent from the filter as a single piece of spherical media could easily clog the drainage outlet.

The pumps used, which can be seen in Plate 18 were 3 micro metering pumps (series II) manufactured by Metering Pumps Ltd. Each consisted of a motor connected through a gearbox driving a shaft at 100 r.p.m. through 6 pump mechanisms and pump heads. Rotation of the shaft in the pump mechanism was transmitted into backward and forward lateral movement of a piston by means of a cam system in the pump mechanism. The piston moved in this manner in a cylinder contained in the pump head unit and thus by a series of valves pumped liquid through the head upwards vertically.

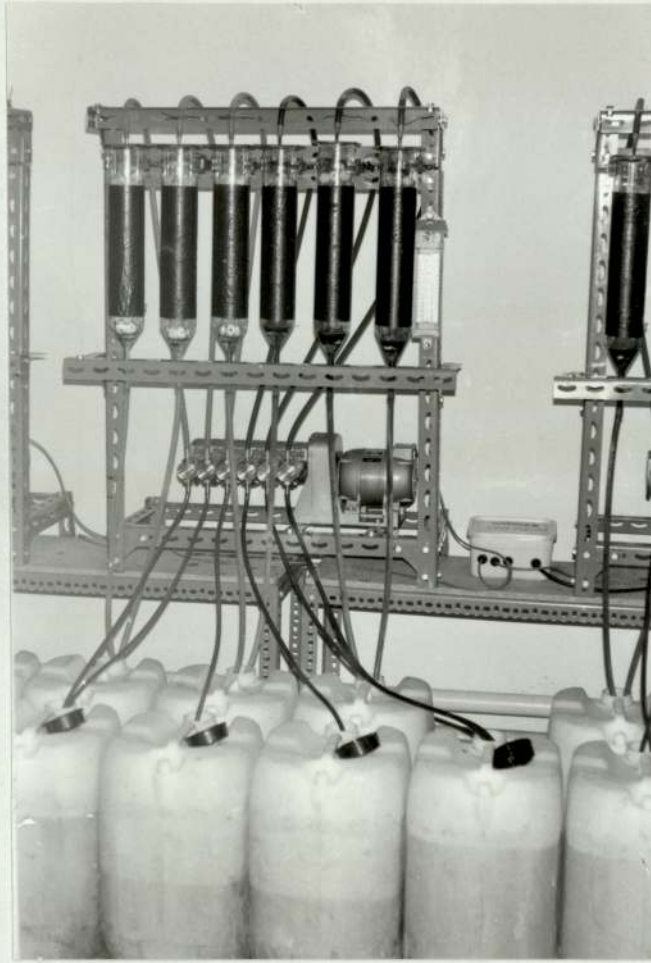


Plate 17. Laboratory scale filter system - general view

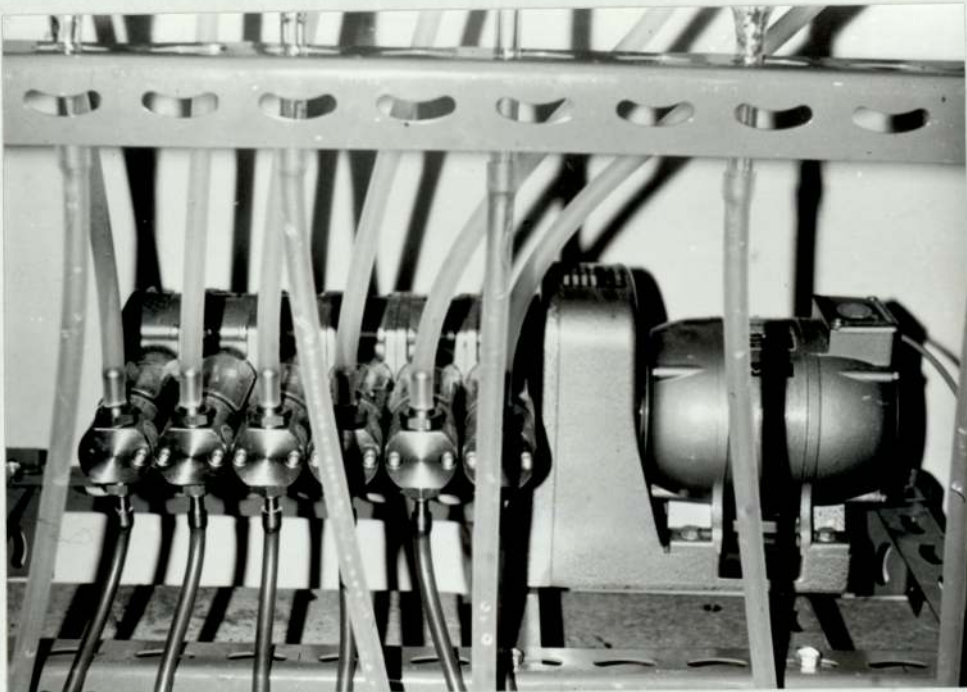


Plate 18. Micro - metering pump as utilised in laboratory scale filter system

The stroke of each piston was controlled by a micrometer thimble situated on the rear of each mechanism unit. Thus the flow through each pump head could be varied independently of the flow through other pump heads. The range of flows could be varied between 0 — 3000 mlhr⁻¹ continuous.

Prior to the start of the experimental work all of the 18 pump heads were calibrated by means of measuring flow passing through the pump heads at a range of micrometer settings from 0.5 — 10 at intervals of 0.5 units (see Figs 7, 8 and 9). In practice all of the pumps were connected through a Sangamo Weston short interval timer to give the required duration and frequency of dosing, also the micrometers were set to give the required hydraulic loading on a daily basis taking into account the duration and frequency of dosing. The micrometer reading corresponding to the specific required flow rate in ml hr⁻¹ was read off from the calibration graphs and the micrometers were set accordingly.

The feed and drain system to the pumps and from the filters involved using a series of 25 litre polythene containers. The feed tubes from 2 pump heads drew in sewage from each single container. The ends of these feed tubes in the containers were fitted with nylon mesh covered funnels to act as strainers to protect the pumps. From each pump head a tube served a single filter which was connected by a tube to an effluent collection container (see Plate 19). The effluent collection containers were of a similar capacity to the feed containers also each container received effluent from 2 filters. The capacity of the feed and effluent containers necessitated refilling of the feed and emptying of the effluent containers every 2 days.

II Experimental conditions

The experimental work was carried out on these filters in 2 phases. In the first phase the filters were commissioned on the 6th September 1977 on the same organic loadings as were applied to the main filters at the works (both primary and

Fig.7. Calibration graph of flow against micrometer setting – Pump A

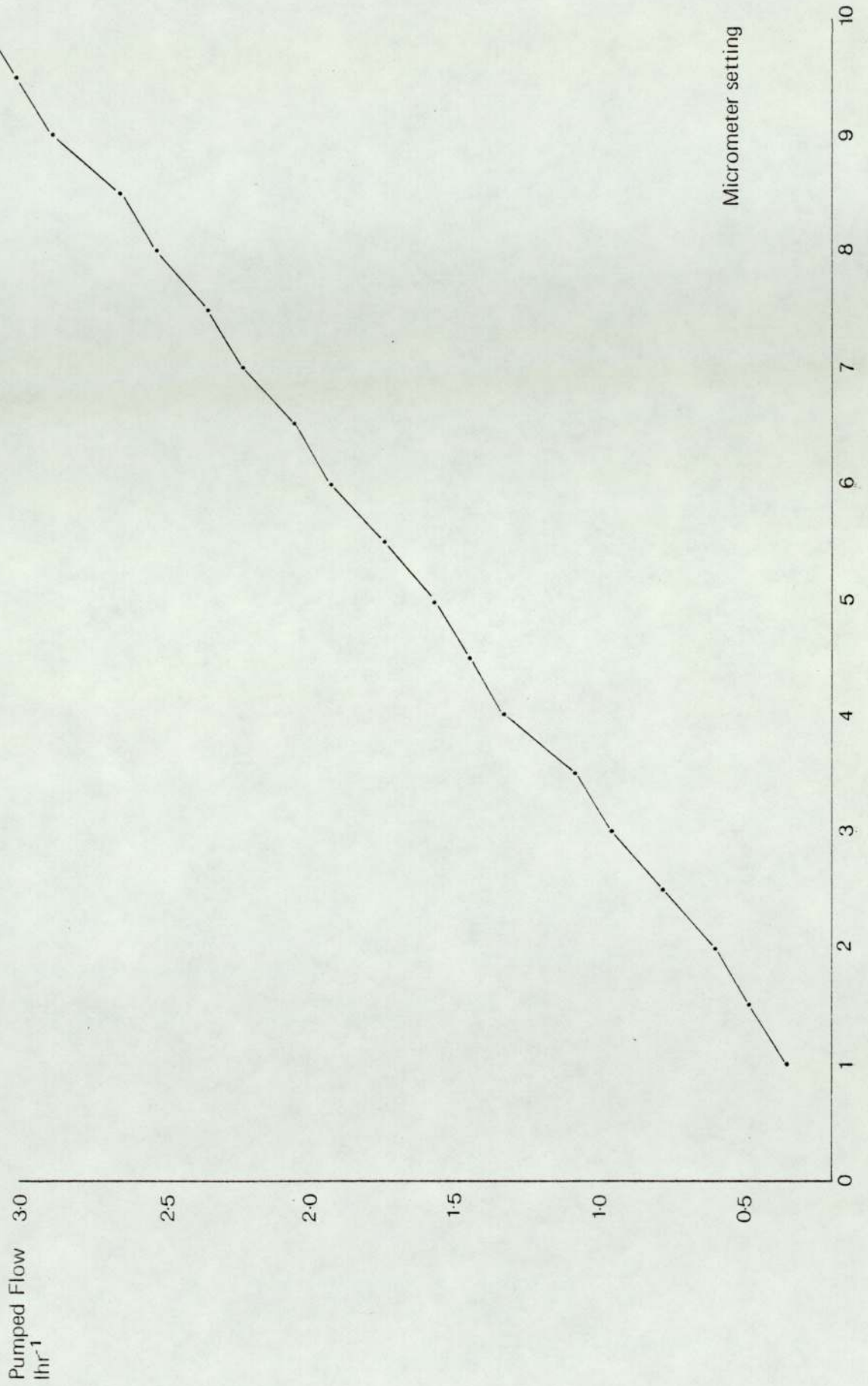


Fig.8. Calibration graph of flow against micrometer setting — Pump B

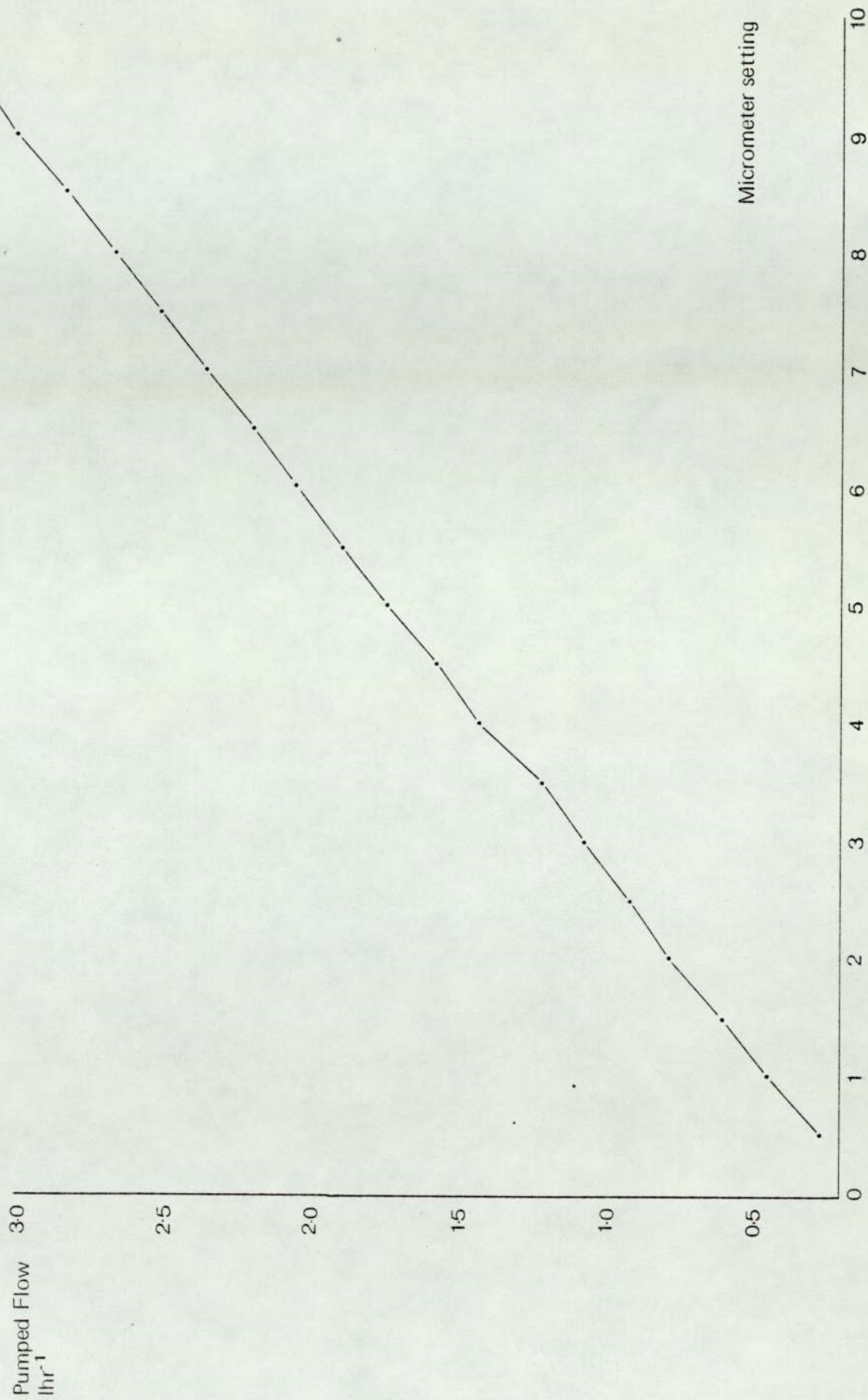
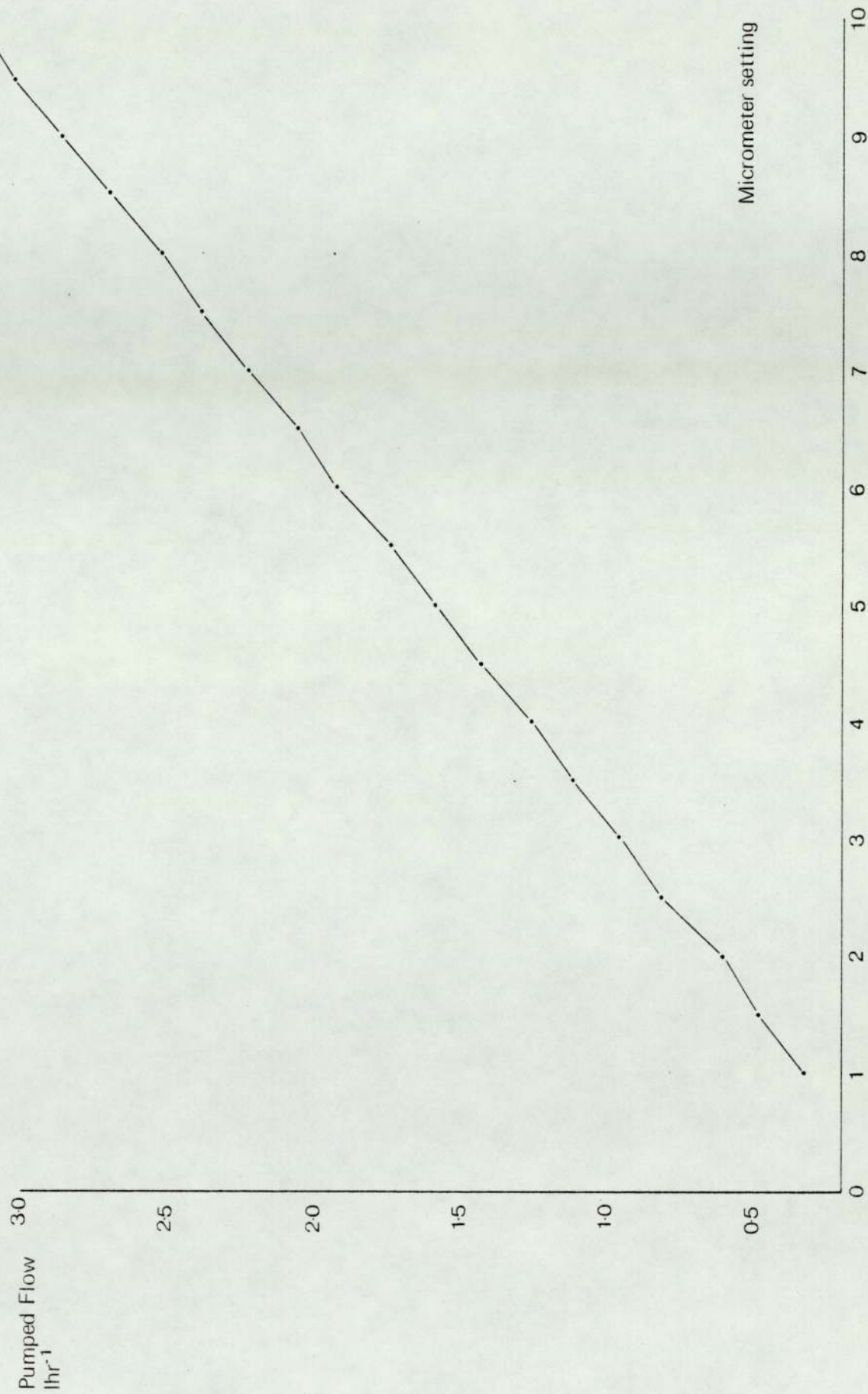


Fig.9. Calibration graph of flow against micrometer setting — Pump C



secondary). 9 of the 18 were subjected to a loading of $0.15 \text{ Kg BOD m}^{-3} \text{ day}^{-1}$ whilst the other 9 were subjected to an organic loading of $0.02 \text{ Kg BOD m}^{-3} \text{ day}^{-1}$, the hydraulic loading in both cases was $0.82 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$ and this figure was based on the hydraulic load received by the main works over the year March 1976 to February 1977. The dosing frequency was 15 minutes to compare with the situation on the main filters. The temperature of the experimental room was set at 20°C and the daylength at 12 hours.

A summary of the experimental work and conditions on the laboratory filters can be seen in Table 7. In detail it was as follows:-

In the first experiment 6 filters were seeded with 25 M. hygroscopicus larvae, 6 with 25 P.alternata larvae and the remaining 6 were seeded with 25 of each species, each group of 6 was split into 2 by the different hydraulic loadings. The larvae were washed from media from Tamworth Water Reclamation Works in the case of M. hygroscopicus and Burntwood Water Reclamation Works in the case of P. alternata. On the 18th – 20th October the filters were dosed with 10 mg l^{-1} solution of the insecticide Actellic EC50 after which a second experiment was started this time seeding the filters with 50 larvae in a similar fashion as before. This experiment was terminated on 23rd November when the loadings to the filters were altered. On 9 of the filters the loading was reduced from 0.15 to $0.05 \text{ Kg BOD m}^{-3} \text{ day}^{-1}$ and on the other 9 filters the loading was increased from 0.02 to $0.12 \text{ Kg BOD m}^{-3} \text{ day}^{-1}$. A third experiment was started on these loadings after dosage with a 10 mg l^{-1} solution of Actellic EC50 on the 28th November. On the 12th December the filters were seeded with larvae in a similar fashion to the second experiment i.e. 50 per filter. Phase one of the experimental work ceased on 16th January when the filters were rested and certain modifications were made which will be described later.

Phase two of the experimental work started with the fourth experiment on the 8th

Table 7. Summary of experimental work on laboratory scale filters

Phase 1

Fly trapping method	—	Sticky plates
Organic loading	—	0-15/0-02 m ³ m ⁻³ .day ⁻¹ until 23-11-77 then 0-05/0-12 m ³ m ⁻³ .day ⁻¹
Hydraulic loading	—	0-82 m ³ m ⁻³ .day
Dosing frequency	—	15 minutes
Temperature	—	20° C (3 Oct.), 15° C (25 Oct.), 12° C (23 Nov.)
Day length	—	12 hours

Experiment no.	Starting date	Seeding per filter	Termination date
1	3-10-77	25 larvae	18/20-10-77
2	25-10-77	50 larvae	28-11-77
3	12-12-77	50 larvae	16-1-78

Phase 2

Fly trapping method	—	Net covers
Organic loading	—	0-05/0-12 m ³ m ⁻³ .day ⁻¹
Hydraulic loading	—	0-82 m ³ m ⁻³ .day ⁻¹
Dosing frequency	—	15 minutes until 25-4-78 then 5 minutes
Temperature	—	15° C
Day length	—	12 hours

Experiment no.	Starting date	Seeding per filter	Termination date
4	8-3-78	25 pupae	28-6-78
5 (continuously dosed with insecticide)	28-6-78	none	17-8-78

February when the filters were recommissioned on the same loadings as in the third experiment of phase one however the temperature of the room was changed to 15°C. They were seeded each with 25 pupae (instead of larvae) in a similar fashion as before i.e. 25 M. hygropetricus on 6 filters, 25 P. alternata on another 6 filters and 25 of each species on each of the remaining 6 filters. These sections of 6 were subdivided into 2 by the loading arrangement, therefore all results expressed are in triplicate. On the 25th April the dosing frequency was altered from 15 to 5 minutes and on the 28th June this experiment was terminated. The fifth experiment started on the 28th June when 6 of the 18 filters were subjected to a feed containing 10 mg l⁻¹ of Actellic M20 for a period of 50 days continuously. This was the final operational change before this series of experiments ceased.

III Fly trapping

Two methods of fly trapping were utilised using these filters. In phase one of the experimental work the system utilised was sticky perspex plates in a similar fashion to the system utilised on the pilot filters. These consisted of perspex squares of dimensions 70 x 70 mm placed on top of the filters containing an aperture through which a feed pipe passed through thus allowing uninterrupted dosing to the filters. A 20 mm diameter net covered vent aperture was also provided in the plate. These plates were greased and subsequently cleaned in a similar manner to the method described for the main and pilot filters. A framework of 6 filters utilising this system can be seen in Plate 20. The distribution system beneath the plate consisted of a glass tube leading to a polypropylene "T" piece suspended some 25 mm above the surface of the media. A close up of this system can be seen in Plate 21. Fly trapping was continuous during the experiments in phase one. Thus the plates were removed for counting, cleaning and greasing after each experiment and left in place for the duration of each experiment.

The second method of fly trapping carried out in phase two of the experimental work utilised a system of net covered wire framed extensions fitted to the top of each

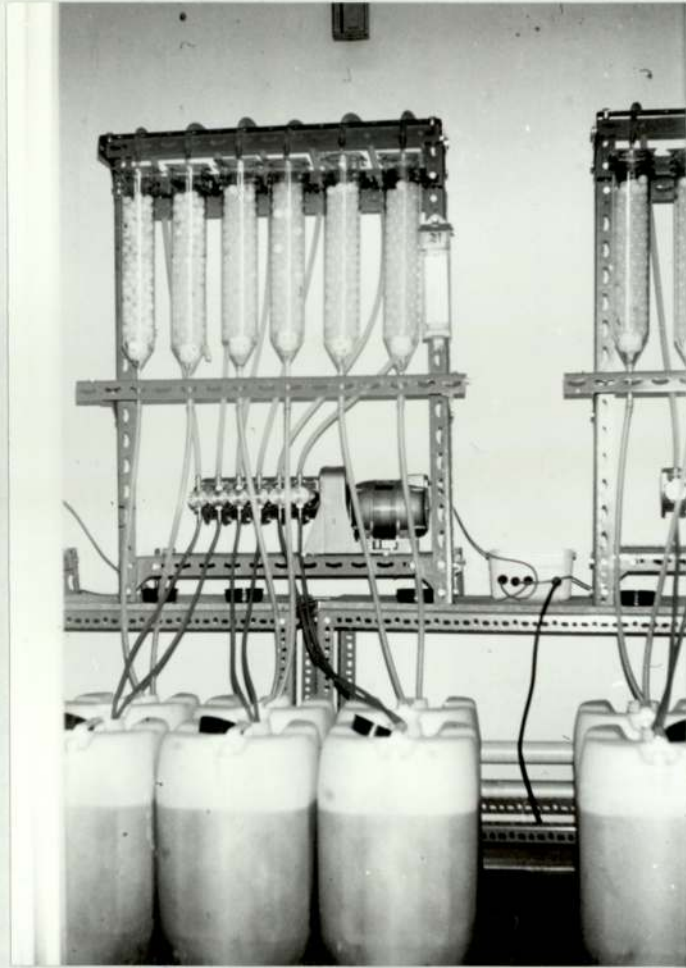


Plate 19. Feed and draw system and associated pipework of laboratory scale filters. Vessels in the foreground contain feed and those in the background contain effluent.

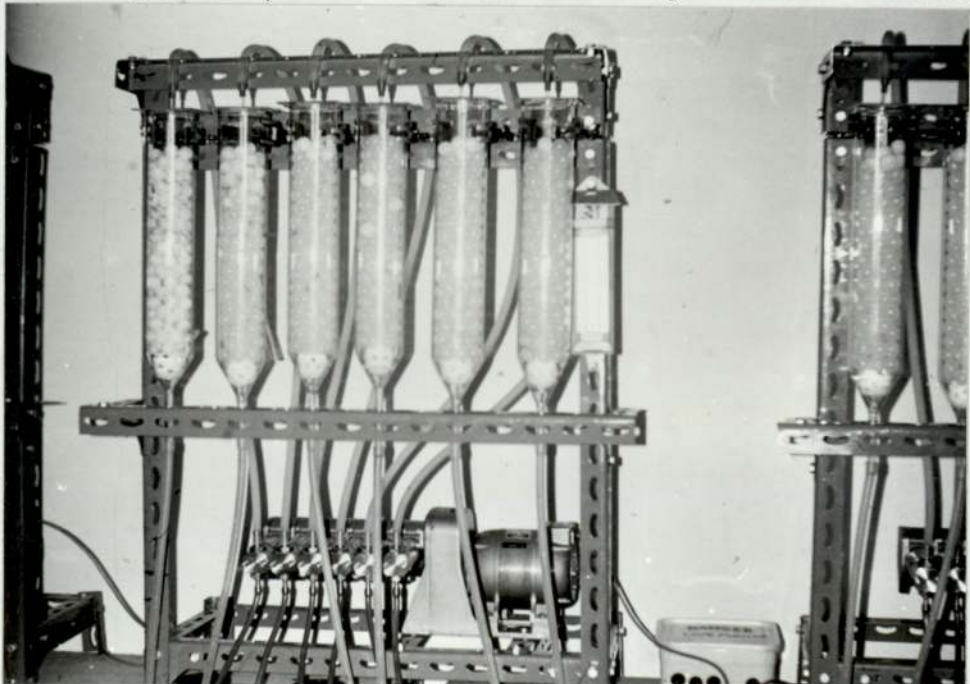


Plate 20. Laboratory filters with sticky plate fly trapping system

filter some 300 mm high and 63.5 mm in diameter. Each filter was fitted with such a system allowing flies to leave the filter, breed in the enclosed space above the filter and return to the filter to lay eggs. This system in place on a framework of 6 filters can be seen in Plate 22. Dosing of the sewage to the filters was achieved using a polypropylene "T" piece with one limb blocked set vertically on its side passing through an aperture in the net cover (see Plate 23). Thus dosing was carried out uninterrupted by the fly trapping apparatus throughout the experiments. Observations of the number and types of flies were taken daily during phase two of the work, flies counted were those in the nets above the filters and those on the top of the surface media. All results were taken in triplicate.

IV Effluent sampling and analysis

The effluent was collected from these filters for various analyses during both phase one and phase two of the experimental work. The method of effluent collection was very simple as all filters were completely isolated from their neighbours and their design allowed easy access to the specific tubes. Waste tubes from each filter were directed into separate 500 ml containers. The operation of the filters was completely unaltered by effluent sampling as the effluent tubes could simply be moved from the large effluent collection containers and placed in the sampling containers when sampling was carried out. The length of the period of sampling was sufficient to allow filling of a 500 ml container from each filter at the specific hydraulic loading of $0.82 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$ i.e. 159 minutes. This procedure was followed for the analysis of effluent during phase one of the experimental work. The analysis carried out was confined to suspended solids determinations and the method followed was that described in H.M.S.O. (1972). The reason for investigating suspended solids levels was to observe any fluctuations that may have been apparent before and after subjecting film laden filters to grazing, thus sampling was carried out before and after seedings. This procedure was carried out 3 times to investigate the effects of each seeding during phase one of the work.

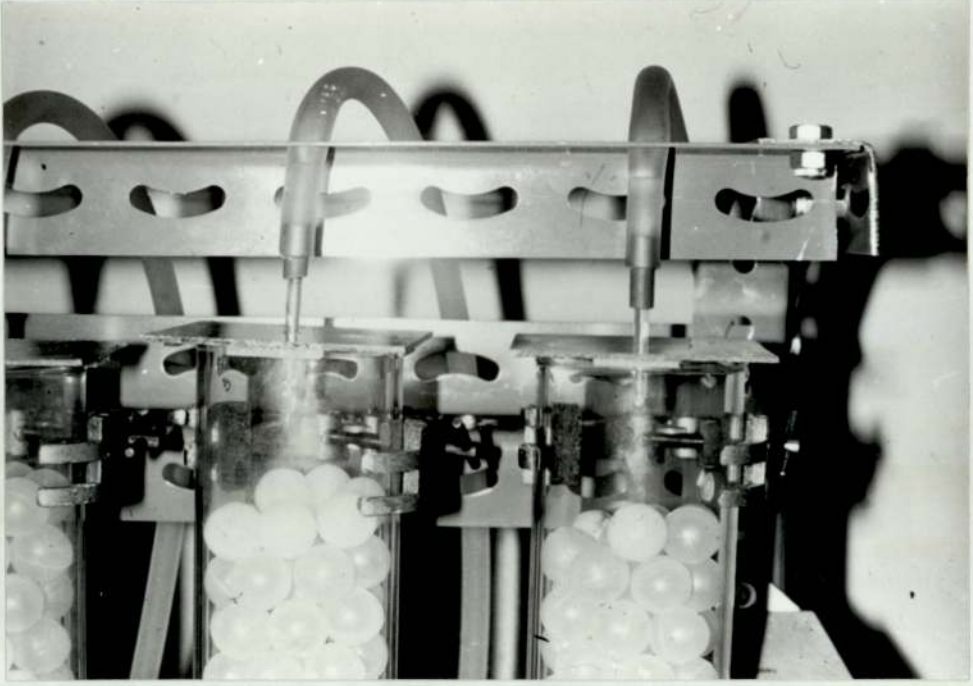


Plate 21. Close up of sticky plate fly trapping system

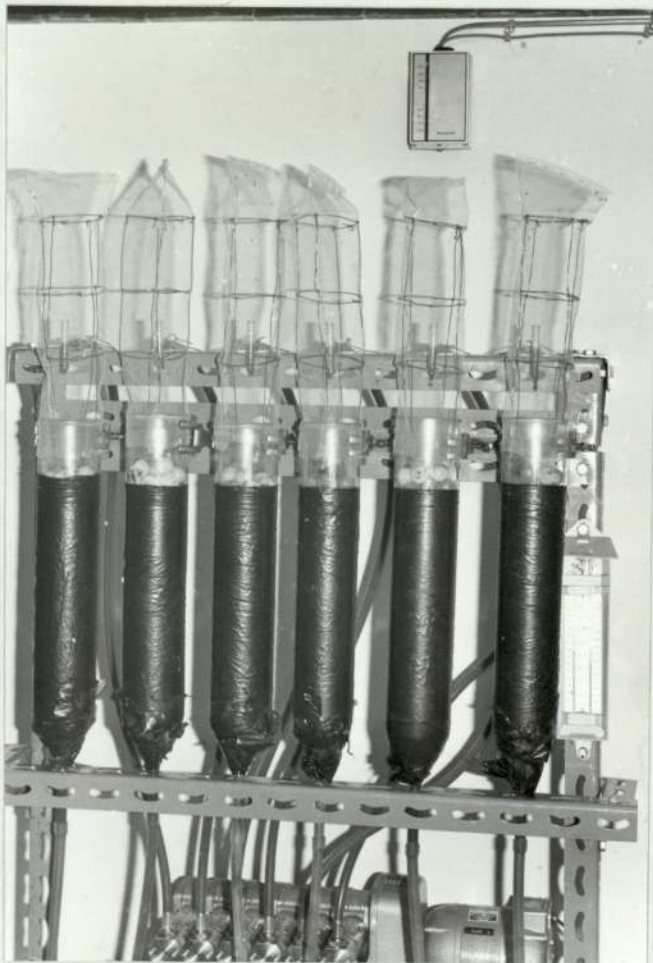


Plate 22. Laboratory scale filters with net fly trapping system

The effluent sampling during phase two of the experimental work was carried out in a similar manner to the above method except that sampling was not carried out in triplicate as a more comprehensive analysis was carried out on these samples and time would not permit analysis of triplicates. Therefore on sampling all of the effluent tubes from each group of 3 were directed into one 500 ml sampling container, thus samples collected were a mixture of each of the 3 triplicates in each group, also the length of the period of sampling was correspondingly reduced to 53 minutes compared to 159 minutes with the previous method as 3 filters were filling 1 sample container. Sampling was carried out to investigate the effects of insecticide application on filter efficiency. This was repeated with a weekly frequency for 6 weeks prior to the filters being subjected to a feed containing 10 mg l^{-1} of Actellic M20. Sampling was carried out for 8 weeks after this time during which the insecticide was added to the filter feed containers at each refilling. Samples of the feed to both sets of filters were also included in the analysis also specific samples of the feed were taken for determination of Actellic M20 levels, firstly when Actellic was added to the feed, secondly after 1 day in the feed container and thirdly after 2 days in the feed container. The procedure for fixing Actellic in the sample was to add 5 ml of cyclohexane and shake the resultant mixture. The sample could then be left without degradation for transit and analysis on gas chromatography equipment (Pye Unicam). The analysis during phase two of the work included determinations of suspended solids, filtered BOD, pH, NH_3 and TON and this was carried out by Severn-Trent Water Authority staff in the divisional laboratories. As before suspended solids, filtered BOD, and pH analyses were carried out using classical methods described in H.M.S.O. (1972). whilst TON and NH_3 determinations were carried out using a chemlab auto analyser.

Toxicity tests

I Introduction to the method

The method used was similar in many ways, except in detail, to the method described

in Green et al. (1975) who subjected enchytraeid worms to a series of industrial pollutants. Basically this method involved subjecting the organisms to the toxicant and observing the organisms for mortality at increasing time intervals, the results of which are plotted on log probability paper. This method is based on the classical method described by Bliss (1937) and subsequently has been used with fish by many workers examples of whom include Lloyd (1960) and Jackson and Brown (1970). Other methods are available for dealing with toxicity data such as the method described by Litchfield and Wilcoxon (1949) however this method was not chosen as it was considered more tedious to process results also the information taken from the results is not always as accurate as with the other method.

The basic method followed involved placing samples of larvae of the particular species being investigated in petri dishes lined with filter paper previously soaked in the insecticide solution and then observing the mortality. Much preparation was needed prior to the start of the experiment and this along with the experimental technique will be described.

II Experimental technique

This involved work in the following areas:-

- (a) Collection and preparation of larvae
- (b) Preparation of petri dishes
- (c) Preparation of bathing and insecticide solutions
- (d) Toxicity test experiment
- (e) Processing of results

(a) Collection and preparation of larvae

The larvae and worms used for the toxicity tests were collected from various works in the divisional area of the water authority depending on which species was being investigated. Samples of the medium were chosen which were particularly well

endowed with the species involved, these were then carefully washed off in tap water into a bucket with as little physical contact with the larvae as possible. The washings were then sieved and washed into a counting tray where they were sorted prior to placing in petri dishes for the experiment. It was attempted to choose larvae in the same instar for each experiment by size relationships. Usually the larvae were collected and washed in the morning ready for the start of an experiment in the early afternoon in order to keep the time between collection and the start of the experiment to a minimum.

(b) Preparation of petri dishes

The containers used in the test were polystyrene petri dishes (53 mm internal diameter of base) lined with Whatman GF/A filter paper (55 mm diameter) as a substratum. Work done by Green et al. (1975) described problems with enchytraeid worms crawling out of the test solution on to the base of the lids, these workers used a barrier of ICI "fluon" P.T.F.E. solution applied around the top rims of the bases. This procedure was followed initially when the bases were inverted and dipped in a 5 mm depth of 10% fluon solution and then dried in a warm oven, however this barrier did not seem to have its desired effect therefore this procedure was dropped after the first batch of tests. It was noticed that the larvae did in fact crawl up out of the test solution but did not remain on the lid, they crawled back into the dish, therefore it seemed that the movement was just normal exploratory behavior. The movement of the worms out of the test solution and remaining on the lid in the case of Green's work may have been due to the worms trying to escape from an unnatural flooded environment because the filter paper was completely soaked and solution flooded over the surface. In the case of this experiment sufficient solution was used to soak the filter paper and none was allowed to remain on the surface. A comparison of the sizes of dishes and the volume of solution used in each case is as follows.

	Size of petri dish (diameter)	Area of petri dish	Volume of Solution used	Theoretical depth of solution assuming no filter paper
Tamworth Expt.	53 mm	2206 mm ²	2 ml	0.90 mm
Green et al. 1975	75 mm	4418 mm ²	5 ml	1.3 mm

Thus in the case of Green et al. (1975) the volume of solution used may have been in excess of that required to soak the filter paper and may have flooded the surface causing the worms to react by crawling out and staying out.

(c) Preparation of bathing and insecticide solutions

In order to minimise physical stresses on larvae an optimum solution for bathing larvae would be one with similar qualities to that found in its natural habitat from the viewpoint of osmotic potential and hardness. Workers in the past have used 0.01% Knop solution for growth and maintenance of worms (Kamemoto and Goodnight 1956) and also a Knop solution for a basic solvent for all insecticide dilutions in toxicity tests on worms (Whitten and Goodnight 1966). Green et al. (1975) used artificially hardened water as a basis for the test solutions which was the solution used for toxicity tests here. It was decided to use a constant hardness of 250 mg l⁻¹ of calcium carbonate throughout the experiments which compares well with the figures obtained for the average calcium hardness of the water supply in the Tamworth area for 1977 which was 242 mg l⁻¹ with a range of 224 — 270 mg l⁻¹ (South Staffordshire Water Works Company figures).

The 250 mg l⁻¹ calcium carbonate solution was used as a basis for all insecticide dilutions and was prepared from 3 stored stock solutions which were made up as follows:-

Solution A	CaCl ₂ ·2H ₂ O	530g)) in 2l
	NaCl	47g)	
	NaNO ₃	12.5g)	
Solution B	MgSO ₄ ·7H ₂ O	234g)) in 2l
	Na ₂ SO ₄ (anhydrous)	125g)	
Solution C	NaHCO ₃	105g	in 2l

The solutions above are all made up in 2l of distilled water and are stored in darkened bottles.

If 1ml volumes of solution A and B are mixed with 5 ml of solution C and made up to 1l with distilled water the resultant solution will have a total hardness of 250 mg l⁻¹ as CaCO₃. Different hardnesses may be prepared by varying the volumes of the solutions A, B and C or by adding the solutions to a volume of water of known hardness.

The insecticide dilutions were prepared from a concentrated solution of Actellic M20 containing 2×10^5 mg l⁻¹ of the active ingredient pirimiphos-methyl. For all dilutions a large initial volume of the concentrate was used to minimise error, e.g. 10 ml of concentrate was added by syringe to a volumetric flask and topped up to 1 litre with hardened water, the resultant solution contained 2000 mg l⁻¹ of pirimiphos-methyl. If 10 ml of this solution was added to another volumetric flask and topped up to 1 litre with hardened water the resultant solution contained 20 mg l⁻¹ of pirimiphos-methyl which was a useful concentration to base any further dilutions. Fresh dilutions were used for each experiment and the concentrate was stored in a refrigerator in the dark as Actellic M20 is liable to degradation by many methods including photolysis.

(d) Toxicity test experiment

20 larvae were placed on the filter paper in each petri dish which had previously been moistened with 1 ml of 250 mg l^{-1} calcium carbonate solution. Altogether 60 larvae were used for the control and 60 for the specific insecticide concentration at each concentration used i.e. triplicates of dishes containing 20. Prior to the start of the experiment the larvae in the dishes was placed in an incubator at 15°C (the temperature at which the experiments were to be carried out) for approximately 1 hour to acclimate to the conditions.

At the start of the experiment (time 0) a further 1 ml of the 250 mg l^{-1} calcium carbonate solution was added to the control dishes and 1 ml of the specific insecticide solution (twice as strong as the final concentration required, to compensate for the 1ml of solution already present in the dish) was added to each experimental dish. The larvae in the dishes were returned to the incubator and they were removed and scored for mortality at semi-logarithmic time intervals which were usually 25, 45, 70, 125, 200, 350 and 600 minutes etc from time 0. The actual time sometimes varied slightly in practice.

Initially a number of toxicity tests were repeated using dried film as a substrate including the filter paper. The reason for this experiment was to investigate the effects of film on insecticide uptake by the larvae. The procedure was exactly the same as for the tests describe above, both control and experimental dishes were used with 20 larvae per dish in triplicate, the exception was that 1 gm of dried film was added to each dish before the hardened water was added.

The procedure for the preparation of the dried film was as follows. A basket of media was removed from a primary filter on the works and the separate pieces of media were scrubbed with a stiff brush in a small volume of water. The washings were then agitated and transferred to evaporating basins which were left to dry

* Another method used for treating the results was that described by Litchfield and Wilcoxon (1949). This involved calculating the percentage mortality results in 24 hours (from the log/probability plots) and plotting these against their respective concentrations on log/probability graph paper. From these plots and the nomographs contained in Litchfield and Wilcoxon (1949) 24 hour LC50's with 95% confidence limits could be calculated.

overnight in an oven at 105°C. The resultant dried film was removed from the dishes and thoroughly pulverised using a pestle and mortar after which the resultant powdered film was stored in an air tight container.

The method of measuring mortality was to note the response of the larvae to a stimulus. The stimulus was repeated gentle prodding with a seeker and the positive response was considered to be an avoidance reaction. The interpretation of death was difficult due to the nature of the insecticide used which is known to affect the nervous system causing twitching and muscular spasms which continue after the positive response has ceased. Therefore it was considered that a true positive response was that if a larva gave an avoidance reaction to successive stimuli (this was scored 0), if the larva reacted to the initial stimulus and not to successive ones it was scored 0.5 and if the larva would not react to the prodding stimulus it was scored 1.0. In some cases in the latter situation the larva continued to twitch but they were considered dead and scored 1.0. These interpretations of mortality were used for all species to give comparisons however it must be noted that different species give different magnitudes of response and in some cases needed to be detected with the aid of a binocular microscope using X30.

(e) Processing of results

The results obtained for the mortality of experimental larvae were corrected for any deaths which may have occurred in the control larvae by subtracting the control score from the experimental score. These results were then averaged and treated graphically according to the method* of Bliss (1937). This involved plotting the cumulative percentage mortality against time for each concentration on log/probability graph paper. From the resultant graph which is usually a series of straight lines it was possible to read off the median survival time for the larvae at each concentration and these values were plotted against their specific concentrations on log/log graph paper, the resultant graph being a curve which

becomes nearly asymptotic to the time axis. From this graph it is then possible to read off the 24 or 48 hour LC50's which are the units quoted in this type of work.

Miscellaneous experimental methods and method development

I Suction fly trapping

Investigations were carried out into the diurnal emergence of sewage filter flies at 1 hourly intervals throughout 24 hour periods using an insect suction tap designed by Taylor (1951).

This trap which was also used by Hawkes (1961a) consisted of a 229 mm diameter Vent Axia fan mounted on a framework underneath which was mounted a gauze cone terminating in a perforated collection tube (see Plate 24). The flies caught were sucked into the trap and down into the collection tube. The trap of flies throughout 24 hours was segregated into hourly catches by means of a solenoid operated release mechanism beneath the fan which caused a disc to drop down an axial rod at hourly intervals thus isolating the previous hour's catch. These discs were covered on one side with cloth which was soaked in pyrethrin prior to the start of the experiment to prevent trapped flies escaping from one sample to the other or up to the cone. Pyrethrin solution was poured into a semi circular perspex trough and the discs were revolved on their carrying spindle in the solution to soak them thoroughly. The discs on their carrying spindle, the perspex trough and the collection tube can be seen in Plate 25. The discs were then replaced in their collection tube which was inverted on the axial rod emerging from the bottom of the trap. The discs were then loaded in the top of the trap by pushing them upwards with a metal tube, then the collection tube was secured, the base fitted and the trap was ready for use.

The interval of disc dropping was variable as a standard Sangamo Weston short interval time was used, in practice the interval was set at 1 hour and 24 discs were used. At the end of the trapping period the collection tube containing the discs was

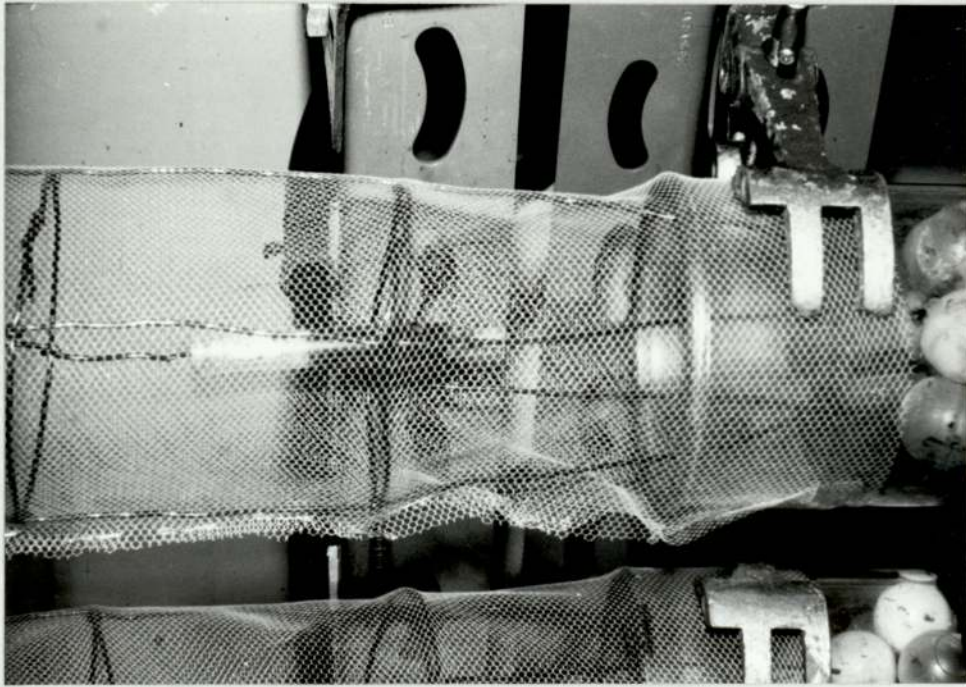


Plate 23. Close up of dosing system within net covers

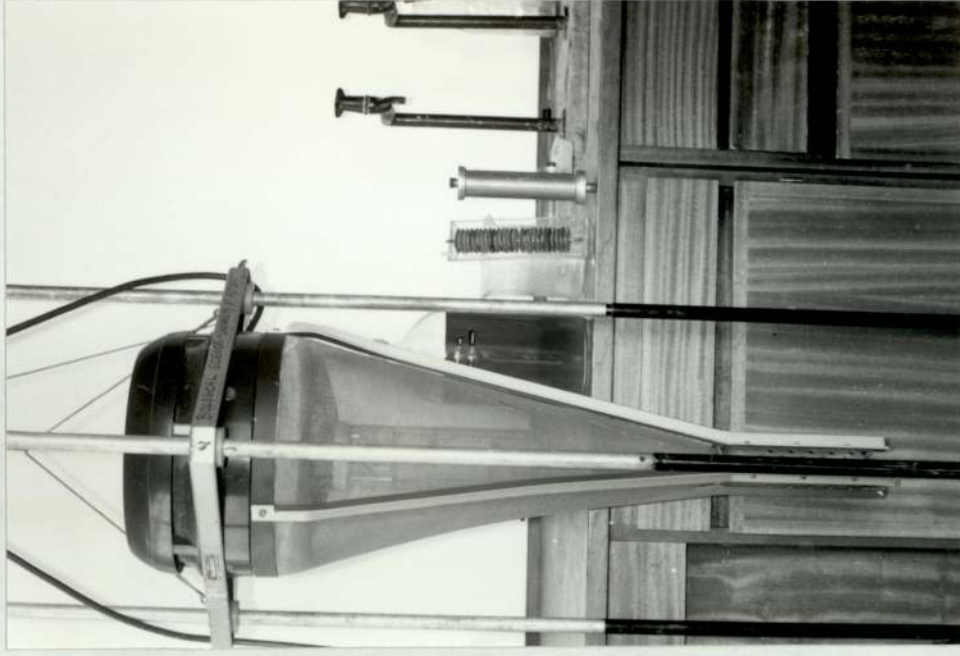


Plate 24. Suction fly trap

removed and the flies caught in each hour were counted.

The trap, when in use was sited at a corner of the administration block at the works where M. hygropetricus frequently swarmed during peak emergence times. This was situated some 137 m from the nearest biological filter. Altogether 3 runs were attempted with this apparatus, 2 of which caught enough flies to give useable results, this was obtained during peak fly emergence times on the main filters.

II Preservation of specimens

Certain samples of specimens were retained for reference throughout the duration of the project. These included adults, pupae and larvae of common filter flies and miscellaneous specimens such as various springtails and worms. The method for preservation of these reference specimens was as follows. In the case of larvae and pupae a similar method to the one described by Bryce and Hobart (1972) was used whereby specimens were killed by immersing in boiling water and then stored in a solution of 70% methanol containing a small amount of glycerine. If close examinations of larvae were required the head capsule was softened by dipping in boiling potassium hydroxide solution for a few seconds and the larvae were mounted ventral surface uppermost on a slide with polyvinyl lactophenol as a mounting medium.

In the case of adult flies a similar method was utilised. They were killed and stored by immersing in a 70% methanol and glycerine solution. The reason for boiling larvae prior to killing was to prevent the gut contents discharging which is found when live larvae are placed in methanol. No such problem was noted with the adults therefore it was considered that boiling was unnecessary. If close examination of the mouthparts or genitalia was required the method of killing and preserving described by Colyer^{and Hammond} (1968) could be used. This involved killing with ethyl acetate which leaves the flies limp for a while (unlike the effects of methanol) and leaves the mouthparts

and genitalia exposed, thus in this condition the flies could be mounted by the method of celluloid dissolved in amyl acetate as described by Colyer^{and Hammond} (1968) and further examination would be possible.

III Culturing techniques

Prior to the start of the toxicity tests already described it was attempted to investigate culturing techniques for M. hygropetricus, P. alternata and P. severini to find favourable conditions for development. Other workers have investigated culturing techniques e.g. Lloyd et al. (1940) who used a whole range of insects including P. severini, P. alternata, O. minimus, M. longitarsus, M. hirticollis and O. perennis and Hawkes (1951a) with S. fenestralis. In the case of Lloyd et al. (1940) cultures were kept in jars on pads of waterlogged cotton wool and the food provided was surface film growth from the filters which had been scalded and kept aerated until its tendency to putrefy was reduced, a whole range of temperatures were used. Hawkes (1951a) cultured S. fenestralis larvae from eggs on filter paper resting on wetted absorbant cotton wool in petri dishes. The food used in this case was sterilized centrifuged activated sludge. The pupae and adults in this case were cultured in 50 x 25 mm (2 x 1 in) glass tubes containing moist cotton wool. The method used in this project was similar to the method used by Hawkes (1951a). It consisted of using polystyrene petri dishes (53 mm based internal diameter) lined with moistened Whatman GF/A filter paper (55 mm diameter). Constant conditions were provided by an incubator. In the case of M. hygropetricus mating couples were collected from beneath a swarm of males and they were then transferred to the dishes. When the egg masses appeared the adults were removed and food was provided in the form of film from the main filters which had been boiled and then frozen for easy storage. With P. alternata and P. severini adult females were chosen and placed in the dishes. No problem was found with inducing P. severini to lay viable eggs as it is parthenogenetic however to obtain P. alternata eggs one had to rely that some pregnant females were chosen in the sample of adults, or that both males and females

were chosen allowing mating to take place in the dish. The dishes were incubated at a constant temperature and removed at intervals through the life cycle for observations. Using this method and by keeping food supply at an optimum and not letting the dishes dry out it was found possible to culture these insects through from eggs to the adult stage with no problems of septicity.

IV Timing of insecticide dosage to achieve optimum effect

An interesting development of methods was the timing of insecticide doses in relation to the prevalent life cycle stage present in the main filters in order to obtain the maximum success with a single dose of insecticide. This was calculated not by media sampling which proved to be unreliable but by estimating the filter temperature in relation to the known sewage and air temperature and from this calculating the life cycle time. Thus if the life cycle time was estimated it was possible to calculate from the previous peak emergence time forward and predict the optimum time to dose with insecticide.

The previous peak in emergence was shown by the fly trapping results and it was assumed that eggs had been laid when the peak was receding thus indicating the starting date of the new generation.

The formula utilised for calculating the time needed for life cycle completion according to Uvarov (1931) is expressed as follows.

$$D = \frac{C}{T-K}$$

where D = development time (days)

C = thermal constant for particular species being investigated (day degrees)

T = Temperature ($^{\circ}\text{C}$)

K = Minimum threshold of development temperature for particular species being investigated ($^{\circ}\text{C}$)

Thus from the thermal constant data provided by Lloyd (1937) and Lloyd et al. (1940), (see Table 1) it was possible to predict at what stage the life cycle would be in at any time from a previous reference date knowing the temperature. Using this method insecticide dosing was regulated and the results will be given later.

V Statistical analysis

All of the statistical tests used in this project were carried out on an Olivetti programma 101 desk computer. The specific tests employed which will be described in the results section involved calculations of means, standard errors of means, also 't' tests, analysis of variance and calculation of correlation coefficients.

VI Temperature measurement

Measurements of settled sewage and filter effluent temperatures, minimum and maximum air temperatures and wet and dry bulb temperatures were taken daily from February 1976 to April 1978. A Stephenson's screen was utilised for the observations of minimum, maximum, wet and dry bulb temperatures. The air temperature was calculated by taking the average of the means of the daily minimum and maximum temperatures. Settled sewage and primary filter effluent temperatures were initially taken by suspending a thermometer in a distribution chamber and recording the result at a set time each day. The inaccuracies of this method were appreciated and from October 1977 a temperature chart recorder was installed to record the settled sewage temperature. This consisted of a Rototherm 7 day motion chart supplied with information by a mercury in steel temperature probe which was constantly immersed in the settled sewage.

Measurement of the secondary filter feed temperature was carried out from February

1976 to August 1977 by reading a thermometer suspended in a distribution trough as above, recording ceased in August 1977 as investigations on these filters were complete.

Measurement of pilot filter temperatures was initially carried out by taking the pilot filter effluent temperature daily however as from December 1977 a similar chart recorder to the one described previously was installed with the temperature probe inserted through the filter wall and into the media of one of the filters, thus achieving a more accurate representation of pilot filter temperature and allowing investigations into diurnal variations in filter temperature.

The measurements described above concerning the main filters and air temperatures can be seen in Figs.35 - 37. The primary filter effluent temperature as used in life cycle time analysis can be seen in Figs.48-50 and the pilot filter effluent and filter temperatures can be seen in Fig.82.

Results of experimental work and discussion

I Full scale filters

A The colonisation of filters

1) Micrograzers

The colonisation and succession of micrograzers in newly commissioned filters was studied for a period of 7 weeks starting 20 days from the actual date of commissioning. The results of this study can be seen in Table 8, these results were processed, grouping the organisms into peritrichous ciliates, holotrichous ciliates and flagellates allowing examination of the numbers of these individual groups in relation to time from commissioning (see Fig. 10).

It can be seen from the results shown in Fig. 10 that initially very high numbers of flagellates were found however their numbers dropped sharply some 3 weeks from commissioning and never recovered. The numbers of ciliates (both peritrichous and holotrichous forms) remained low and showed no drastic changes during the period of study. As far as can be determined work specifically concerned with micrograzers on filters has not been well documented, Barker (1942) dealt with their seasonal incidence but not their succession as such. The work in this field seems to have been restricted to dealing with larger organisms and most work on the subject of micrograzer succession has been restricted to the activated sludge venue examples of which have been quoted by Agersborg and Hatfield (1929) and Horosawa (1950) who noted three distinct stages during the development of a good condition sludge. These stages involved a succession from a community dominated by flagellates through holotrichous ciliates to a situation where peritrichous ciliates dominated. The theory behind this succession, according to McKinney and Gram (1956) was explained by the low energy requirements of the flagellates allowing them to dominate early in the succession, however ciliates would dominate later because of their ability to

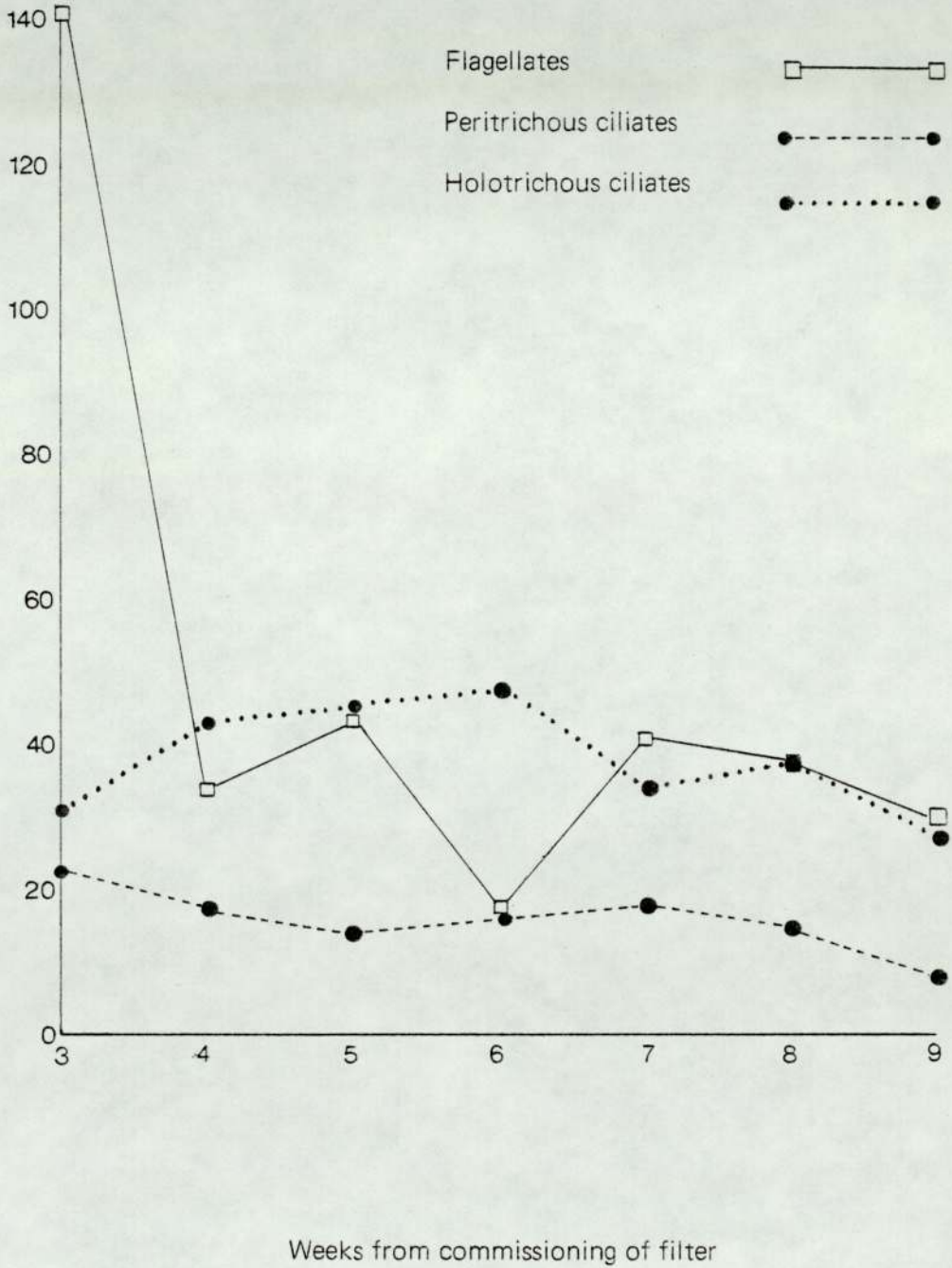
Table 8. Micrograzer population levels on a newly commissioned filter

Micrograzers present in sample	Mean numbers of micrograzers (per 1ml of washings *)						
	from 3 - 9 weeks from commissioning						
	3	4	5	6	7	8	9
<u>Vorticella</u> spp.	800	525	975	1300	1700	1225	775
<u>Colpidium/Glaucoma/Chilodonella</u>	2555	2400	2425	1675	950	1450	1025
<u>Epistylis/Charchesium</u>	2350	2225	1325	1325	875	975	525
<u>Paramecium</u> spp.	150	375	175	700	700	725	475
<u>Litonotus</u> spp.	2200	3975	3975	4075	2575	2375	1725
<u>Amoeba</u> spp.	—	25	—	—	—	—	25
<u>Prorodon teres</u>	—	—	275	750	250	525	525
<u>Coleps hirtus</u>	—	—	400	500	1025	975	625
<u>Opercularia</u> spp.	—	—	—	—	300	150	—
Flagellata	20200	5425	6875	3700	6475	6025	4800
Rotifera	—	25	25	75	25	50	75
Nematoda	875	575	825	1050	1050	850	900

* For details on the washing water and media volumes used refer to the methods section.

Fig. 10. Graph showing succession of protozoa on a newly commissioned filter

Protozoa numbers per
1l of media ($\times 10^5$)

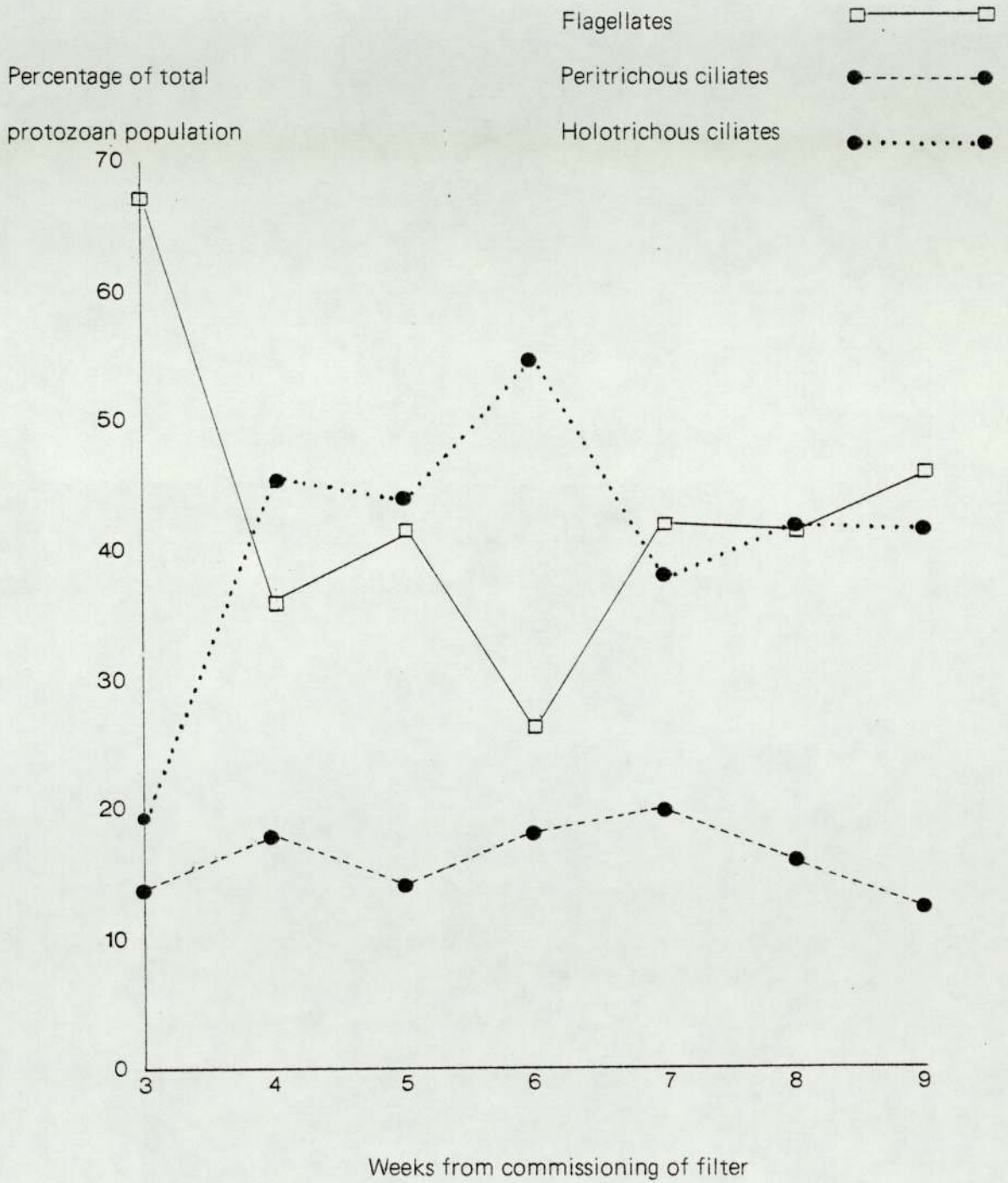


assimilate food more rapidly.

Curds (1966) expressed results of protozoa counts in a succession study as the numbers in each individual group, as a percentage of the total protozoan population and his results showed an initial drop in the numbers of flagellates followed by a peak in the numbers of free swimming ciliates and then an alternation in peaks between attached and crawling ciliates. The theory behind this succession according to Curds (1966) hinged on nutrition being a major factor. More recent work by Curds (1971 and 1973) has simulated these succession curves on a computer by taking theoretical considerations of the growth kinetics, nutrition and settling properties of the organisms concerned and upon the flow rates of an activated sludge plant.

The results shown in Fig. 10 can be further processed in a similar manner to Curds (1966) results and the resultant graph of percentages of each group in relation to the total protozoan population against time can be seen in Fig. 11. It can be seen from these results that the flagellate population was declining when sampling commenced 3 weeks from commissioning and it seemed to level out at approximately 4% of the total population level unlike the case of Curds (1966) where the population of flagellates had almost disappeared after 3 weeks. Also the population levels of holotriches did seem to increase with time however the population of peritriches did not show any definite trends. It should be appreciated here that it is difficult to compare the succession of micrograzers in activated sludge with that in percolating filters, as the two environments are so dissimilar. It would be expected that in activated sludge any succession would occur much more rapidly than in a percolating filter, as in the former the relevant organisms are provided from the time of commissioning via the seeding sludge (when used), however in the case of percolating filters a specific time is needed for film build up and the relevant organisms have to arrive by chance via either the settled sewage feed or some other vector. These facts could explain the lack of any specific trends shown by these results, also it is

Fig. 11. Graph of specific protozoan types as a percentage of the total protozoan population against time



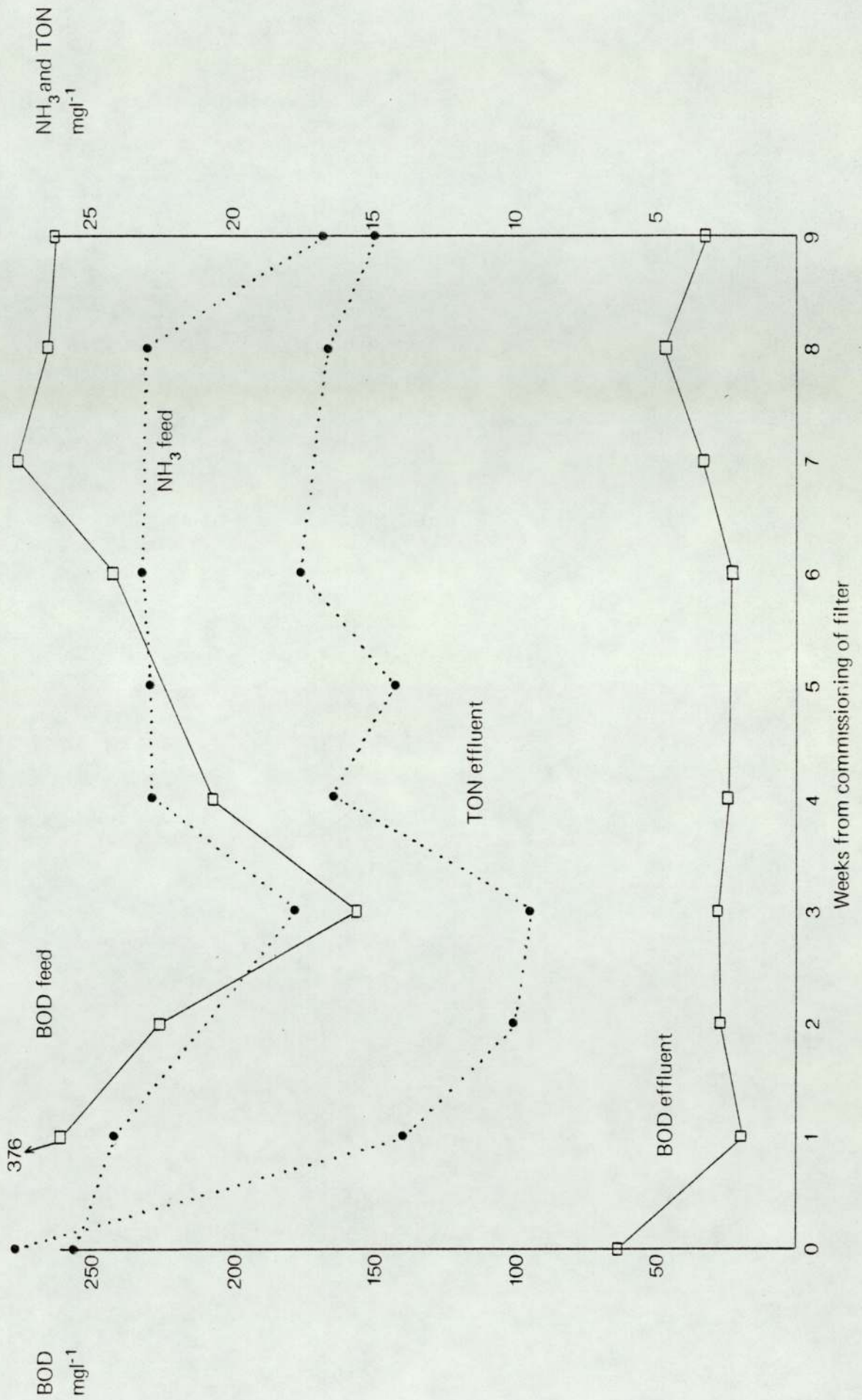
appreciated that a much longer study would be needed to recognise any successions in filters as they take so long to mature, obviously since the last sample was taken 10 weeks from commissioning the filters would be by no means mature.

The reason why sampling was terminated was that the effluent quality declined over the 3 months during the investigation and it was decided to make operational changes to the works. The fact that peritrich numbers failed to rise may be correlated with the production of an unsatisfactory effluent as such a correlation between species structure of activated sludge and the quality of the effluent produced has been suggested by Curds and Cockburn (1970). They found high numbers of flagellates were associated with the production of an unsatisfactory effluent. A strict comparison between activated sludge and filters cannot be drawn here as filter stratification has to be taken into account i.e. flagellates are found in upper half where loads are higher and peritriches lower down where conditions improve however it is not unreasonable to assume that the zone of flagellate domination would increase if purification efficiency declined and vice-versa with the peritrich zone. The purification efficiency of the filter concerned regarding BOD levels in feed and effluent and NH_3 levels in the feed and TON (total oxidized nitrogen) levels in the effluent from October – December 1975 can be seen in Fig. 12 (it should be noted that the figures given for the effluent are those obtained after settlement in humus tanks). The reason for unsatisfactory effluent production was probably due to a combination of the high loading and commissioning the filters at the onset of winter.

(ii) Macrograzers and adult fly emergence

The fact that 4 filters were commissioned at the start of the project gave an opportunity to study the order of colonisation and build up of macroinvertebrate populations on these filters. Macrograzer determinations commenced 3 weeks from the time of commissioning (Oct.9th) and continued until Dec.17th. It is appreciated that a longer period would be necessary to fully investigate colonisation however the

Fig. 12. BOD, NH_3 and TON levels of feed and effluent on single filtration October – December 1975



operational changes referred to earlier forced an early termination to this study.

The first macrograzer to appear some 4 weeks from commissioning were larvae of M.hygropetricus. A few days later 2 species of enchytraeid worms were found being Lumbricillus rivalis^{Levinsen} and Enchytraeus albidus^{Henle} also small numbers of M.hygropetricus pupae were found, representing approximately 10% of larval numbers. The next grazer to appear was the collembolan Hypogastura viatica which were found in small numbers after the fifth week however numbers increased gradually. Two other common grazers Orthocladus minimus and Psychoda severini were both observed on very few occasions in negligible numbers. The early appearance of the macrofauna described is not unusual. Gilcreas and Sanderson (1940) found that the time needed for the appearance of macrofauna could be as short as 50 days and they listed the various means by which macrofauna may be derived i.e. inoculation from neighbouring filters, settlement from the air, in the incoming sewage or via birds. Sources of inoculation in this case may have been provided by the block of 4 filters which were left to dry out when the 4 filters investigated were commissioned. Also it should be mentioned that the 4 filters commissioned were not completely "clean" as they were last in use 3 months previously so it is possible that some residual organisms may have been present at the time of commissioning.

A summary of the condition of the filters 3 months after commissioning as regards macrograzer and film levels compared to the condition of the filters which were used prior to the start of the colonisation investigation can be seen as follows:-

Table 9

	Original filters		Newly commissioned filters after 3 months	
	Mean no. dm ⁻³	% of total population	Mean no. dm ⁻³	% of total population
Enchytraeid worms	93	85.4	5	39.1
<u>M.hygroptericus</u> larvae	12	11.3	5	36.2
<u>M.hygroptericus</u> pupae	4	3.3	3	24.7
Wet solids	5%		28%	
Volatile solids	0.61 gdm ⁻³		1.54 gdm ⁻³	

It can be seen that the overall population structure is very similar in both cases except that a larger proportion of M.hygroptericus were found from the newly commissioned filters which probably reflects the insects' low temperature requirements also it may be due to the absence of large numbers of competitors, such as enchytraeid worms as the progeny of the emergences from the block of rested filters at the start of the experiment were provided with a competitor free habitat which could explain the proportions found.

Adult flies were first trapped using the Solbē type trap 43 days from commissioning, these consisted of M.hygroptericus, P.severini and O.minimus of which in the following weeks M.hygroptericus proved to be by far the most numerous. Of the total number of all three species of flies caught P.severini represented approximately only 0.5% and O.minimus 1%.

Work concerning the order of colonisation of filters by macroinvertebrates has been

carried out by Solbē et al. (1967) giving the results shown in Table 10.

Table 10 Filters commissioned Oct. 1963, first sampling March 1964

<u>Species</u>	<u>Date recorded</u>
<u>Psychoda severini</u> (Diptera)	March 1964
<u>Hypogastura viatica</u> (Collembola)	March 1964
<u>Enchytraeus coronatus/minus</u> complex (Enchytraeidae)	March 1964
<u>Lumbricillus rivalis</u> (enchytraeidae)	March 1964
<u>Metriocnemus hygropetricus</u> (Diptera)	May 1964
<u>Psychoda alternata</u> (Diptera)	June-July 1964
<u>Eiseniella tetraedra</u> (Lumbricidae)	August 1964
<u>Dendrobaena subrubicunda</u> (Lumbricidae)	August 1965 then early 1966

As in this case sampling did not start until 6 months after commissioning a direct comparison is difficult as it is not known exactly when the initial colonisers found in the first sampling appeared. An interesting point here is that in the case of Tamworth filters the first species to appear was M.hygropetricus, whilst in the work of Solbē et al. (1967) P.severini appeared first. One explanation could be that the seasons of the year in which the filters were commissioned has an effect thereby favouring different species.

It was known that adult M.hygropetricus were present at the time of commissioning the Tamworth filters also Solbē et al. (1967) stated that at the time of commissioning their experimental filters, a neighbouring filter only 2 m away supported a diverse fauna. The operating conditions, media and loadings were quite similar in both cases however the 5 minute periodicity of dosing used in Solbē's work may have favoured

P.severini from the onset but it is possible that organisms present in a neighbouring filter would have some influence on the order of colonisation of a newly commissioned filter, however in the long term it is likely that the operating conditions present on a filter will decide the specific macrofauna present and their population levels.

It was clear that after 3 months of sampling the Tamworth filters were by no means mature; Solbē et al. (1967) stated that it may take as long as a few years before a climax community is reached with populations of such animals as earthworms (see Table 10). Another chance of studying the order of colonisation was offered when the original block of filters (4C, 4A, 3C and 3A) were recommissioned in February 1976 as primary filters of a double filtration system. The order of colonisation of macroinvertebrates was recorded during the routine media sampling (see Table 11).

Table 11 **Filters commissioned 9th Feb. 1976**

<u>Organism found</u>	<u>First time recorded in sample</u>
Arachnids	Present before commissioning
<u>Lumbricillus rivalis</u> (Enchytraeidae)	26th February
<u>Enchytraeus albidus</u> (Enchytraeidae)	26th February
<u>Hypogastura viatica</u> (Collembola)	16th March
<u>Metriocnemus hygropetricus</u> (Diptera)	26th March
<u>Leptocera sp.</u> (Diptera)	12th April
<u>Psychoda severini</u> (Diptera)	19th April
<u>Orthocladus minimus</u> (Diptera)	19th April
Coleopterans	26th April
Lumbricids	5th July
<u>Psychoda alternata</u> (Diptera)	9th August
<u>Isotoma viridis</u> ^{Bourlet} (Collembola)	1st September

* Appearance of adult stage of above organisms is recorded.

The early appearance of arachnids and enchytraeids was probably due to their presence before commissioning as shown by samples of media taken at that time. As before M.hygropetricus appeared first, followed by P.severini (in very small numbers) approximately 3 weeks later. P.alternata was not found until August. Again unlike the work of Solbē et al. (1967) M.hygropetricus appeared earlier than P.severini probably due to the inoculation reasons described previously. The reason for the initial appearance of M.hygropetricus followed by P.severini and much later P.alternata may be due to their specific thermal requirements (see Table 1). As the filter was commissioned in February it is likely that M.hygropetricus, a species with a threshold of development of 1°C and a very low temperature mating bar, would be favoured slightly in place of the parthenogenetic P.severini whose threshold for development is 2.2°C . Similarly the late appearance of P.alternata can be explained by their high threshold for development which is 5.9°C and mating bar being 6°C , also it has been stated by Lloyd (1943a) that P.alternata cannot mate successfully at low temperatures.

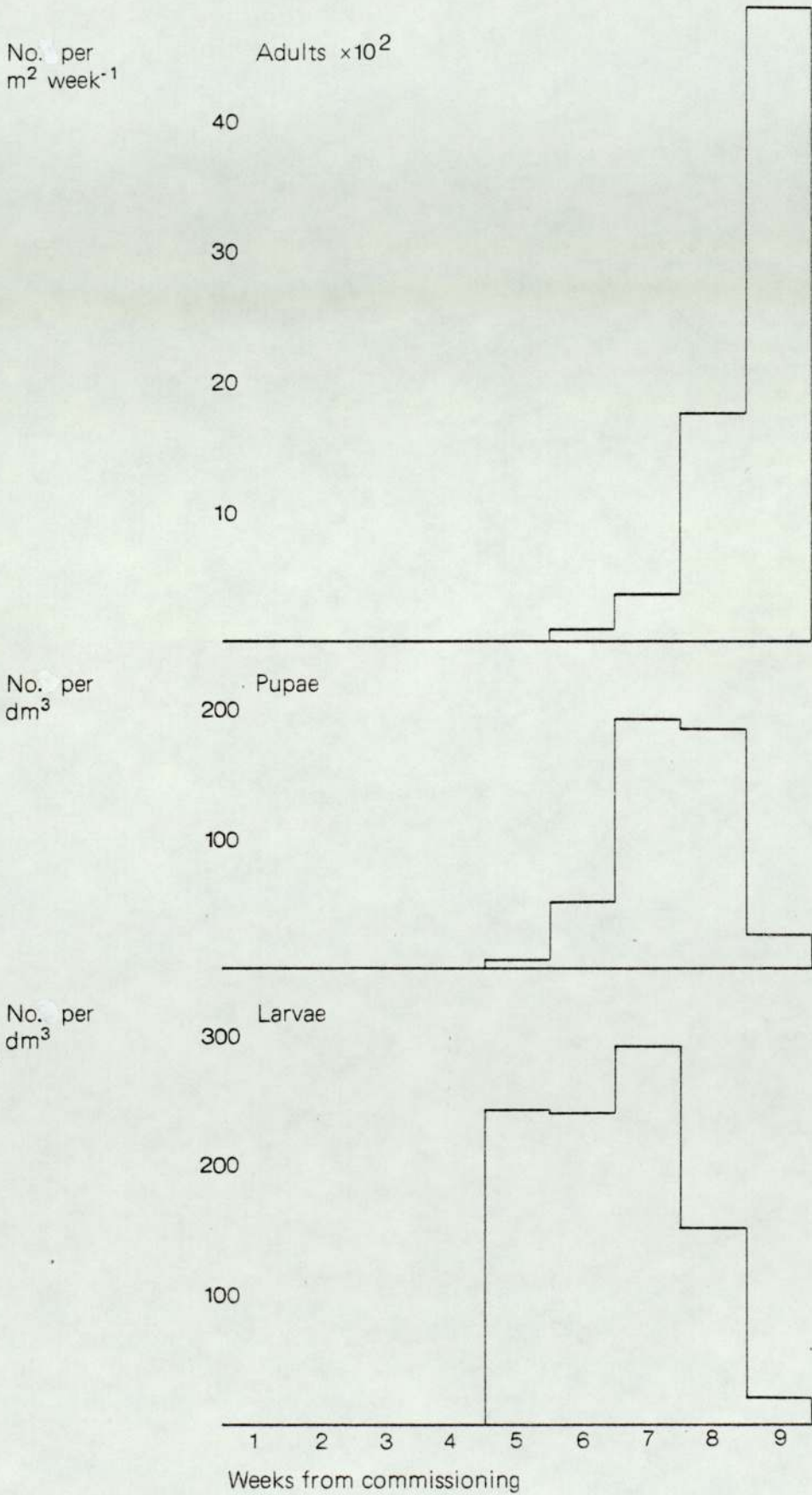
From the results shown in Table 11 it is interesting to note that lumbricid worms took only 5 months to appear on the Tamworth filters compared to 10 months in the work of Solbē et al. (1967). An explanation here could be that either cocoons or adult worms had survived the 4 months resting period in the depths of the filter and had reached sufficient density to appear sporadically in the samples after 5 months. Therefore apart from slight differences most likely due to filter conditions before commissioning, and temperature, these results compare favourably with the results reported by Solbē et al. (1967).

The first 3 months colonisation results showed some interesting trends as regards the relative abundances of different life cycle stages of one particular species namely

M.hygropetricus, these are shown in Fig. 13. This shows well an initial peak in larval numbers followed by a peak in pupal numbers in turn followed by a distinct peak in adult fly emergence. This pattern is particularly evident as the generation of flies represented was the first generation to appear on these filters since commissioning. In other situations where mature filters have been studied interferences in this pattern may show up for a variety of reasons including temperature (Lloyd 1941) and competition from other species (Lloyd 1945).

It was apparent from public complaints that a large emergence appeared on December 6th. Similarly a peak in fly trapping was observed in the same week. By considering the thermal requirements for the development of M.hygropetricus (Lloyd et al. 1940) it is evident that the eggs for this generation were laid soon after the filters were commissioned, an obvious source of inoculation would be the drying out filters which were used previous to October 9th. This is apparent from calculation using Uvarov's (1931) development time formula and the thermal requirements of M.hygropetricus (Lloyd et al. 1940). Thus as the thermal constant (C) is 540 day degrees and the minimum threshold temperature for development (K) is 1°C , the time required for the life cycle to be completed (D) may be calculated knowing the temperature of the filter (T). It was found that a useful estimation of filter temperature was to take the mean of the average air temperature over the period of study (see Fig. 14a). According to Bayley and Downing (1963) the temperature within the filter is closely related to the temperature of the applied sewage however it is clear that the air temperature would have some effect on the filter temperature as the filters are well ventilated and mostly above ground level. Also by considering the dosing periodicity at the works which was 15 minutes it is likely that the filter would be affected by both air and sewage temperatures rather than sewage temperature alone as there is a long period of possible cooling between doses. On calculation the mean filter temperature over the period was estimated to be 9.77°C . By substituting the above information in the formula:-

Fig. 13. Relative abundances of different life cycle stages of *M.hygroetricus* during first generation



$$\begin{aligned}
 D &= \frac{C}{T-K} \\
 &= \frac{540}{9.77-1} \\
 &= 55 \text{ days}
 \end{aligned}$$

The period October 9th — December 3-10th = 55-62 days thus it is apparent that eggs were probably laid at the time of or just after commissioning of this block of filters.

The reason for the commissioning of a new block of filters when the project started stemmed from the infestation of the original block of filters with M.hydropetricus over the summer of 1975. It was considered that by commissioning a new block of filters on a fairly high load (which averaged $0.163 \text{ kg BOD m}^{-3} \text{ day}$ over the 3 months) species other than M.hydropetricus may dominate, for example Psychoda spp. as it has been expressed that high organic loading conditions are unfavourable to chironomids (Lloyd 1945, Tomlinson and Stride 1945). However the reason for the apparent domination of M.hydropetricus from the onset probably has something to do with a variety of factors including the media size, dosing frequency and application method on the filters at Tamworth. Terry (1956) stressed that a combination of loading and media size decided the presence or not of certain species in filters also Lloyd (1945) quoted specific conditions ideal for Metriocnemus development which involved a topping media size of 51-76 mm diameter (2-3 in). The media size on the filters at Tamworth is 50-65 mm which seems ideal for Metriocnemus. Concerning the dosing frequency, Hawkes (1955b) used frequencies of 10.5 — 13.75 min. to control Psychoda also Hawkes (1961b) stated that Psychoda could not withstand the high flushing action associated with low frequency dosing thus it is not surprising that in the case of Tamworth filters which were at a dosing frequency of 15 min.

M.hygropetricus thrived due to an absence of competitors such as Psychoda. The application method on the Tamworth filters consisted of straight through jets at 152 mm intervals which according to Hawkes (1959) allowed a balanced fauna to exist by providing alternate wet and dry areas on the filter. No such wet and dry areas were found on the Tamworth filters, the reasons for this difference probably lie in the different operating conditions present in each study. The dosing frequency in the case of Hawkes' (1959) study was 3 min. which would have produced a much more gentle flow on a unit area of the bed as the distributor arm passed than in the Tamworth case of 15 min. Also the jets in Hawkes' (1959) study pointed vertically downwards from the distributor arm on to the filter surface whereas at Tamworth the jets tilted slightly upwards from the horizontal position. These two factors may explain the absence of alternate wet and dry areas on Tamworth filters as the force of the flow on a unit area of the bed was greater allowing the sewage to splash out as it impinged on the surface media, also it had to travel a much larger distance through the air allowing lateral displacement and breakup of the jet of sewage. Thus it is easy to imagine how no completely dry areas were left on the media between the jets thereby failing to achieve the balanced fauna described by Hawkes (1959).

iii) Film levels and filter efficiency

The film levels as determined by volatile solids over the 3 months from commissioning can be seen in Fig. 14b. This shows clearly how film levels gradually increased and then stabilised at approximately 1.6 gdm^{-3} at the end of sampling. This can be explained by the fact that the filters were commissioned in the autumn and that grazing activity, due to the low temperatures was not sufficient to keep film levels from rising. No problem was encountered with excessive build up and ponding which may be a reflection on the effects of the large media size and the low dosing frequency.

Although film levels were rising they did not reach sufficient levels to discourage

Fig. 14a. Sewage, air and mean sewage/air temperatures October – December 1975

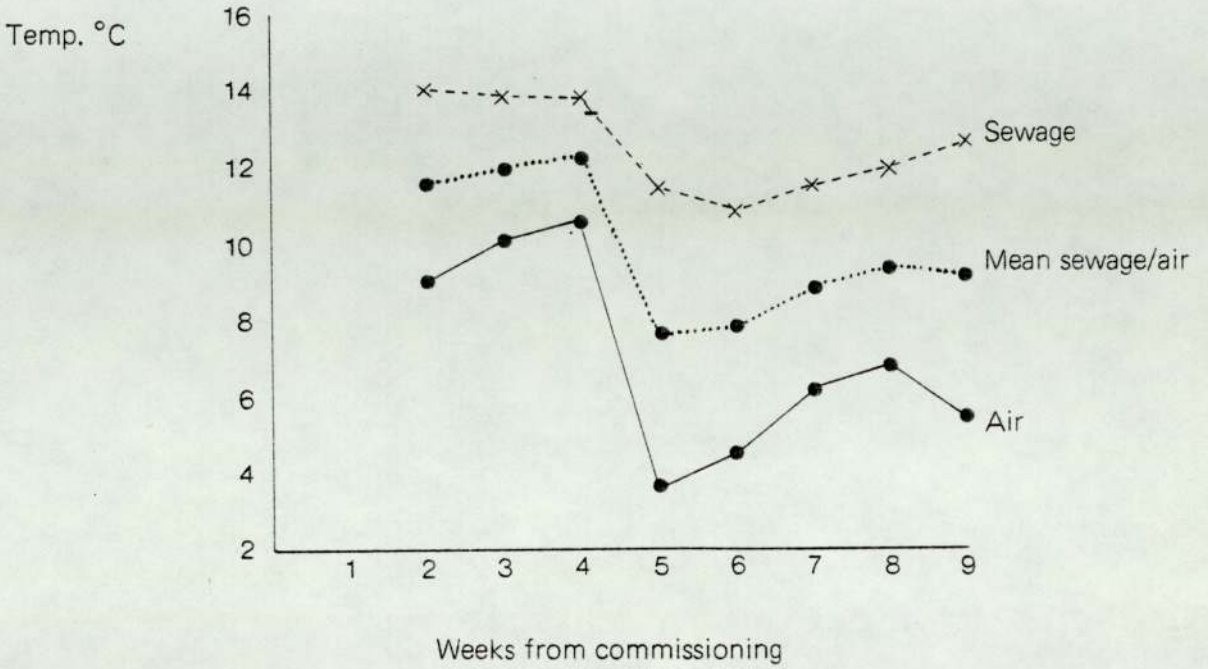
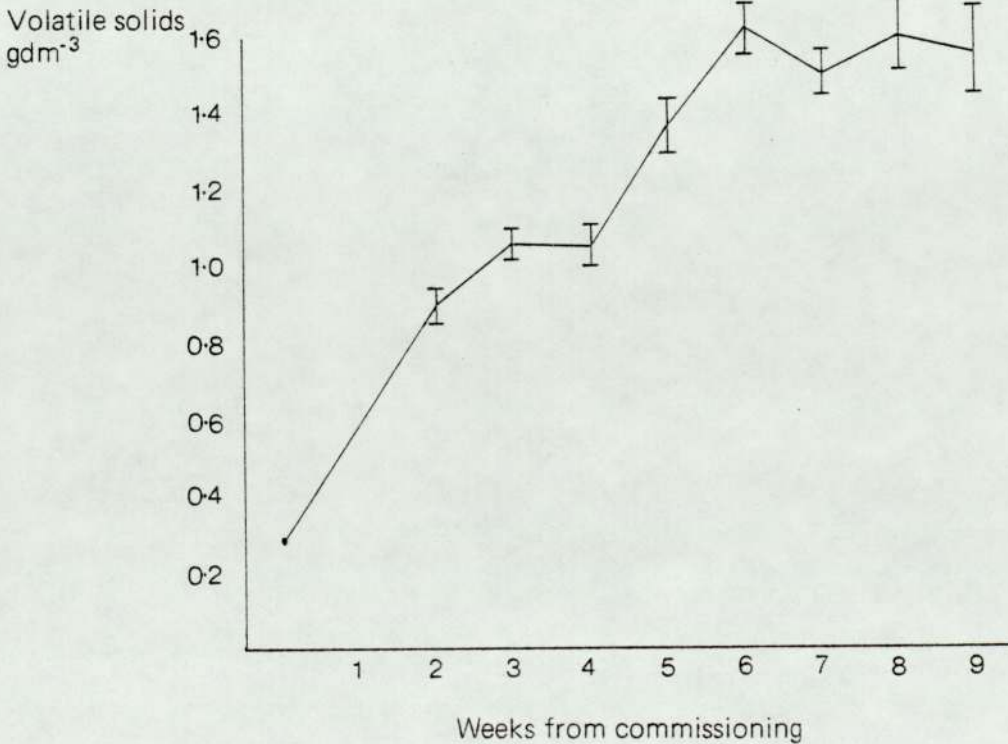


Fig. 14b. Mean volatile solids levels from 4 newly commissioned filters

October – December 1975



M. hygroscopicus as this species thrived during the period of study. It was the view of Tomlinson and Stride (1945) that M. hygroscopicus thrived on clean lightly loaded filters but they were found to survive up to a film level of 3 gdm^{-3} however this has to be looked at in relation to the media size. The size used in their study was 38 mm (1.5 in) diameter pebbles whereas in the case of Tamworth the media is 50-65 mm slag. Thus specific film levels would have different effects on the macrofauna depending on the media size as surface area to volume changes have to be considered. Terry (1956) stated that Metriocnemus preferred "clean" filters, presumably meaning filters whose voidage does not contain too much film therefore as M. hygroscopicus thrived on the Tamworth filters at solids levels over 1.6 gdm^{-3} it is probable that the media size was large enough to retain a sufficient voidage thereby allowing M. hygroscopicus to survive. As the amount of voidage would affect the ventilation this may be a key factor in determining M. hygroscopicus presence. This factor will be dealt with in detail later.

Concerning the build up of filter efficiency work done by Truesdale (Symposium 1960) suggested that newly commissioned filters containing a media of 63.5 mm (2.5 in) slag had matured from a BOD point of view after 5 weeks. Similarly the work of Solbē et al. (1967) using filters which were commissioned in October showed no signs of excessive film accumulation when studied in March of the following year, also although the purification efficiency dropped, as determined by percentage BOD removal during the winter months, it soon recovered in the spring. Therefore if the Tamworth filters were left to operate over the winter it is most probable that efficiency would have increased and film levels would have stabilised or decreased as temperatures increased during spring, so it may have been shortsighted to stop the flow to these filters. One major difference which would have undoubtedly influenced the decision to stop the flow would be that it is obviously more important to do everything possible to maintain the quality of the effluent when there is a large works discharging to a river, as in the case at Tamworth. In the work of Solbē et al. (1967)

it is doubtful whether the effluent quality over winter caused any concern. Also as it was the final effluent sampled and not specifically the filter effluent, the decrease in quality of the final effluent over winter (see Fig. 12) may not be due entirely to the filters, it may have been due to other treatment processes, however as the final effluent rarely reached Royal Commission Standards and a complete block of filters were idle it was decided to use all the filter capacity available as a double filtration system, thus a new batch of experimental work was then carried out on this new system.

B. Fly control by insecticide application

i) Introduction to the insecticide and its application method on filters

The insecticide used on the filters at Tamworth was Actellic M20 (the organophosphate Pirimiphos-methyl in a micro-encapsulated, slow releasing formulation manufactured by ICI Ltd, the chemical structure and formula can be seen in Table 3. This particular formulation contains 200 gdm^{-3} of the active ingredient and consists of micro-capsules each surrounded by a polymeric shell suspended in water. The reason for choosing this insecticide was due to failures encountered with other insecticides in dealing with Tamworth's specific problem, also it is known that Actellic M20 has low mammalian and fish toxicities thereby causing less of a hazard to the environment. It was stated by Thomson (1973) to have an acute oral LD_{50} (lethal dose required to kill 50% of the population) of 2050 mg Kg^{-1} against albino rats. Similarly Woods et al. (1976) quoted 48 hr. TLM's (median tolerance limits) of 1.4, 0.5 and 0.25 mg l^{-1} for common carp, brown trout and rainbow trout respectively. As far as can be established Actellic M20 had not been used in the percolating filter venue prior to the work at Tamworth where it was first used in 1975.

It was applied to 3 of the 4 filters in both the primary and secondary blocks (4C, 3C, 3A and 2C, 1C and 1A). This left one filter in each block (4A and 2A) untreated to act as controls. The method of application was to dose the settled sewage in a distribution chamber directly above the filters at a rate of the concentrate to give a concentration of 5 mgdm^{-3} of the active ingredient in the settled sewage, this was carried out by means of a peristaltic pump. Treatment was carried out for 1 hour on each block of filters and taking into account the dosing frequency this gave the whole filter area 4 complete doses with the insecticide.

Initially when field trials of the insecticide were being carried out one block of filters was dosed and the system was isolated and recirculation was maintained for 7 days whilst the main flow was switched to the other block of filters. This procedure was followed as it was not sure what levels of the insecticide would reach the river. Work of Woods et al. (1976) investigated the fate of Actellic M20 in the filter system at Tamworth and it was found that of the original 5mgdm^{-3} insecticide concentration applied only 15% reached the filters. It was suggested that this low recovery may have been due to adsorption of this insecticide by solids in the distribution chamber and pipework as it is known that pesticides are adsorbed by humic substances (Khan 1972). The low recovery may also have had something to do with the insecticides instability as it has been shown to have a half life of 6 hours in final effluent (Woods et al. 1976). In addition to this the breakdown of a related compound - malathion, has been shown to occur by many means including photolysis and alkaline degradation (Wolfe et al. 1977). Woods et al. (1978) stated the various means by which Actellic M20 is degraded and these included hydrolysis, volatilisation, photolysis and bacterial degradation , easy degradation was shown by investigating the concentration in film levels some 2 hours after treatment which revealed no detectable concentrations.

In order to elucidate the fate of this insecticide Woods et al. (1976) carried out an experiment which involved seeding humus sludge to give a 5mgdm^{-3} concentration of Actellic M20. It was observed that within 1 hour 28% of the original concentration was recovered in the sludge, only 2% of this value being present in the liquor and after 3 hours only 7% of the original concentration was recovered, all of this being present in the solids. Thus it is easy to appreciate the high affinity of the insecticide to humic solids and due to the low recovery levels in the effluent it was decided that in future treatments with the insecticide no recirculation would be necessary and treatment could continue with no interruption in works operation.

A programme of insecticide treatment was then embarked upon as from February 1976 and it continued throughout the project. In all, over the period February 1976 — June 1978, 22 applications of insecticide were made and the effects of these treatments as regards adult fly emergence, macrofauna population and film levels were observed.

ii) Effects of Actellic M20 treatment on adult fly emergence

The trapping of adult flies was carried out from February 1976 to July 1978 using the traps designed by the author (see Plate 7). Up until July 1977 trapping was carried out in triplicate on the primary and secondary filters however after this time the traps were removed from the secondary filters and intensive trapping using 5 traps per filter resumed on the primary filters. The results of these trappings expressed weekly can be seen in the following figures.

Primary filters:-

<u>Filter no.</u>			<u>Year</u>		
			<u>1976</u>	<u>1977</u>	<u>1978</u>
4A	Control	Fig.	15	16	17
4C	Insecticide treated	"	18	19	20
3C	" "	"	21	22	23
3A	" "	"	24	25	26

Secondary filters:-

<u>Filter no.</u>				<u>Year</u>	
				<u>1976</u>	<u>1977</u>
2A	Control	Fig.	27	28	
2C	Insecticide treated	"	29	30	
1C	"	"	31	32	
1A	"	"	33	34	

(a) Primary filters

By comparing the control filter (Fig.15) with the insecticide treated filters (Figs.18, 21 and 24) during 1976 it can be seen that M.hygroptericus emergence levels were reduced on all of the treated filters in relation to the control, conversely P.severini levels on the control filter were lower than those on the insecticide treated filters. The mean percentage reduction of M.hygroptericus on the treated filters compared to the control was calculated to be 75.2%.

In 1977 (Figs.19, 22 and 25) it can be seen that a slight reduction in M.hygroptericus levels is evident on the treated filters in relation to the control (a mean reduction of 59.1%) however the reduction was not so drastic as in the previous year. Similarly levels of P.severini on the control filter were lower compared to the levels found on the treated filters but again the reduction found was not so great as in the previous year.

In 1978 (Figs.17, 20, 23 and 26) M.hygroptericus levels did build up to a higher level earlier on the control filter compared to the treated filters however the whole of the fly season could not be studied as trapping terminated in June, therefore a true reduction in M.hygroptericus levels could not be calculated as in order to be comparable to previous years' results a whole seasons' results would be needed. As

regards P.severini levels no obvious differences could be seen between control and treated filters.

Statistical analysis consisting of unpaired "t" tests were carried out on the results shown in Figs. 15-26 to investigate if any significance could be attached to the reductions in M.hydropetricus numbers found or the increases in P.severini numbers found on the treated filters. Mean monthly fly emergences calculated from the results shown in Figs. 15-26 were used for the purposes of the tests, the results of which are as follows.

Period Feb. 1976 — Jul. 1978

M.hydropetricus

Filter	Mean weekly emergence per m ² (X10 ²)	Paired 't' test Control v. treated filters		
		't' value	'P' value	Significance
4A Control	3.1 ± 0.7	—	—	—
4C Treated	1.3 ± 0.4	2.69	0.1-1%	VHS
3C "	0.8 ± 0.3	3.32	0.1-1%	VHS
3A "	1.3 ± 0.3	2.77	0.1-1%	VHS

P.severini

4A Control	1.0 ± 0.1	—	—	—
4C Treated	2.9 ± 1.4	-1.41	>10%	NS
3C "	1.4 ± 0.4	-1.01	>10%	NS
3A "	2.7 ± 0.9	-1.97	5-10%	NS

± = Standard error of mean (S.E.M.) No. observations = 109

Thus it can be seen that treatment with the insecticide caused a significant reduction in M.hydropetricus levels on all filters (4C, 3C and 3A) and this reduction was shown to be highly significant on one filter (3C). As regards P.severini levels slight increases were found on the treated filters however on analysis these were not

significant. This could suggest that P.severini numbers were allowed to increase when M.hydropetricus were removed on treatment due to reduced competition pressure. These results also show that Actellic M20, when used in the concentrations used to control M.hydropetricus, does not show any detrimental effects on P.severini. By consulting Figs. 15 - 26 the results suggest that the treated filter P.severini levels were in excess of control filter levels only during the first year of operation (Figs. 15, 18, 21 and 24), during 1977 and 1978 the P.severini levels seemed fairly similar from control and treated filters. The reason for this differential effect is not clear however the season of the year during which the filters were commissioned may have had some bearing on the large numbers of P.severini found in the first year. Certain workers (Lloyd 1937, Lloyd et al. 1940 and Solbe et al. 1967) have expressed the season during which P.severini seems to be prolific as February/March — June (see Table 2), thus it is possible that the high numbers of P.severini found during the first year may be due to the fact that the filters were commissioned in February at the onset of P.severini's prolific season allowing development of this species in the absence of large numbers of competitors. The drop in P.severini numbers over subsequent years may have been due to the populations of other grazers becoming sufficiently established over the years to compete with and check numbers of P.severini.

The apparent ineffectiveness of insecticide treatment on P.severini populations may be explained by two possible reasons, or a combination of both. Firstly it has been shown that P.severini larvae reside in greater numbers at a sub-surface level in the filter bed (Hawkes and Shephard 1972). This was borne out by basket sampling which was carried out to a depth of 200 mm and very rarely revealed any P.severini larvae even though the adults were caught occasionally. It was possible that the insecticide, which has an affinity for humic substances (Khan 1972) was adsorbed by the film on the upper layers of media and never reached the P.severini larvae in sufficient quantities to reduce their numbers. Secondly a given concentration of Actellic M20

may be not as toxic to P.severini as it is to M.hydropetricus. It has been expressed by Laverack (1963) that lumbricids can tolerate low concentrations of organophosphate insecticides as their body wall tissue contains large amounts of cholinesterase, thus a large amount of the toxicant is needed to tie up the unusually large concentration of cholinesterase. Psychoda larvae may have amounts of cholinesterase in their body wall however further investigation is necessary to prove this.

There are no obvious physiological differences regarding nervous transmitter substances between Metriocnemus and Psychoda to explain their possible different responses. One morphological difference which may have some bearing may be due to the apneustic nature of the respiratory system of Psychoda, i.e. being closed except for the posterior siphon. However with Metriocnemus, which has open spiracles, it is possible that the insecticide would be assimilated more easily as it is considered to be a contact toxicant.

The reasons for the previous mentioned differential M.hydropetricus reductions in 1976 and 1977 (75.2 and 59.1%) is probably due to problems encountered with the insecticide. During the period June — October 1977 the M.hydropetricus emergences on the treated filters (see Figs. 19, 22 and 25) were rising rapidly with each emergence peak and by October they had in all cases exceeded M.hydropetricus emergence levels on the control filter (see Fig. 16) and continued to do so until January 1978. During this period the form of the insecticide changed from its usual liquid suspension to a semi-solid gel which was very difficult to pump. Instead of flowing smoothly into the settled sewage it flowed in short bursts when the pressure was sufficient to suck up the gel. This gave an intermittent flow of insecticide on to the filter which probably resulted in uneven distribution of the insecticide on to the filters. This fact alone may have explained the rapid rise of M.hydropetricus levels on the treated filters however it was also suspected that the insecticide, in this form,

had a reduced toxicity.

The fact that M.hygropetricus levels, when unchecked after doubtful treatments rose above the control level is not easy to explain. It may be due to increased film levels and therefore food supply on the treated filters (as a consequence of removal of a component of the grazing fauna by previous successful treatments) allowing a build up of M.hygropetricus in excess of control levels however as will be described later no significant increases were found in the film levels on treated filters compared to control, therefore this reason is unlikely. A more plausible explanation, though much more difficult to prove was that treatment with insecticide was sufficient to remove a high percentage of the population however it is likely that more hardy and in some cases resistant members of the population may have survived whilst treatment was fairly successful and when given the opportunity to increase, when the effectiveness of the treatment was suspect, it is possible that the population could have risen to levels above those found on the control filter as shown by the results.

When it was noticed that the form of the insecticide was altered a new batch was ordered but supply problems were encountered and the new batch was not received until late September which was then only sufficient to give a half strength dose (2.5 mgdm^{-3}) to the primary filters and none at all to the secondary filters. As can be seen from Figs. 19, 22 and 25, the half strength dose in September was not sufficient to reduce M.hygropetricus emergence.

(b) Secondary filters

The adult fly emergence from the secondary filters can be seen in Figs.27-34. It is initially apparent that on all four filters there was a peak of P.severini emergence in the spring of 1976 soon after these filters were commissioned. As mentioned in the previous section these filters were used as the main filters on a single filtration system for 4 months prior to their commissioning as the secondary filters of a double

filtration system in February 1976. Thus it is likely that the initial peak of P.severini found during the spring of 1976 consisted of the members of a population which thrived on the high film levels found when the filters were operating on single filtration. Also the drop in P.severini numbers after these peaks may be attributed to the film levels falling from an average of 1.6gdm^{-3} (Fig.14b) found when the filters were on single filtration to an average of approximately 0.5gdm^{-3} during 1976. It has been shown by Tomlinson and Stride (1945) that Psychoda is sensitive to BOD loadings and therefore film levels. They found that Psychoda numbers were directly proportional to the filter loading thus very few were found on lightly loaded filters as was found in this investigation. As P.severini levels were fairly low on all filters after the initial peaks no apparent differences were noted in numbers of this species between control and insecticide treated filters.

A peak in P.severini numbers was found on all filters during the period May — July 1977 which is not easy to explain. Film sampling had ceased by this time thus the emergence cannot be compared to specific film conditions however it is unlikely that film levels varied greatly at this time as the operating conditions during this period were normal.

This peak may have been a result of the weather improving after the generally rough weather conditions apparent during the first few months of the year. During the period January — May 1977 although the mean air temperatures were similar (6.86°C - 1976, 7.33°C - 1977) to the same period in 1976, rainfall was considerably higher and the wind was stronger giving generally unfavourable conditions for fly emergence. The high rainfall can be seen by comparing the total flow to the works over that period in both years as follows:-

Flows $\text{m}^3 \text{ day}^{-1}$

	<u>Jan.</u>	<u>Feb.</u>	<u>Mar.</u>	<u>Apr.</u>	<u>May</u>
1976	15 202	15 670	17 903	14 942	15 143
1977	27 777	32 627	20 780	15 136	19 973

Mean flow (daily) Jan. Mar.

1976	16 258
1977	27 061

Thus it is apparent that the rainfall and therefore the flow was considerably higher in the first 3 months of 1977, in fact the mean of the period Jan — Mar. 1977 was 66.5% higher than the same mean of 1976. In the following months of April and May it can be seen that conditions improved so these facts probably explain the late appearance and sudden peak in P.severini as the bad conditions would probably have caused retarded development thus causing a build up of the species in its longest life cycle stage i.e. the larval stage.

As regards wind velocity it has been shown by Hawkes (1961a) that generally at all temperatures an increase in wind velocity caused a decrease in the aerial density of Sylvicola recorded. Thus it is not surprising that the unfavourable weather conditions found in the first half of 1977 resulted in an inhibition of emergence until the weather conditions were favourable for the first time in May when a large emergence occurred.

Concerning M.hydropetricus emergence from the secondary filters it can be seen by comparing control filter emergence (Figs.27 and 28) with treated filter emergences (Figs.29-34) that generally a reduction of M.hydropetricus emergence was apparent on the treated filters but this was not so great a reduction as was found on the primary

filters. The results were treated statistically and it was found that none of the reductions were significant on the secondary filters. Also by consulting Fig.27 it can be seen that M.hygroptericus did not become successfully established in large numbers even on the control filter in 1976 which may be a result of the low film levels found therein.

It has been stated that M.hygroptericus thrives in clean lightly loaded filters (Tomlinson and Stride 1945 and Terry 1956) however it is possible that the average film levels of 0.5gdm^{-3} were in fact too low in 1976 to allow the small population of M.hygroptericus established to peak in the same manner as described in the case of the primary filters.

A sudden peak in emergence of M.hygroptericus in May - Jul. 1977 was found, similar to that described for P.severini, in fact by consulting Figs. 28, 30, 32 and 34 it can be seen that on all four filters the peaks of M.hygroptericus emergence follow closely those obtained for P.severini. This seems to back up the theory that weather conditions were the main factor in causing this as it is very unlikely that both P.severini and M.hygroptericus would develop to maturity at exactly the same time in 4 separate filters.

As in the case of the primary filters the mean percentage reduction of M.hygroptericus on the treated filter compared to the control filter was calculated. The initial results given are those for the secondary filters, the primary filter results follow in parentheses, 1976 - 69.9% (75.2%), 1977 - 61.0% (59.1%). As before the most successful reduction was achieved during the first year of insecticide treatment, in the second year the mean reduction dropped off by approximately 10%. One of the previous reasons suggested for the primary filters centred round the insecticide consistency altering does not figure in this case as trapping from these filters ceased in July 1977 and the first and only treatment with the affected insecticide was

applied in that month so this suggests that some other factor may have caused the decreased percentage reduction in 1977. The factor concerned here could well be as described for the primary filters in being the selection of disease free more hardy populations by means of regular insecticide dosing over the previous year, or more importantly resistance to the insecticide could well have been building up by means of selection of possible resistant genes in the population.

It is generally accepted that treatment with insecticide is not so effective on filters with high film accumulations (Tomlinson et al. 1949, Hawkes 1951a, 1955c and 1957) however the mechanisms behind this effect were not explained. It is likely that it is due to the high film levels removing a quantity of the insecticide applied by the process of adsorption or absorption, or a combination of both, thus giving a lower effective concentration beneath the surface. It should be stated that in these cases the film levels reported were much higher than the levels encountered in this study.

If the mean percentage reduction of M.hygropetricus results are looked at it can be seen that a higher reduction was obtained on the primary filters than the secondary filters in 1976 and both reductions were fairly similar in 1977. The reason for these effects is not clear but it seems that film levels do have some bearing on these results as in 1976 film levels on the primary filters were generally much higher than the secondary filter levels however in 1977 up until sampling ceased film levels on the primary and secondary filters were fairly similar in being around the 1.5gdm^{-3} level (see Figs. 42 and 47). However clearly the argument that the effectiveness of the control decreases with film accumulation cannot be put forward here as the opposite effects were observed. One reason for these results may be that Actellic M20 was acting not just as a contact effective insecticide but was being ingested via the film and thus reaching the larvae via two routes. It should be mentioned here that the quality of the film in both situations was vastly different, the primary filter film was thick, mainly fungal and bacterial therefore containing large amounts of water

whereas the secondary filter film was thin, mainly bacterial and did not have a high water content. Although the grazing habits of larvae are not known it is most probable that grazing activity would be higher on the primary filter. Therefore it is likely that in the case of the primary filters the insecticide was reaching the larvae by body contact as film levels were high also it was probably being rapidly ingested as population levels and grazing activity were high whereas in the case of the secondary filters the film levels were not so high giving less body surface contact of the larvae with the insecticide affected film also it is likely that grazing activity was less for the reasons already described. Therefore considering these factors it can be appreciated how a reduced control was found on the secondary filters.

iii) Effects of Actellic M20 on macrograzer populations and volatile solids levels

The media sampling carried out during the period of insecticide treatment involved investigations into macrograzer population levels and volatile solids levels on 4 filters, one control and one insecticide treated on both the primary and secondary filter blocks. This sampling was carried out from Feb. 1976 — Feb. 1977. The results of the investigations can be seen in the following figures.

Primary filters

	<u>Control</u>	<u>Insecticide treated</u>
<u>M.hydropetricus</u> larvae and pupae	Fig. 38	Fig. 39
Enchytraeid worms	" 40	" 41
Volatile solids	Fig. 42	

Secondary filters

	<u>Control</u>	<u>Insecticide treated</u>
<u>M.hydropetricus</u> larvae and pupae	Fig. 43	Fig. 44
Enchytraeid worms	" 45	" 46
Volatile solids	Fig. 47	

(a) Primary filters

By consulting Figs. 38 and 39 the effects of insecticide treatment on M.hydropetricus larvae and pupae can be seen. It is apparent that generally larval and pupal numbers were lower on the treated filter than on the control. Statistical analysis was applied to these results and the reduction in both cases was found to be significant.

	Mean weekly counts per dm ³	Paired 't' test 't' value	Control v. treated filter 'P' value	Significance
Larvae				
Control	6.1 ± 1.7	—	—	—
Treated	2.3 ± 1.6	4.11	<0.1%	VHS+
Pupae				
Control	2.2 ± 0.8	—	—	—
Treated	0.1 ± 0.1	2.61	1-2%	HS

± = S.E.M. No. observations = 39

Obviously as the insecticide is specifically active against larvae it is hardly surprising that a significant reduction was found however these results indicate that even with regular insecticide dosing the larval population can increase and create a potential nuisance. This can be seen in Fig. 39 where in Aug./Sep. a sudden build up in larval numbers occurred on the treated filter with numbers exceeding control levels which resulted in a peak emergence of adults on the treated filters, (see Figs. 18, 21 and 24). The reason for this build up is quite simple and hinges on the susceptibility of only one stage of the life cycle; the larval stage, to insecticide treatment. It can be seen from Fig. 39 that during the preceding months to the large build up, insecticide dosing was carried out each time just after a peak in the larval numbers. Thus it was likely that when this was carried out larval numbers were falling as the larvae were pupating and emerging as adults. It was established in toxicity test work to be described later that pupae were resistant to the action of Actellic M20, the reason for this probably lies in their mucus ensheathment which would hinder the uptake of the insecticide by contact routes, also they do not graze therefore the insecticide would not enter via oral routes. Similarly it is obvious that adults would not be affected by insecticide treatment as they are not grazers and they quickly move away from the target site when conditions are favourable. Therefore it is likely that the timing of the insecticide dose was not judged correctly to coincide with the peak in

the vulnerable larval stage so this allowed larval numbers to rise steadily with each generation.

After this large peak in September, 2 insecticide doses were applied, the first of which probably had no effect as it was immediately after the peak and most of the larvae would probably have pupated and emerged, however the second one was timed to coincide with the initial development of the progeny of the preceding peak and was most successful in preventing a build up in larval numbers as can be seen by comparing Figs. 38 and 39, where the peak in larval numbers in Sept./Oct. on the control filter cannot be seen on the treated filter. Similarly another successfully timed dose in late October preceded the build up in larvae found on the control filter in November thus preventing a build up in numbers on the treated filter. Thus it can be seen how the concept of correct timing of dosing is most important in insecticide control measures and this subject will be dealt with in more detail later.

It was found that levels of M.hygroptericus larvae, when unchecked by incorrect dosage timing on a treated filter exceeded control levels in a similar manner to results described previously for the adults. The reason for this may be similar to that described previously i.e. the selection of more hardy individuals or it may be due to increased availability of food supply. For example if numbers of M.hygroptericus were kept artificially low the grazing activity would be less and it is likely that film levels would rise, therefore if M.hygroptericus were allowed to increase due to unsuccessful treatment it is likely that they would increase in excess of control levels due to the larger food supply available.

If an unnatural ecosystem is set up by repeated insecticide treatment this may enable other organisms to increase which would otherwise be controlled by their competitors. Such a situation may have been occurring on the treated filters in the case of enchytraeid worms as it can be seen from Figs. 40 and 41 that their

* Statistical analysis of volatile solids levels from control and insecticide treated filters.

Filter	Mean weekly volatile solids gdm^{-3}	Paired 't' test 't' value	Paired 't' test 'P' value	Control v. treated filter Significance
Control	1.6 ± 0.1	—	—	—
Treated	1.6 ± 0.1	0.11	>10%	NS

\pm = S.E.M. No. observations = 41

population levels were more erratic and in some cases higher but not significantly higher than the control filter levels. The erratic nature of enchytraeid worm levels could possibly be explained by the repeated removal of their competitor M.hygroetricus by insecticide treatment resulting in low interspecific competition pressures and allowing an increase in numbers. The reason why enchytraeid worms never dominated the community for any length of time was probably due to the incomplete control of M.hygroetricus whose numbers rose slightly between treatments or after badly timed treatments exerting a competition pressure on the worms thereby reducing their numbers.

Therefore from these results it can be assumed that treatment with Actellic M20 does not detrimentally affect the worms, a similar result with DDT and Gammexane was found by Tomlinson and Jenkins (1947), also when control of M.hygroetricus is successful it seems that enchytraeid worm populations will rise slightly to compensate for the removal of a component of the grazing fauna thus minimising any solids build up. A similar situation was described by Hawkes (1955c) who stated that enchytraeid worms in a treated filter were slightly more common than in a control filter.

As regards volatile solids, the levels on a treated filter can be compared with control levels by consulting Fig. 42. It is apparent that the two filters were remarkably similar in solids content in fact on statistical analysis* no significant difference could be detected between the two suggesting that insecticide treatment did not cause any film accumulation problems. These problems have been expressed by other workers most notably Tomlinson et al. (1949) who had problems with ponding on some insecticide treated filters. Conversely Hawkes 1955c working with Sylvicola found volatile solids levels on treated filters to be lower than on control filters. It could be suggested that the removal of Sylvicola allowed other more active grazers to increase. Hawkes (1955c) found increased numbers of collembolans and these have

been shown to be active grazers (Parkinson and Bell 1919 and Bell 1926). However a more important result from this work was that it was found that BHC actually acted as a fungicide reducing the thick fungal film (Hawkes - private communication). As regards collembolans these were hardly ever recorded and no increases were found on the Tamworth filters.

By consulting Fig. 42 it is apparent that insecticide treatment, when applied on a filter whose solids levels are rising can cause sharp peaks in solids levels which in some situations may cause problems. Such peaks were obtained in April prior to sloughing and in Nov./Dec. and in both cases the solids levels on the treated filter exceeded that on the control filter however no ponding problems were encountered which are probably a reflection on the operating conditions on the filters. In other conditions where for example the media size is smaller or the dosing frequency higher timing of the insecticide doses and measurement of film levels would need to be monitored closely to avert any possible problems of film accumulation.

It has been expressed that spring unloading of a filter can be delayed by insecticide treatment (Hawkes 1951b) by suppression of grazing activity however this was not found on the Tamworth filters. From Fig. 42 it can be seen that sloughing occurred on both filters at the same time even though insecticide was being applied at that time. This is probably explained by the actively grazing enchytraeid worm population increasing thus preventing solids accumulation.

The effects of temperature on film accumulation can be seen by comparing Figs. 35 and 36 with Fig. 42 and various workers e.g. Heukelekian (1945), Hawkes and Shephard (1975) and Graaf (1976) have studied these effects.

It was stated by Hawkes and Shephard (1975) that temperature has an effect on film levels even in the absence of grazers and the most significant way in which it does

this is by affecting the microbial activity. Also Heukelekian (1945) expressed that the summer fall in solids levels which can be seen in Fig. 42 was due to bacterial oxidation of the film and not to increased grazing action. From Fig. 42 it can be seen that the expected pattern of a winter build up in film followed by a spring slough and low summer levels was followed. However during the year there were 3 sharp drops in film levels, one at the end of August, one at the end of September and the next at the beginning of January. By comparing the timing of these with the variations in settled sewage feed temperature in Figs. 35 and 36 it can be seen that each drop in film levels was preceded by an increase in that temperature. It seems from these results that temperature is probably a more important factor than grazing activity in controlling film levels as the treated filter film levels follow for the most part similar trends to control filter levels. However grazing activity must play some role as in both cases the temperature was falling and the solids levels were rising (Feb./March and Oct./Nov. 1976) and these solids levels on the treated filter rose above those on the control filter. Therefore high film levels brought about by low temperatures can only be increased by insecticide treatment.

(b) Secondary filters

The levels of M.hydropetricus larvae and pupae on insecticide treated and control filters on the secondary block can be compared by consulting Figs. 43 and 44. As in the case of the primary filters a reduction in larval numbers was noticed on treated filters except for a single peak in early September which was found on all filters both primary and secondary, control and treated. The occurrence of a single peak in larval numbers as seen in Fig. 44 without a build up of previous generations is unusual as it seems to suggest that the larvae found in the peak were the offspring of parents originated from other filters. No significant numbers of larvae were found in that filter previously therefore it is most likely that the parents of these larvae originated from the primary block of filters as it can be seen from Fig. 39 that during the period July, August and September 1976 insecticide doses were ill timed, generally after a

larval peak, resulting in large emergences from all primary filters (see Figs. 15, 18, 21 and 24) so the chances of cross-infection to the secondary filters would have been high.

The importance of correct timing of the insecticide dose can be appreciated by noting that just before the larval peak in Fig. 39 the filters were treated with insecticide which completely checked the impending emergence on the treated filters (see Figs. 29, 31 and 33), this should be compared to the control emergence in Fig. 27.

Generally it can be seen that larval and pupal levels were lower on these filters compared to the primary filters which suggests that food supply is an important factor here in limiting macrograzer population build up as film levels were much lower on these filters (see Fig. 47) than on the primary filters (see Fig. 42). Similarly with enchytraeid worms it can be seen from Figs. 45 and 46 that population levels on the secondary filters were much lower than on the primary filters (Figs. 40 and 41). Unlike the primary insecticide treated filter, enchytraeid populations on the secondary treated filter did not seem higher or more erratic when compared to their respective control filters. It is possible that M.hygroscopicus larval populations were so small that when removed no differences were noticed with the enchytraeid worm population or more likely that the numbers found in the samples were too small to notice any significant changes.

The film levels on an insecticide treated and control filter in the secondary block can be seen in Fig. 47. It can be noticed that the classical pattern of seasonal film level variation with temperature as was seen with the primary filters was not apparent. Film levels remained fairly constant throughout the year except for a slight rise in the winter. The obvious reason for this is the extremely low organic load received by these filters which averaged $0.02 \text{ Kg BOD m}^{-3} \text{ day}^{-1}$ over the study period compared to $0.155 \text{ Kg BOD m}^{-3} \text{ day}^{-1}$ for the primary filters, thus any growth changes due to

temperature would still occur but they would be of a smaller magnitude to those found with the higher organic loadings.

This can be seen by comparing the volatile solids levels on the primary filter (Fig. 42) with the secondary filter (Fig. 47). It can be seen that in both cases peaks occurred in April/May, August and the winter months interspersed with drops in the solids levels in June and Sep./Oct. Therefore as film conditions, loadings and macrograzer population levels were completely different on these filters this shows the importance of temperature in controlling film levels.

As regards the effects of insecticide treatment on film levels on the secondary filters it can be seen from Fig. 47 that in some cases but not always treated filter film levels increased after treatment generally in excess of control filter film levels. The cases when this increase was noted was at the end of April, middle of June, middle of July and at the end of November. If the temperatures during these periods are investigated on Figs. 35 and 36 it can be seen that although no drastic differences in the secondary filter feed temperature was noted, each period seemed to coincide with a drop in the mean air temperature, so again the implications of insecticide dosage when temperatures are falling are stressed. The reason why air temperature seems to be more important than sewage feed temperature in controlling film levels on the secondary filters than on the primary filters is probably due to the low hydraulic load received by these filters, on average $0.424 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$ compared to $0.743 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$ for the primary filters over that period. Obviously air temperature would control to a greater degree on filters with low hydraulic loadings.

iv) Life cycle time analysis and effects of timing of insecticide dose

The effects of ill timed insecticide treatments have already been described. However to give constructive comments on the optimum time for dosing with the particular insecticide utilised it is necessary to analyse the life cycle time in relation to

temperature and relate these to the timing of the insecticide dose in day degrees and the percentage reduction in fly emergence obtained on the treated filters. This analysis was carried out for the fly emergences obtained on filters 3C (insecticide treated) and 4A (control) and can be seen in the following tables.

Table 12a

Life cycle period analysis in relation to temperature

<u>Generation no.</u>	<u>Average effluent</u>	<u>Theoretical life</u>		<u>Actual life</u>	
	<u>temperature</u>	<u>cycle period</u>		<u>cycle period</u>	
	(°C)	<u>day</u>	<u>day⁰</u>	<u>day</u>	<u>day⁰</u>
1 (19 Apr. – 31 May)	13.92	42	540	42	540
2 (31 May – 12 Jul.)	19.15	30	540	42	762
3 (12 Jul. – 10 Aug.)	19.13	30	540	29	526
4 (10 Aug. – 1 Sept.)	20.36	28	540	22	426
5 (1 Sept. – 6 Oct.)	17.97	32	540	35	594
6 (6 Oct. – 6 Nov.)	15.42	37	540	31	448

Table 12b

Analysis of success of insecticide dose in relation
to the time of application of the insecticide

<u>Date eggs laid</u>	<u>Date of insecticide dose</u>	<u>Period between eggs</u>		<u>% decrease in fly emergence control – treated</u>
		<u>days</u>	<u>day⁰</u>	
19 Apr.	13 May	24	310	94.7
31 May	16 Jun.	16	290	93.3
12 Jul.	14 Jul.	2	36	42.1
10 Aug.	12 Aug.	2	36	58.0
1 Sept.	2/20 Sept.	1/19	17/323	97.6
6 Oct.	20 Oct.	14	202	94.8

Initially it can be seen from Table 12a that the actual life cycle periods for 4 of the generations in 1976 compare fairly favourably with the theoretical life cycle periods (540 day⁰ for M.hygropetricus) as calculated using the formulae described by Uvarov (1931) which has been previously described. The other 2 generations which do not fit well with their theoretical life cycle periods (no. 2 and 4) can be explained as follows. In generation 2 the actual life cycle period of 42 days was much longer than the calculated theoretical period of 30 days, also in generation 4 the actual period of 22 days was shorter than the theoretical period of 28 days. It is attempted to explain these differences by considering the mean air temperatures shown in Fig. 35.

Firstly considering generation 2 during the period 31 May — 12 Jul. It can be seen from Fig. 35 that very steep rises in air temperature were apparent during this period when in fact the mean air temperature exceeded the sewage temperature in late June/early July which is quite unusual as it was the only time in the 3 year project when this occurred. Then by consulting Fig. 15 it can be seen that approximately 3 weeks after the peak emergence on 31 May a second emergence occurred followed by a slight drop and then the predicted emergence of 12 July. It is likely that the extreme temperatures during this period caused a rapid increase in development of the progeny from the 31 May emergence along with development of residual larvae and pupae within the filter causing a prolonged emergence over the warm period. From Fig. 15 it can be seen that the mid point of this prolonged emergence was around 28 June, thus the period 31 May — 28 June is 28 days which corresponds well to the theoretical time of 30 days. Calculations for the next emergence however were taken from 12 July as this was the end of the prolonged emergence and the latest possible time at which eggs would be laid for the next generation.

Secondly by considering generation 4 during the period 10 Aug. — 1 Sept. it can be seen from Fig. 35 that during this period another steep rise in air and sewage temperatures occurred which would probably have had the same effect as above in

causing a rapid increase in development thereby reducing the life cycle period from the expected 28 to 22 days.

It should be mentioned here that although in some workers' opinions the sewage feed temperature determines the filter temperature (Bayley and Downing 1963), it was found by comparing certain temperatures including those of the effluent, the feed, the mean air temperature and the mean of the air temperature and the feed that the most accurate representation of filter temperature was the effluent temperature. Thus for all calculations the effluent temperature was utilised. This temperature can be seen over the 3 year project period in Figs. 48-50.

From the results described in Table 12a it is apparent that in normal weather conditions the effluent temperature can be used in estimating life cycle periods however during extremely hot weather conditions as were experienced in the summer of 1976 it is likely that filter temperatures, especially near the surface would increase in excess of the effluent temperature due to solar radiation as the surface filter temperature would only be affected by the sewage temperature for a short period in every 15 minutes. Thus it is not surprising that an unusually rapid development of the insects ensued in these high temperature conditions. As regards insecticide dose it can be seen from Table 12b that good percentage reductions of fly emergence on the treated filters compared to the control were obtained on all but two emergences. On both of these occasions (July & August) dosing was carried out either during or just after the emergence. This would have had a negligible effect as the insecticide is not active against pupae or adults which would be the main life cycle stages present at that time. However it is apparent that dosing, if applied when the bulk of the population is in the larval stage, is most successful in reducing M.hygropetricus adult emergence. Therefore as the time required for development of the larval stage of M.hygropetricus is 440 day⁰ (Lloyd et al. 1940) it would be most effective to dose at the mid point in this stage i.e. 220 day⁰ from the time of egg

incubation, when the bulk of the population would be in the larval stage. This can be calculated back to the time of the previous emergence by adding 63 day⁰ which is the time necessary for hatching of the egg (Lloyd et al. 1940). Therefore in order to obtain maximum success with one insecticide dose it is necessary to dose 283 day⁰ from the time of egg laying i.e. the previous emergence. In order to do this it is important that regular media sampling, fly trapping and temperature recording are carried out to ensure a successful programme of insecticide treatment.

v) Effects of Actellic M20 on the purification efficiency of filters

Due to the common effluent collection chambers from each block of 4 filters it was impossible to sample the effluent from individual filters to investigate any effects that insecticide treatment may have on purification efficiency. The only comparison that could be made was one of the filter efficiency prior to the commencement of the project to the efficiency during the insecticide treatment programme however this is made difficult as during the time of works commissioning in 1971 to the commencement of the project the works was subjected to many operational changes and various insecticide trials with malathion and gammexane. The only period during this time when double filtration was in operation was Aug. 1974 — Mar. 1975. Therefore it was decided to compare the BOD removal (settled sewage — final effluent) between this period and Aug. 1976 — Mar. 1977 when a similar system of double filtration was in operation.

An average removal of 95.0% was obtained for Aug. 1974 — Mar. 1975 against 95.2% for Aug. 1976 — Mar. 1977, therefore this infers that no detrimental effects were caused by Actellic treatment, however since the full history of trial insecticide treatments during the early period is not known no conclusion can be drawn from this.

It should be mentioned here that some workers have noticed different efficiencies with and without certain organisms. Hawkes (1955c) found that on controlling

Sylvicola in a filter with gammexane the efficiency of that filter increased beyond that of the control filter, however it was explained that high fungal film levels on the control filter caused this. The general view is that without grazers filters are less efficient as shown by Solbē et al. (1974) however it has been shown that other grazers can perform undisturbed by treatment with insecticide allowing efficiency to be retained if specific target insecticides are used such as Actellic M20.

Further studies were made into filter efficiency during insecticide treatment on laboratory scale filters and these will be described later. Other work to be described later includes toxicity tests using Actellic M20 on various filter fauna.

On the subject of the persistence of Actellic M20 in the environment it is unlikely that this would be a problem as it is broken down quickly by many means. According to Woods et al. (1976) it is not persistent and the specific toxic levels to some fish were quoted.

Fig. 15. Adult fly emergence from filter 4A (Control)

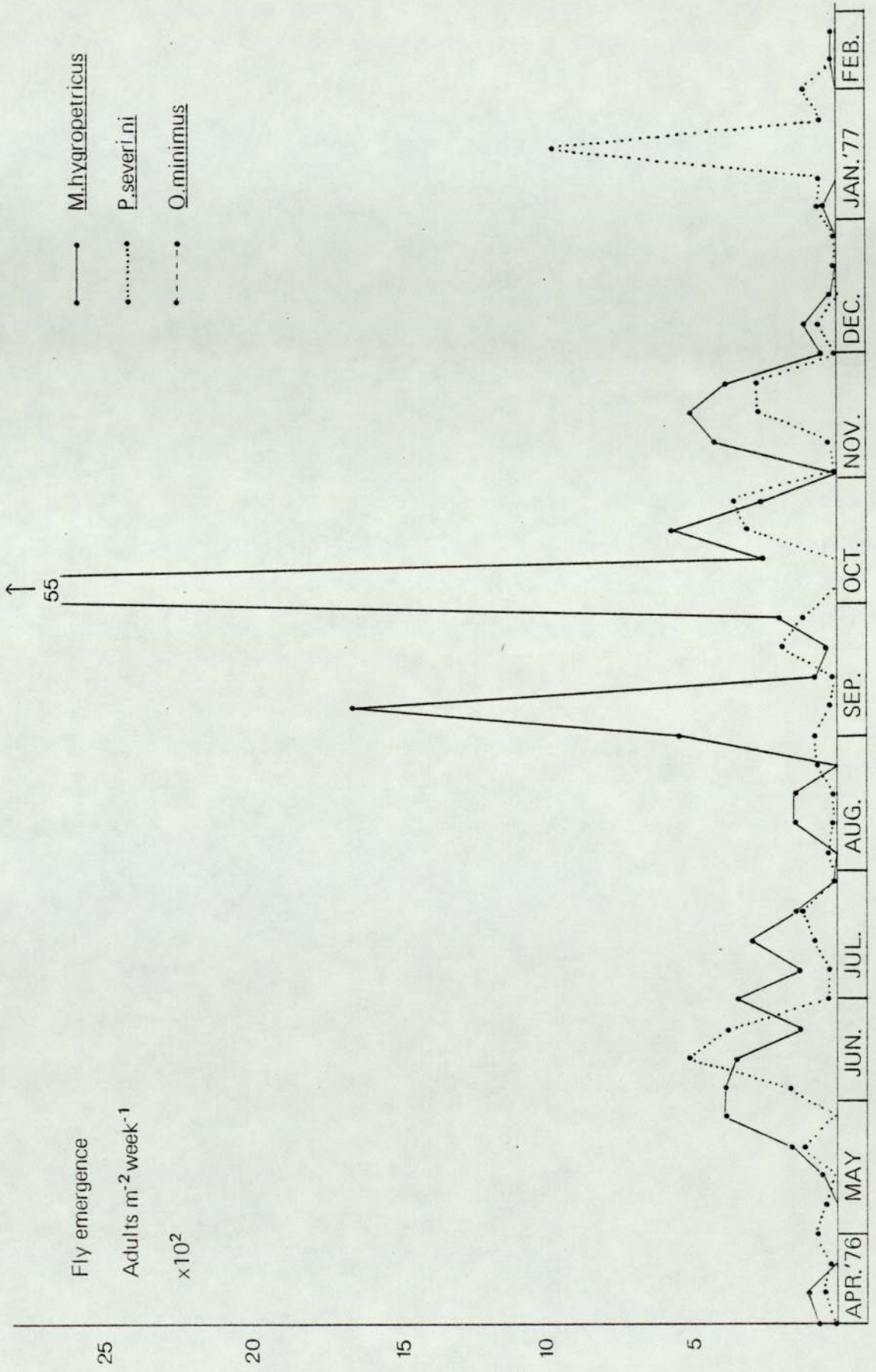


Fig. 16. Adult fly emergence from filter 4A (Control)

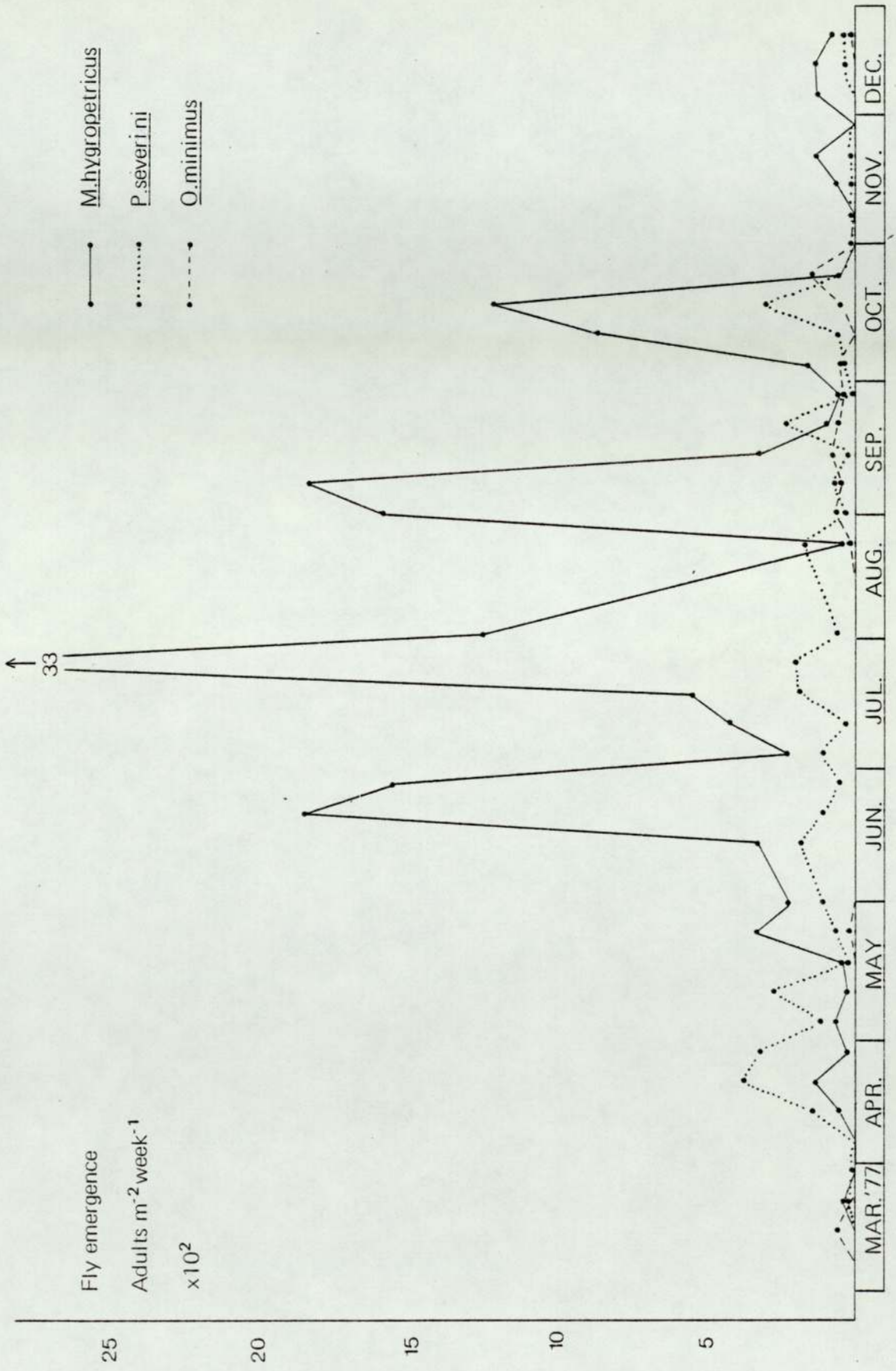


Fig. 17. Adult fly emergence from filter 4A (Control)

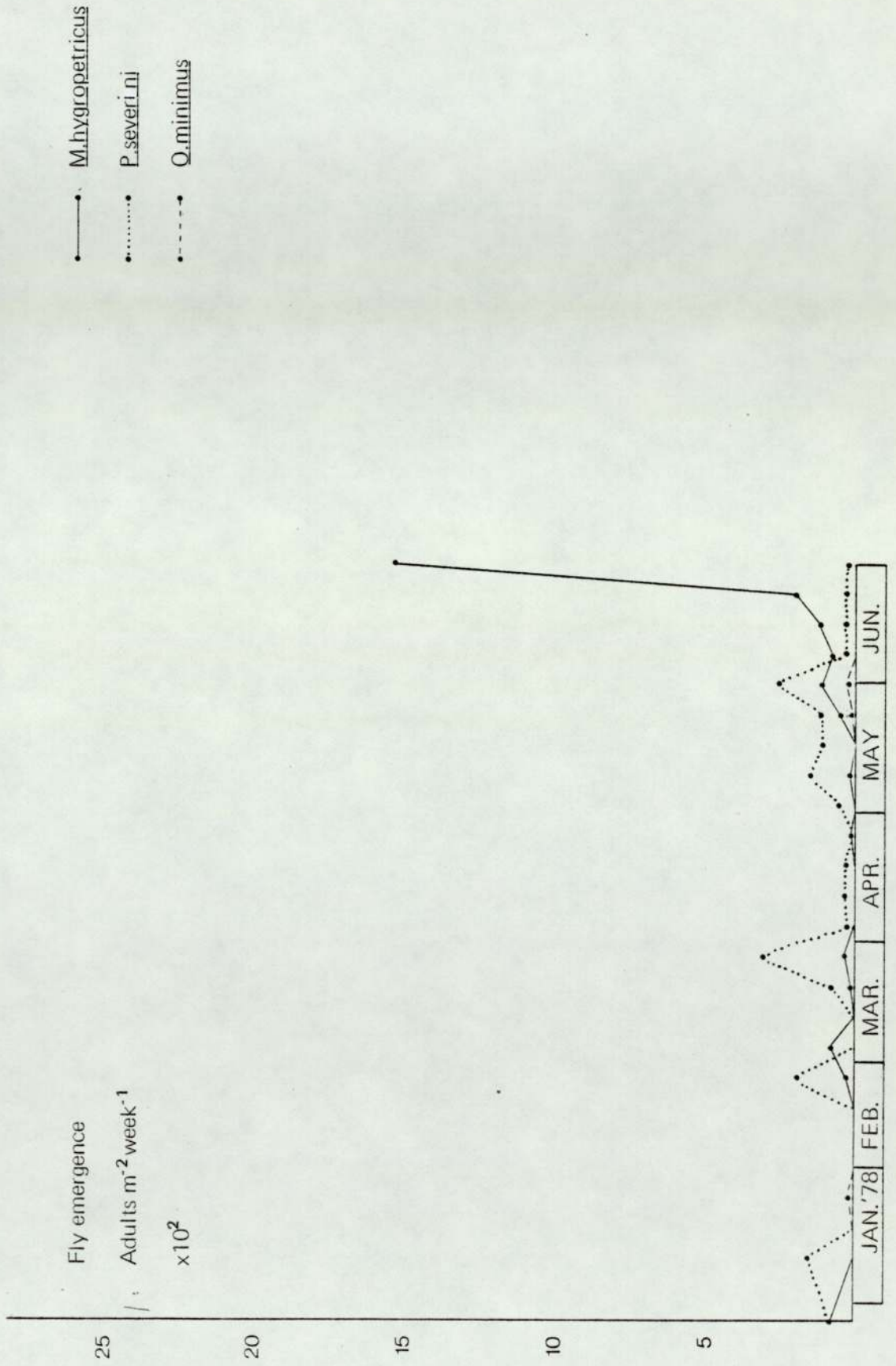


Fig. 18. Adult fly emergence from filter 4C

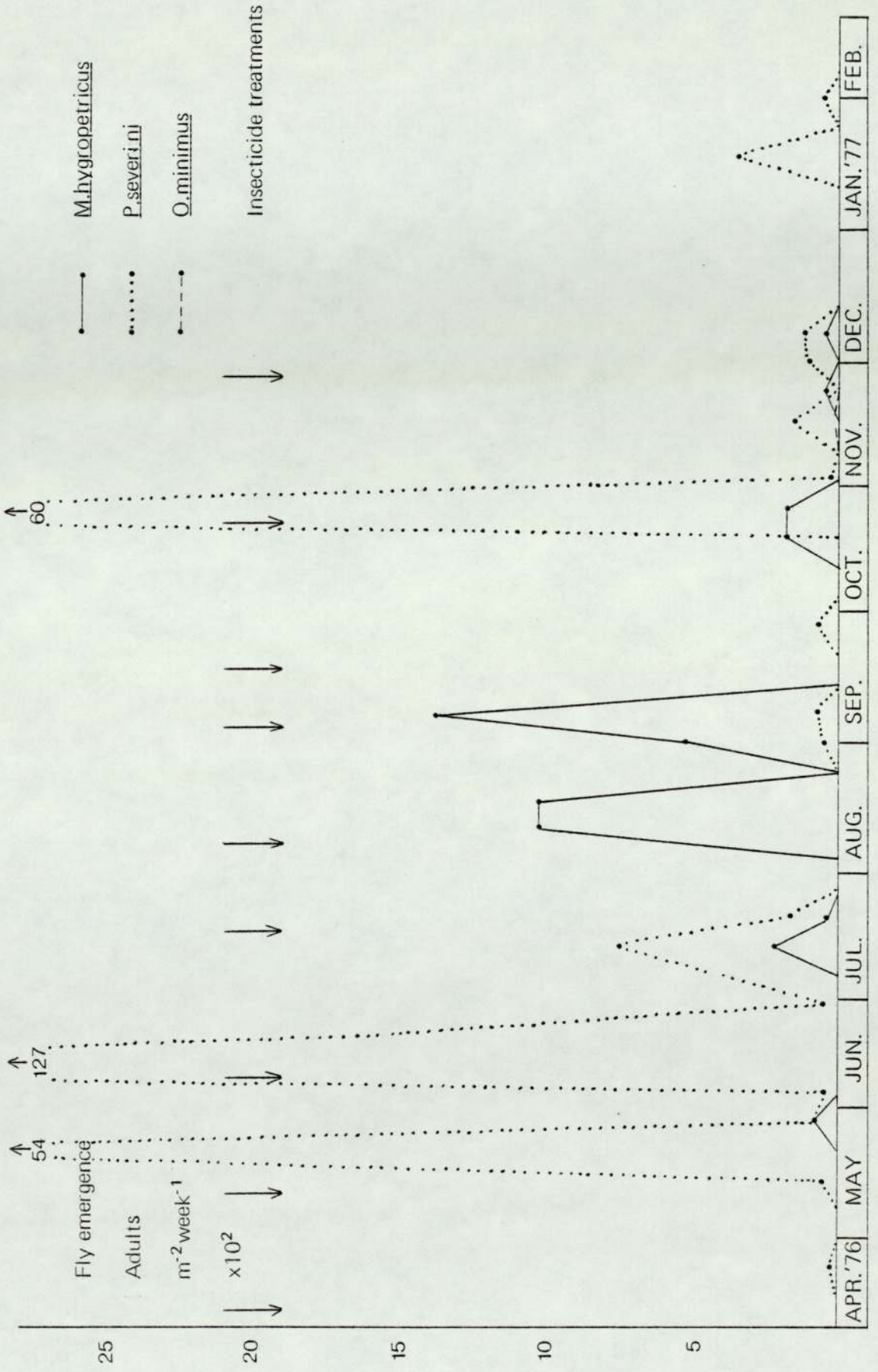


Fig. 19. Adult fly emergence from filter 4C

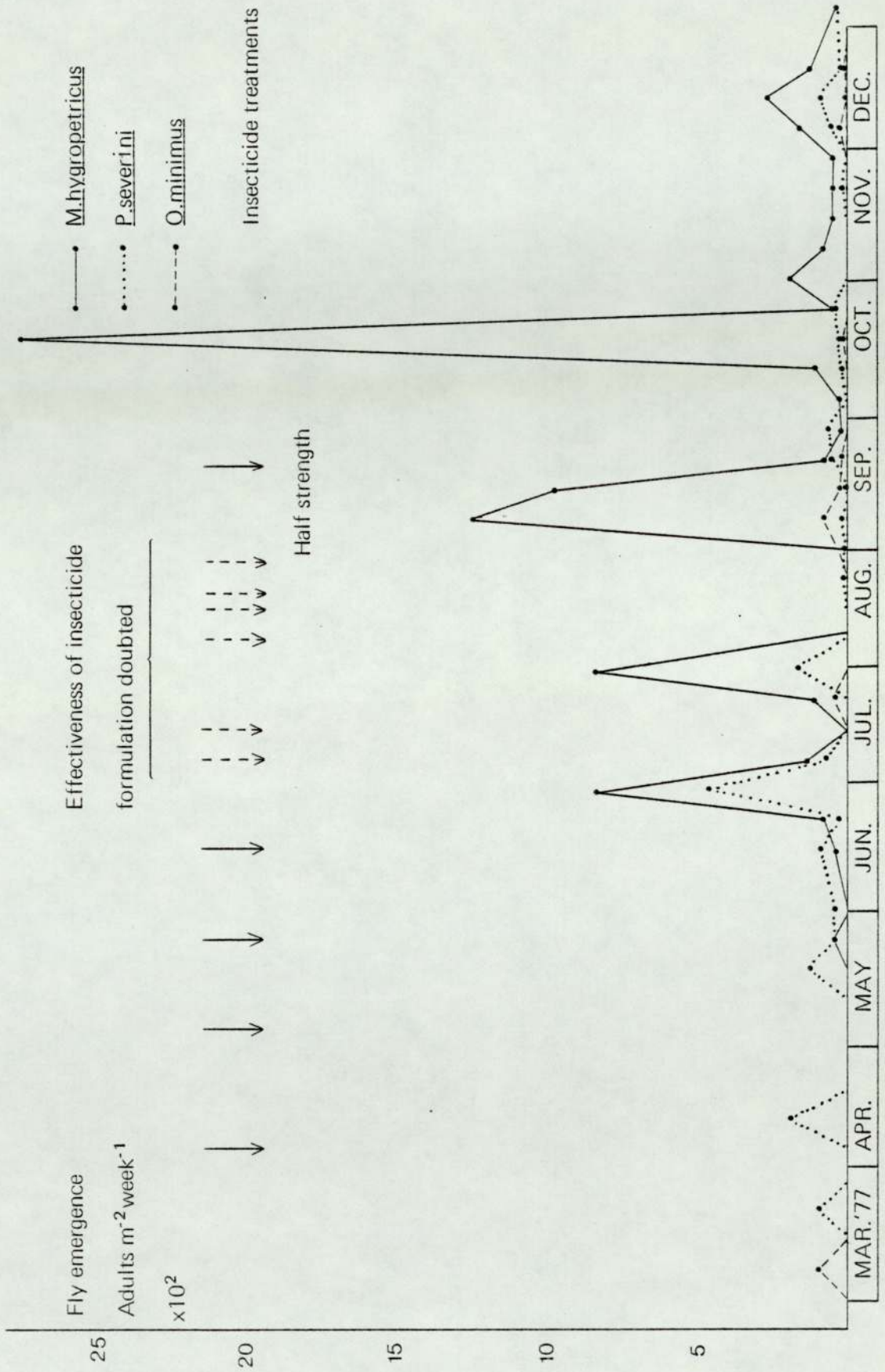


Fig. 20. Adult fly emergence from filter 4C

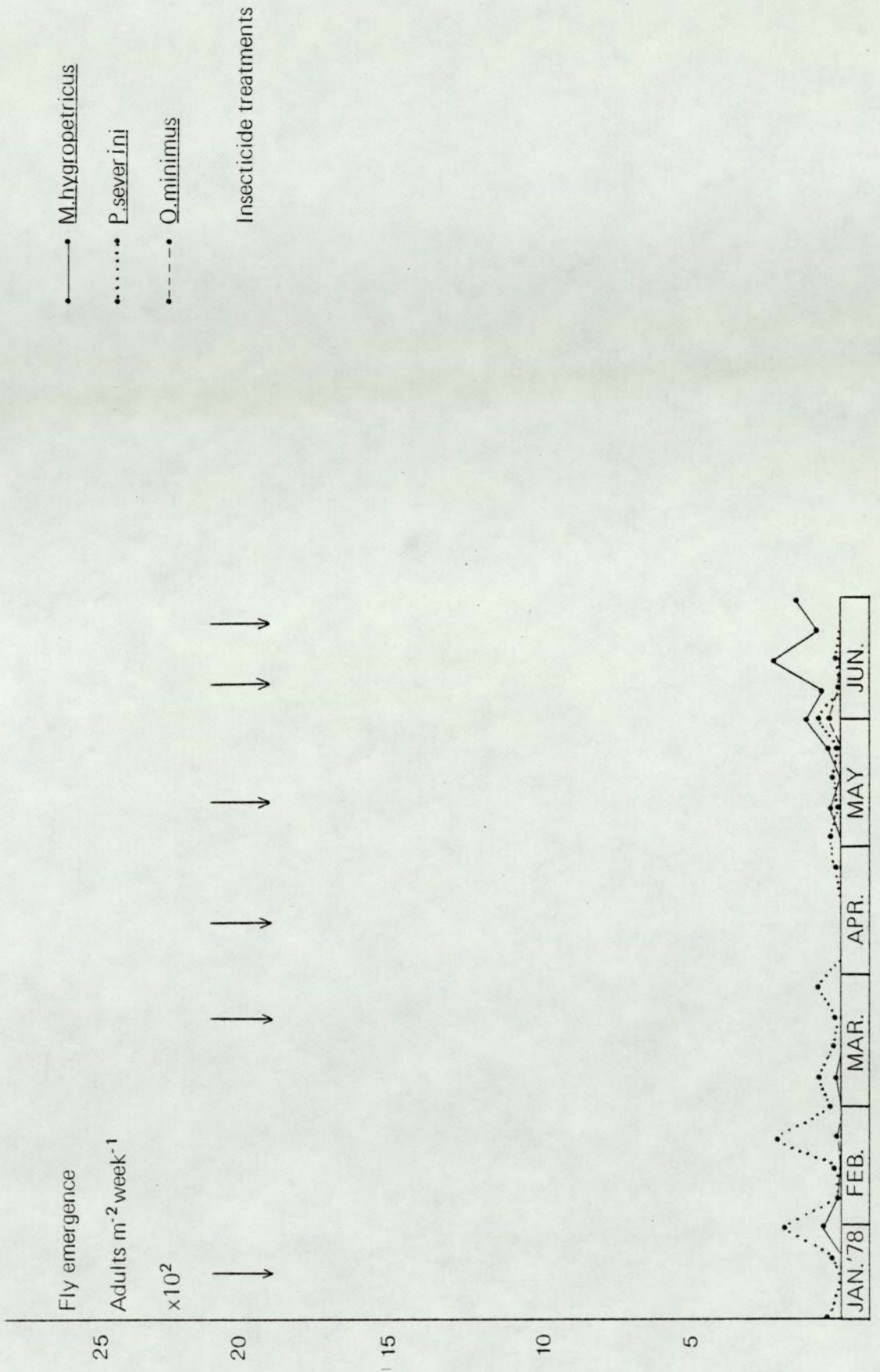


Fig. 21. Adult fly emergence from filter 3C

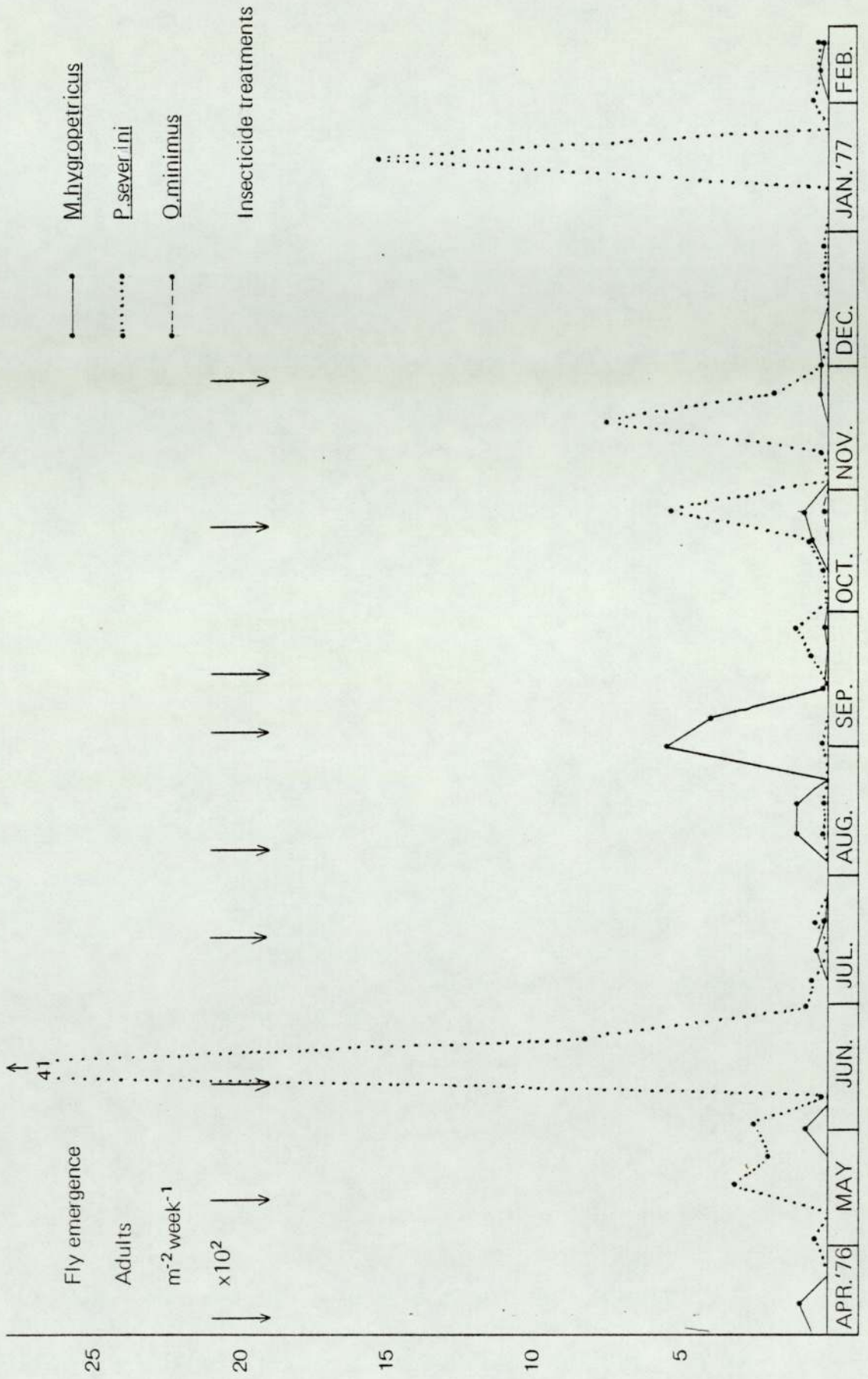


Fig. 22. Adult fly emergence from filter 3C

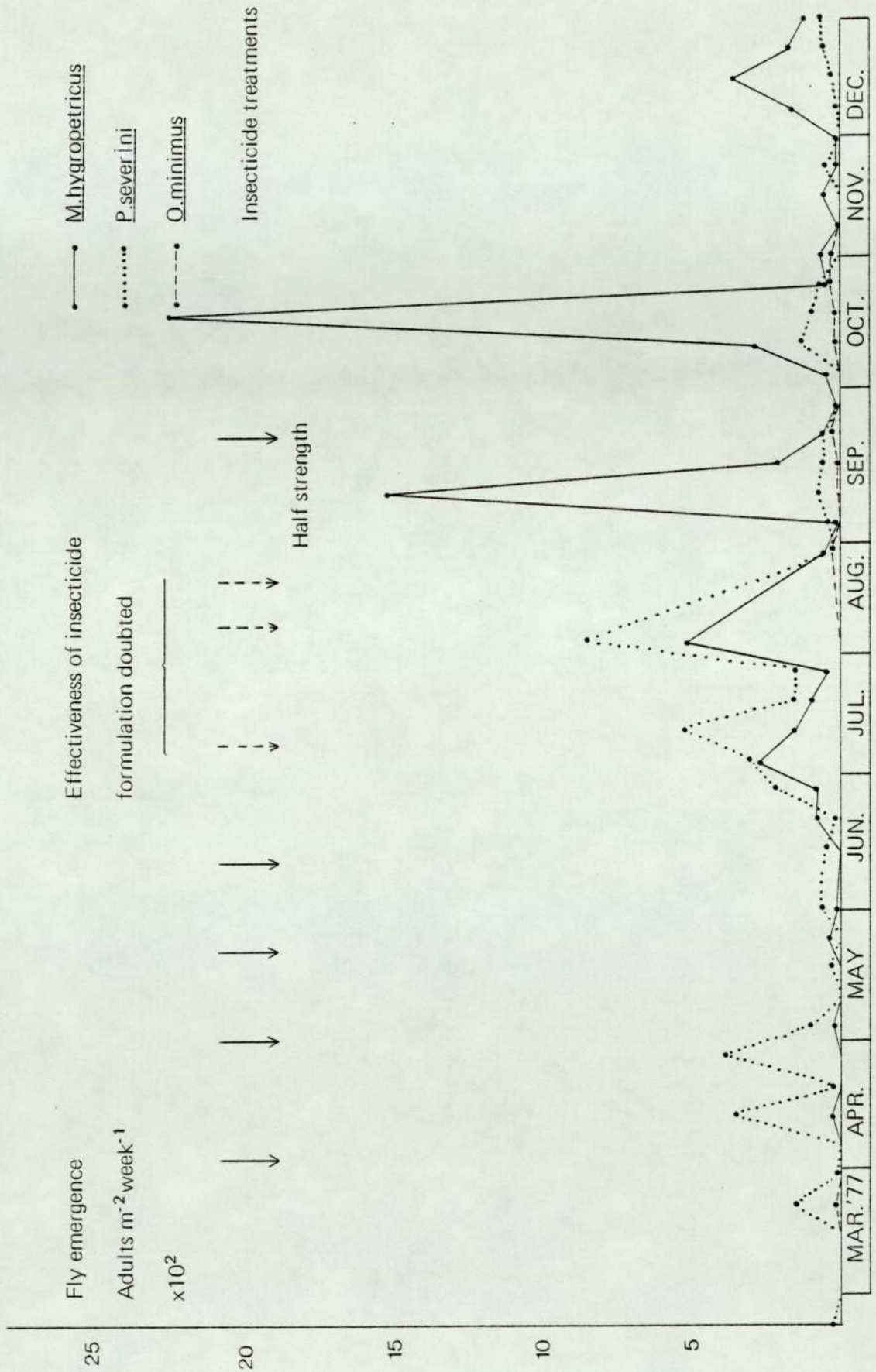


Fig. 23. Adult fly emergence from filter 3C

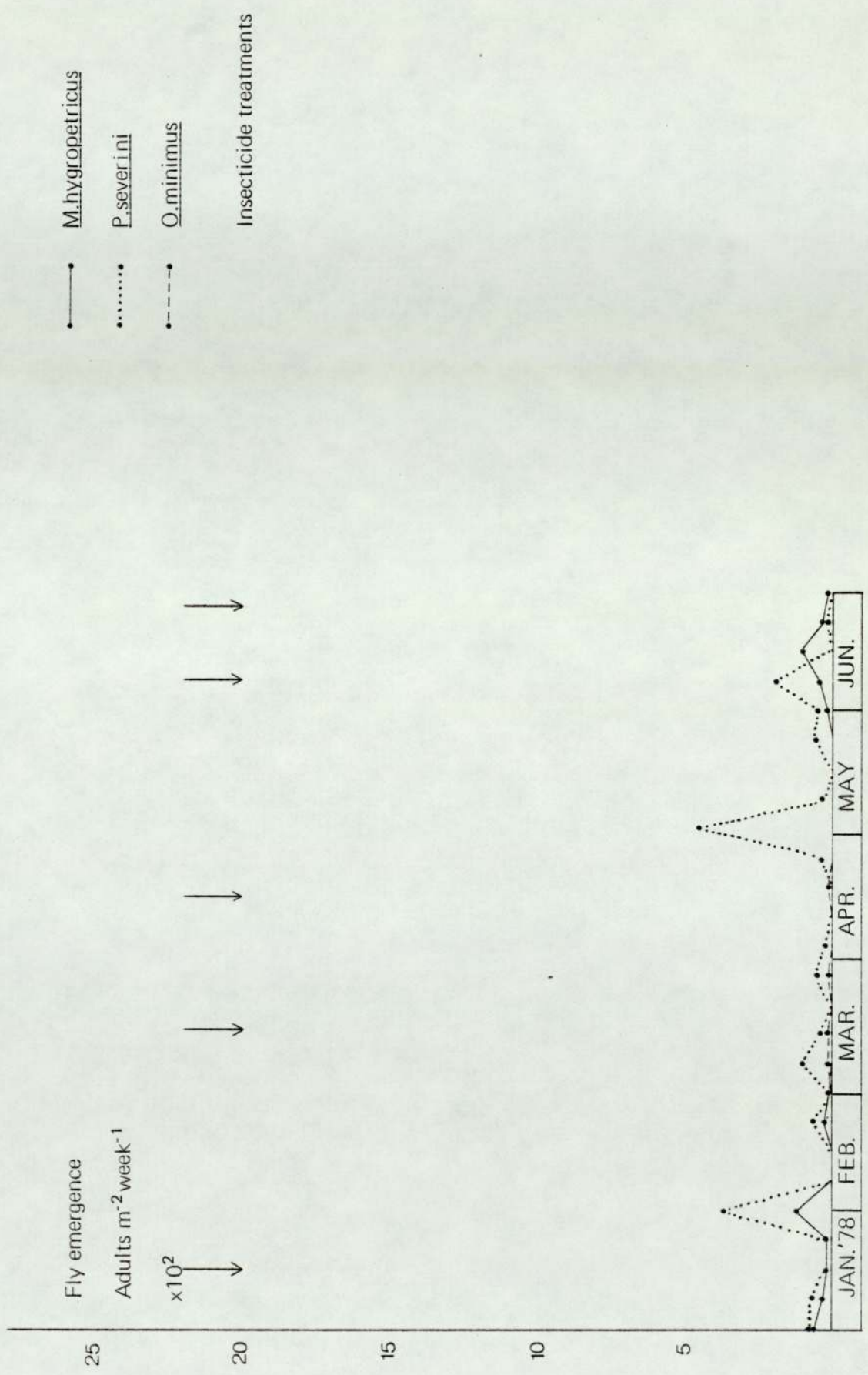


Fig. 24. Adult fly emergence from filter 3A

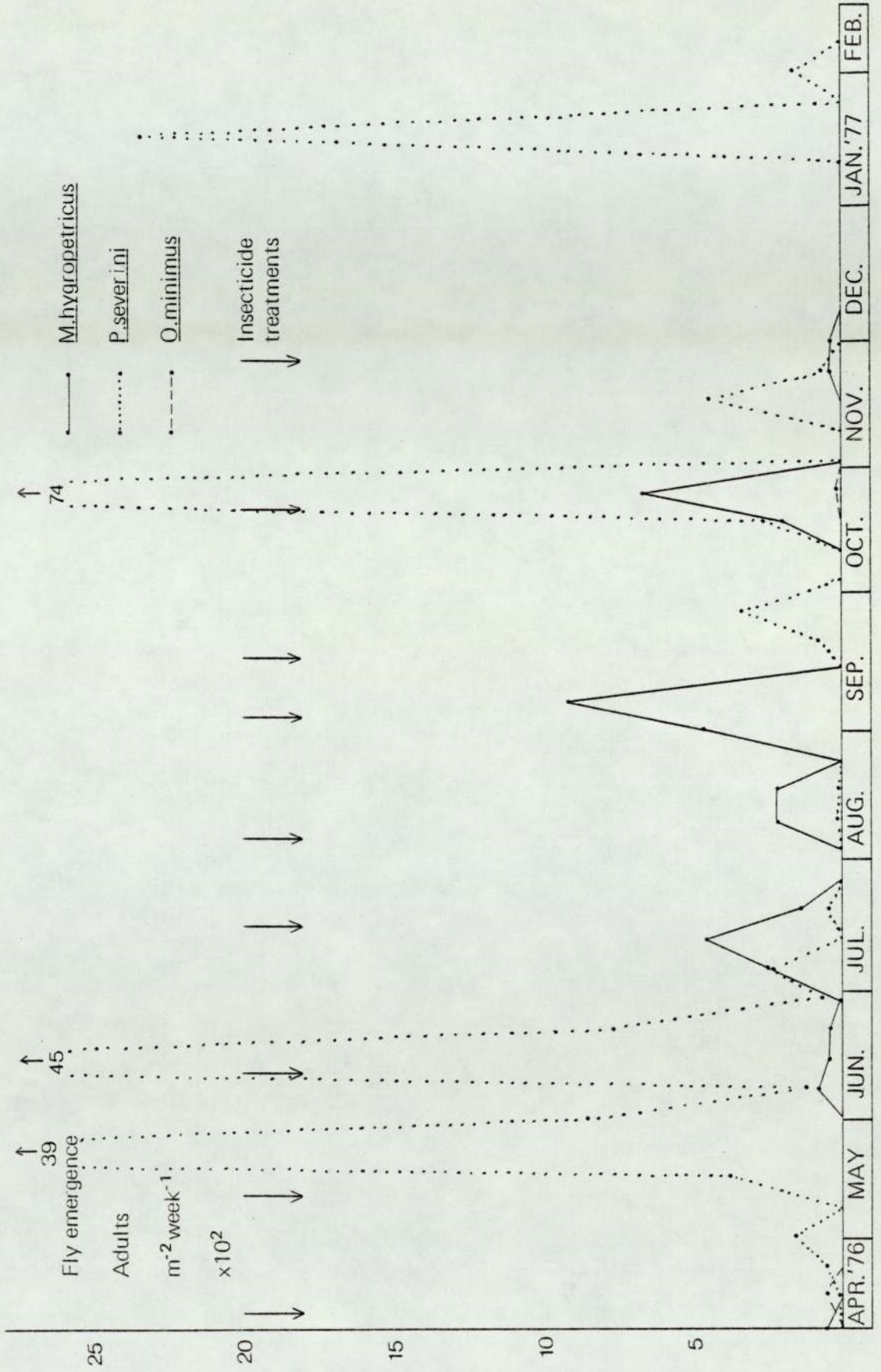


Fig. 25. Adult fly emergence from filter 3A

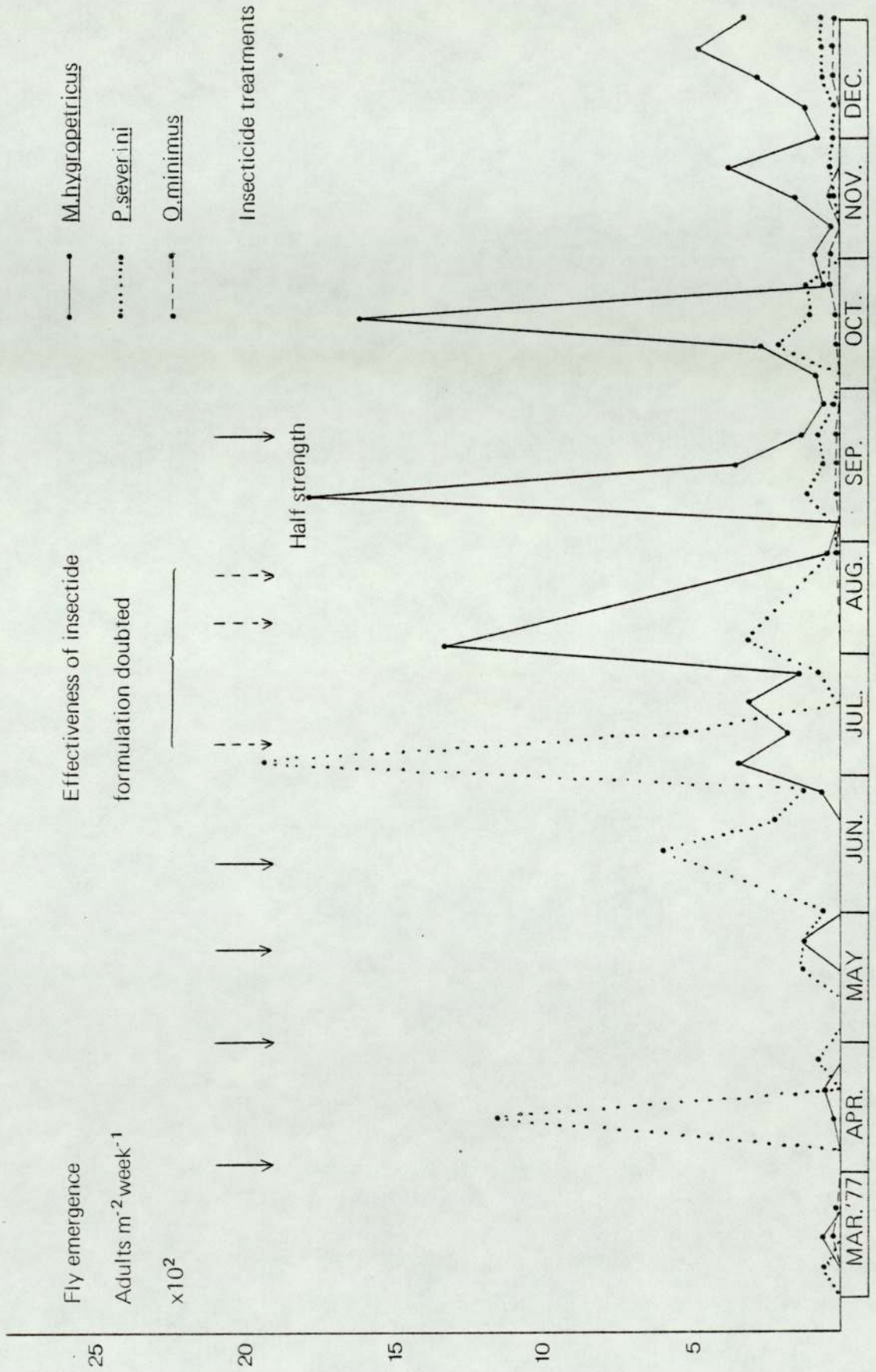


Fig. 26. Adult fly emergence from filter 3A

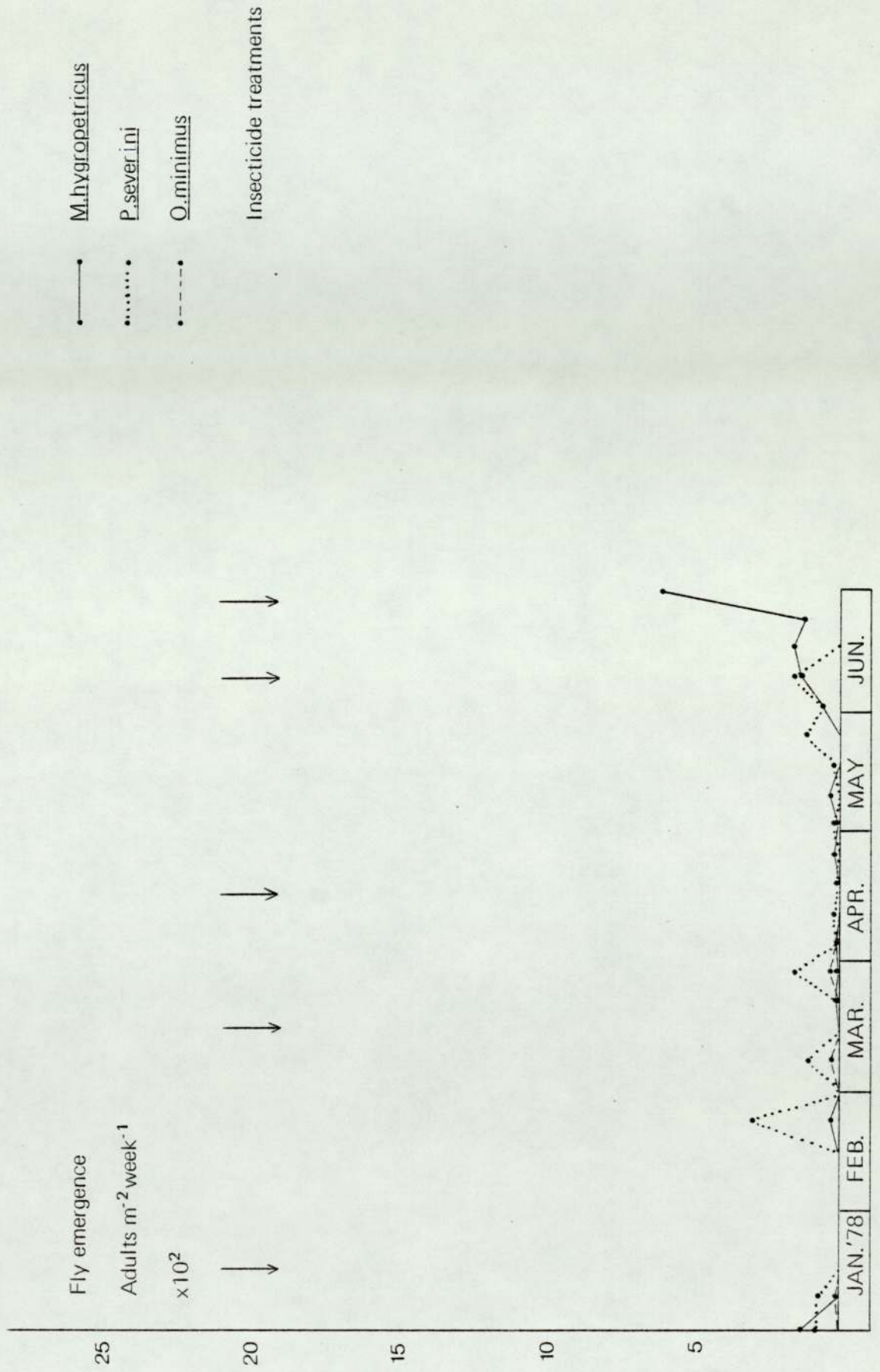


Fig. 27. Adult fly emergence from filter 2A (Control)

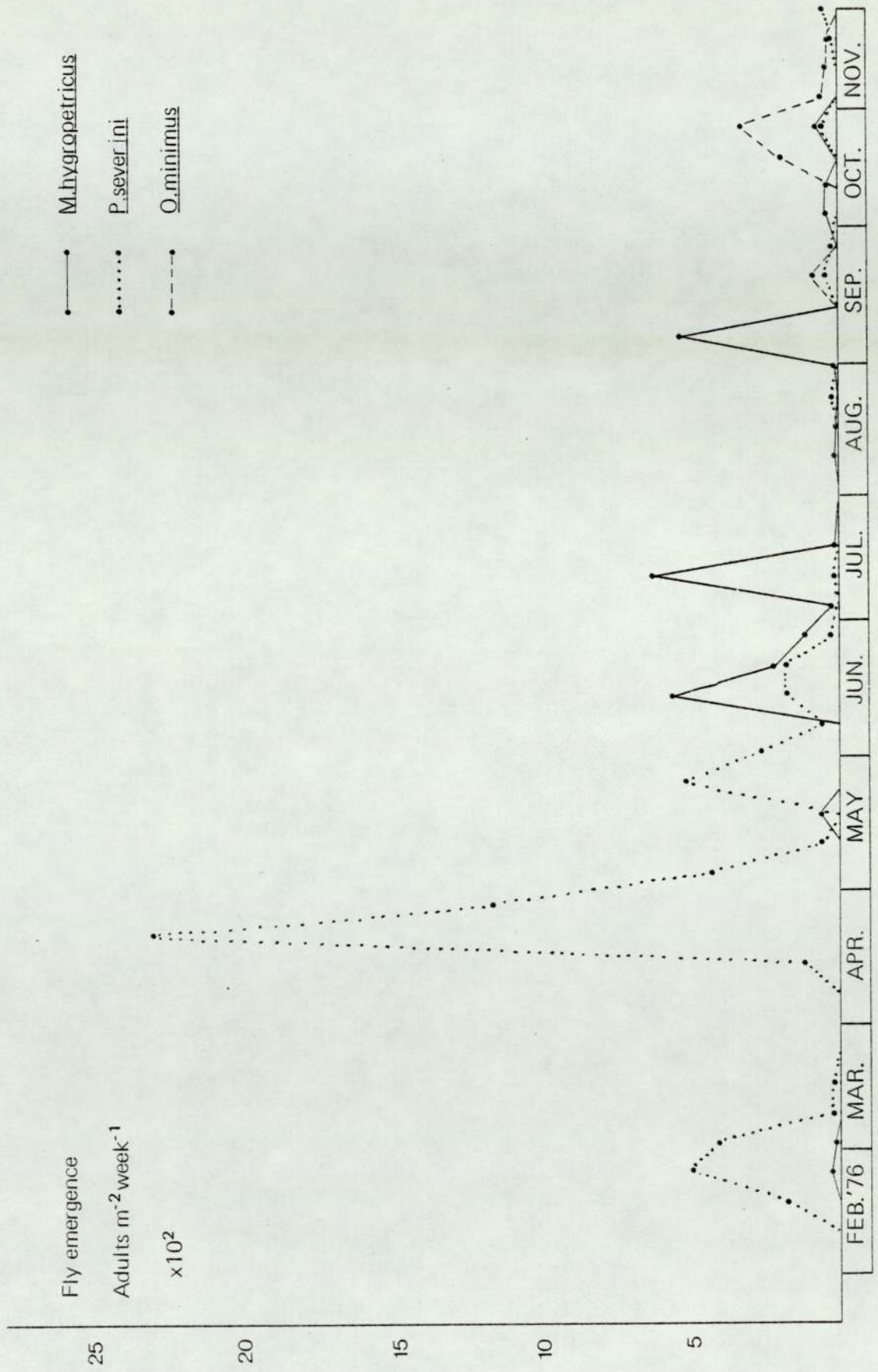


Fig. 28. Adult fly emergence from filter 2A (Control)

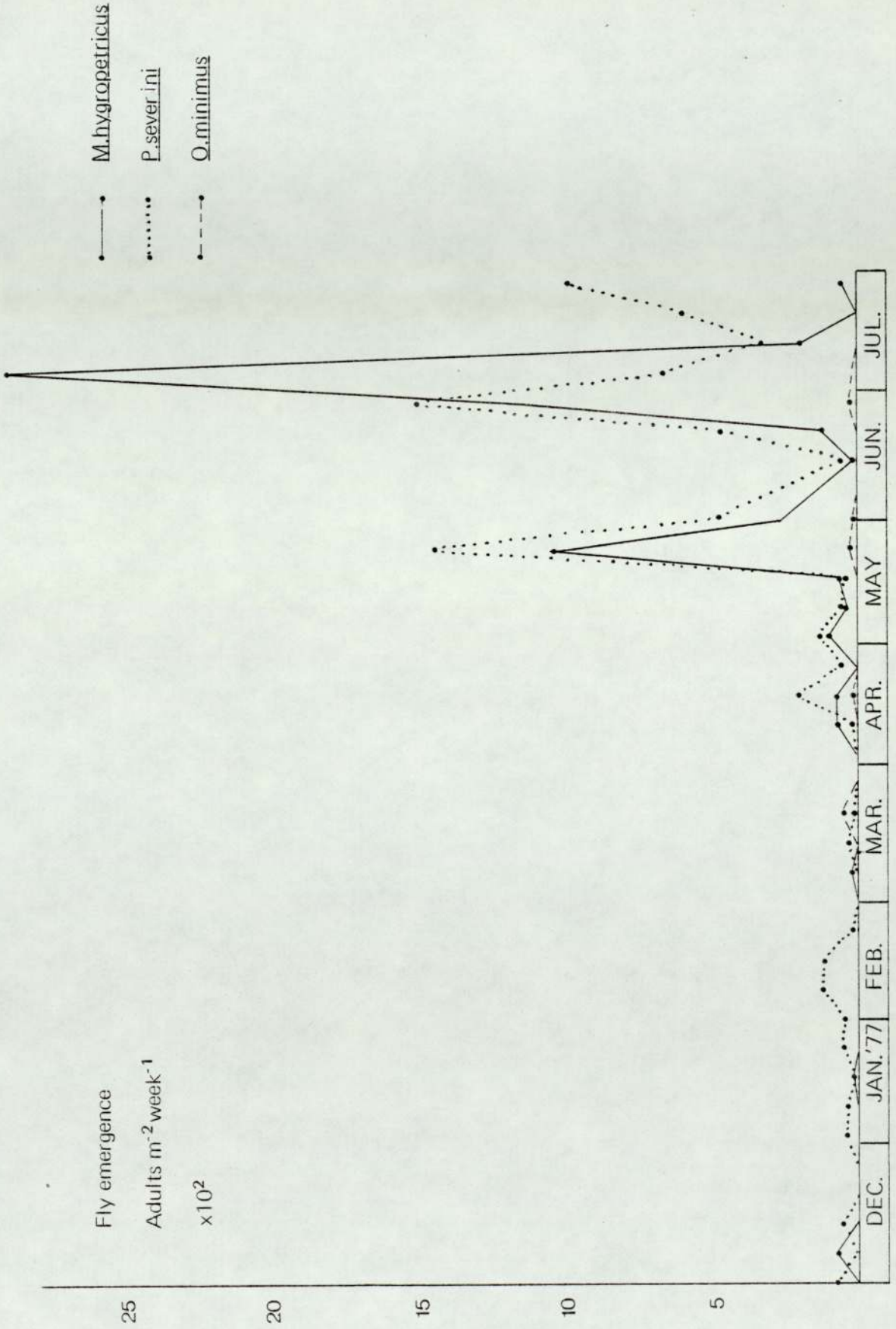


Fig. 29. Adult fly emergence from filter 2C

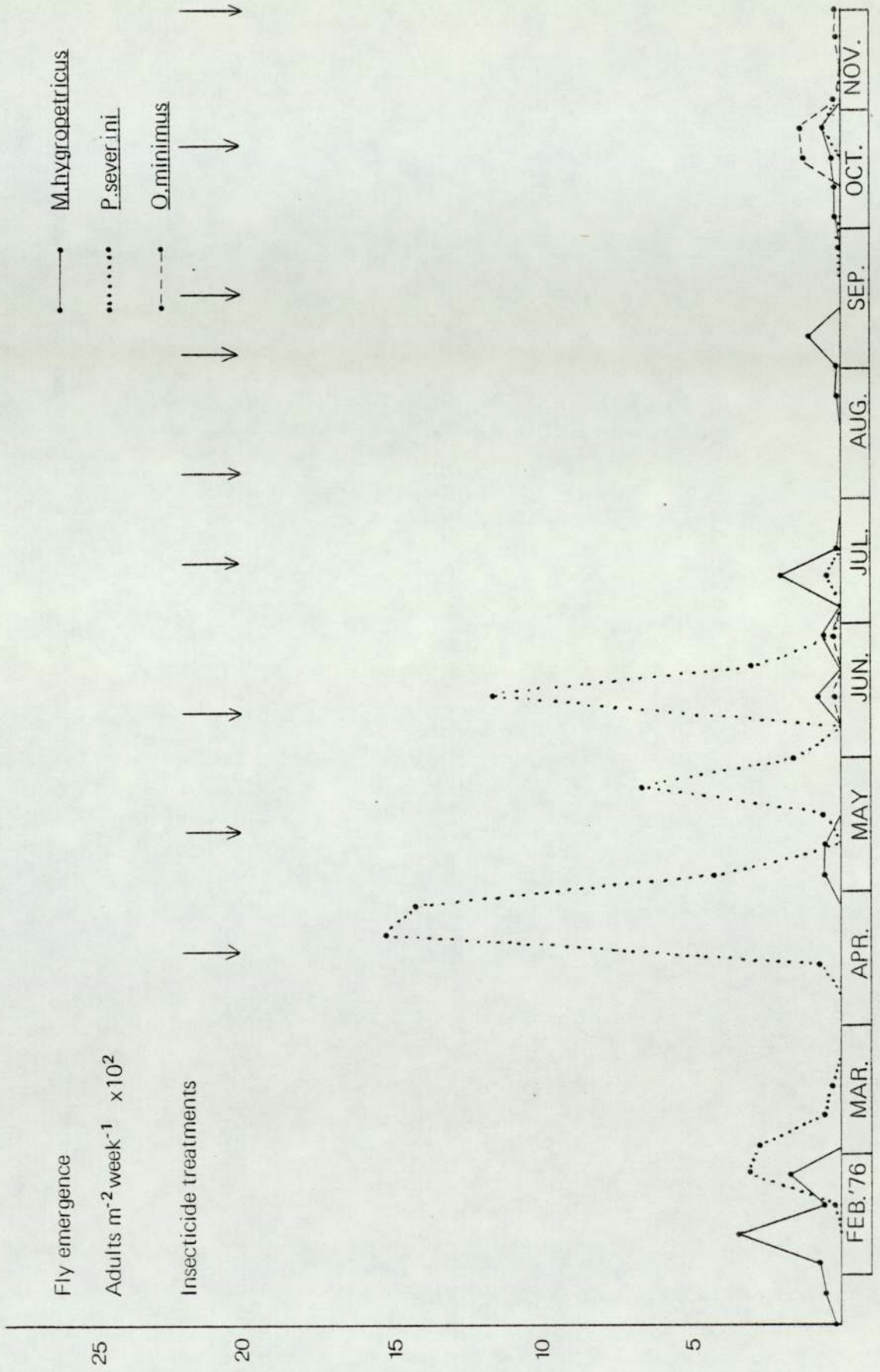


Fig. 30. Adult fly emergence from filter 2C

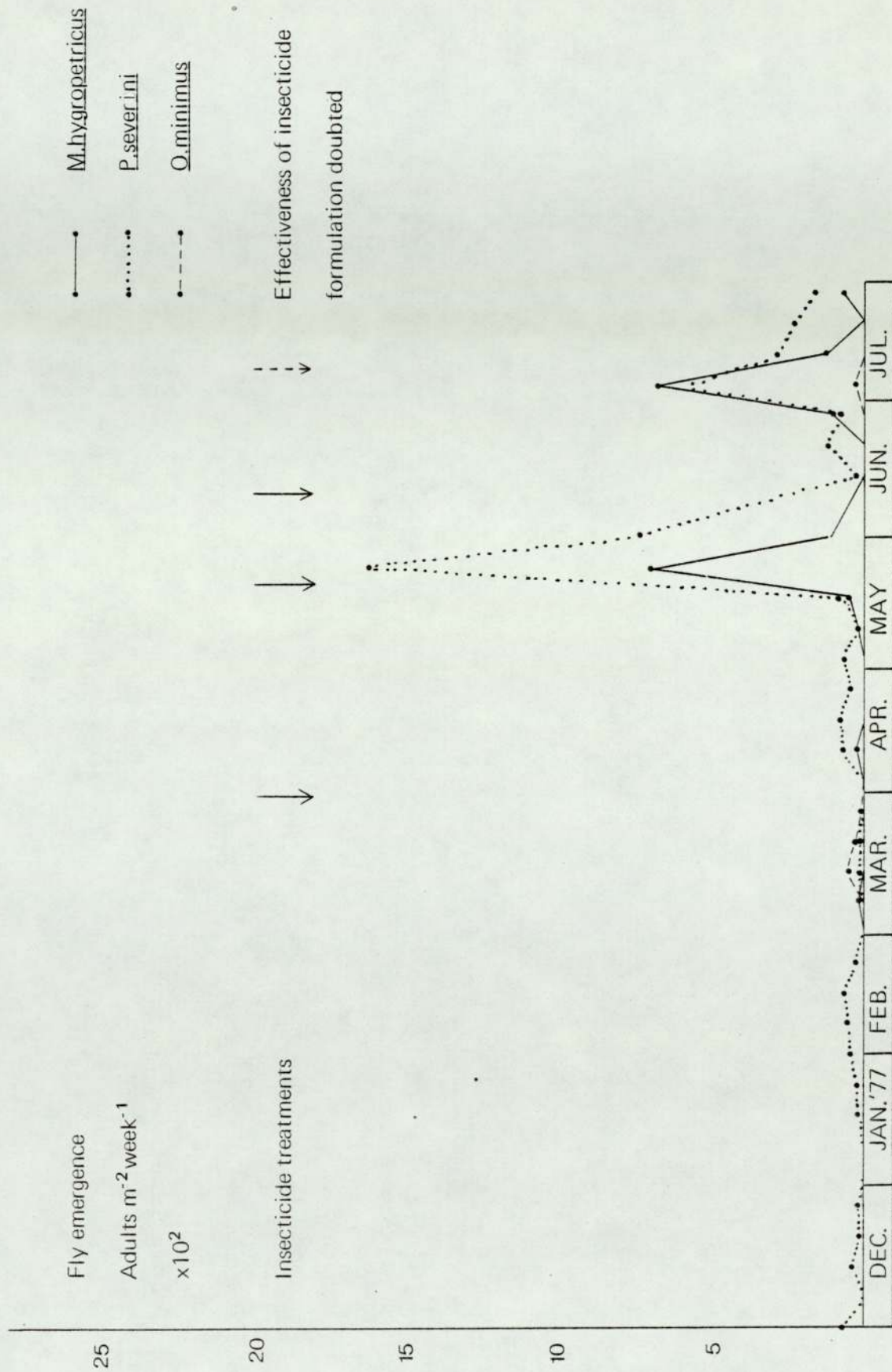


Fig. 31. Adult fly emergence from filter 1C

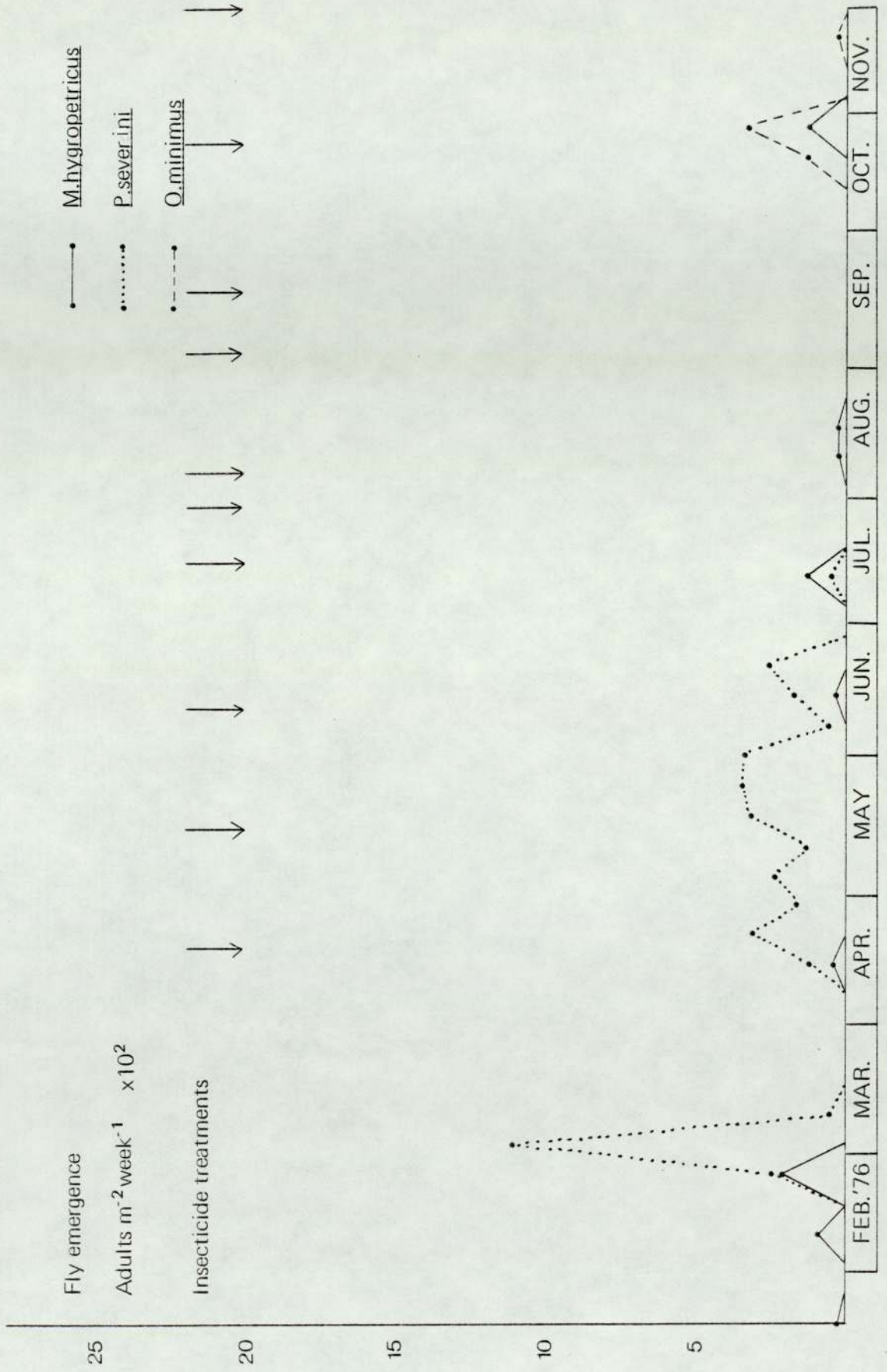


Fig. 32. Adult fly emergence from filter 1C

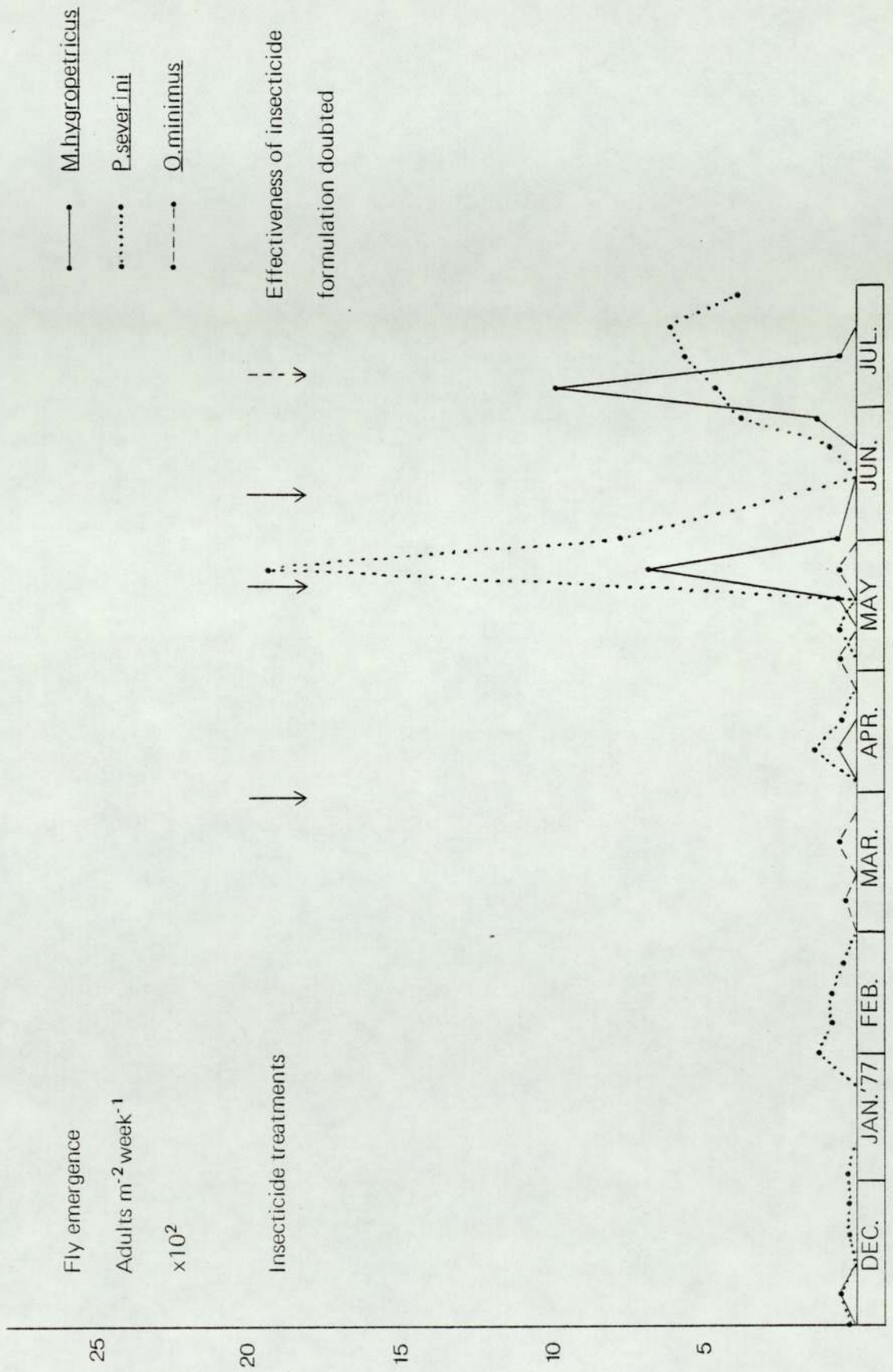


Fig. 33. Adult fly emergence from filter 1A

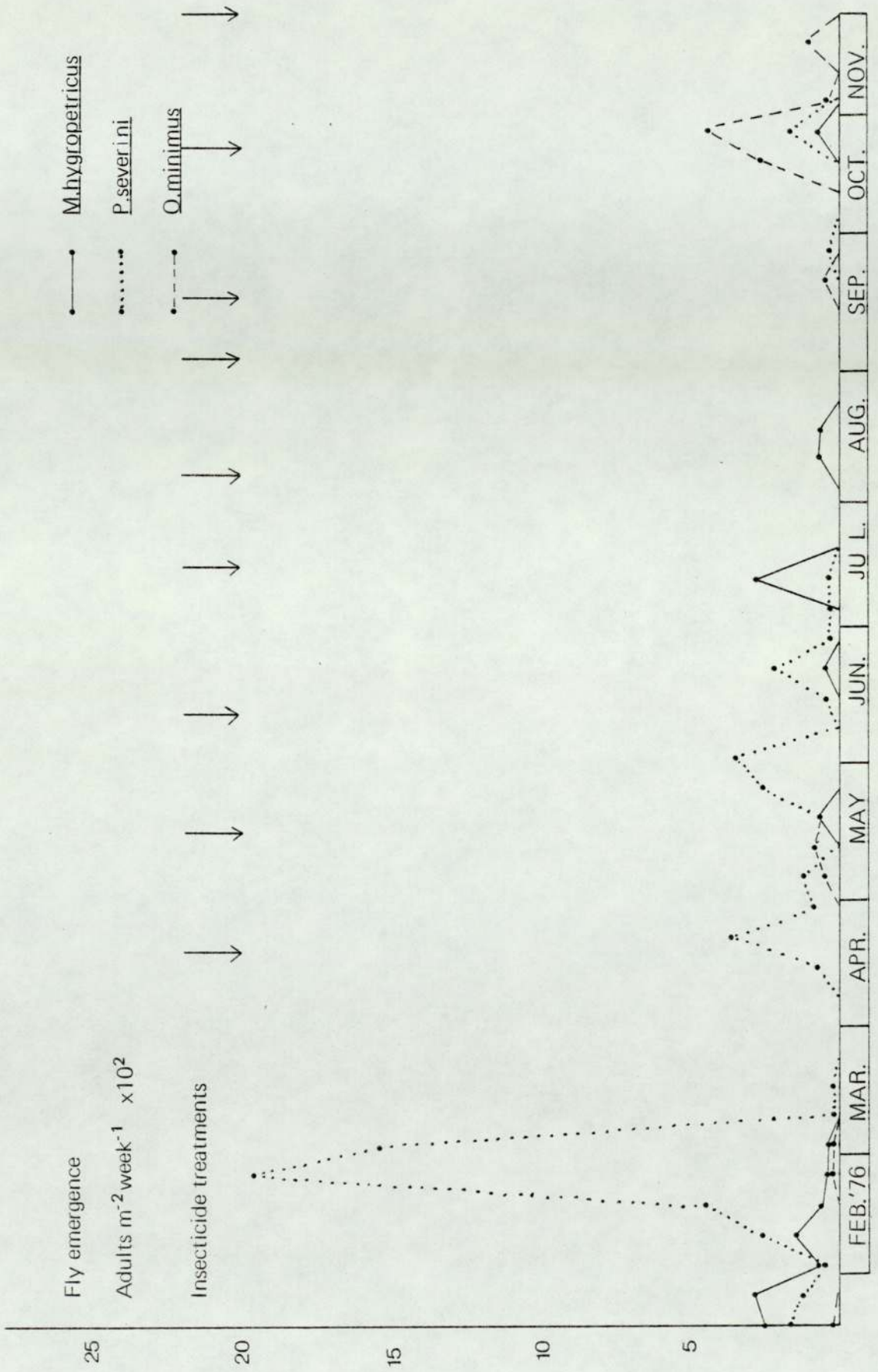


Fig. 34. Adult fly emergence from filter 1A

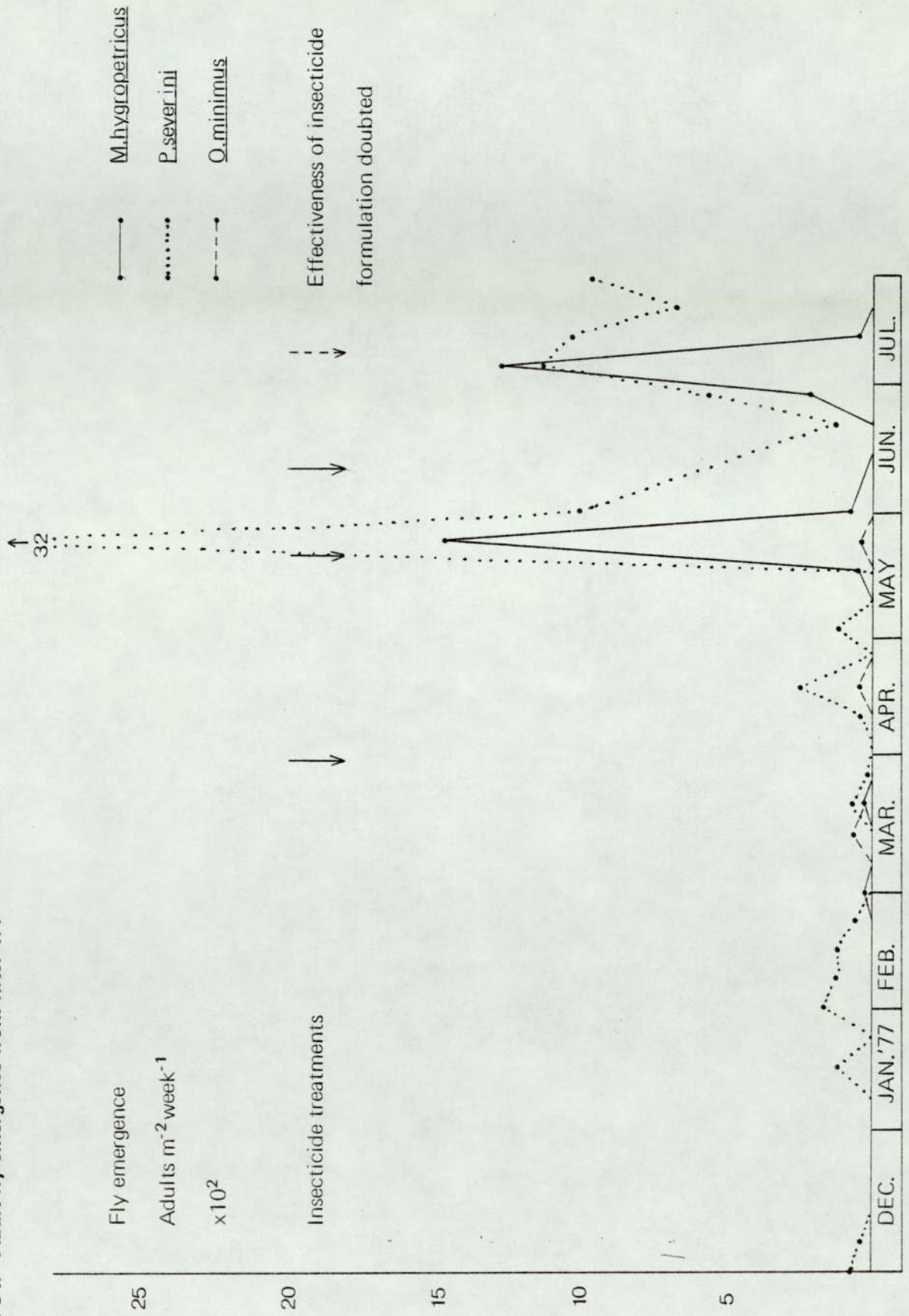


Fig. 35. Mean weekly temperatures February — October 1976

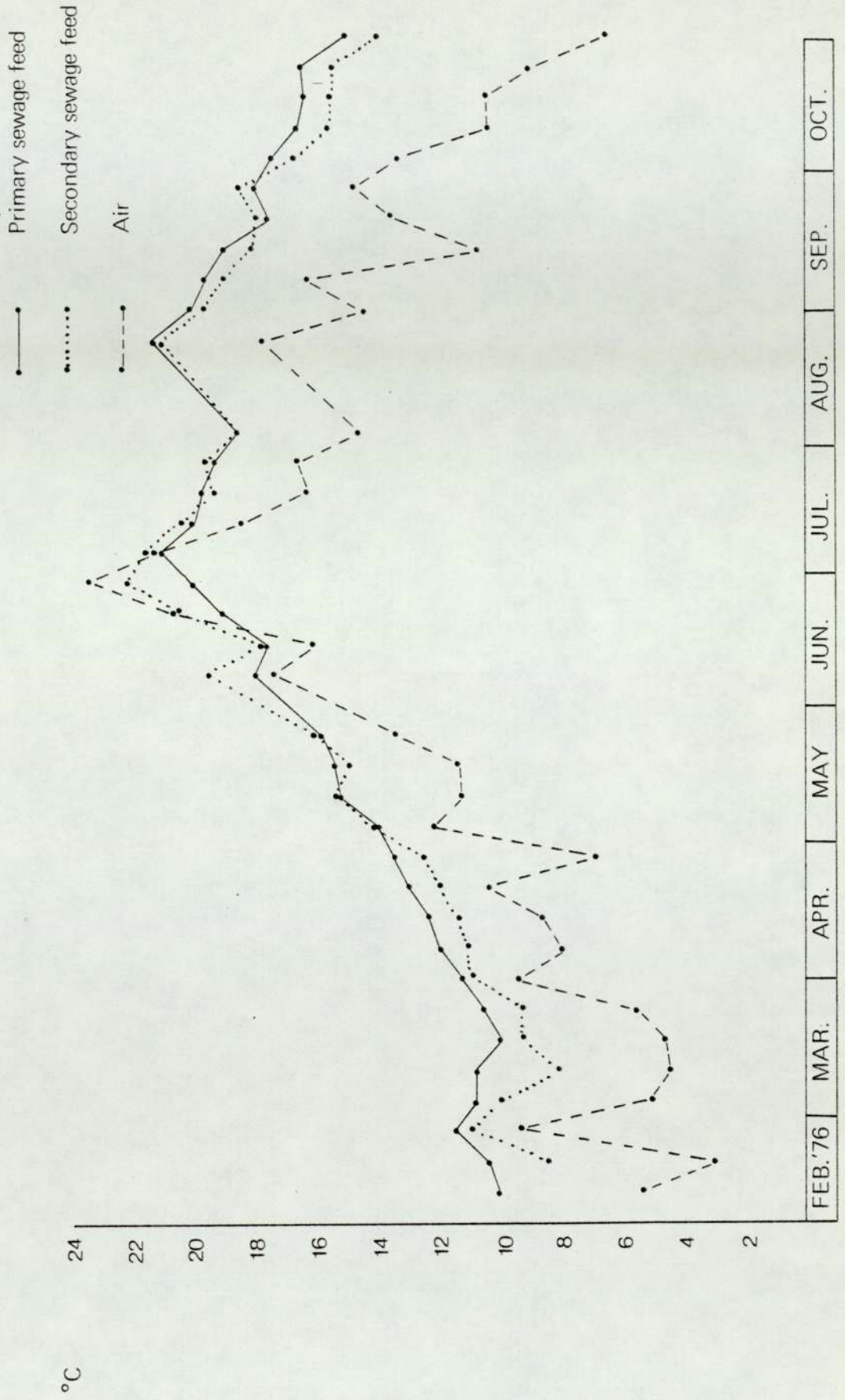


Fig. 36. Mean weekly temperatures November 1976 — August 1977

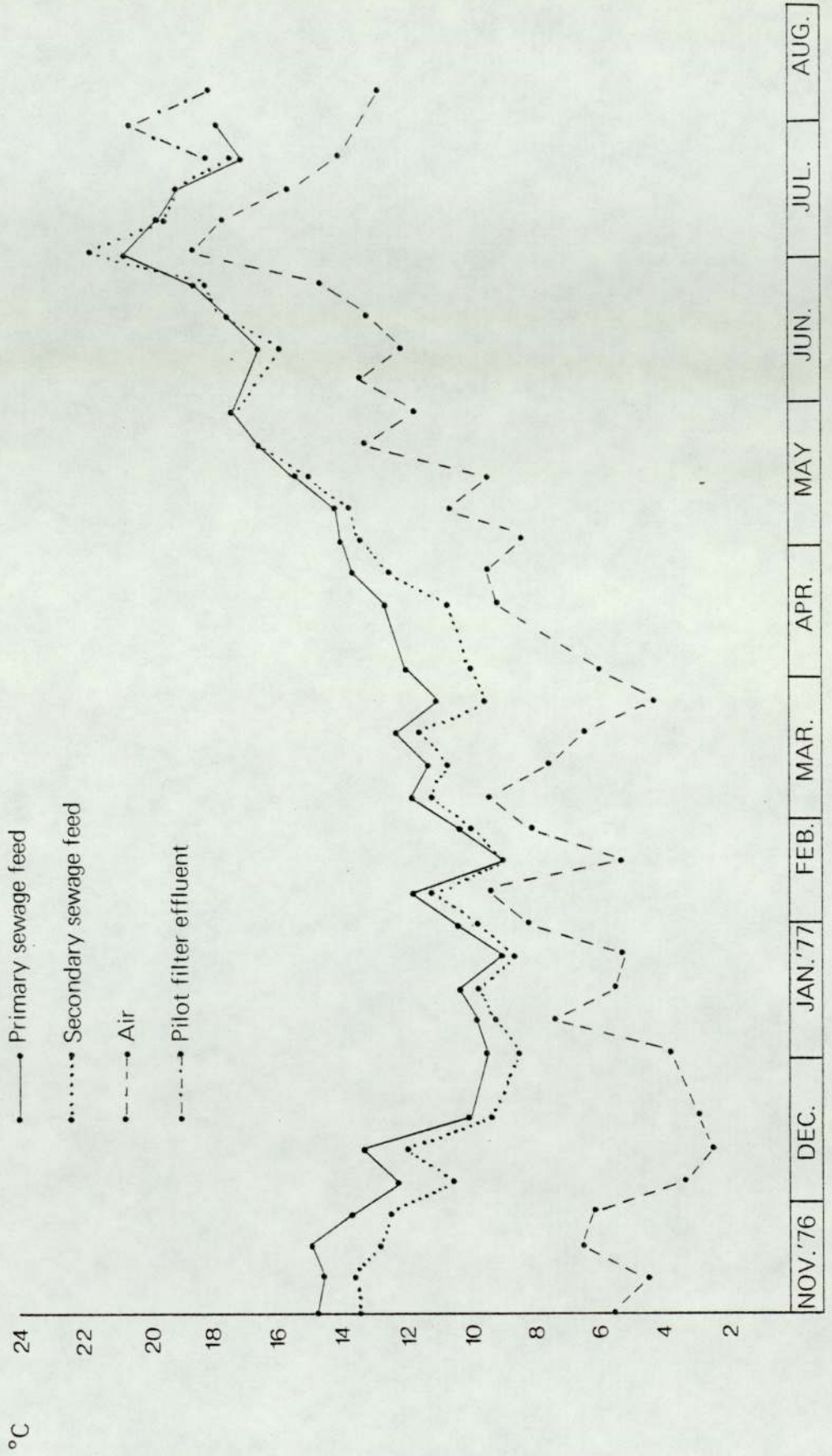


Fig. 37. Mean weekly temperatures August 1977 - April 1978

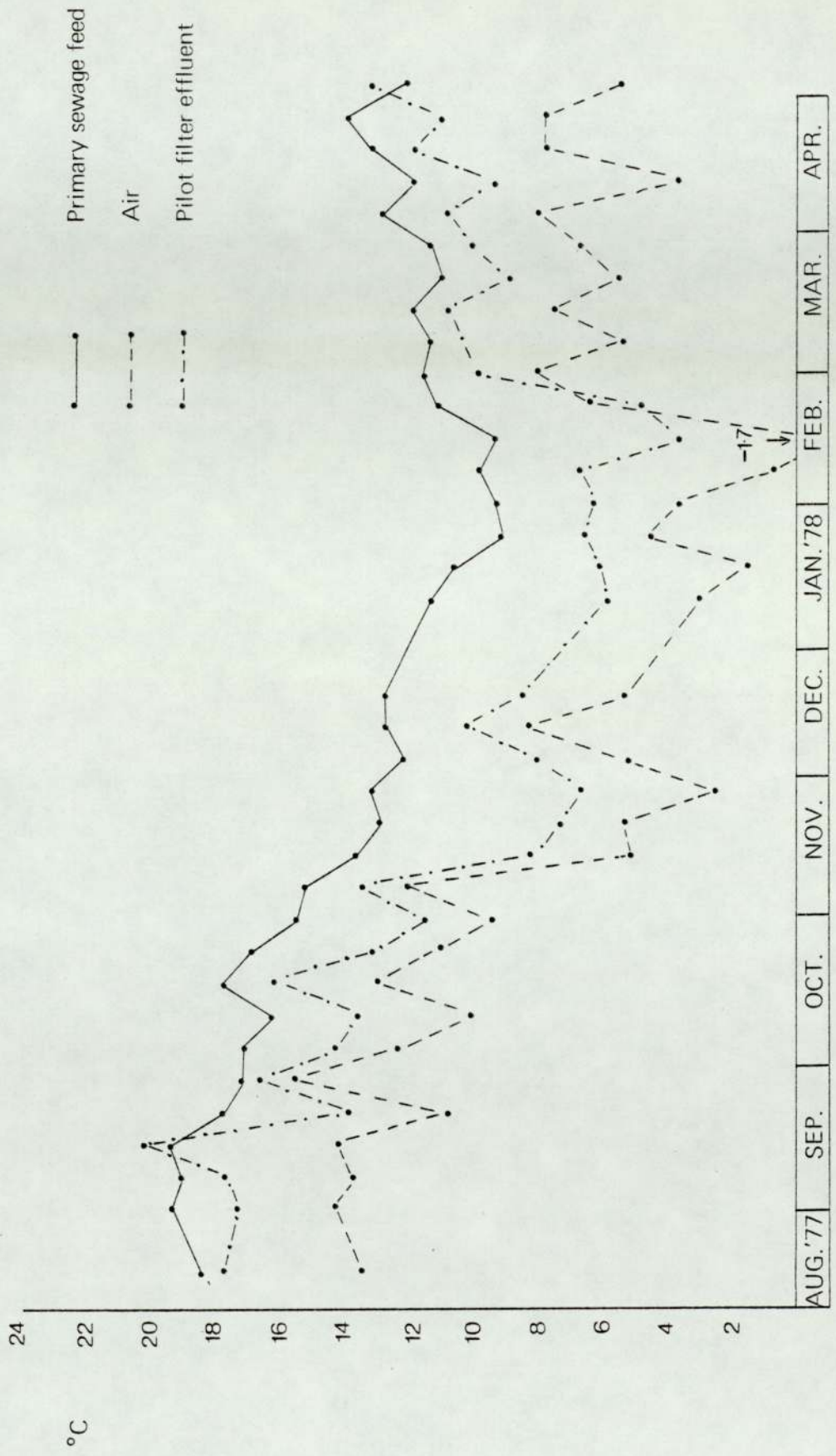


Fig. 38. Mean weekly numbers of *M. hygroplitricus* larvae and pupae — Control filter (primary block)

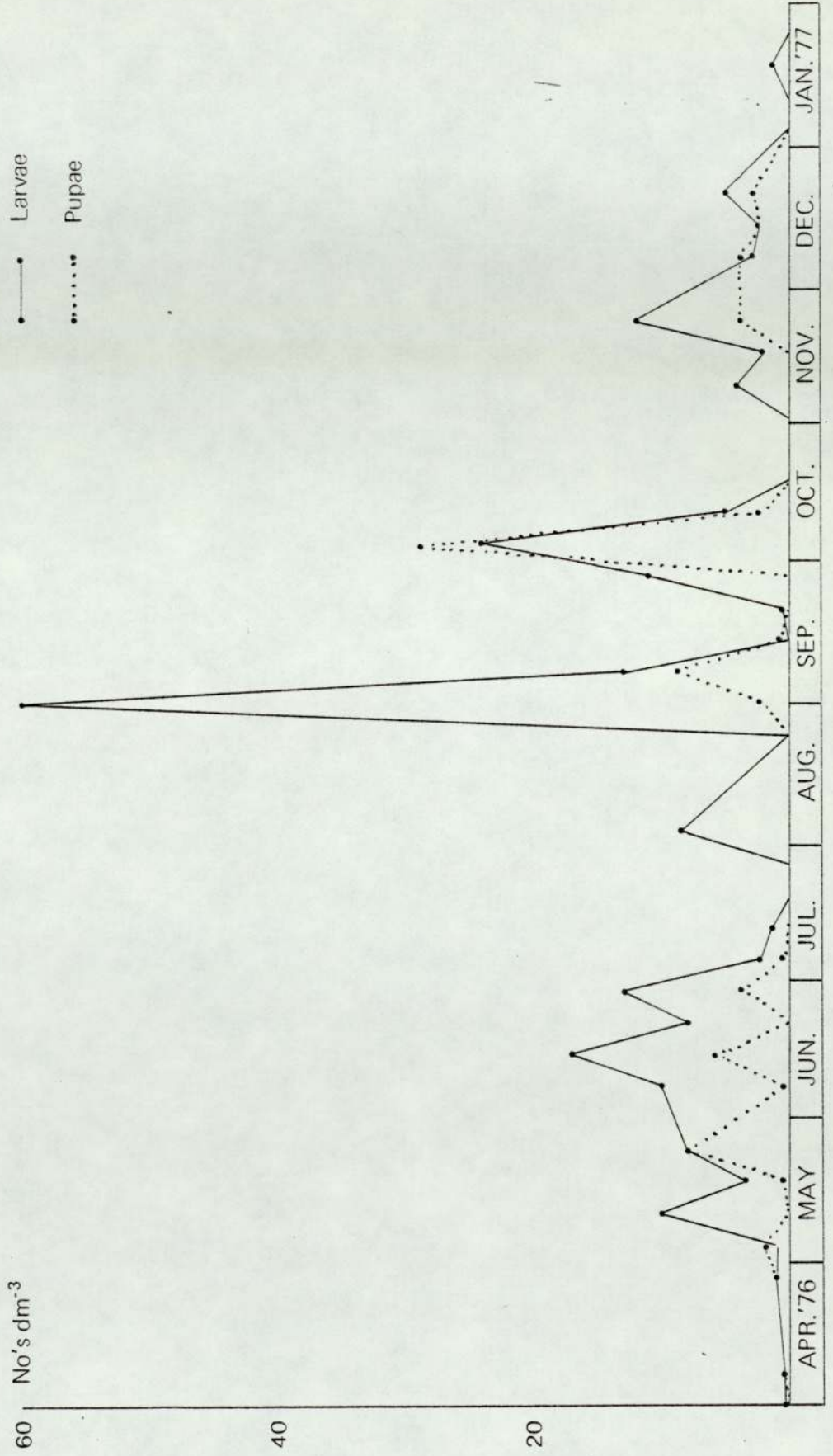


Fig. 39. Mean weekly numbers of *M. hygropliticus* larvae and pupae — Insecticide treated filter (primary block)

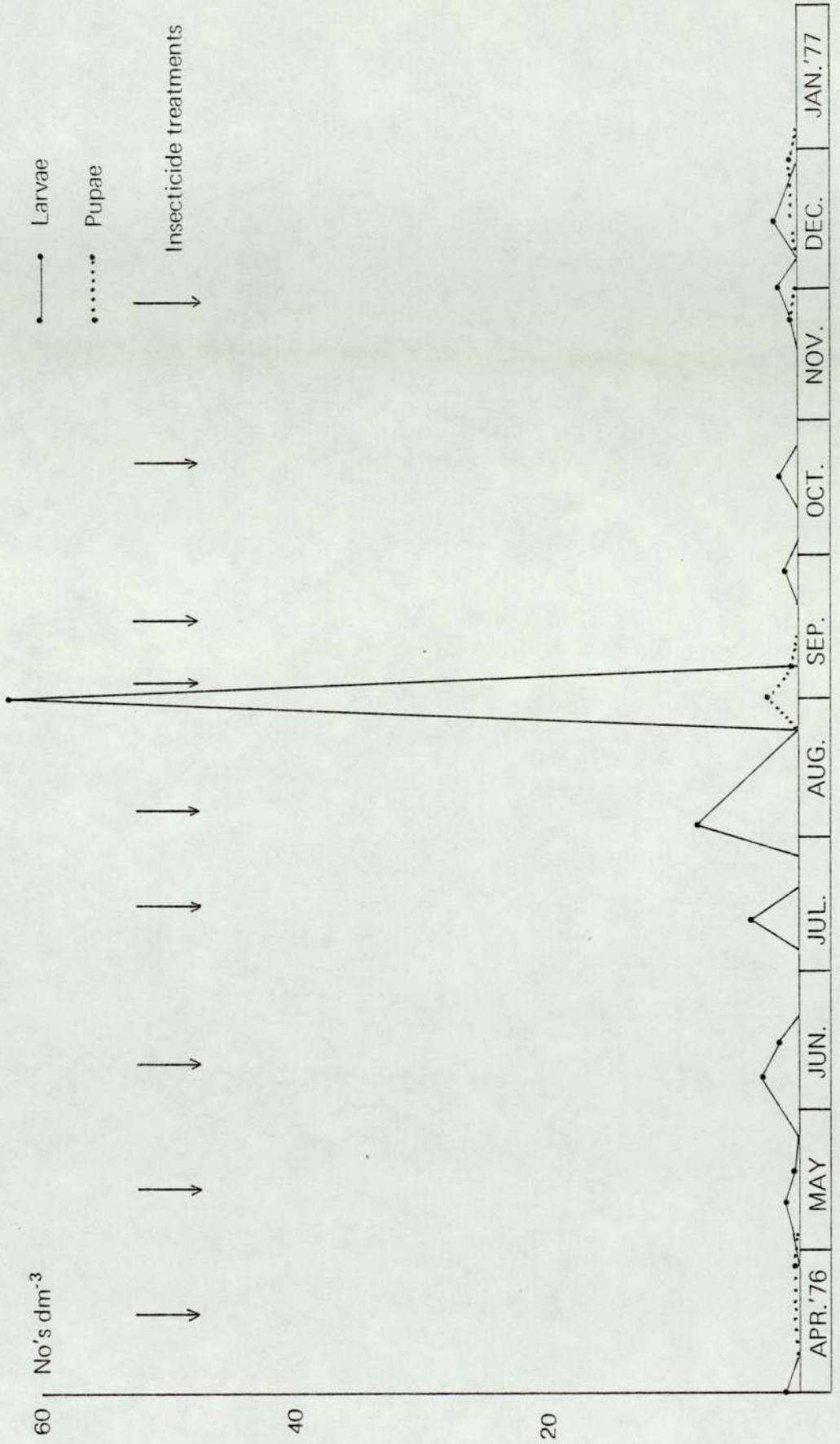


Fig. 40. Mean monthly enchytraeid worm numbers (primary block)

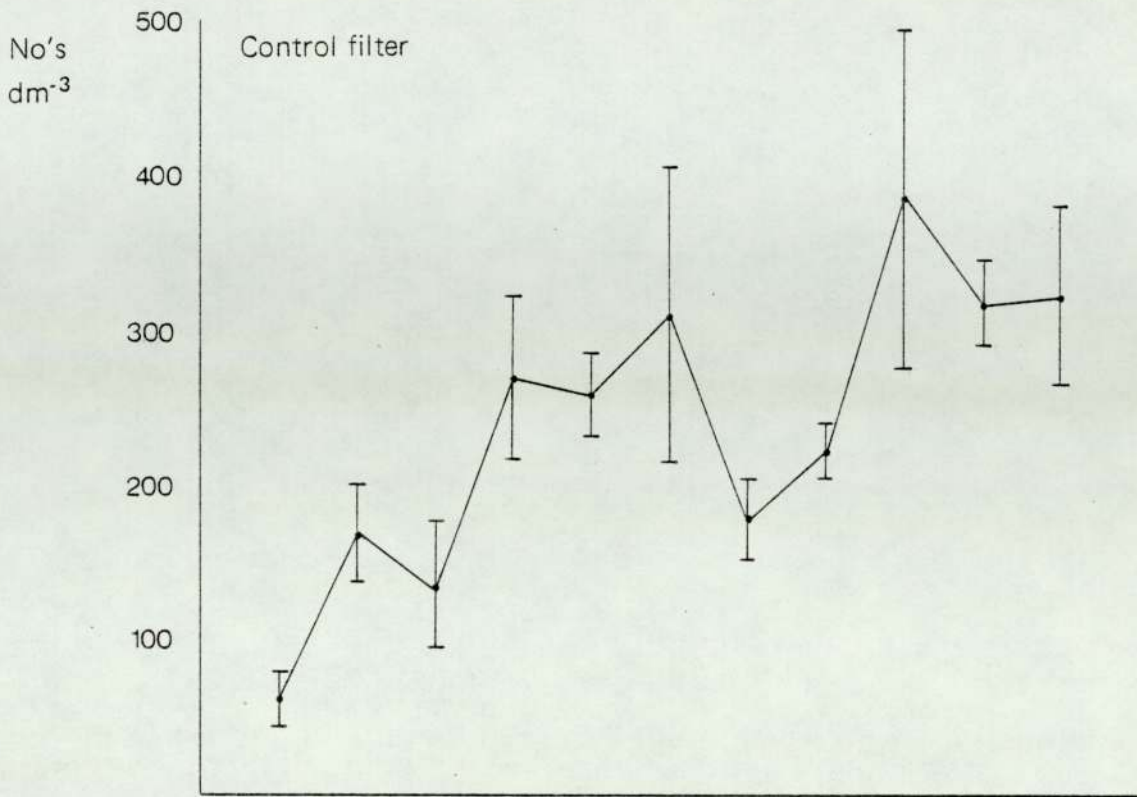


Fig. 41.

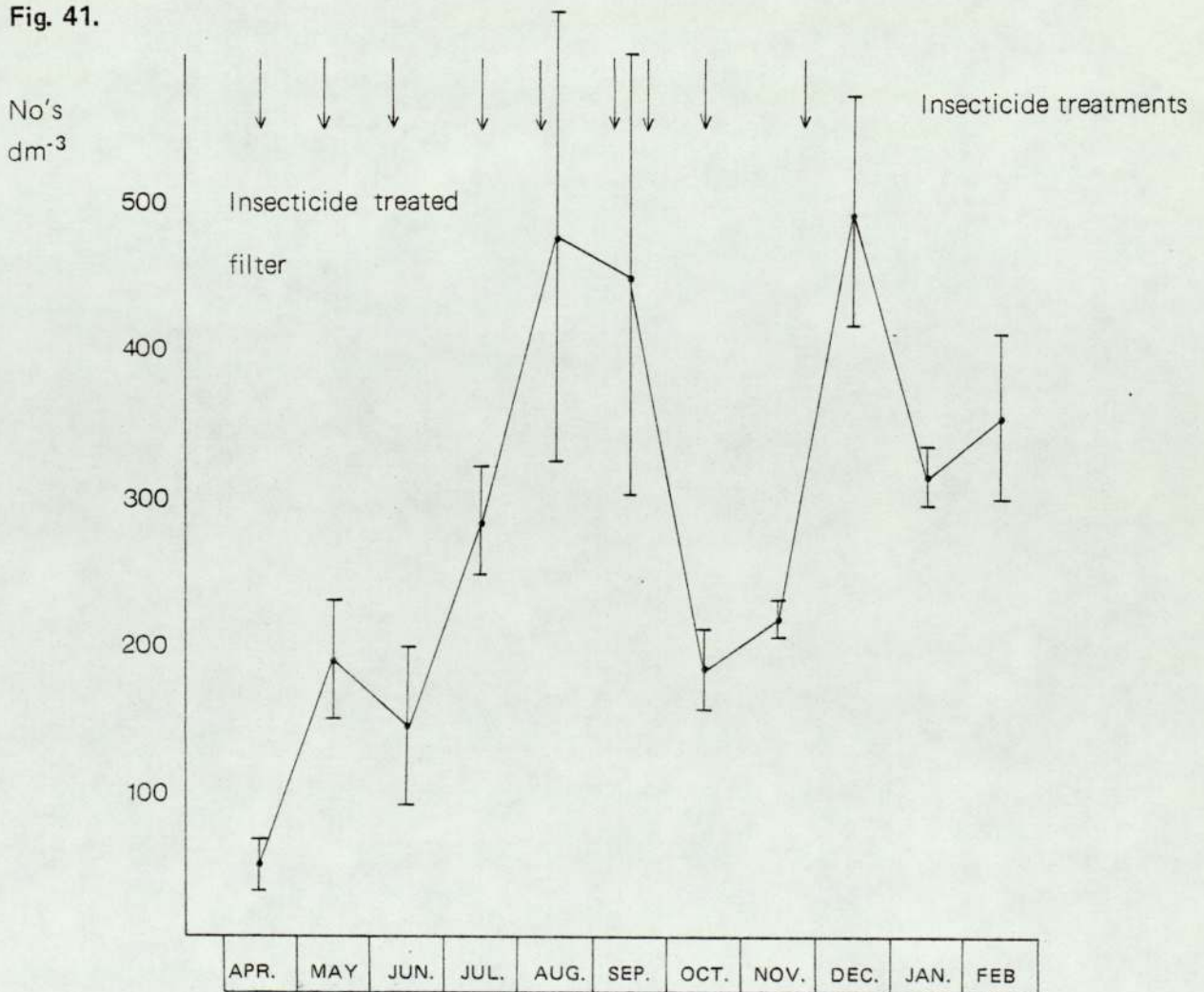


Fig. 42. Mean weekly volatile solids on control and insecticide treated filters (primary block)

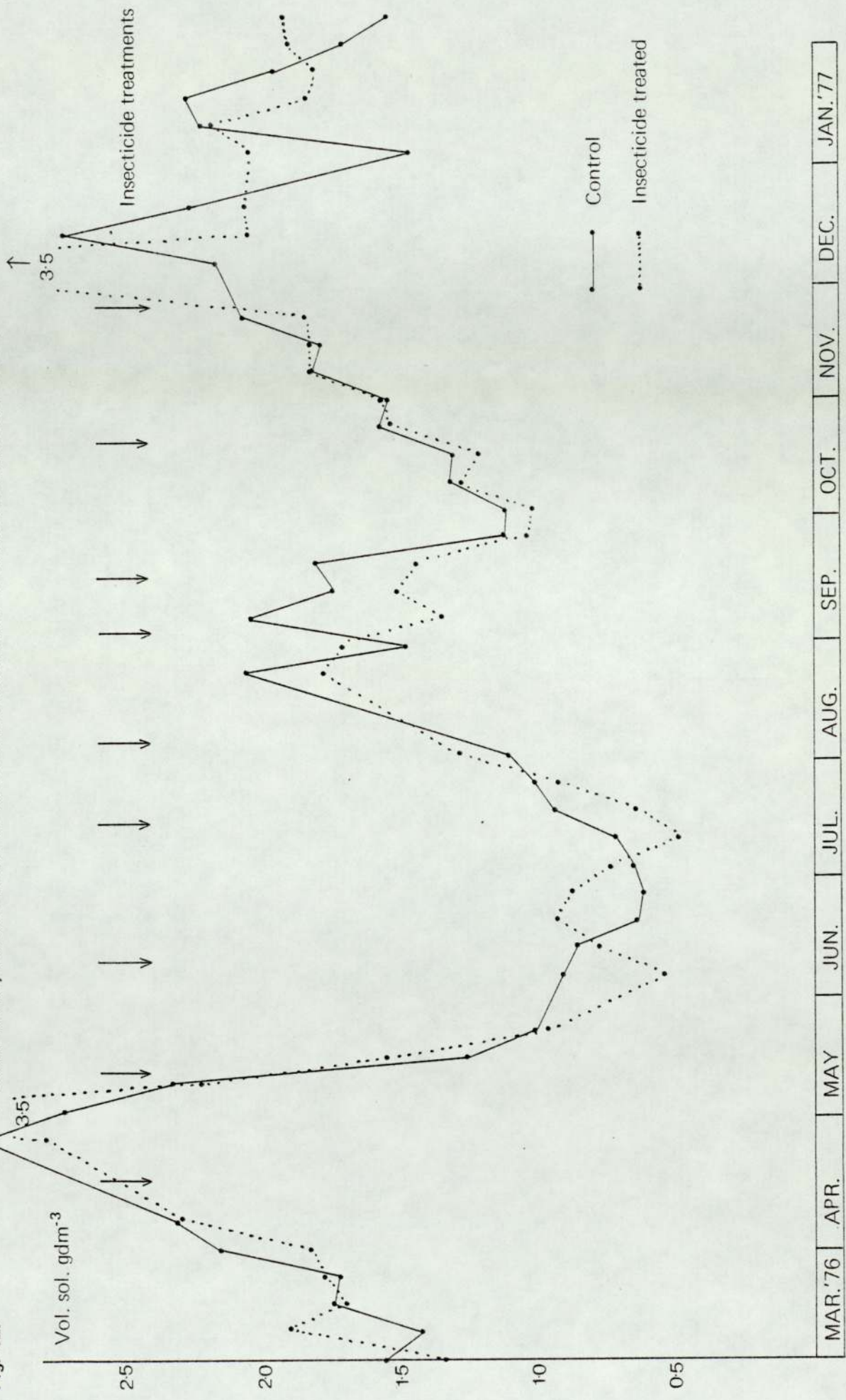


Fig. 43. Mean weekly numbers of *M. hydropetricus* larvae and pupae — Control filter (secondary block)

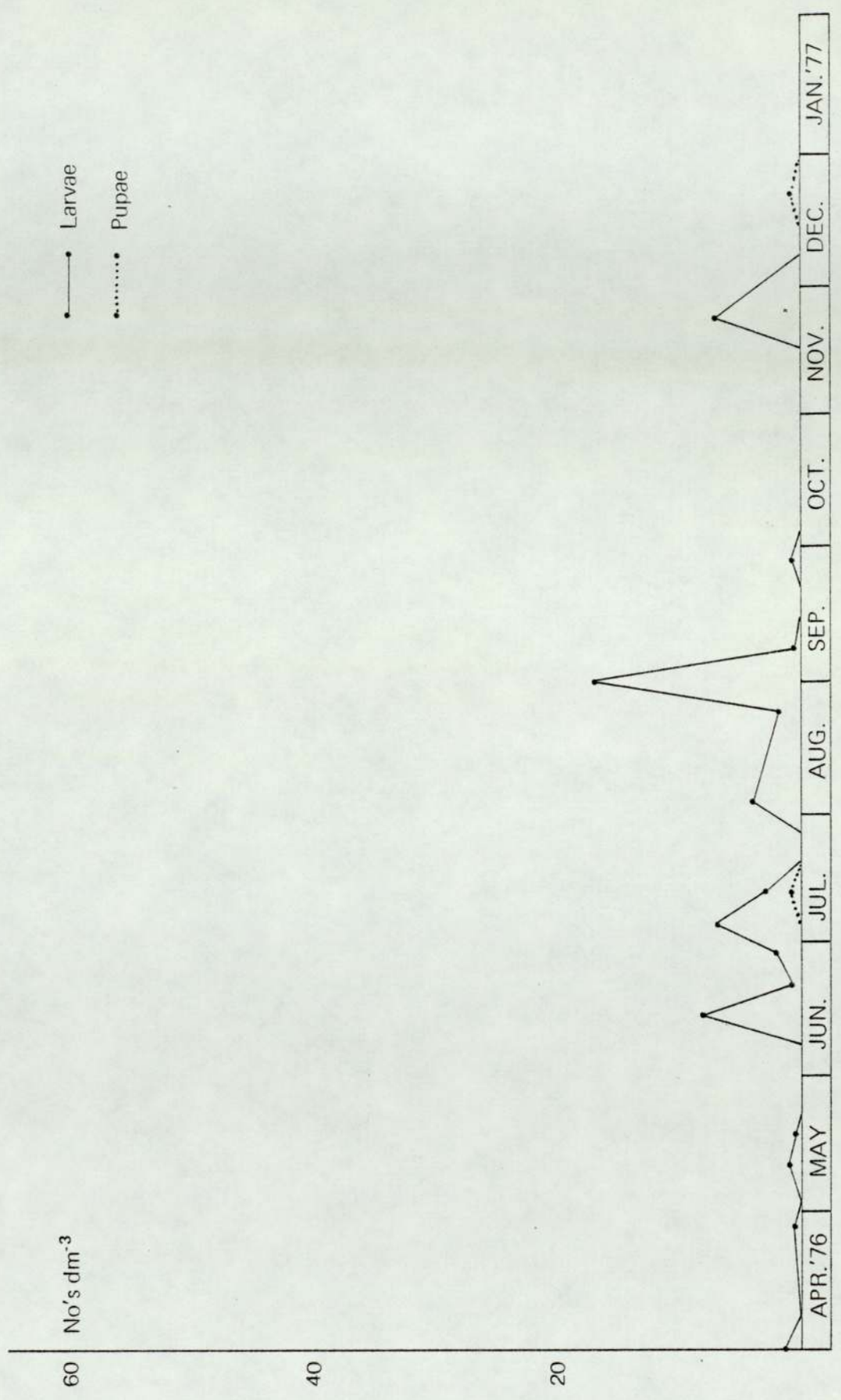


Fig. 44. Mean weekly numbers of *M. hygropetricus* larvae and pupae — Insecticide treated filter (secondary block)

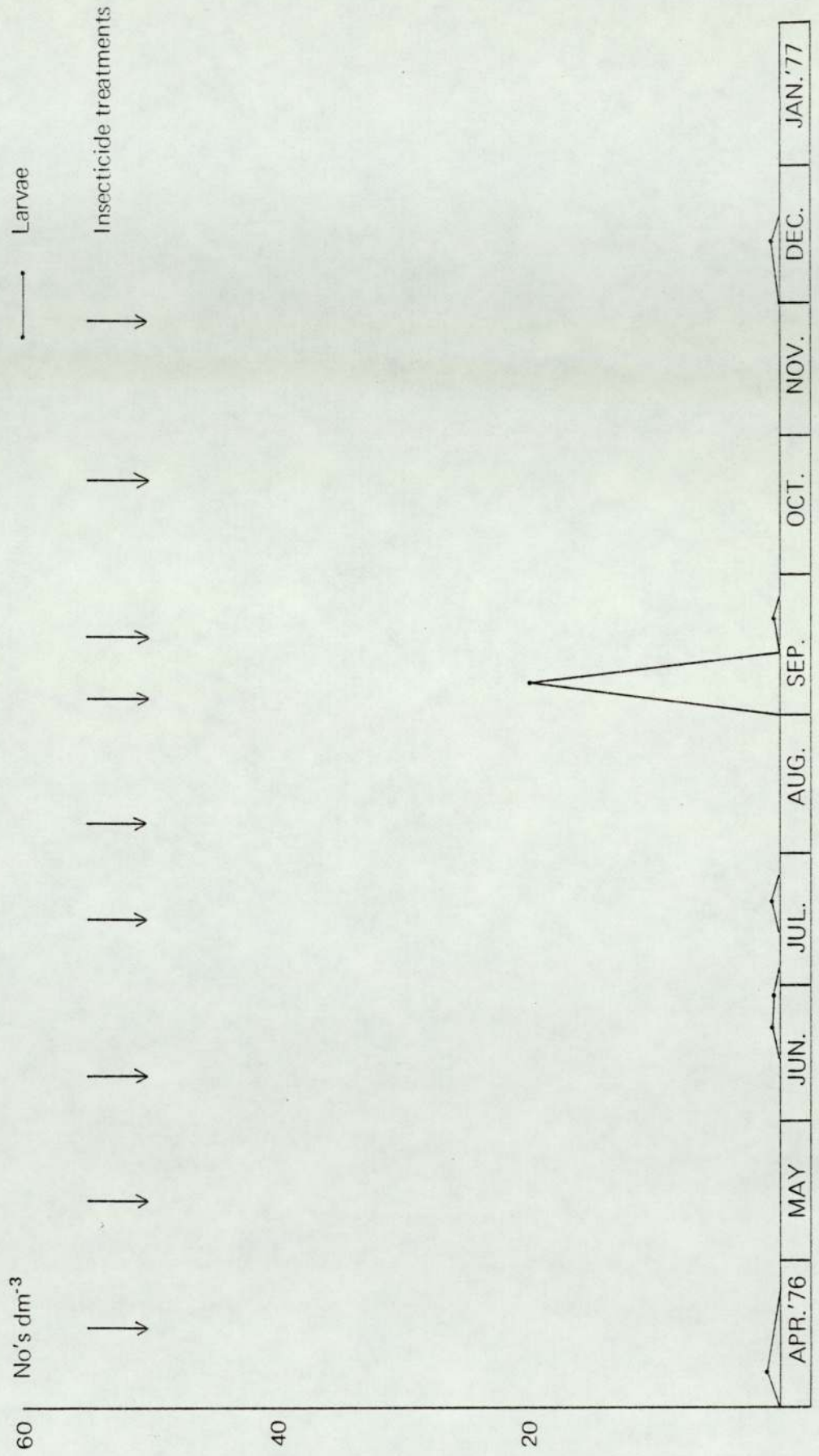


Fig. 45. Mean monthly enchytraeid worm numbers (secondary block)

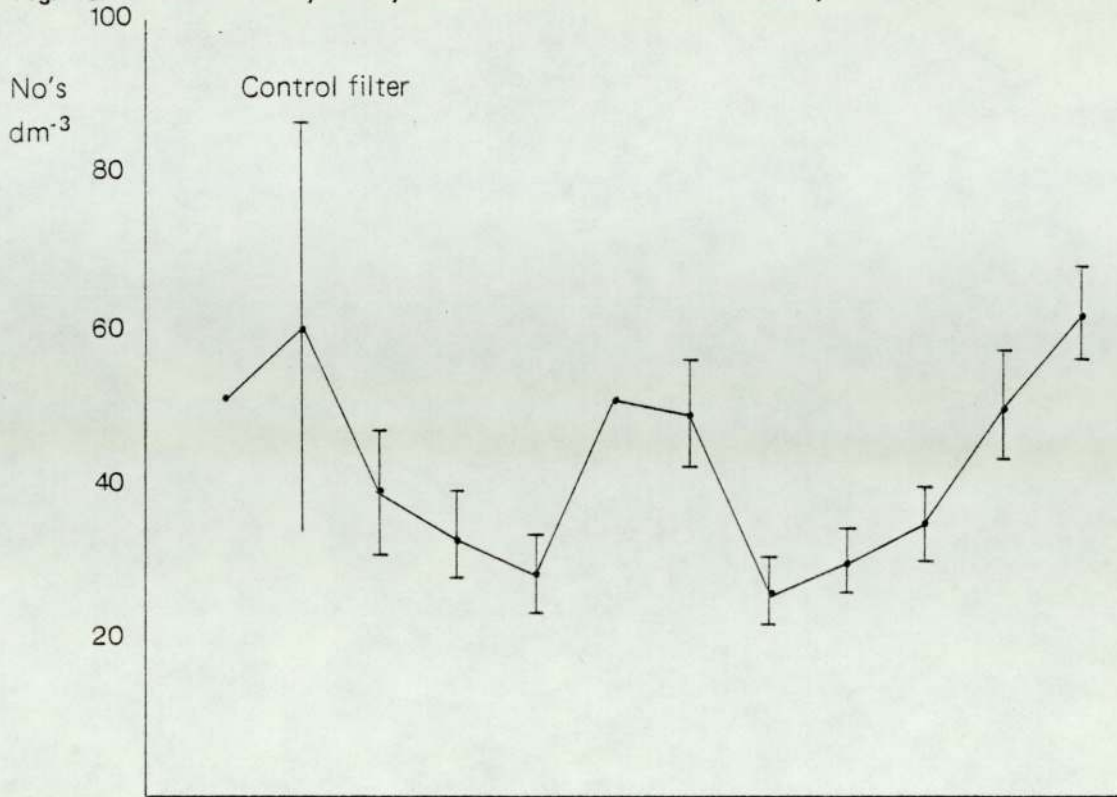


Fig. 46.

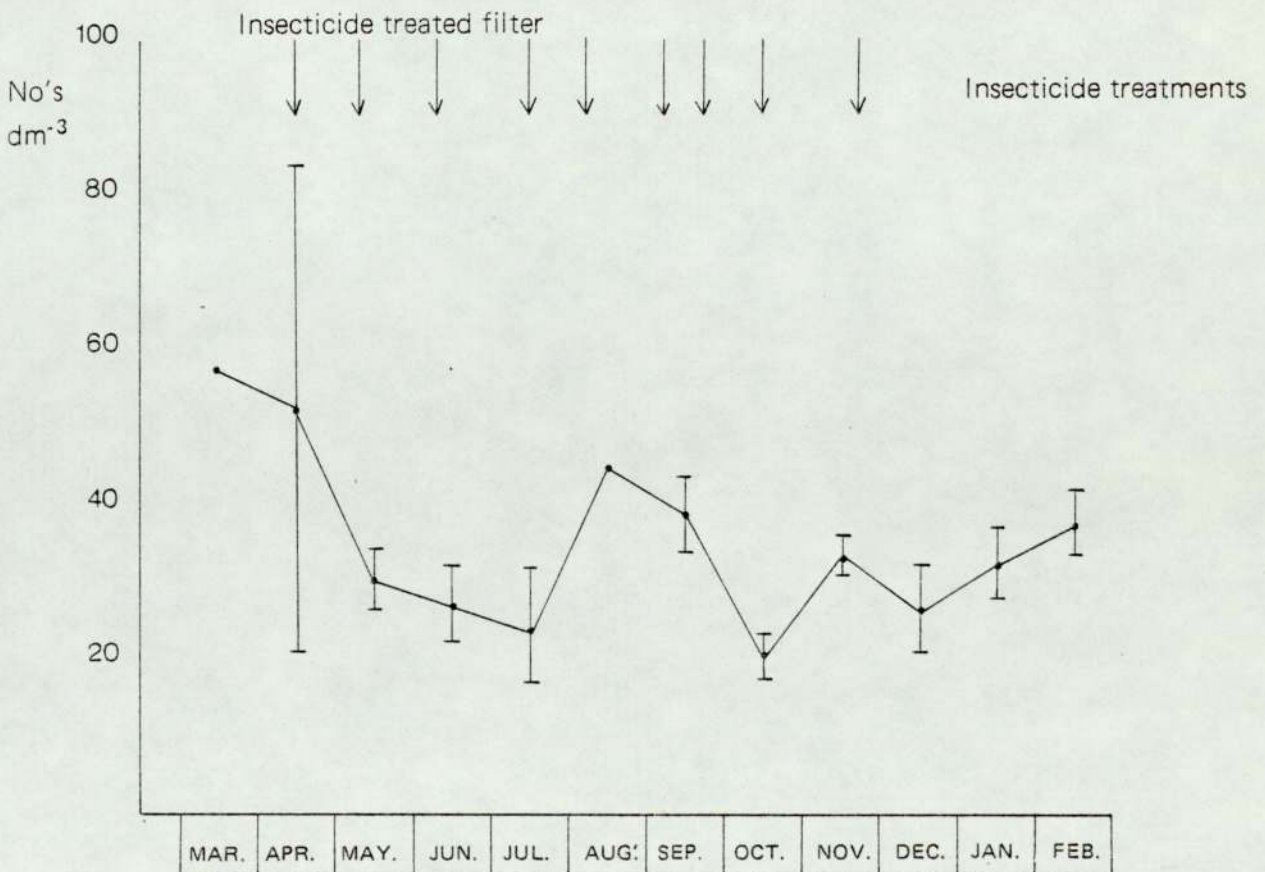


Fig. 47. Mean weekly volatile solids on control and insecticide treated filters (secondary block)

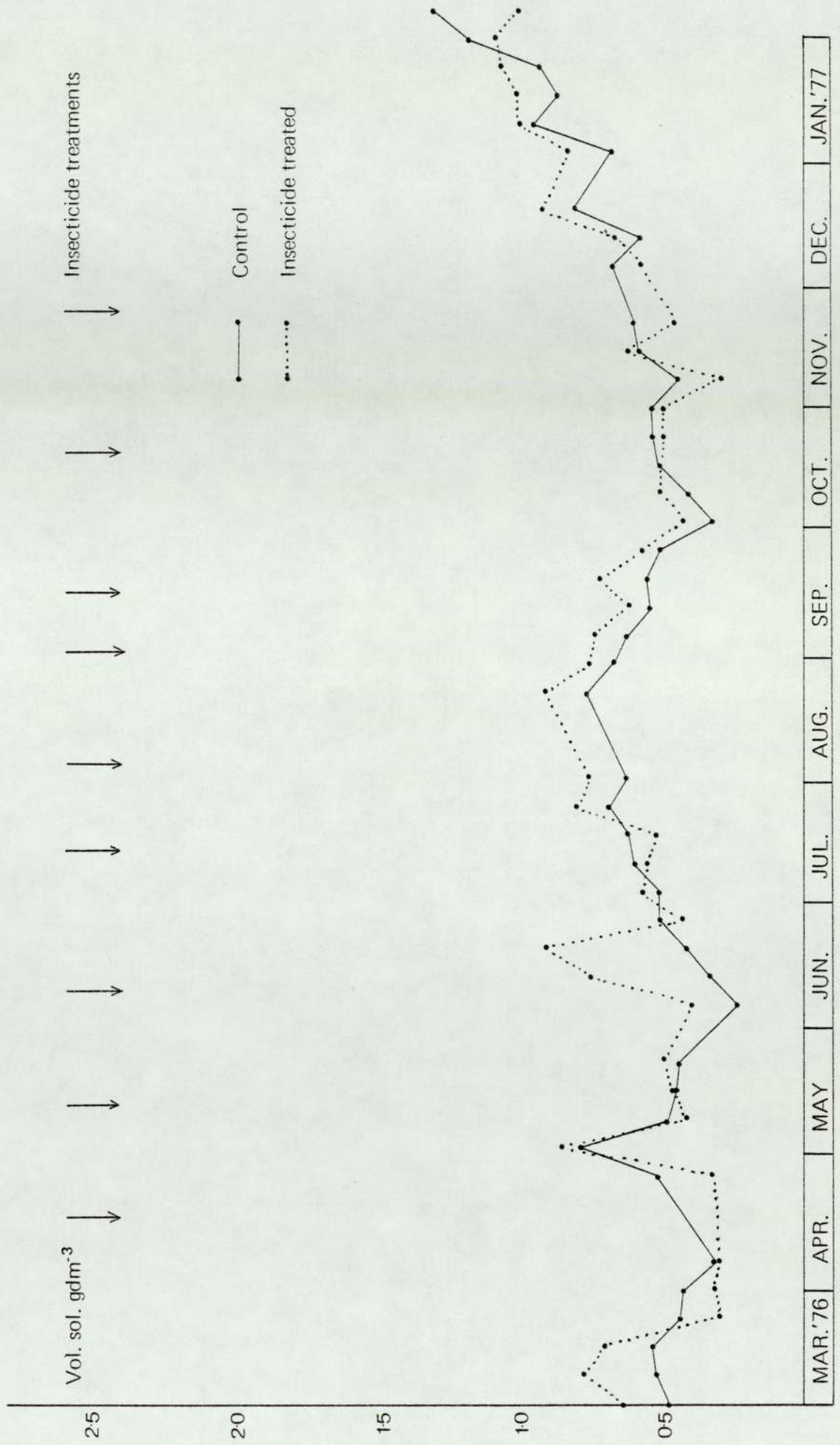


Fig. 48. Mean weekly temperatures — primary filter effluent as used in life cycle analysis February — October 1976

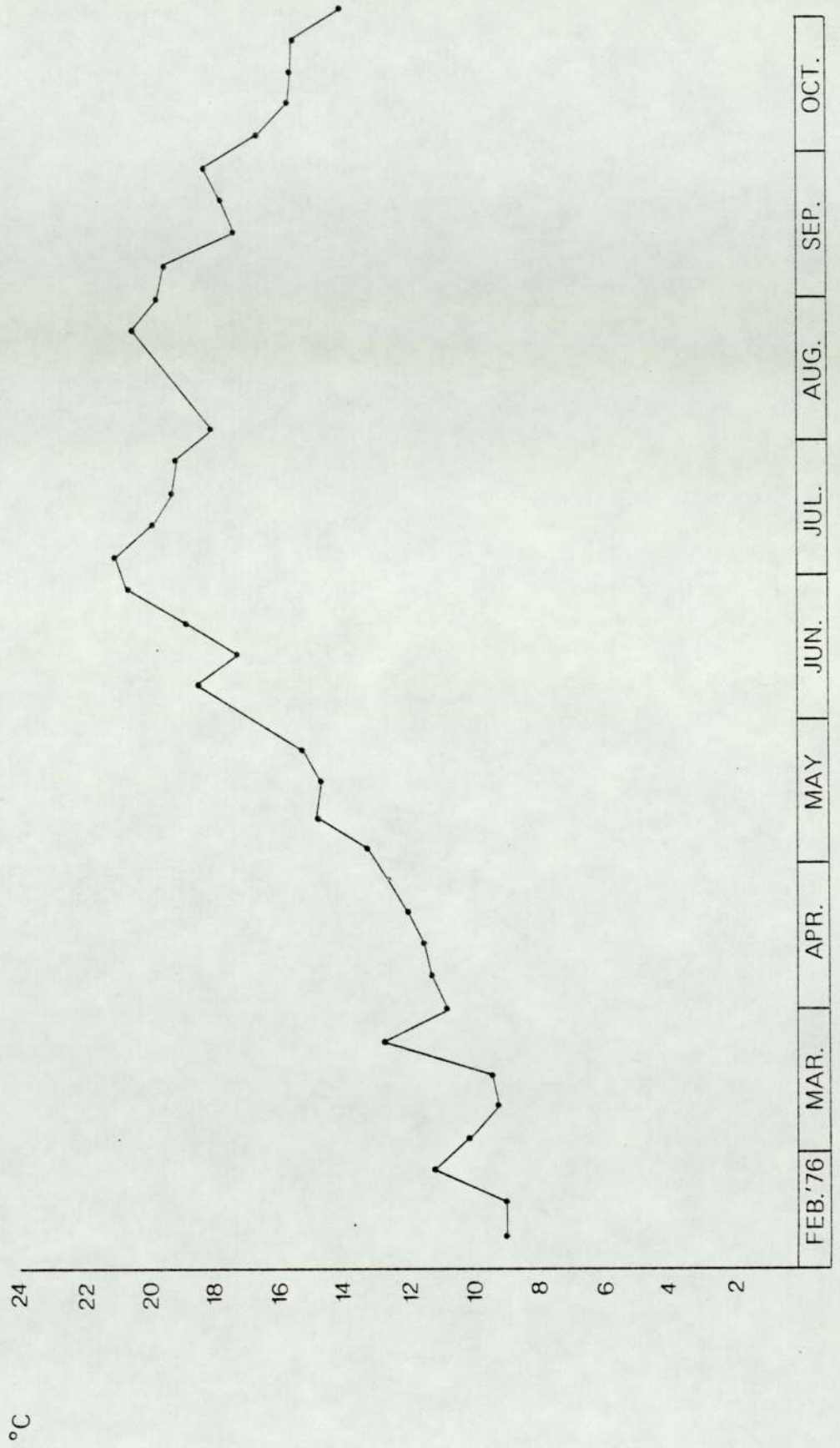


Fig. 49. Mean weekly temperatures — primary filter effluent as used in life cycle analysis November 1976 — August 1977

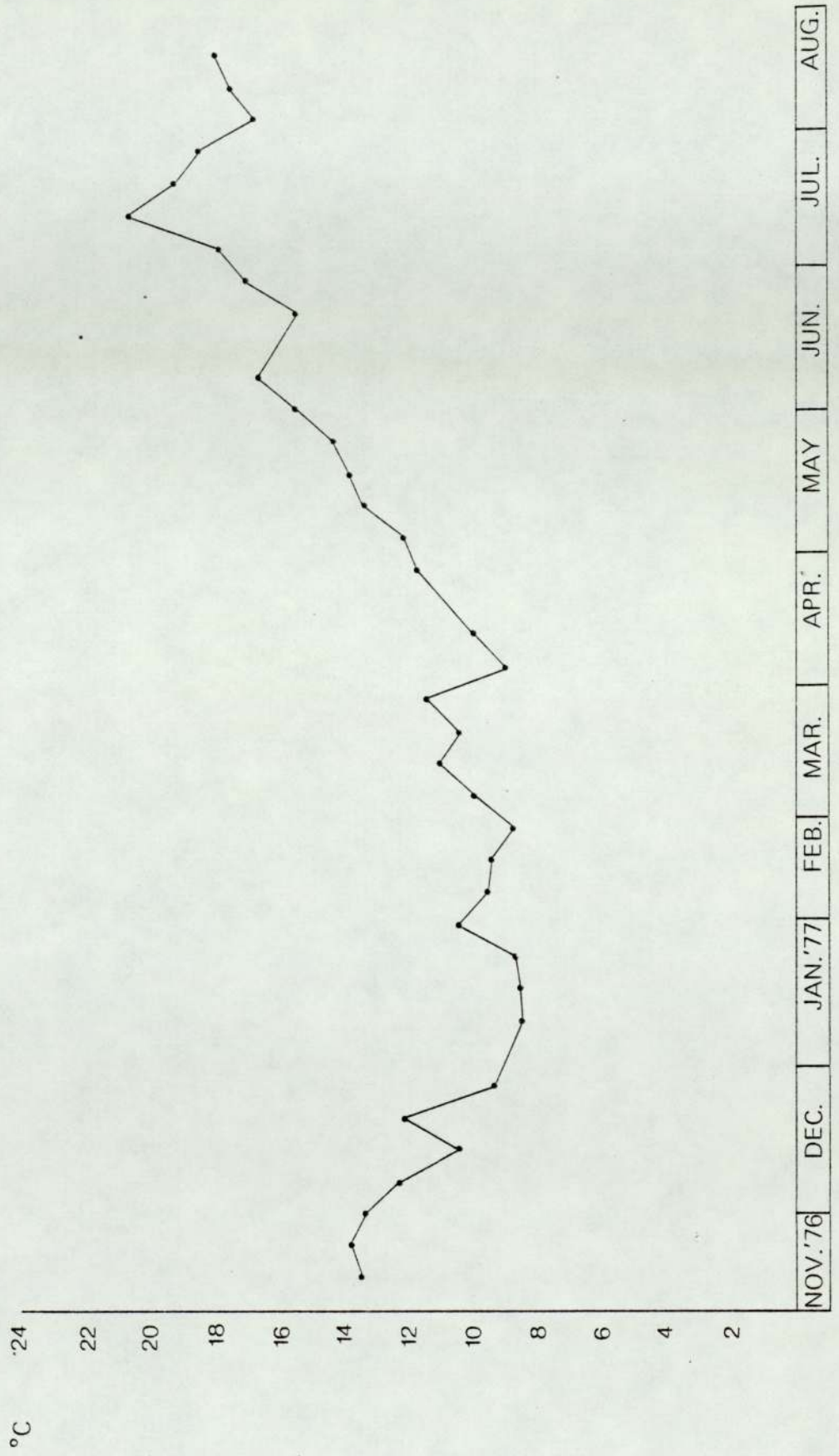
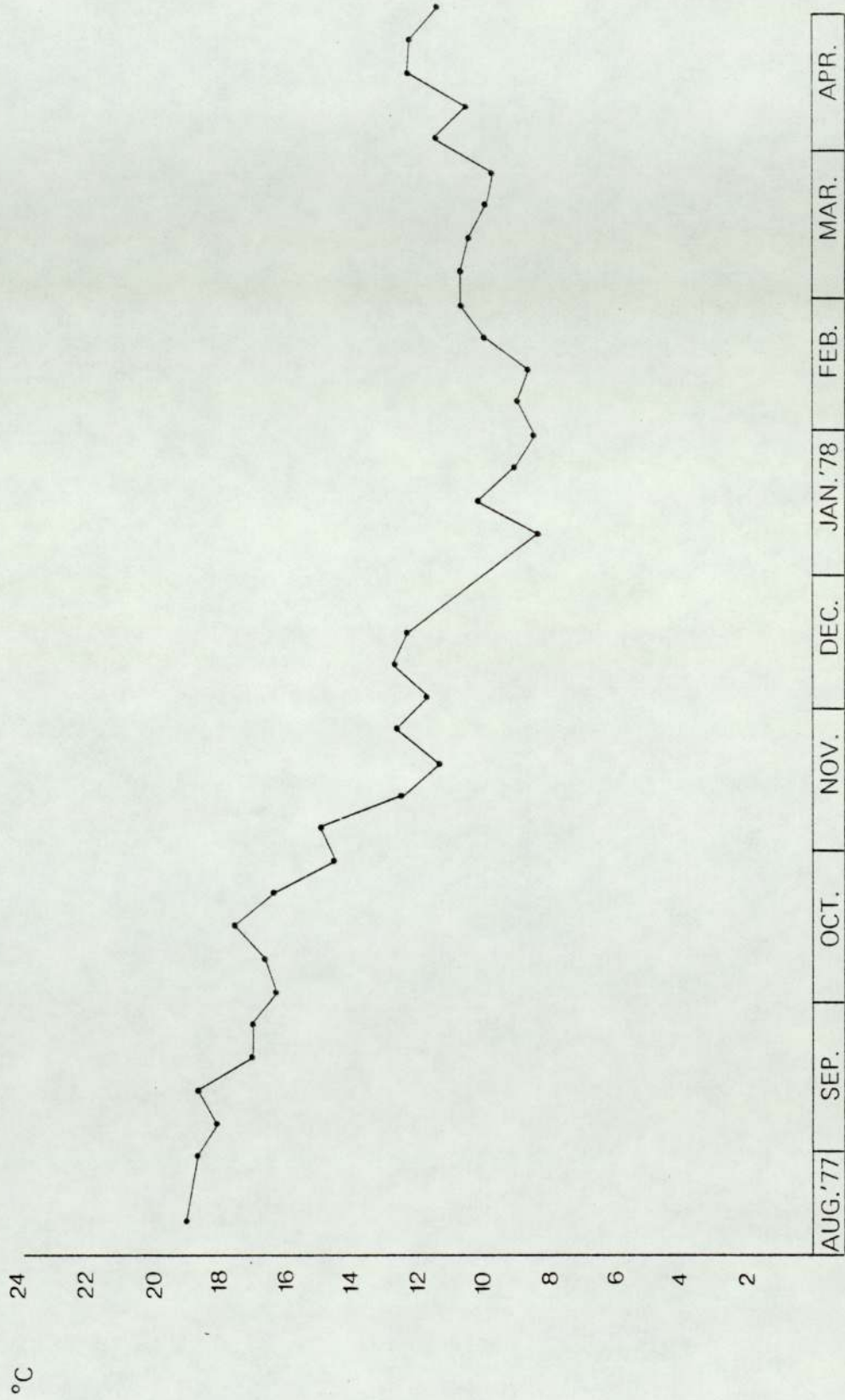


Fig. 50. Mean weekly temperatures — primary filter effluent as used in life cycle analysis August 1977 — April 1978



C. Fly control by operational modifications

i) Introduction to the modifications utilised

At the time of starting this investigation it was considered that insecticidal control had shortcomings, was not ecologically satisfactory and that more natural forms of control via changing operational conditions would be more attractive for long term control measures. It was therefore decided to investigate the feasibility of operational methods of fly control, even though at the time of starting these experiments insecticidal control was being utilised successfully.

The modifications used to investigate operational methods of fly control have already been described in the methods section. To repeat these consisted of:-

- 1 Double loading (hydraulic and organic).
- 2 Double dosing frequency 15 — 7.5 mins.
- 3 Altering application method of sewage on to filter by fitting splash plates.
- 4 Application of small media topping (230 mm depth, 19 mm diameter) to surface of filter.
- 5 A combination of 3 and 4 above, small medium topping impinged on with sewage via the splash plate apparatus.

The reasons for choosing these specific modifications were as follows.

1 Double loading

Many workers in the past observed that different loadings tended to favour different fly species. These instances can be referred to in the Literature review section.

Generally it is accepted that film levels as decided by many factors including

loadings, are the main factor in deciding the presence or not of certain species, also it was the general view of many workers such as Tomlinson and Stride (1945) and Terry (1956) that Psychoda levels increase directly with loading and that Metriocnemus preferred clean lightly loaded filters. Therefore it was decided to alter the loading on a portion of the main filters.

It should be appreciated that it would be very difficult to increase the organic loading without increasing the hydraulic loading as this would entail changing the strength of the sewage, therefore the only possible way in which the organic loading could be increased was to increase the total flow on to the experimental portion of the filter. A system was used whereby the flow from 4 nozzles was redirected to impinge on an area of filter already served by 4 nozzles (see plate 1), thus the flow and therefore the hydraulic and organic loads received by that portion of the filter underneath the modification were doubled.

Since according to some workers large hydraulic loads inhibit fly emergence (Edmonson and Goodrich 1943, Lloyd 1945, and Bruce and Merkens 1970) the inadvertent doubling of the hydraulic load may have been to some advantage however this will be discussed later.

2 Double dosing frequency

As regards dosing frequency most work in the past has been concerned with its effects on filter efficiency (Tomlinson and Hall 1955, W.P.R. 1956, Stanbridge 1956 and 1958 and Cook and Crame 1976) and its effects in reducing film accumulation (Tomlinson and Hall 1951, Hawkes 1957 and Peach 1957). Concerning fly control Hawkes (1955 a and b) has described how low dosing frequencies could control fly emergences by two possible methods. Firstly low frequency dosing has been shown to keep film levels low by giving long periods for possible negative film growth between doses (Hawkes 1961b), thus this may limit macrofauna populations by reducing their

food supply. Secondly the effect that low frequency dosing has on the scouring action may play a part here. Obviously as the dosing frequency decreases the dose of sewage experienced by a unit area of filter is maintained for a longer period in the dosing cycle. This then would have the effect of increasing the scouring action which has been shown to reduce both surface film levels (Holtje 1943 and Bridge-Cooke 1959) and fly emergence (Hawkes 1955b).

It has been suggested by Barritt (1939) that the scouring action found with low frequency dosing may result in a monopoly of the filter by 2 or 3 species which obviously allows these species to build up in excess of normal levels due to the exclusion of competitors. This has been shown by Hawkes (1961b) who found worm domination of low frequency dosed filters. Also it seems likely that the prehensile enchytraeids and chironomid larvae would be retained with the exclusion of competitors in filters subjected to high scouring forces. It has already been found that these organisms can withstand much higher scouring forces than other species (Styles 1975).

Therefore it was decided to increase the dosing frequency on a portion of the main filters with the intention of increasing film levels and species diversity in order to provide conditions suitable for competitors of Metriocnemus to survive. A simple way to do this without altering the speed of the distributors was found to be to divert the flow from 4 nozzles on one half of the distributor arm used during the "outward" journey to the other half used on the "homeward" journey resulting in a dosing frequency of 7.5 min. For further details on this system the methods section (p. 51) should be referred to and the apparatus for achieving this can be seen in Plate 2.

3 Application method

In the past studies have been made to investigate the optimum application system from the point of view of achieving a balanced grazing fauna whilst retaining the purification efficiency (Hawkes 1959). The reason for requiring the balance was to prevent nuisance species from increasing by allowing their competitors to survive.

It was the view of Hawkes (1959), when comparing certain application methods that 152 mm (6 in.) spaced straight through jets provided the best conditions of the methods tested and achieved a balanced grazing fauna by providing alternate "wet" and "dry" areas. At Tamworth the nozzle spacing is identical however no tracking was noticed and it is clear that the grazing fauna is not balanced therefore it is likely that other factors are preventing the desired achievement such as the large media size and the high scouring forces. It was suspected that that high scouring action due to the low dosing frequency on the main filters caused the enchytraeid and chironomid dominated community therefore it was decided to fit a splash plate over a series of nozzles to "fan" the sewage out into a sheet thus softening the force with which it impinged on the filter and covering the entire width of the experimental strip with sewage thus physically hampering fly emergence. Niani (1955) showed that flies could be controlled physically by "blanket" spraying with sewage. It was hoped that this system would encourage competitors of Metriocnemus such as psychodids to survive successfully. Thus a splash plate was fitted to alter the flow pattern from 8 consecutive nozzles on the distributor arm and its effect on this flow pattern can be seen in Plate 3.

4 Small media

Much work has been carried out in the past on the effects of media size on filter efficiency, film accumulation and fly emergence and this has been discussed fully in the literature review section. To repeat the general opinion is that expressed by Lloyd (1945) in that larger media favours Metriocnemus and smaller media Psychoda. The

mechanisms behind this according to Learner (1975) probably arise from different respiratory requirements of the two insect larvae being affected by the different film levels per unit volume found with different sized media.

As it has been found that Metriocnemus prefers large media it is hardly surprising that they have been found in large numbers with the exclusion of other species at Tamworth as the media is 50-65 mm in diameter. Similarly other factors already described including the loadings and dosing frequency also favour Metriocnemus therefore this probably accounts for their presence in large numbers.

Regarding media it was decided to increase the film levels per unit volume and reduce the voidage by applying small medium to the surface of the filter with the hope of preventing Metriocnemus emergence physically and encouraging other species to thrive in the high film conditions e.g. Psychoda. The small medium was applied in a similar fashion to that described by Tomlinson and Stride (1945) in being a 229 mm (9 in.) depth of 19 mm ($\frac{3}{4}$ in.) diameter smooth pebbles placed in a pre-excavated portion of the main filter. These authors found that the effects of this were physical, i.e. less flies of all species emerged from filters subjected to this treatment. The small medium in place on the filter can be seen in Plate 4.

5 Small media and splash plate

At the onset of applying small medium it was noticed that "tracking" was more apparent on this area giving completely "dry" areas between the jets of sewage which are obvious sites for fly emergence. This was apparent as lateral displacement of the sewage when it impinged on the medium was clearly greater on the large medium causing a fairly evenly wetted filter compared to the small medium area which gave the "tracking" effect.

Therefore it was decided to investigate these effects by positioning the previously

described splash plate apparatus over a portion of the small media section to alter the flow pattern to that portion. The intention here was to give even coverage of the filter with sewage thus leaving no "dry" areas for fly emergence and to give a thick even layer of subsurface film in hope of discouraging Metriocnemus emergence physically and favouring Psychoda via the high film levels.

ii) Effect of operational modifications on fly emergence

The results of the investigations into the above mentioned modifications on fly emergence can be seen in the following figures. Trapping was carried out weekly in triplicate from February 1976 to June 1978.

<u>Primary filters</u>	<u>M.hydropetricus</u>	<u>P.severini</u>
Double load	Fig. 51	Fig. 52
Double dosing frequency	" 53	" 54
Small medium	" 55	" 56
Small medium and splash plate	" 57	" 58
Splash plate alone	" 59	" 60

Secondary filters

Double load	Fig. 61	Fig. 62
Double dosing frequency	" 63	" 64
Small medium	" 65	" 66
Small medium and splash plate	" 67	" 68
Splash plate alone	" 69	" 70

These results were then treated statistically by applying "t" tests to investigate the reductions in M.hydropetricus and increases in P.severini emergences found. The

Table 13 continued
P.severini

Filter modification	Mean weekly emergence per m ² (×10 ²)	Paired 't' test Control v. Modified areas		
		't' value	'P' value	Significance
Primaries				
Control	1.0 ± 0.1	—	—	—
Double load	0.7 ± 0.1	2.14	2.5%	S
Double dosing frequency	3.0 ± 0.4	-4.61	<0.1%	VHS+
Small medium	0.8 ± 0.1	1.43	>10%	NS
Small medium and splash plate	0.9 ± 0.1	0.57	>10%	NS
Splash plate alone(Apr.'76-Aug.'77)	1.7 ± 0.3	-1.64	>10%	NS
Splash plate alone(Aug.'77-Jun.'78)	0.5 ± 0.1	1.06	>10%	NS
Secondaries				
Control	2.0 ± 0.5	—	—	—
Double load	1.8 ± 0.5	0.93	>10%	NS
Double dosing frequency	2.4 ± 0.7	-1.12	>10%	NS
Small medium	0.6 ± 0.3	3.83	<0.1%	VHS+
Small medium and splash plate	1.2 ± 0.3	3.23	0.1-1%	VHS
Splash plate alone	2.4 ± 0.7	-0.83	>10%	NS

± = S.E.M.

No. observations = 109 (primaries) 67 (secondaries)

mean monthly fly emergences for each modification were compared to the control filter emergences (see Table 13).

Table 13

Filter modification	<i>M. hygropetricus</i>		<i>P. severini</i>		Significance
	% change from control	"t" value (52°F)	"t" value (53°F)	"P" value	
		Mean weekly emergence per m ² (X10 ²)	Paired 't'test 't' value	Control v. Modified areas 'P' value	
Primaries					
Control		3.1 ± 0.7	—	—	—
Double load	-54.70	1.4 ± 0.3	2.81	0.1-1%	VHS
Double dosing frequency		1.0 ± 0.2	3.11	0.1-1%	VHS
Small medium		0.8 ± 0.2	3.51	<0.1%	VHS+
Small medium and splash plate		0.6 ± 0.1	3.58	<0.1%	VHS+
Splash plate alone (Apr.'76-Aug.'77)		3.9 ± 0.8	-0.22	>10%	NS
Splash plate alone (Aug.'77-Jun.'78)		0.6 ± 0.1	2.45	1-2%	HS
Secondaries					
Control		1.2 ± 0.5	—	—	—
Double load	-60.34	0.5 ± 0.2	2.01	≈5%	≈S
Double dosing frequency		0.4 ± 0.2	2.19	2-5%	S
Small medium		0.4 ± 0.2	1.63	5-10%	NS
Small medium and splash plate		0.3 ± 0.1	2.14	2-5%	S
Splash plate alone		0.9 ± 0.5	2.21	2-5%	S

± = S.E.M. No. observations = 109 (primaries) 67(secondaries)

(a) Primary filters

i) Double load

By consulting Figures 51 and 52 the effects of double loading on adult fly emergence in relation to control can be seen.

It can be seen that a reduction in M. hygropetricus emergence was apparent over the

experimental period which on analysis proved to be very highly significant ($P = 0.1 - 1\%$), however P. severini levels did not rise as expected, in fact in some cases they were higher from the control filter than from this modification. It would be expected that a filter on such a loading ($0.3 \text{ Kg BOD m}^{-3} \text{ day}^{-1}$) would support a large population of Psychoda and very few chironomids (Lloyd 1945, Tomlinson and Stride 1945 and Terry 1956). However, it is likely that the results obtained were influenced by the increase in hydraulic load which was experienced along with the increase in organic loading. The high organic load would probably have had the effect of increasing film levels, however this would not have occurred immediately sub-surface as the increase in hydraulic load would probably have pushed the peak in sub-surface film to a lower level. Also any increase in Psychoda that may have been apparent due to the increased film levels would probably have been reduced as it is known that they have to migrate towards the surface to pupate, and it is probable that the scouring action would have been great there due to the high hydraulic load. The inability of Psychoda to withstand high flow rates has been shown by Styles (1975) where rates of 29 mls^{-1} were sufficient to displace Psychoda pupae compared to rates of $60-80 \text{ mls}^{-1}$ for chironomids.

Similarly, a greater reduction in M. hygropliticus emergence levels would be expected if the organic load alone was increased. A significant reduction was obtained which suggests that either the increase in sub-surface film levels was sufficient to discourage Metricnemus or the scouring action was high enough to displace their pupae which is not so likely, as very high flow rates are required (Styles 1975).

2 Double dosing frequency

The effect of increasing the dosing frequency from 15 to 7.5 min on fly emergence can be compared to control emergence in Figures 53 and 54.

It can be seen that the reduction in M. hygropetricus emergence over the three years on the modified area was considerable, and this was matched by a corresponding increase in the numbers of P.severini caught from that area. On statistical testing it can be seen that the M.hygropetricus reduction was very highly significant ($P < 0.1\%$) whilst the P.severini increase was very highly significant ($P = < 0.1\%$). These results fit in well with other people's work (Hawkes and Shephard 1972) and can be explained by the effects that dosing frequency has on the scouring action of the sewage on the filter and by its effects on film levels.

It has already been stressed that as the dosing frequency increases, the force with which the sewage impinges on the media would decrease giving rise to less scouring action and higher surface film levels. These conditions therefore would tend to favour Psychoda in place of Metriocnemus as the film levels would be higher and the scouring action slight, factors both of which are generally known to favour Psychoda. Thus, this may account for the increase in Psychoda emergence from the modified area. The relationship between scouring forces and dosing frequency has been described by Hawkes (1955a) and Cook and Crame (1976).

Regarding M. hygropetricus emergence a plausible explanation for the reduction may be that their numbers were reduced as a result of increased interspecific competition from the increase in P. severini levels, however the fact that film and therefore food levels were not limiting seems to suggest that competition was not playing a major part here. Such a factor may be reduced ventilation due to high surface film levels giving rise to depletion in oxygen levels immediately under the surface as described by Learner (1975) however it is difficult to imagine that this would occur on 50-65 mm media, very high solids levels would be needed to cause this. A more likely explanation for the decrease of M. hygropetricus would probably be its reliance on cutaneous respiration compared to P. severini's respiratory siphon system which allows it to respire in high film conditions with only its siphon

projecting out of the film. Therefore, considering these factors, this modification was most successful in controlling M.hygropetricus emergence, however the increase in P.severini levels has to be taken into account as these, being non-migratory, may not be a nuisance to surrounding habitations but have been shown to be a nuisance to plant operators, and have caused public health problems (Johnson 1914, Ordman 1946 and Steinhaus and Brinley 1957).

3 Small media

The effect of small medium topping on adult fly emergence compared with control filter fly emergence can be seen in Figures 55 and 56. M.hygropetricus emergence was reduced in a similar fashion to the double dosing frequency section and likewise it was found to be ^{very} highly significant ($P = < 0.1\%$), however P. severini emergence did not increase and was similar to control levels.

The explanations for the reductions in M.hygropetricus emergence on the small medium probably lie in the small interstices size found with such medium not being of a sufficient size to allow successful emergence of M. hygropetricus. Learner (1975) suggested that depletion in oxygen levels caused reduced larval development and this could well have been occurring here, as it was noticed that the majority of the film in this medium was found in a band between 152 and 229 mm (6 and 9 in) deep and on many occasions this film was quite thick, such a sub-surface accumulation would be expected with a dosing frequency of 15 minutes. The drastic reduction in M.hygropetricus numbers agree well with a similar experiment carried out by Tomlinson and Stride (1945).

It is interesting to note that P. severini levels did not rise corresponding to the M. hygropetricus reduction suggesting that the medium size may have been controlling Psychoda also. A similar result was expressed by Tomlinson and Stride (1945) who found that 19mm ($\frac{3}{4}$ inch) medium did not have much effect on Psychoda, however

Hawkes and Jenkins (1951) working with 16 mm ($\frac{5}{8}$ inch) medium actually found a reduction in Psychoda emergence, indicating a critical size of media (subject to the thickness of the film covering) which physically prevents Psychoda from emerging. It is to be expected that a smaller media is required to physically prevent Psychoda from emerging than Metriocnemus because of the size differences in body length and wingspan between the two insects. The fact that there was not an increase in P. severini does not rule out that competition is definitely not occurring between this species and Metriocnemus, it was noticed that P. severini larval and pupal levels were very high from media samples at the bottom of the 229 mm (9in) depth section suggesting that the thick film conditions present there prevented M. hygropetricus from surviving and allowed P. severini to build up in numbers, but the build up never manifested itself in an increase in fly emergence. This is quite plausible as P. severini, being parthenogenetic, can produce offspring without leaving the filters. In order to explain why the build up in larval and pupal numbers never manifested itself in an increase in adult emergence, it is necessary to consider that the larva has to migrate near to the surface to pupate and emerge successfully and this journey through approximately 152 mm (6 in) of small medium which was displaced with every sewage dose would be a practically impossible task considering the inability of Psychoda to cope with high scouring forces.

Therefore considering the above results small medium seemed quite a good method of controlling fly emergence however the problems of tracking, allowing fly emergence from the "dry" areas needed solving and some investigation was obviously needed to clarify its effect on filter efficiency considering the thick film accumulation.

4 Small media and splash plate

The effect on fly emergence of applying sewage to small medium via the splash plate apparatus to reduce the tracking effects can be seen compared to control in Figures 57 and 58.

As with the small medium and normal application modification a drastic decrease in M.hygroptericus emergence resulted, the greatest reduction of all of the modifications so far described was obtained and it was found to be very highly significant ($P = < 0.1\%$). Similarly P.severini levels were very similar to control. The fact that this modification was more successful than small medium alone in reducing M.hygroptericus emergence infers that the splash plate apparatus was successful in spreading the flow pattern out and overcame the tracking effect. The explanations for the M.hygroptericus and P.severini emergence levels are probably the same as above as large P.severini larval and pupal populations were found in the depths of this section on media sampling. This then suggests that small medium when completely covered with each sewage dose is most successful in controlling M.hygroptericus emergence however as above its effect on purification efficiency needs to be ascertained as a build up in solids was noted in the depths of this section.

5 Splash plate

The effects of the splash plate modification on fly emergence (when impinging on normal 50 - 60 mm media) can be compared with control by consulting Figs. 59 and 60. This investigation was split into 2 parts, the first part from February 1976 to August 1977 and the second part from August 1977 to June 1978 during which time a sprinkling of small medium (19 mm, as used in the other modifications) was added to the surface of the filter.

Dealing with the first half of the experiment it can be seen from Figures 59 and 60 that M.hygroptericus emergence was generally above that found on the control filter, also P.severini emergence was fairly similar to that found on the control filter. The reason for applying this modification was that it was thought that spreading the flow pattern out would decrease the scouring action and favour competitors of M.hygroptericus such as P.severini however clearly this did not occur. The means by which the desired control was to operate was via increased film levels however it can

be seen by comparing this filter (Fig. 81) with control (Fig. 42) that although an increase was noted it was only slight. Therefore it is likely that the increase in film was not sufficient to discourage M.hygroptericus from surviving successfully and that the increase in film and therefore food supply was however sufficient to allow M.hygroptericus levels to rise above those of the control filter, in fact the average increase over the period studied was 16.42%.

The reason why P.severini never dominated here is not clear as both the scouring action was reduced and the film increased both of which should serve to increase its levels. It is suspected that any increase in P.severini may have been disguised by competition from the increased numbers of Metriocnemus which are known to have strongly carnivorous habits (Lloyd et al. 1940) and compete with psychodids.

As the modification produced more M.hygroptericus than control it was decided to modify it further in August 1977 by adding small medium to the surface. It was suspected that the 229 mm (9in) depth was more than was required for effective control of M.hygroptericus as if the process was physical in blocking the insects' easy egress from the filter it was reasoned that such a depth was not necessary as a sprinkling on the surface between the larger pieces of media would achieve the same objective. Therefore it was decided to sprinkle a portion of the main filter, subjected to sewage application via the splash plate, with small medium investigations into the effects of this constituted the second half of the experiment.

By consulting Figures 59 and 60 the fly emergence from this section can be compared with control. It can immediately be seen from Figure 59 that on application of the small medium M.hygroptericus emergence dropped and remained very low during the rest of the investigation. Compared to control the mean reduction from September 1977 to June 1978 was 81.09% and this was greater than the result obtained for the small medium (229 mm depth) and splash plate for the same period which was 70.26%.

No specific trends were shown with P.severini emergence (Fig. 60).

The success in controlling M.hygroptericus from this area seems to indicate that the mechanism is physical in preventing easy egress of the adult flies. This is postulated as the bulk of the film would lie, not at the surface, but at a sub-surface level on the 50-65 mm grade media thus providing identical film conditions at that level, as found on the control area. Therefore the only possible barrier to emergence of these flies would be the surface sprinkling of small medium

The reason why the reduction was greater for this area than the 229 mm depth area is not clear. One reason may be that the surface media on the 229 mm depth area had lower solids levels due to mechanical abrasion between the media particles when they were displaced with each dose of sewage, thus favouring M.hygroptericus survival, however a more feasible explanation would be that the actual space for emergence i.e. open interstices at the surface would be greater with a uniform covering of 19 mm medium than with a mixture of large pieces of media interspersed with 19 mm medium

Therefore of all of the modifications, the surface sprinkling of small medium filling the interstices between the larger pieces of media combined with a splash plate to give an even wetted area seems to give the best reduction in M.hygroptericus emergence.

(b) Secondary filters

The same modifications as have been described for the primary filters were investigated on the secondary filters and it can be seen from Table 13 that M.hygroptericus reductions were apparent compared to control and in only three cases was the reduction significant to the $P=2.5\%$ level, one was the small medium 229mm (9 in) depth and splash plate. However it must be realised here that M.hygroptericus emergence from the secondary filters was only 37.17% of that obtained from the

primary filters during the period of study (see Figs. 15, 16, 27 and 28). Therefore fly emergence levels were very low and any differences would be correspondingly small and would need a long period of study to show significance.

Obviously with the secondary filters different factors would need to be considered in explaining fly reductions as the film levels were so low, 37.10% in comparison with the primary filters over the period March 1976 to February 1977 (it is interesting to note that M.hydropetricus emergence from the secondary filters was 37.17% of that from the primary filters though it is doubtful whether any special significance can be attributed here). The main factors operating on these filters would most likely have been the very low film levels and the mechanical scouring effect.

1 Double loading

It can be seen from Figure 61 that M.hydropetricus emergence was reduced (slightly significantly) compared to control and that P.severini emergence (Figure 62) was fairly similar to control. In this case it is likely that the extra hydraulic load caused by doubling the flow resulted in even lower film levels due to the increased scouring action thereby reducing the total food supply available. This may explain the reduction in fly emergence. The lower film levels can be seen by comparing Figs. 76 and 77.

2 Double dosing frequency

It can be seen from Figs. 63 and 64 that M.hydropetricus emergence was reduced and P.severini slightly increased compared to control. It can therefore be postulated that in this case the film levels were increased sufficiently to favour P.severini slightly at the expense of M.hydropetricus. This slight increase in film levels can be seen by comparing Figs. 76 and 78 however the increase was not significant. This seems to suggest that competition is occurring between the two species and that P.severini fares best in increased film conditions.

3 Small media

As above this modification produced a slight reduction in M.hydropetricus emergence, compared to control (Fig. 65) however unlike above P.severini levels, most notably in 1977 were less than those found on the control filter (Fig. 66) suggesting that they were either being controlled physically by the media size or through solids levels. It was the view of Hawkes & Jenkins (1951) that inhibition of Psychoda was only found on media less than 16 mm ($\frac{5}{8}$ in) diameter so it is doubtful whether 19 mm ($\frac{3}{4}$ in) media would have had much effect here, also an increase in volatile solids levels (see Figs. 76 and 79) was not apparent therefore it is unlikely that these factors were the cause.

The most likely explanation for the reduction could probably lie in the different characteristics of the film due to the low hydraulic loads found on these filters. It was noticed that the film covering over the surface of the small medium generally consisted of a thick algal slime physically covering most of the open interstices, also this type of film was completely different from primary filter film in having a high water content and being found only on the surface media. On the primary filters no such surface film was observed which was thought to be due to mechanical abrasion of the stones with each other due to the high hydraulic loads applied. On the secondary filters the hydraulic load was usually only 50% of that experienced by the primary filters as not all of the primary humus tank effluent was pumped on to the secondary filters. This may have allowed thick surface film to develop and affected fly emergence physically.

4 Small media and splash plate

As in the case of the primary filters this modification produced the highest reduction in M.hydropetricus emergence than any other modification and its effects compared with control on that fly species can be seen in Figure 67 and on P.severini in Figure 68. The reduction was in fact significant to the 2-5% level. The reasons for this

modification's increased success have been described earlier and rely on complete wetting of the filter surface with sewage leaving no "dry" areas which in this case produced an even algal film over the surface thereby hindering fly emergence. As above P.severini levels were also decreased slightly which suggests inhibition of this fly also.

5 Splash plate

It can be seen by comparing fly emergence from this area with control (Figures 69 and 70) that this modification had a slight effect on M. hygropetricus emergence. Solids levels were not affected therefore it can be deduced that on the low hydraulic loads apparent on the secondary filters the splash plate did not alter the flow pattern sufficiently to change film levels and drastically affect fly emergence. The surface sprinkling of small medium was not added to this modification as when this alteration was conceived sampling from these filters had ceased.

iii) Effects of operational modifications on macrograzer populations and volatile solids levels

Basket media samples were taken from March 1976 to February 1977 in order to investigate the effects of the previously described modifications on macrofauna and volatile solids levels. The only results expressed graphically concern enchytraeid worm and volatile solids levels both on the primary and secondary filters. M. hygropetricus and P.severini larvae and pupae were found on sampling and are included in Table 14.

Table 14

Mean weekly abundance of fly larvae and pupae per dm³ over 12 months

(a) Primary filters

Modification	<u>M. hygropliticus</u>		<u>P. severini</u>	
	Larvae	Pupae	Larvae	Pupae
Control	5.09	1.84	0.04	-
Double load	0.55	0.15	-	-
Double dosing frequency	2.77	0.36	2.83	0.31
Small medium	3.53	0.27	7.58	3.31
Small medium and splash plate	10.29	0.71	10.79	3.45
Splash plate	4.13	0.62	0.17	0.07

(b) Secondary filters

Control	1.22	0.04	0.16	-
Double load	0.28	-	0.20	-
Double dosing frequency	0.29	0.05	0.34	0.15
Small medium	3.86	0.15	1.69	0.08
Small medium and splash plate	1.27	-	6.18	0.28
Splash plate	0.45	0.13	0.30	0.10

The results shown in Table 14 are interesting as they show that the specific modifications which cause a drop in adult fly emergence (refer to Table 13) do not necessarily produce a similar drop in the larval and pupal numbers. Such an example is the small medium and splash plate modification on the primary filters where the maximum decrease in M.hygropetricus emergence was found, however from Table 14

it can be seen that this modification showed the largest populations of larvae of that species, a similar situation was shown by the small medium modification on the secondary filters. These results seem to disagree with the idea put forward by Learner (1975) that high film levels per unit volume (as found on small media) may have a physiological effect in limiting oxygen levels thus inhibiting larval development as the maximum number of larvae were found in these conditions. It is more likely that the effects of small media are mainly physical in preventing easy egress of the flies from the filter which has been also suggested by Learner (1975).

Another interesting point from these results is that the maximum number of larvae were found in the samples from the small medium and small medium with splash plate modifications showing that the actual surface area of film laden media was the greatest for these modifications which is to be expected as these give a higher surface area to volume ratio than in the normal media situation.

The reason why these results were not plotted graphically was that generally throughout the experiment basket sampling results were very sporadic and hardly ever consistent. For example from a specific modification on one sampling occasion many larvae may have been found whereas on another occasion none may have been found. The small size of the sampling baskets in relation to the media size may have some bearing on these findings but also it should be remembered that results from these baskets represent the organisms found from only the top 155 mm of the filters depth. Thus any grazers present below these levels would not be recorded. It was therefore considered that monitoring of adult fly emergence was by far the most accurate means of assessing the effects of the modifications applied as this parameter reflected the sum total effect of the modifications on the whole of the filter whereas basket sampling was restricted to showing the surface effects of the modifications.

Table 15 continued
Primary filters – Volatile solids

Filter modification	Mean monthly Paired 't' test Control v. modified areas			
	volatile solids gdm ⁻³	't' value	'P' value	Significance
Control	1.7 ± 0.2	—	—	—
Double load	2.0 ± 0.2	-2.25	2-5%	S
Double dosing frequency	2.0 ± 0.3	-2.92	1-2%	HS
Small medium	2.7 ± 0.4	-3.72	0.1-1%	VHS
Small medium and splash plate	2.6 ± 0.4	-3.12	≈1%	HS-VHS
Splash plate alone	1.9 ± 0.3	-0.92	>10%	NS

± = S.E.M. No. observations = 12

The following graphs indicate the results of the enchytraeid worm and volatile solids determinations during the sampling period, these are expressed as monthly means.

<u>Modification</u>	<u>Enchytraeid Worms</u>	<u>Volatile Solids</u>
Control	Figs. 40/45	Fig. 76
Double load	" 71	" 77
Double dosing frequency	" 72	" 78
Splash plate	" 73	" 79
Small medium	" 74	" 80
Small medium and splash plate	" 75	" 81

These results were then treated statistically in the usual manner to investigate if there was any significance in the changes.

The results of the "t" tests applied can be seen in Table 15.

Table 15

<u>Primary filters</u>	<u>Enchytraeid worms</u>	<u>Volatile solids</u>
Primary filters — Enchytraeid worms		
Filter modification	Mean monthly counts per dm ³	Paired 't' test Control v. modified areas
		't' value 'P' value Significance
Control	239.6 ± 28.8	—
Double load	254.7 ± 47.3	-0.56 >10% NS
Double dosing frequency	426.1 ± 65.3	-3.51 0.1-1% VHS
Small medium	655.5 ± 110.4	-4.88 <0.1% VHS+
Small medium and splash plate	768.0 ± 117.2	-5.65 <0.1% VHS+
Splash plate alone	410.6 ± 77.2	-2.90 1-2% HS

± = S.E.M. No. observations = 11

Table 15 continued
Secondary filters — Volatile solids

Filter modification	Mean monthly Paired 't' test Control v. modified areas			
	volatile solids gdm^{-3}	't' value	'P' value	Significance
Control	0.6 ± 0.1	—	—	—
Double load	0.6 ± 0.1	0.78	>10%	NS
Double dosing frequency	0.7 ± 0.1	-0.81	>10%	NS
Small media	0.6 ± 0.1	0.84	>10%	NS
Small media and splash plate	0.8 ± 0.2	-1.30	>10%	NS
Splash plate alone	0.7 ± 0.1	-0.82	>10%	NS

\pm = S.E.M. No. observations = 12

Table 15

Primary filters		Encrusted worms		Volatile solids	
Modifications	% change from control	't' value	'P' value	% change from control	't' value
Double load	+ 7.62	0.22	>10%	+ 18.26	1.42
Double dosing frequency	+ 78.48	2.09	0.1 - 1%	+ 21.10	1.18
Small media	+178.49	3.22	0.1 - 1%	+ 61.13	2.68
Small media and splash plate	+222.60	2.82	> 0.1%	+ 24.14	2.26
Small media alone	+ 72.48	1.87	2 - 10%	+ 12.78	0.62

Table 15 continued
Secondary filters – Enchytraeid worms

Filter modification	Mean monthly counts per dm ³	Paired 't' test 't' value	Control v. modified areas 'P' value	Significance
Control	42.1 ± 4.1	—	—	—
Double load	27.1 ± 10.3	1.76	>10%	NS
Double dosing frequency	61.3 ± 13.9	-1.55	>10%	NS
Small medium	43.3 ± 7.6	-0.18	>10%	NS
Small medium and splash plate	41.7 ± 12.3	0.03	>10%	NS
Splash plate alone	50.4 ± 14.5	-0.61	>10%	NS

± = S.E.M. No. observations = 11

(a) Primary filters

1. Double loading

It can be seen by comparing Figs. 76 and 77 that the volatile solids on this modification were very similar to those obtained on the control filter. This suggests that any solids build up due to the increased organic load on this modification was compensated for by the removal of film by scouring action due to the increased hydraulic load. It would be expected that this would cause no change in enchytraeid worm levels as the food supply remained constant. This was in fact found and can be seen by comparing Fig. 71 with control filter levels in Fig. 40.

2. Double dosing frequency

The volatile solids obtained on this modification can be compared with control by consulting Figs. 76 and 78. The rapid build up in solids in March/April culminated in ponding of this area. The reason for this was that at first no provision was made to reduce the flow from each nozzle used, therefore this area, as well as being dosed at twice the frequency was receiving twice the organic and hydraulic loads, so it is not surprising that an excessive accumulation of film built up. This situation was rectified by altering the nozzle size on each of the 8 nozzles involved in this

modification to give approximately 50% of the original flow from each nozzle. This portion of the filter was then rested for 2 months to allow film levels to fall before recommissioning.

After this time it can be seen from Fig. 78 that volatile solids were generally slightly higher over the remaining months than on control however it can be seen from Table 15 that this increase was not significant. The theory of the effects of dosing frequency was described by Hawkes (1961b) and this can be used to explain the increase in solids found when the dosing frequency was increased here. It is obvious that with a higher dosing frequency the mechanical scouring action would be less as a unit volume of sewage would fall on a larger area of filter than it would have done on a lower dosing frequency. This reduced scouring action would therefore cause an increase in film levels. Similarly an increase in film levels could also be due to nutritional effects also described by Hawkes (1961b). In order to appreciate this it is necessary to consider the contact time between doses of sewage held in the film as it has been expressed by Hawkes (1961b) that long periods between doses controls film by producing starvation conditions and film growth may well pass into an endogenous phase towards the end of the dose cycle. Therefore if the sewage is applied with a greater frequency, even though the total loading is unchanged, it is likely that the sewage held on the filter would still be of sufficient strength to actively contribute to the growth of the film by the time the next dose arrives. Therefore the total growth rate of the film would be higher because the endogenous phase would never be reached.

These changes in film levels described would not always be obvious. Considering a filter without grazers these above effects would probably cause a considerable increase in film levels however as it can be seen from Figs. 40 and 72 the enchytraeid worm levels on this modification were considerably higher than on control (this increase was found to be very highly significant ($P = 0.1 - 1\%$) therefore the

increased grazing activity would probably have the effect of disguising any drastic film level changes, however it is clear that some factor must have caused the rapid increase in worm levels which could well be increased film levels.

3 Small media and small media with splash plate

The two modifications above were combined together for this discussion as due to the size of the sample baskets (200 mm) in relation to the nozzle spacing (155 mm) it was impossible to sample from "wet" and "dry" areas separately in the small medium and normal application method modification as the samples were subject to interference from the overlap between the two areas.

The effect of small medium on solids levels can be seen by comparing Figs. 79 and 80 with control levels in Fig. 76. The solids increase was quite considerable and was found to be ^{highly} significant ($P \approx 1\%$) in the small medium and splash plate case and v. highly significant ($P = 0.1-1\%$) in the small medium and normal application method case. The difference between the two modifications seems to suggest that the splash plate has its desired effect in spreading the flow pattern out, thus giving a lower overall solids level than in the normal application case where the sample baskets were placed directly under the nozzles.

The increase in solids obtained can be explained by the larger surface area per unit volume available for microbial growth offered with this medium. Although some workers have experienced problems with excessive film accumulation and ponding with small media (Hawkes and Jenkins 1955) none was experienced here. It was assumed that no problems were found due to the very large populations of actively grazing enchytraeid worms found on these areas. It can be seen by comparing Figs. 74 and 75 with Fig. 40 that large increases in enchytraeid populations were experienced and from Table 15 it can be seen that these increases were very highly significant ($P < 0.1\%$). Similar results were found by Hawkes and Jenkins (1955) who

found the highest enchytraeid worm and solids levels on the smallest of 4 grades of media studied. The large increase in enchytraeid numbers can simply be put down to an increase in their food supply due to higher film levels and a greater surface area per unit volume for grazing activity with these modifications. Also there may be exclusion of their competitors such as M.hygroetricus larvae. It should be mentioned here that although P.severini adult emergence was reduced large larval populations were noted at all sampling times thus indicating that they were likewise preventing excessive film accumulation.

It has previously been mentioned that some high film levels were experienced in the lower portions of samples from the small medium and normal application method modification during winter suggesting that sub-surface ventilation problems may have been occurring. Similar seasonal problems with ponding were experienced by Hawkes and Jenkins (1955) however no decline in effluent quality was noted by these workers which may be a result of interference from other areas of unaffected filter where normal purification was occurring. This highlights the difficulty in sampling effluent from any small portional modification on a large filter.

It should be mentioned that no high level film problems were found on the portion of small medium served by the splash plate which suggests that thick sub-surface sub-jet film conditions were responsible for this problem before, and that spreading out the film over the whole section by using the splash plate prevented local sub-jet film accumulation.

In conclusion the absence of film accumulation problems generally with these modifications can possibly be put down to the increase in grazing activity and the effects of low frequency dosing by scouring and the nutritional effects described earlier.

4 Splash plate

The effect of the splash plate with normal media on volatile solids levels can be seen by comparing Fig. 81 with control in Fig. 76. Although an increase was apparent it was not significant (see Table 15) and it can be seen that the seasonal peaks in solids levels found on the control filter due to temperature and sloughing effects were exaggerated with the splash plate modification. In a similar fashion generally higher solids levels were experienced by Hawkes (1959) with a splash plate when studying a range of application methods.

An increase in enchytraeid worm levels was also apparent from this area, it was highly significant ($P = 1 - 2\%$). This can be seen by comparing Fig. 73 with control in Fig. 40. This increase can simply be explained by an increase in food supply due to the lessened scouring action and more even spreading of sewage giving an even covering of film.

The large increase in enchytraeid worms here raises the question whether competition is occurring between these and fly larvae as it can be seen from Figs. 15-17 and 59-60 that fly emergence was also increased over control for this area. Obviously if competition was apparent it would be expected that an increase in the strongly carnivorous M.hydropetricus and P.severini would result in a decrease of enchytraeids. The reason for this slight increase in enchytraeids is therefore not sure but it may be that competition is not so severe on this modification as the spread of film is greater giving more physical space for grazing activity.

(b) Secondary filters

With the secondary filters control film and macrograzer levels were so low that any changes brought about by the modifications were correspondingly low. It can be seen from Table 15 that slight solids increases were present on all of the modifications but in no case was the increase significant. Similarly with enchytraeid worms in no

cases were the increases significantly above control.

1 Double load

It can be seen from Figs. 76 and 77 that solids levels on this section were slightly lower than control, similarly enchytraeid worm levels were lower (see Figs. 71 and 40). This decrease was found to be not significant ($P = > 10\%$). The differences expressed as mean percentages can be seen from Table 15.

The decrease in solids and enchytraeids can be explained by the increase in scouring action due to the double hydraulic load received by this area. The decrease due to the scouring action would probably override any increase due to the increased organic load therefore this would result in a decreased food supply thus explaining lower levels of enchytraeids found.

2 Double dosing frequency

It can be seen from Figs. 76 and 78 that the solids levels did rise on this modification, similarly an increase in enchytraeid levels was also apparent (see Figs. 72 and 40), however from Table 15 it is apparent that none of these increases was significant.

Unlike the above modification double dosing frequency would have the effect of increasing film levels due to the same reasons described for the primary filters, i.e. less scouring action and nutritional effects due to available growth periods and sewage strength. Thus it is not surprising that the slight increase in film found here caused an increase in enchytraeid worm levels.

3 Small media and small media with splash plate

The effects of these modifications on volatile solids can be seen by comparing Figs. 79 and 80 with Fig. 76. These followed the control levels except for a slight accumulation of film in the winter months.

It may be expected that a greater rise in solids would be apparent, as found in the primary filters, however unlike that case the sewage strength was so weak that any increase probably only manifested itself in the surface layer of medium as it was found that the sub-surface medium was devoid of visible film. No great changes in the enchytraeid worm populations were found as can be seen by comparing Fig. 40 with Figs. 74 and 75.

4 Splash plate

From Figs. 76 and 81 and Table 15 it can be seen that the splash plate caused a slight increase in film levels, similar results were obtained concerning enchytraeid worms (see Figs. 40 and 73).

It is obvious that spreading the flow out would give a decreased scouring action which would then cause a slight increase in film and therefore available food. This explanation can be used to explain the slight increase in enchytraeid worm levels apparent from Table 15.

iv) Effects of modifications on purification efficiency of filters

It is appreciated that although a certain modification may have its desired effect in controlling fly nuisance it may detrimentally affect the purification efficiency of the filters which were designed to operate to maximum efficiency on the specific conditions found on the works.

As it was impossible to segregate the effluent from the normal filter and the modified area no study could be made on the effect of these modifications on the purification efficiency of the main filters. Therefore it was decided to construct a series of pilot filters as described in the methods section with the main intention of investigating the effects of the most efficient modifications, found on the main filters (from the fly control viewpoint), on filter efficiency.

Due to operational difficulties the only modifications from the main filters which could be investigated easily were the small medium(229 mm) and normal application method modification, the small medium(229 mm) and splash plate modification and the small medium (surface sprinkling) and splash plate modification. The effects of these will be discussed in detail in the pilot filter section.

As regards the remaining modifications, the double loaded and splash plate with normal media sections proved to be unsatisfactory from a fly control viewpoint therefore it was considered unnecessary to investigate their effects on purification efficiency.

Unfortunately the double dosing frequency modification which was most successful in controlling M.hygropetricus was too difficult to simulate in the pilot filter system as a completely separate feed, timer and pump system would have been required, therefore it is only possible to theorise on the effects of that modification on purification efficiency. Many workers have studied effects including Tomlinson and Hall (1955), Hawkes (1957 and 1961b), Stanbridge (1958), Novelli (1975) and Cook and Crame (1976) and there are conflicting views on its effects on filter efficiency. Cook and Crame (1976) suggested that continuous dosing gave the best efficiency with shallow filters, in other cases a dosing regime of 5 minutes on and 5 minutes off was found to be most efficient. Other workers (Tomlinson and Hall 1955, Hawkes 1957 and Stanbridge 1958) were of the opinion that it is film accumulation that causes a drop in efficiency, and controlling film accumulation by low frequency dosing serves to increase filter efficiency. When considering filter efficiency retention time has to be taken into account and this is drastically affected by dosing frequency (Cook and Katzberger 1977). According to Stanbridge (1956) the most efficient frequency is 10-15 min. on a clean filter and this efficiency was reduced at lower frequencies (a similar result was expressed by W.P.R. (1956). However Hawkes (1961b) was of the opinion that it was not directly dosing frequency that determined efficiency, this was

coupled with film accumulation and in order to control solids levels it may be necessary to give dosing frequencies lower than 10-15 min. The resultant loss in efficiency due to retention time being more than compensated for by an increase in efficiency due to loss of film accumulation.

As regards the change from 15 to 7.5 min. on the modification in question it is known that the solids increased slightly so this may have caused a drop in efficiency. However enchytraeid worm levels were very highly significantly higher and it was the view of Solbe et al. (1974) that filter performance and most notably nitrification was better in the presence of grazers. Other views were put forward by Stover et al. (1976) who were of the opinion that carbonaceous micro-organisms, when actively growing, produced metabolites which detrimentally affected nitrifying micro-organisms. Thus high film levels may reduce nitrification however opposite views were put forward by Hockenbury et al. (1977) who stated that nitrifiers were not affected by actively metabolising heterotrophs.

Thus it is possible to see how conflicting ideas are held on filter efficiency, not just on the effects of dosing frequency alone, but on film accumulation generally. It is probably reasonable therefore to say that the effect of changing the dosing frequency from 15 to 7.5 min. may only very slightly affect efficiency assuming film levels did not rise too rapidly and grazing populations were active.

The arguments for and against the above modifications compared to the use of insecticide control measures in order to minimise fly nuisance will follow in the next section.

Fig. 51. Effects of double loading on M.hygropetricus emergence (primary filters)

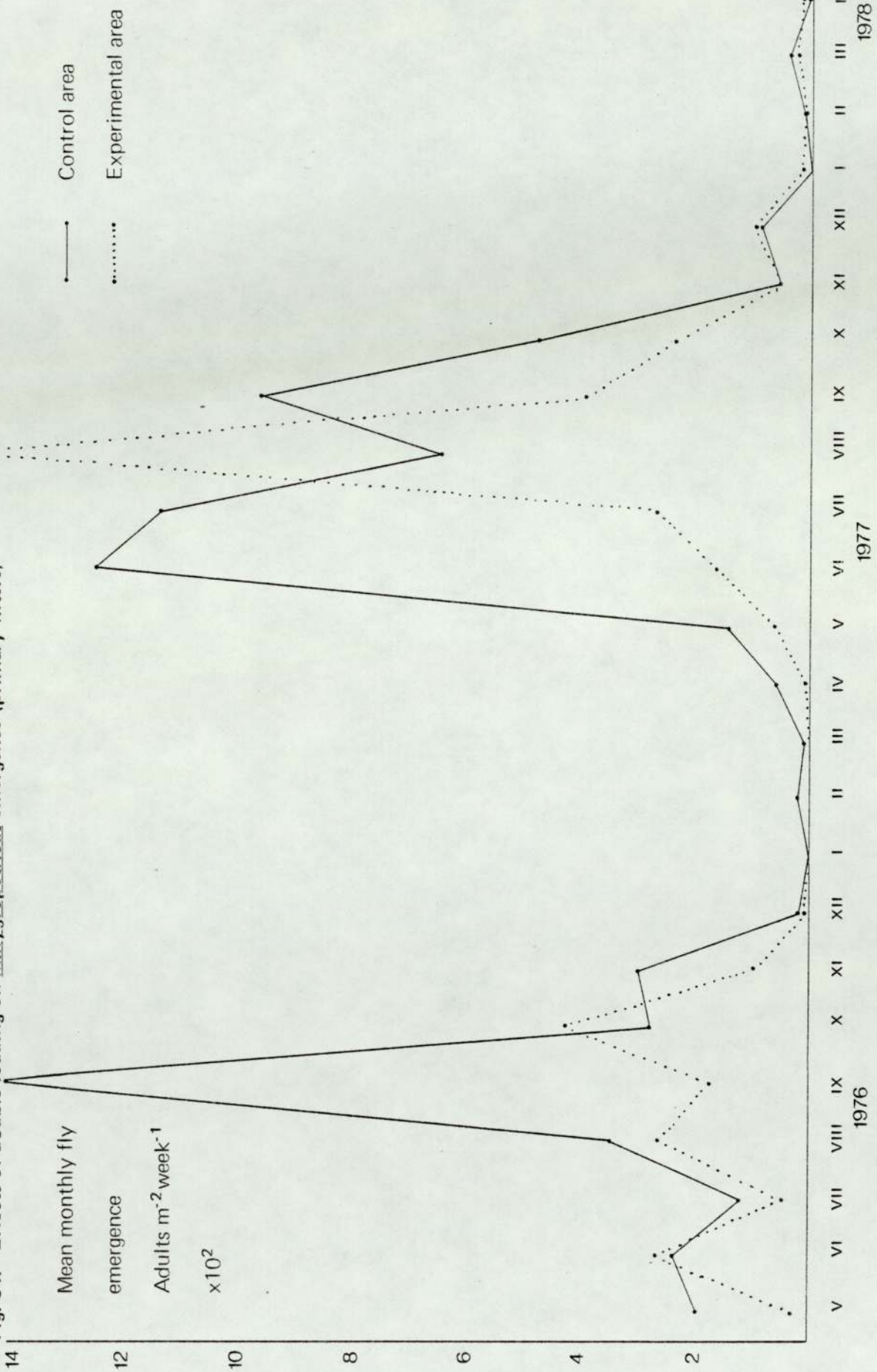


Fig. 52. Effects of double loading on *P. severini* emergence (primary filters)

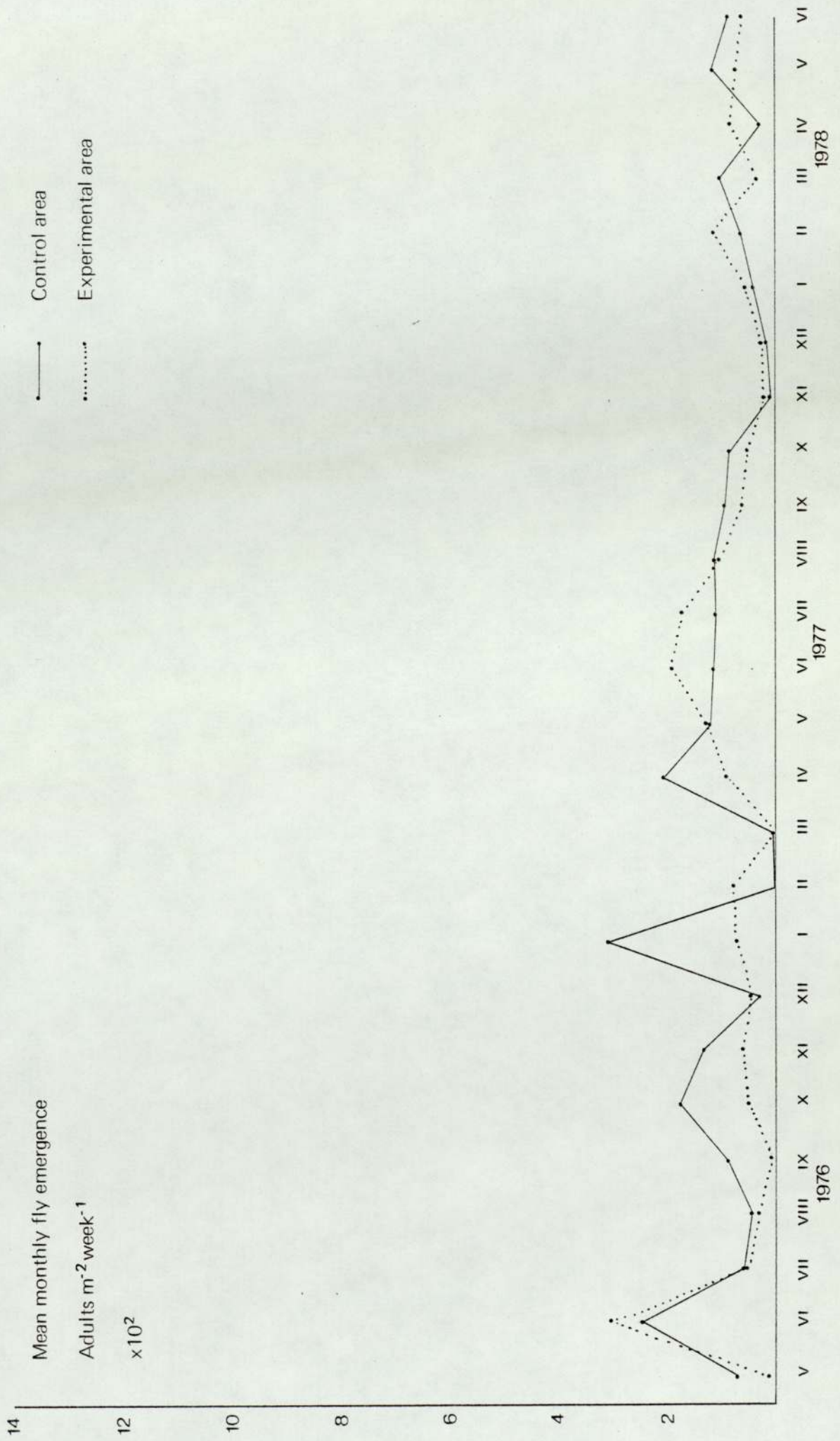


Fig. 53. Effects of dosing frequency on *M. hygroplitricus* emergence (primary filters)

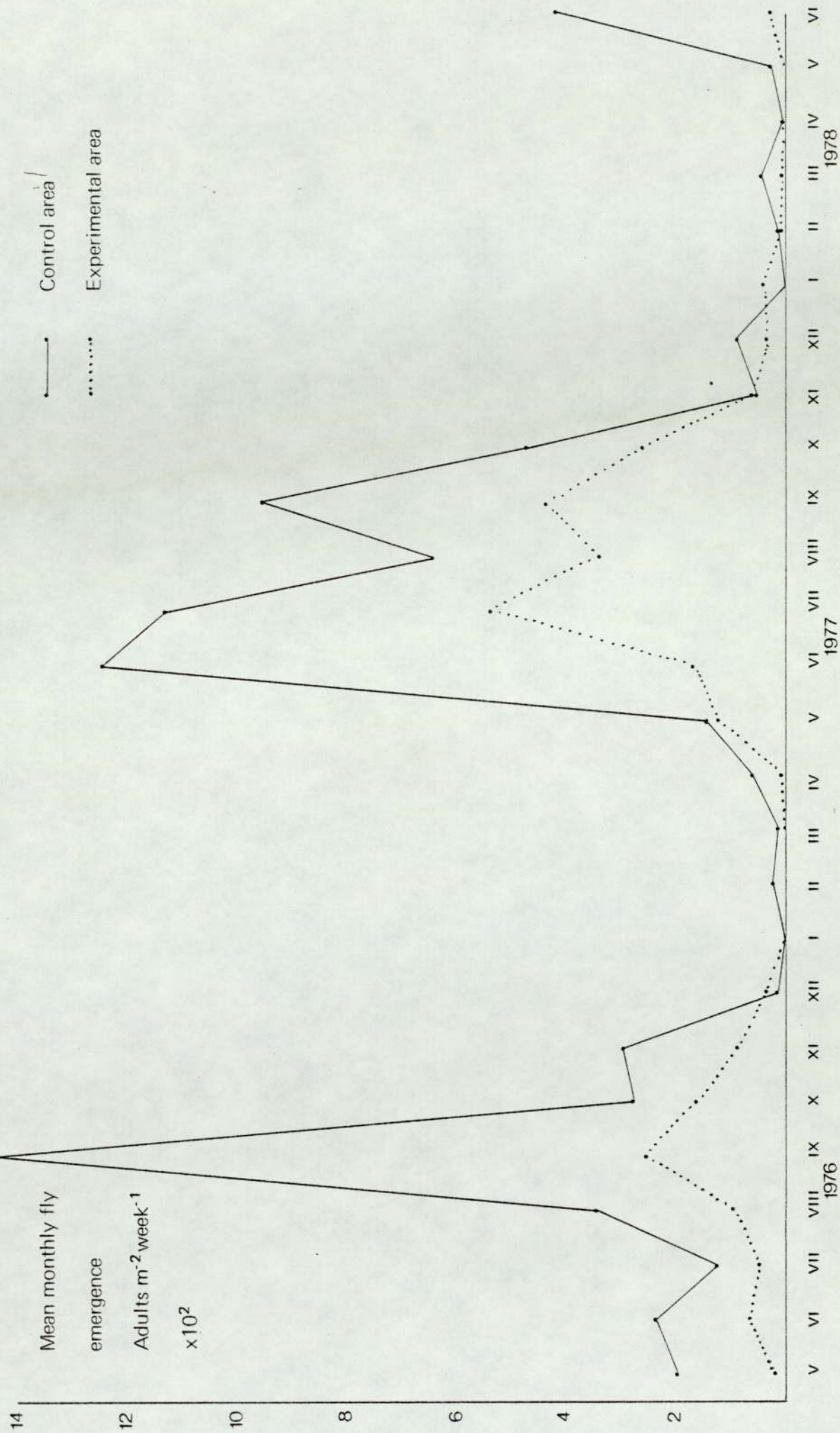


Fig. 54. Effects of dosing frequency on *P.severini* emergence (primary filters)

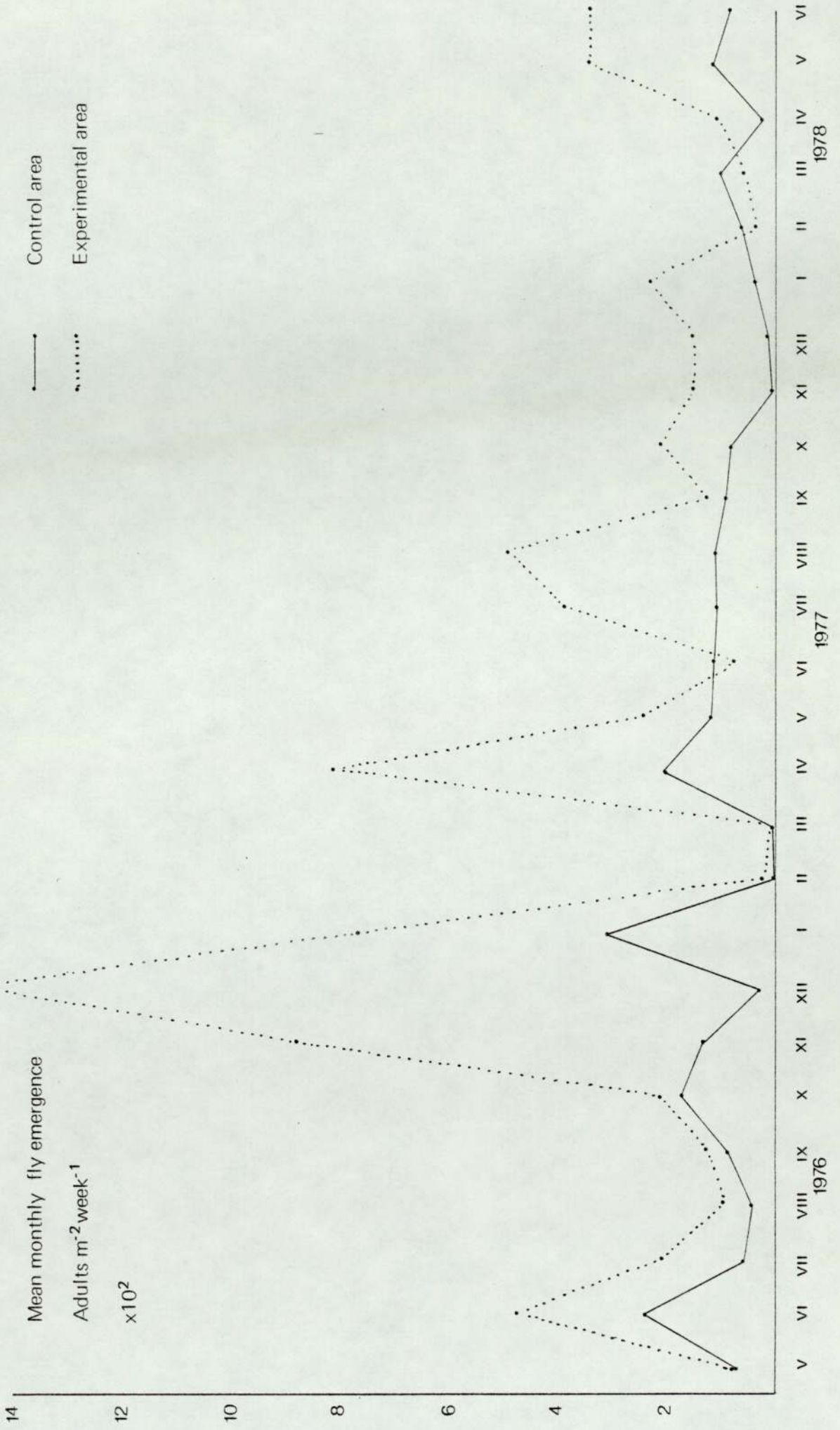


Fig. 55. Effects of small media (229 mm. depth) on M.hygropetricus emergence (primary filters)

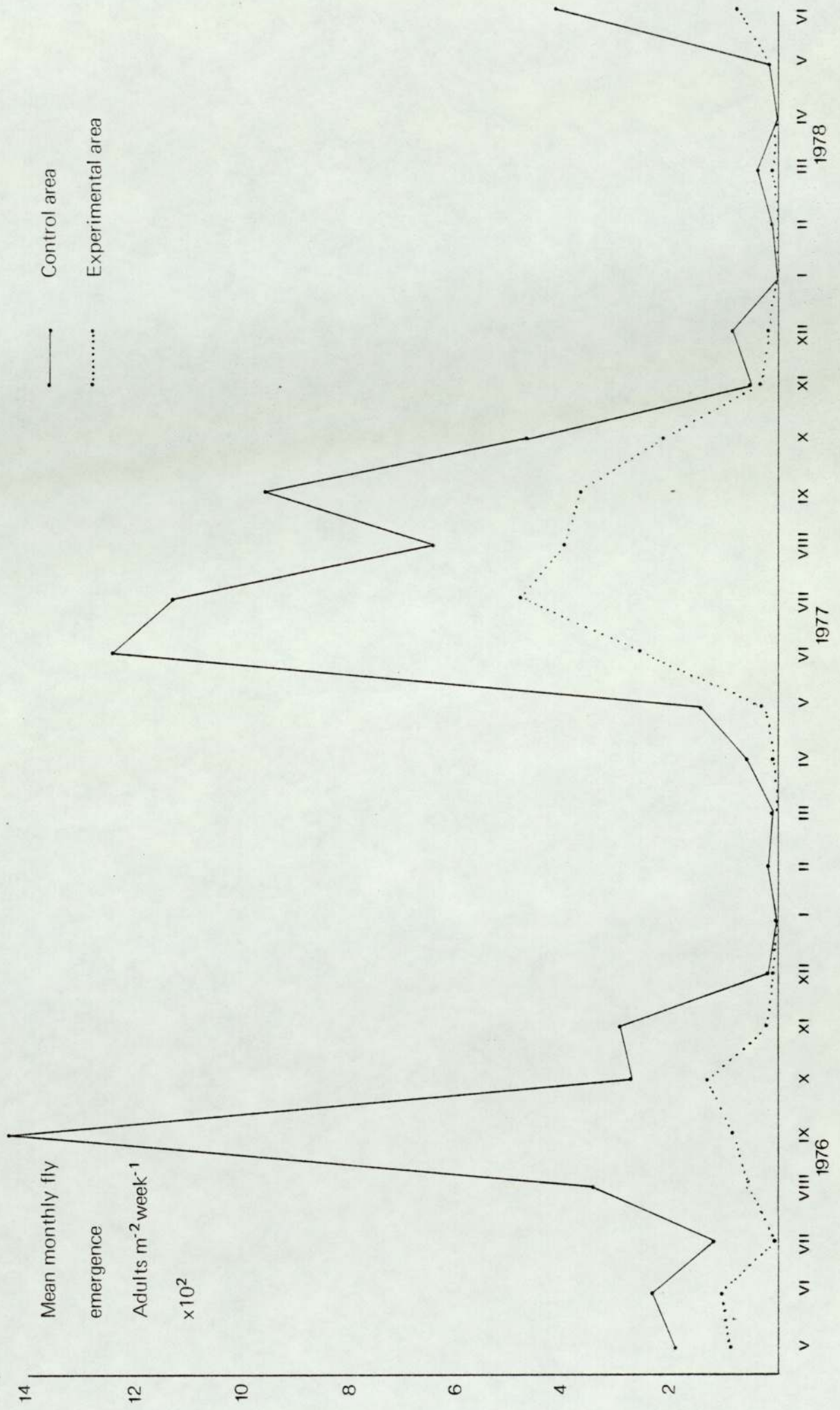


Fig. 56. Effects of small media (229 mm. depth) on *P.severini* emergence (primary filters)

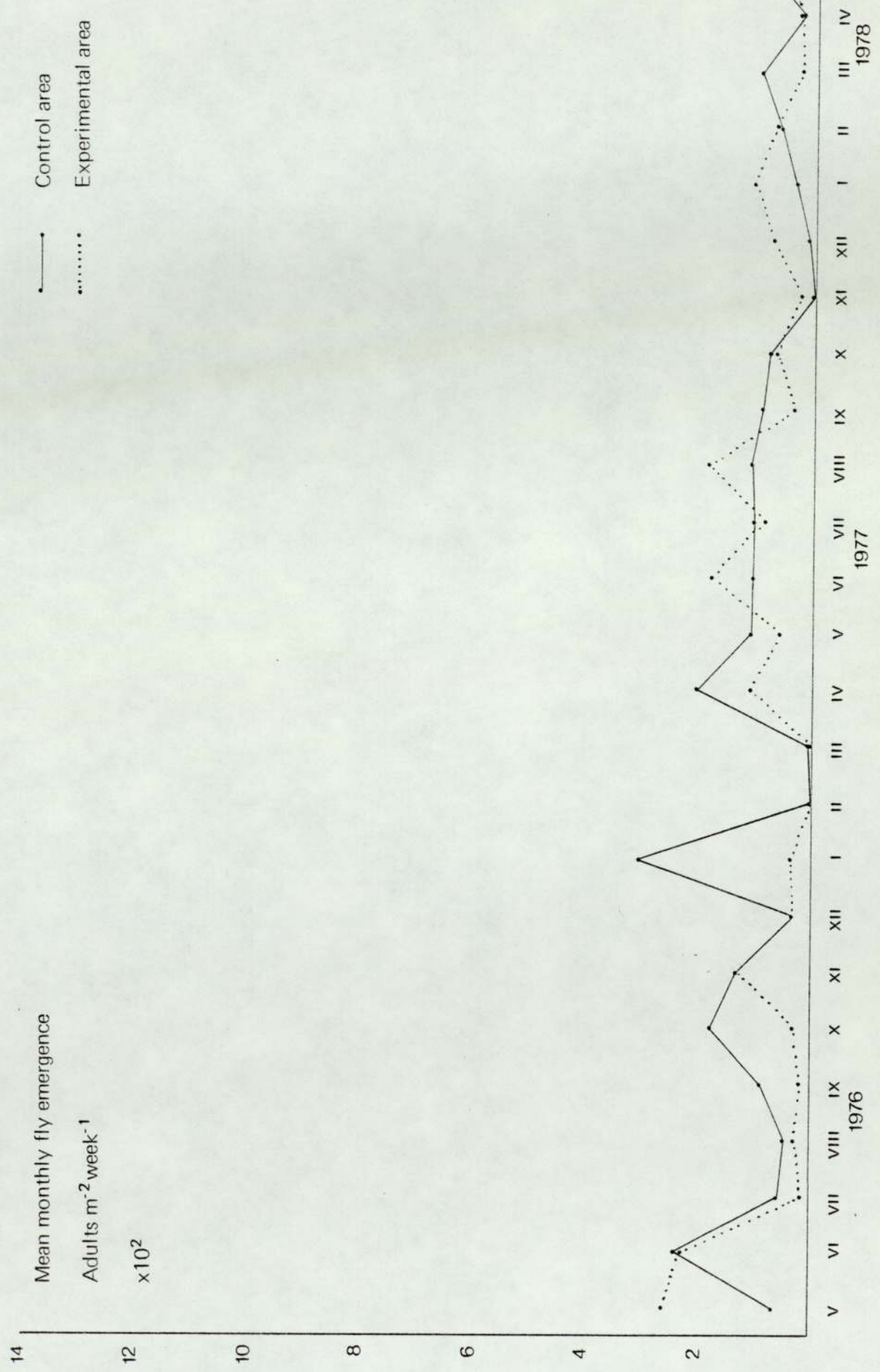


Fig. 57. Effects of small media (229 mm. depth) and splash plate on *M. hygropliticus* emergence (primary filters)

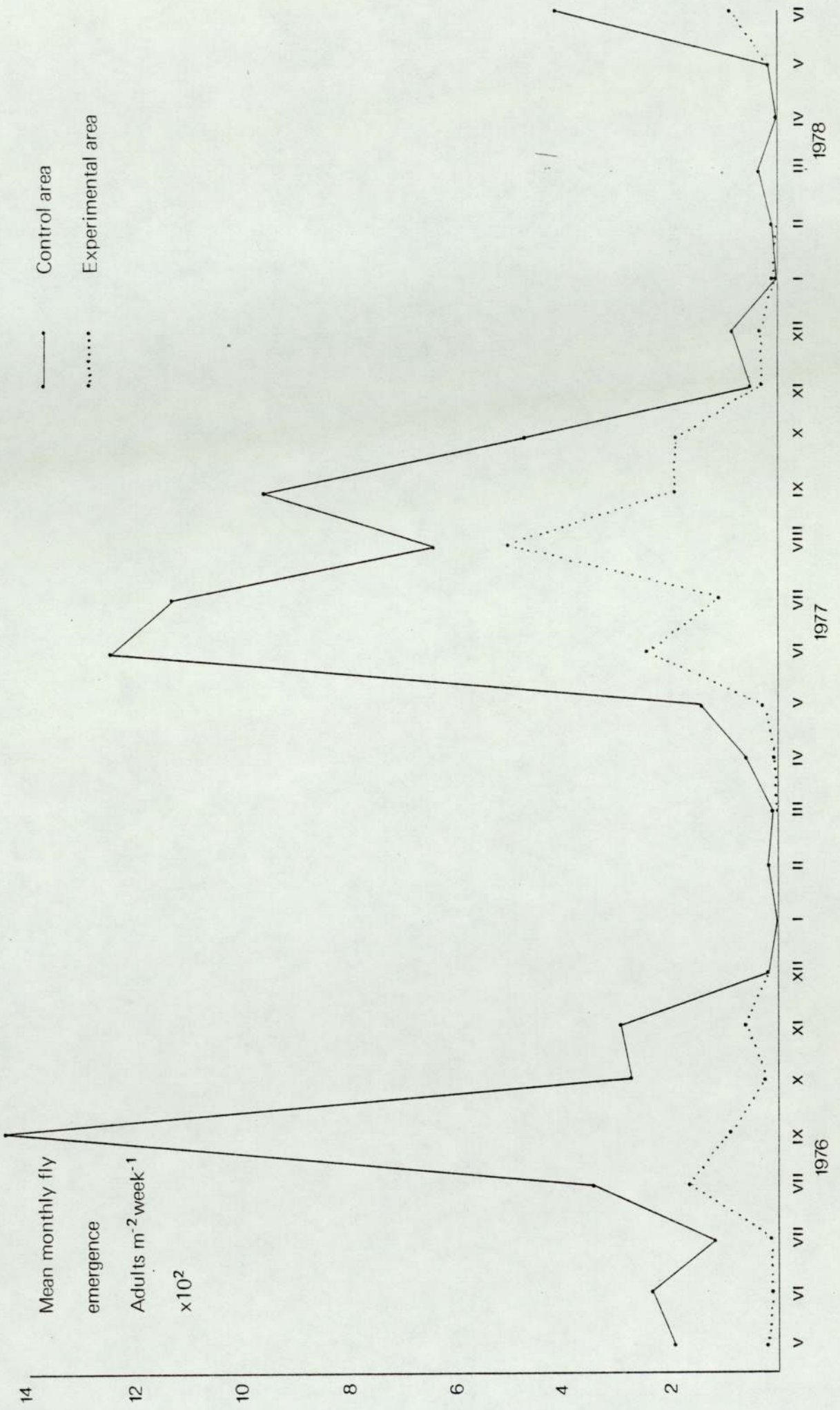


Fig. 59. Effects of splash plate on *M. hygropliticus* emergence (primary filters)

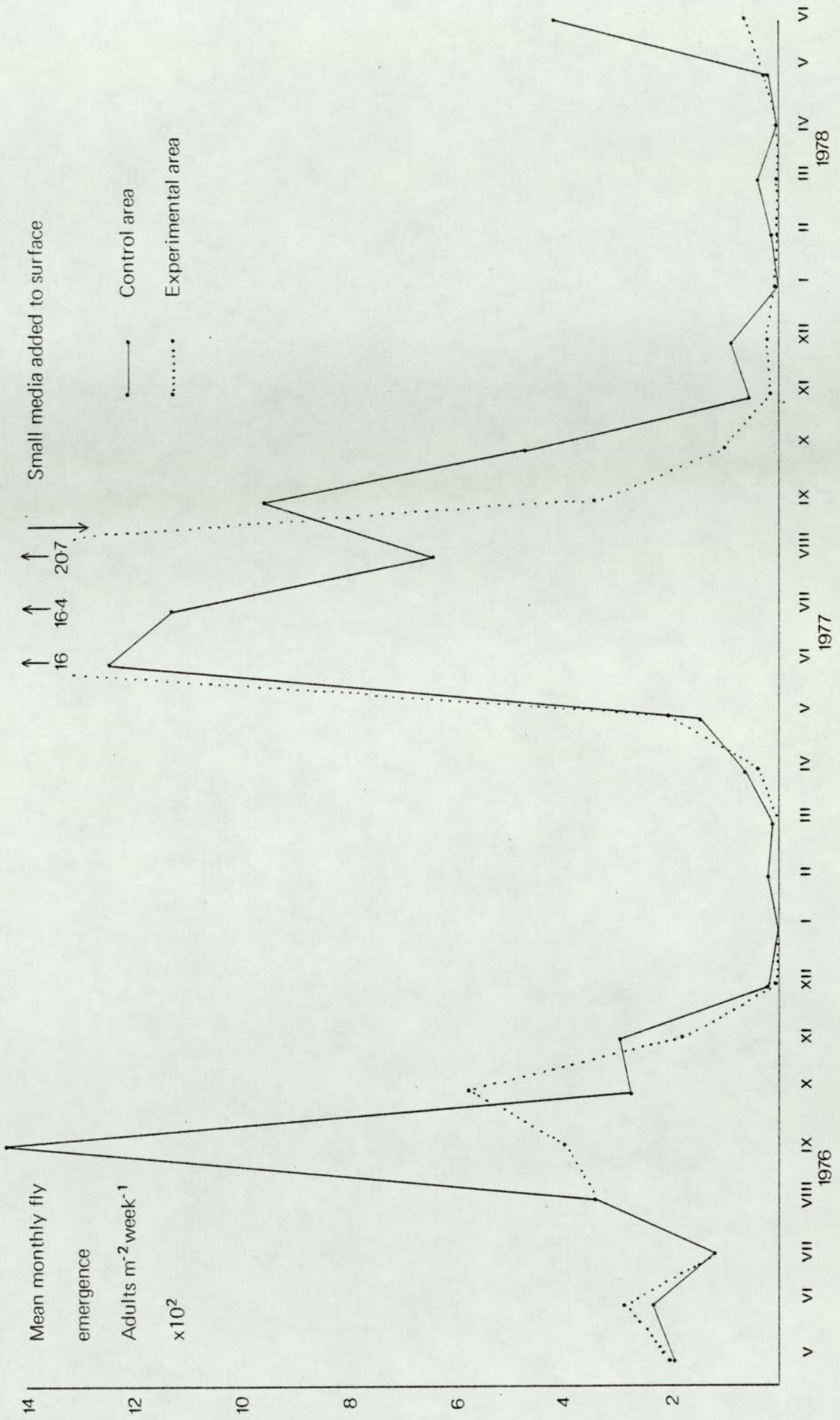


Fig. 60. Effects of splash plate on *P. severini* emergence (primary filters)

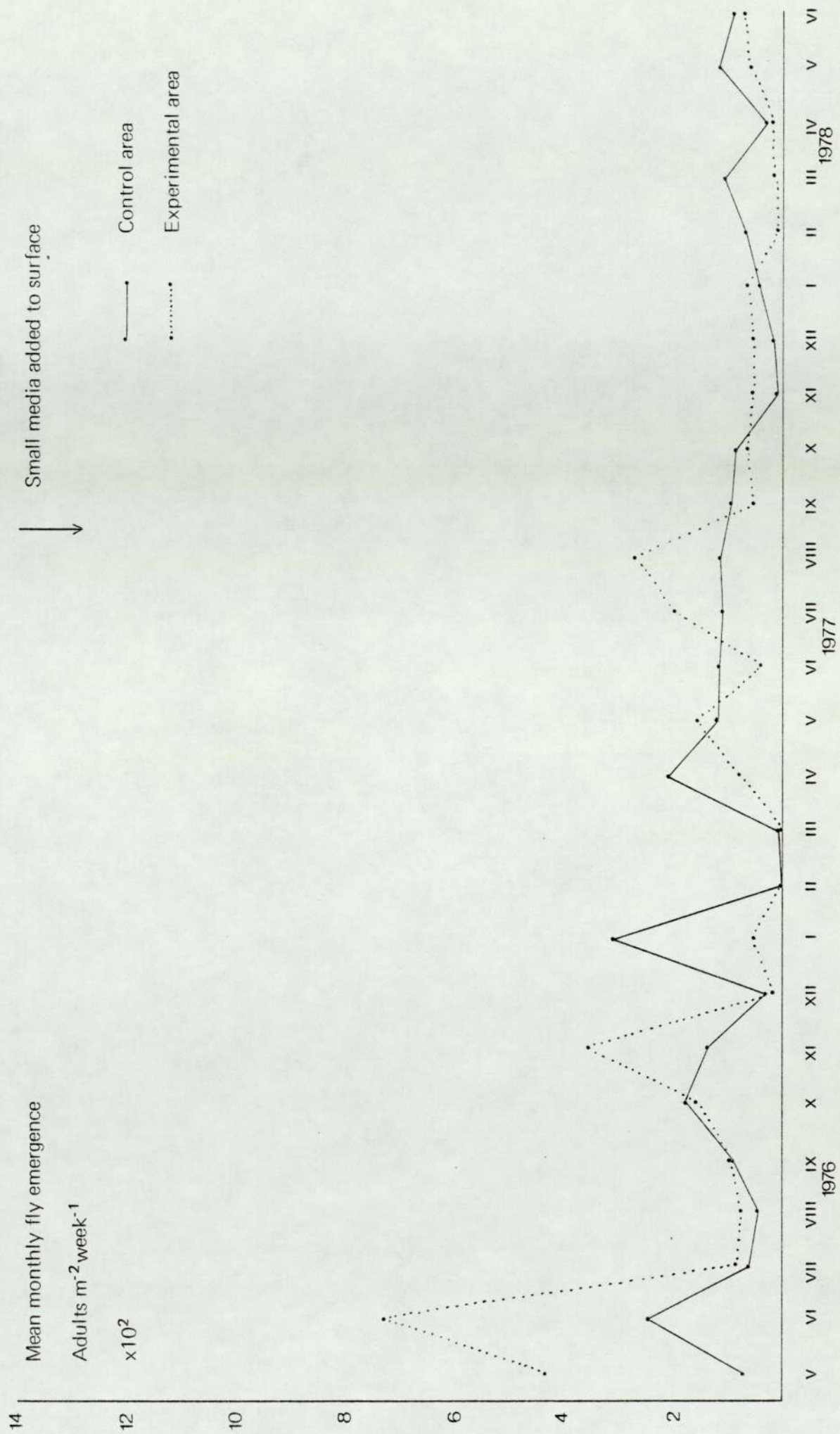


Fig. 61. Effects of double loading on *M.hygropetricus* emergence (secondary filters)

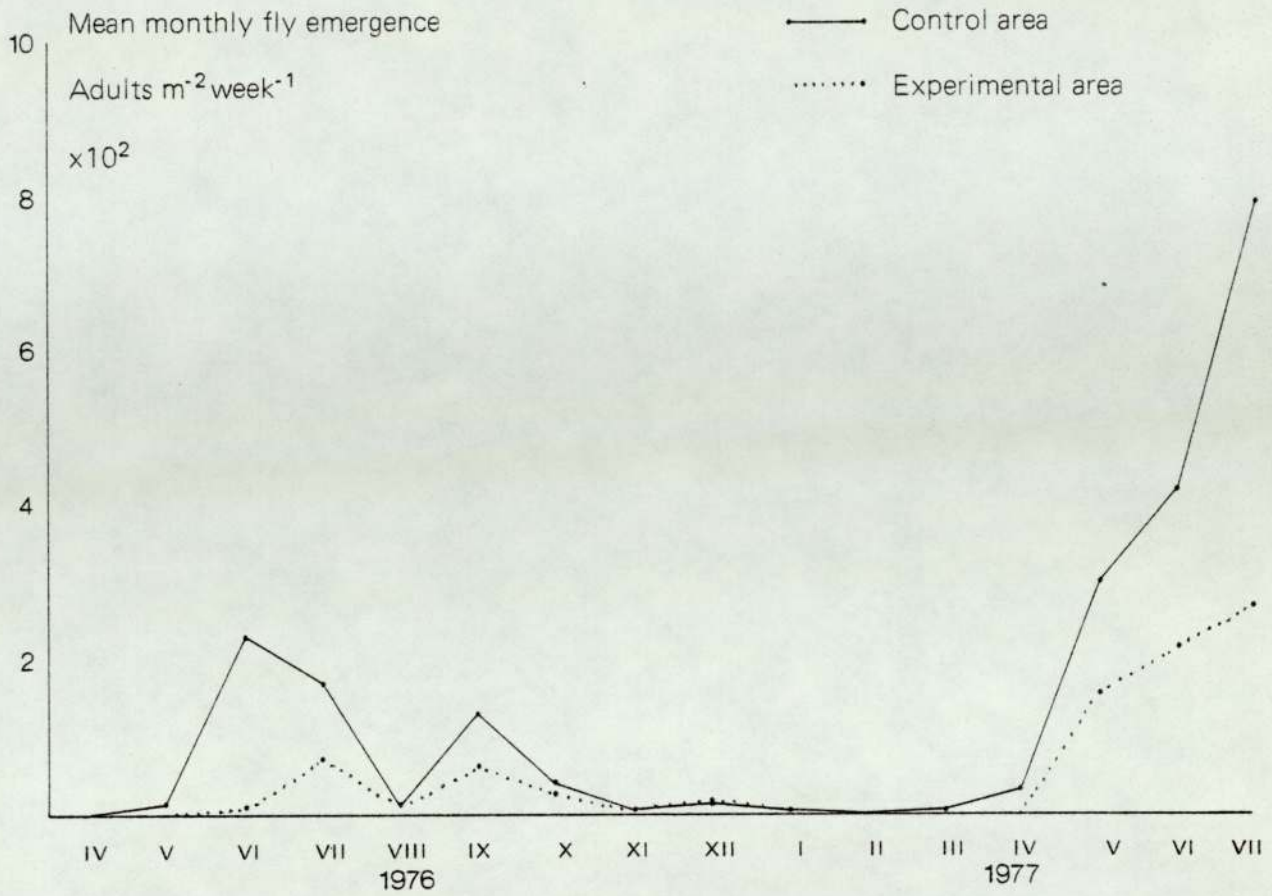


Fig. 62. Effects of double loading on *P.severini* emergence (secondary filters)

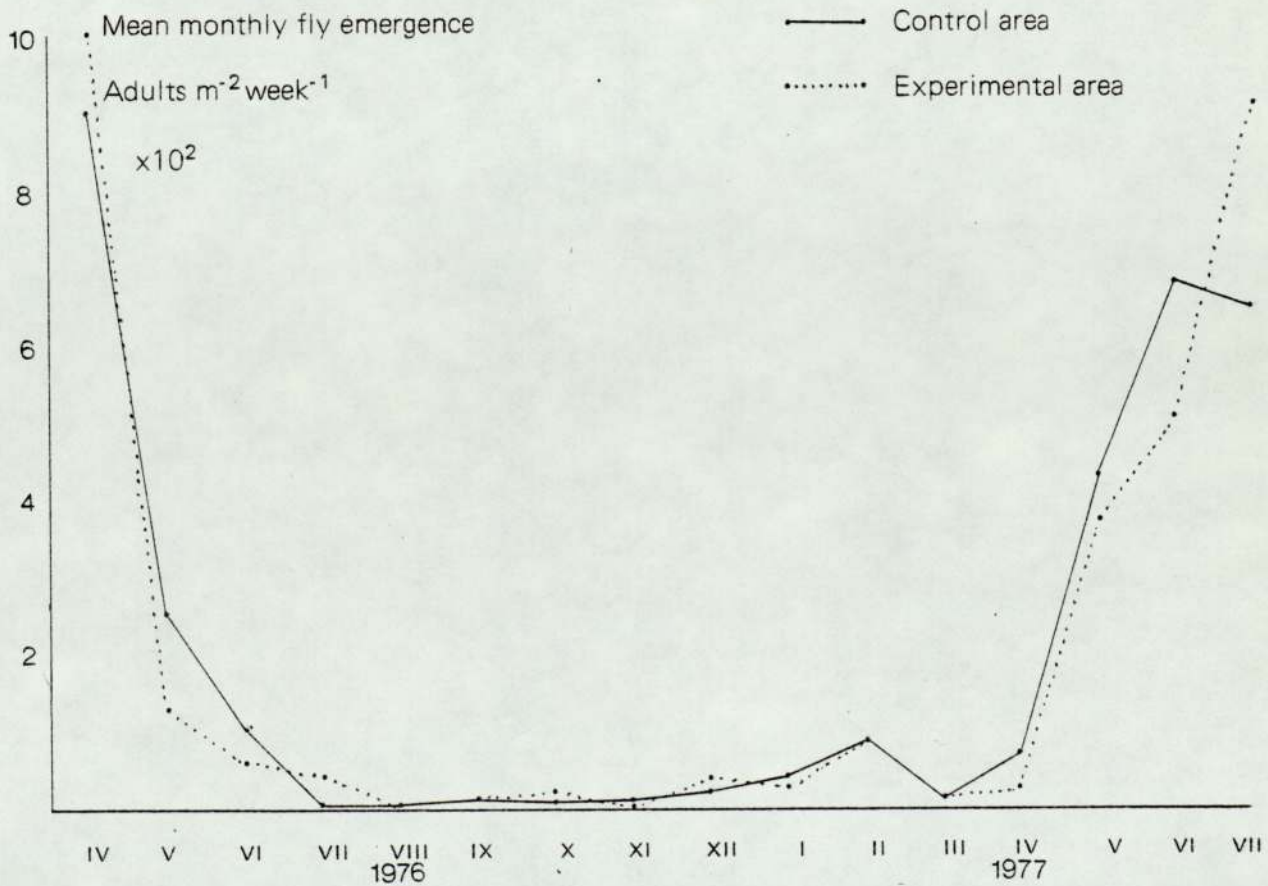


Fig.63. Effects of dosing frequency on M.hygroetricus emergence (secondary filters)

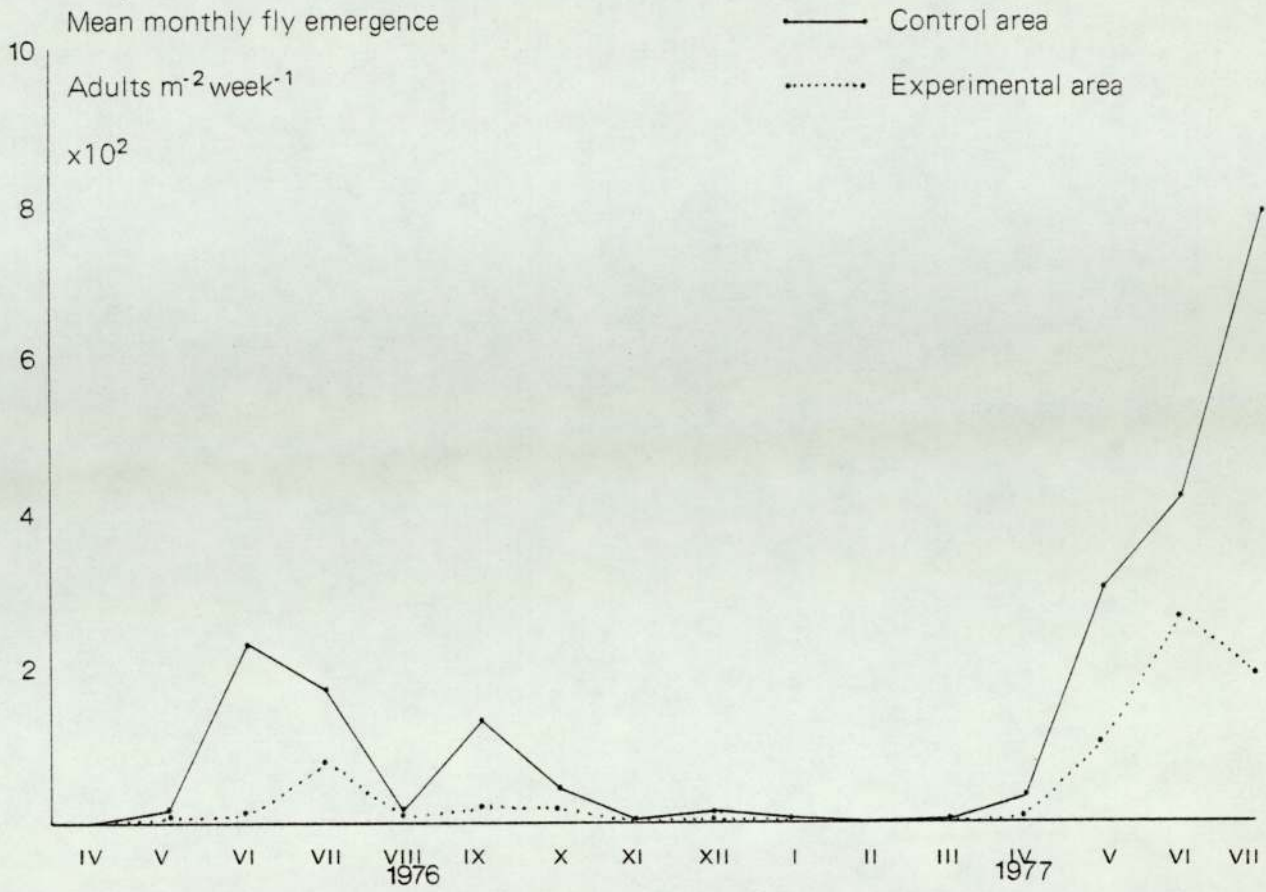


Fig. 64. Effects of dosing frequency on P.severini emergence (secondary filters)

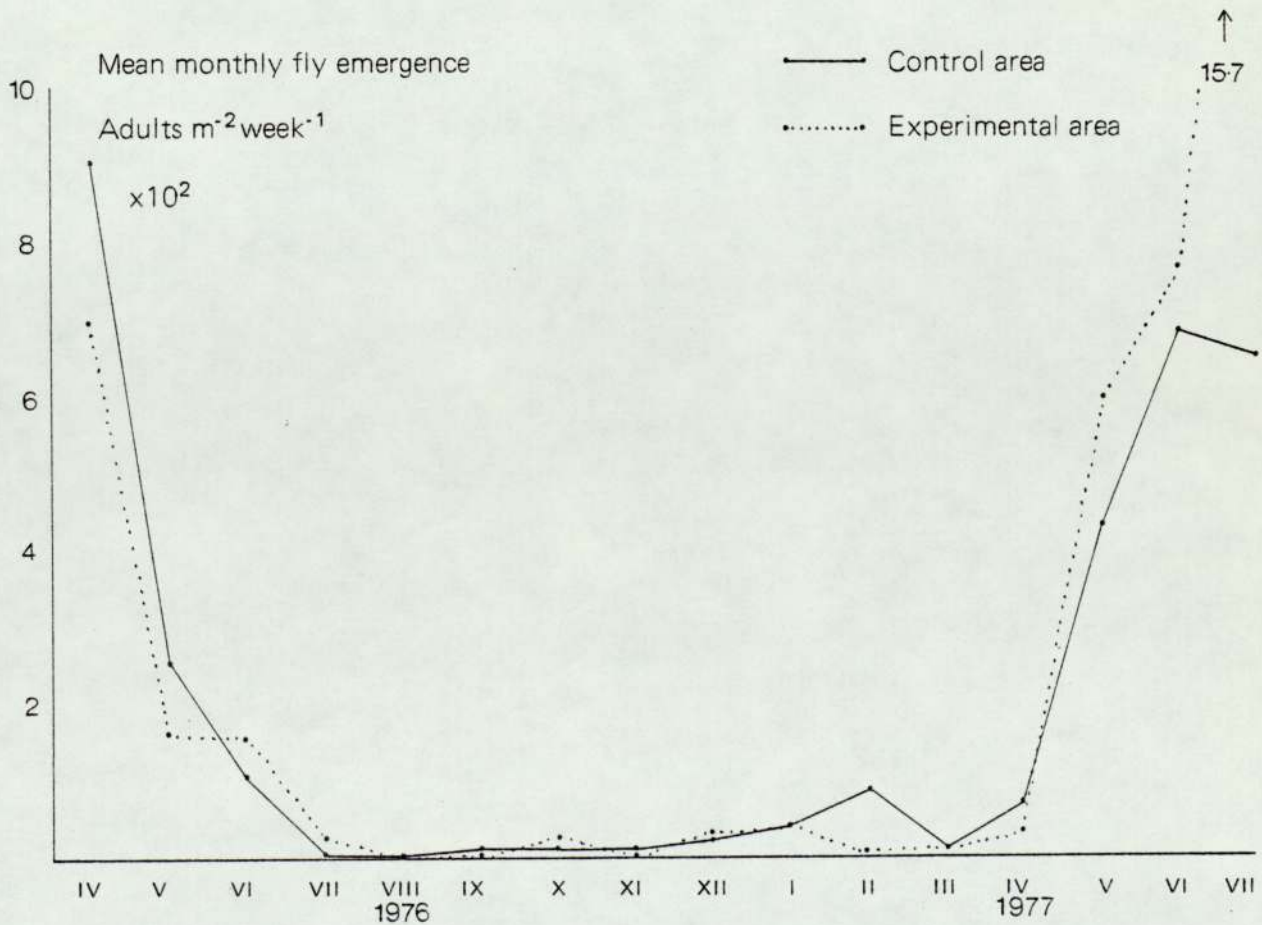


Fig. 65. Effects of small media (229 mm. depth) on *M.hydropetricus* emergence (secondary filters)

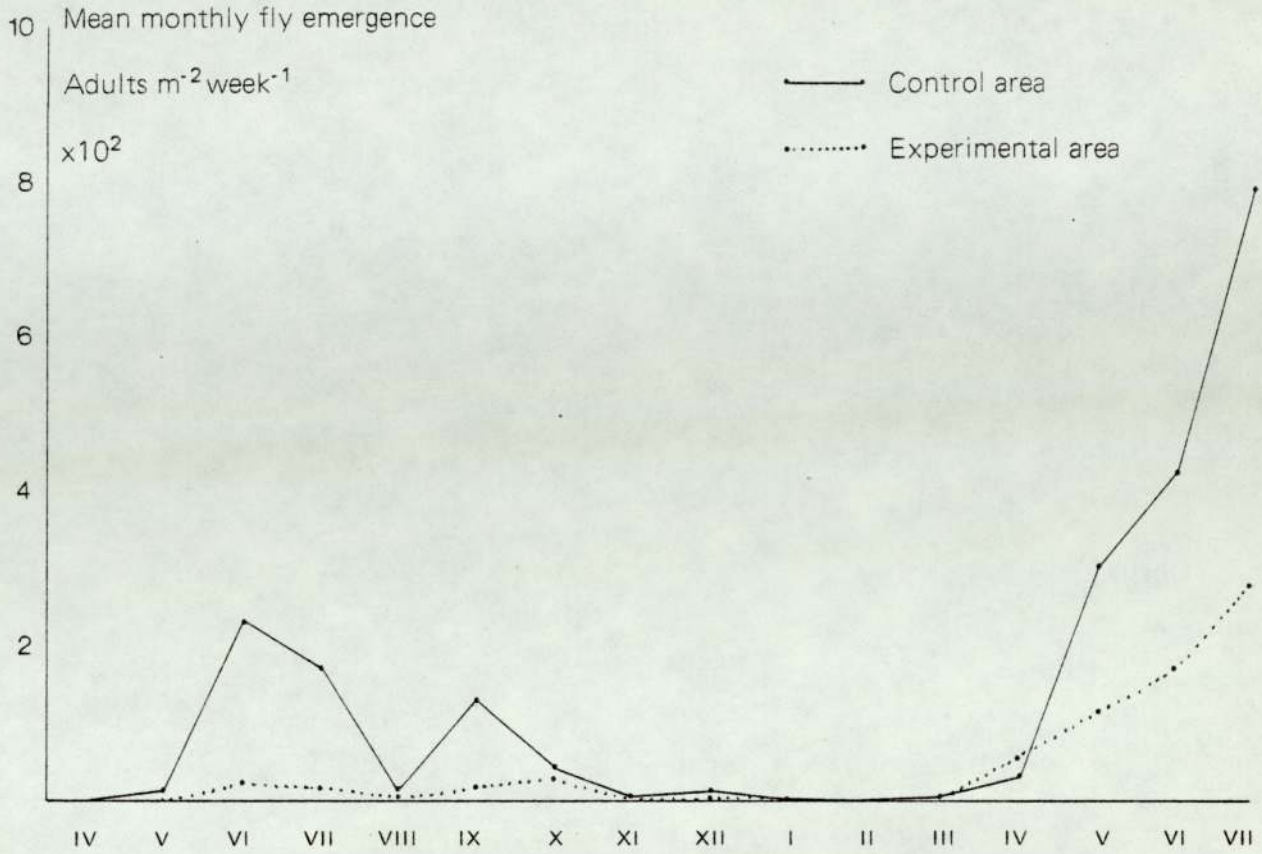


Fig. 66. Effects of small media (229 mm. depth) on *P.severini* emergence (secondary filters)

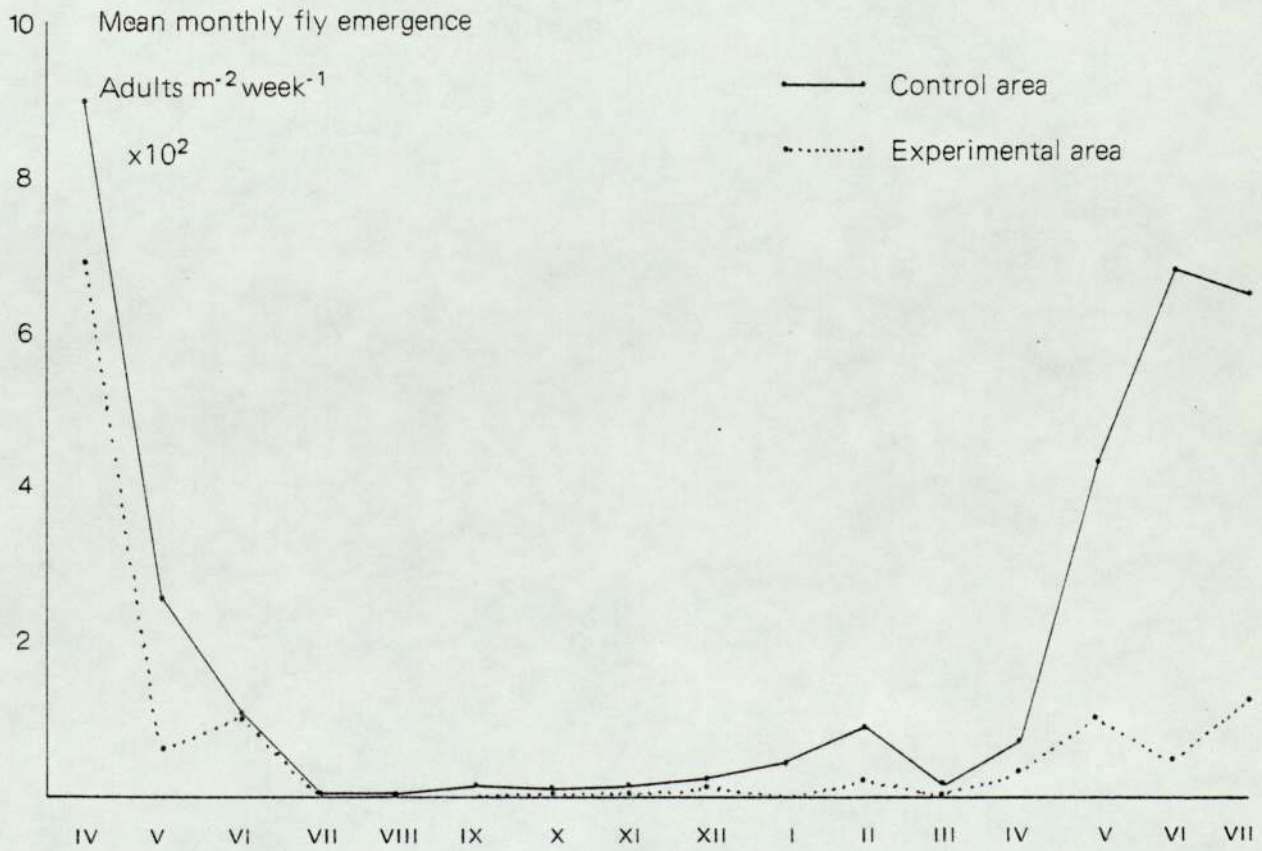


Fig. 67. Effects of small media (229 mm. depth) and splash plate on *M.hydropetricus* emergence (secondary filters)

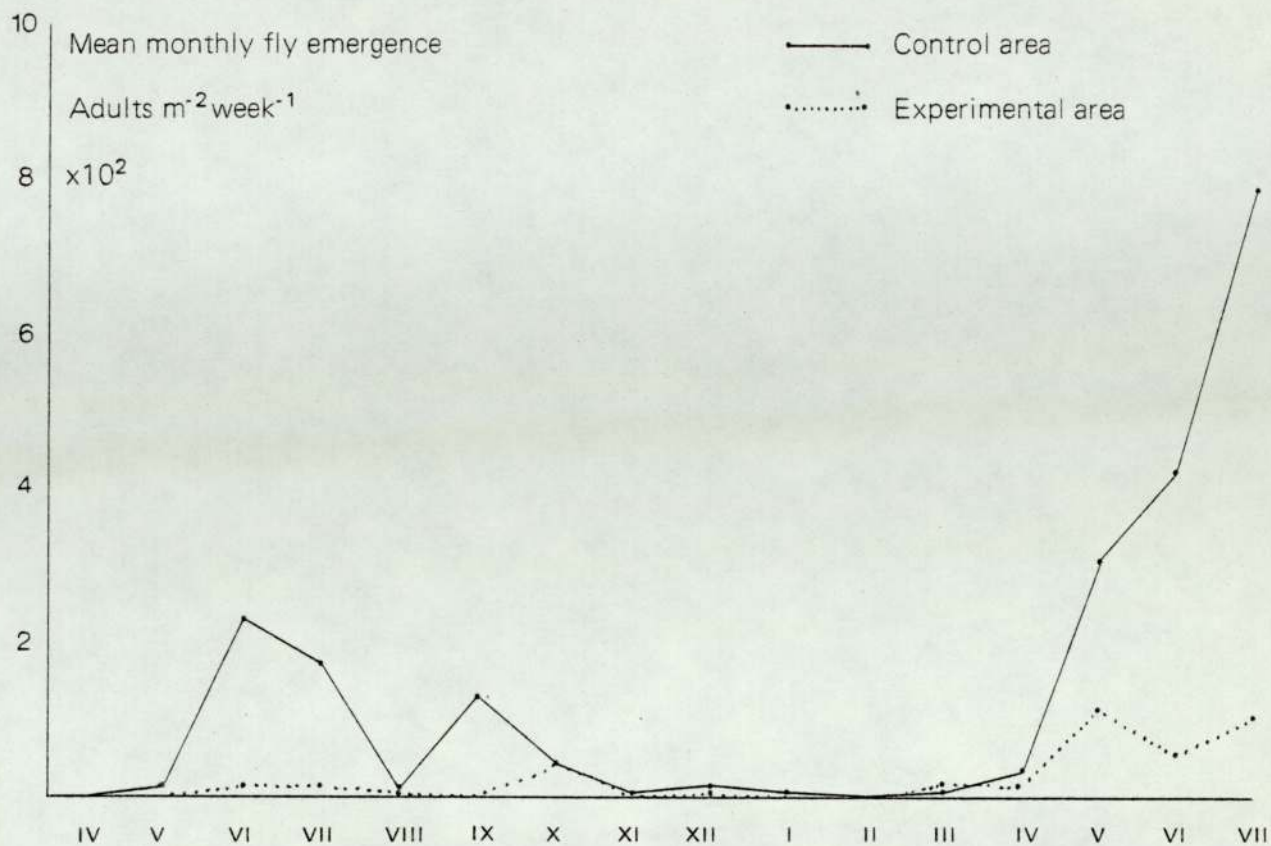


Fig. 68. Effects of small media (229 mm. depth) and splash plate on *P.severini* emergence (secondary filters)

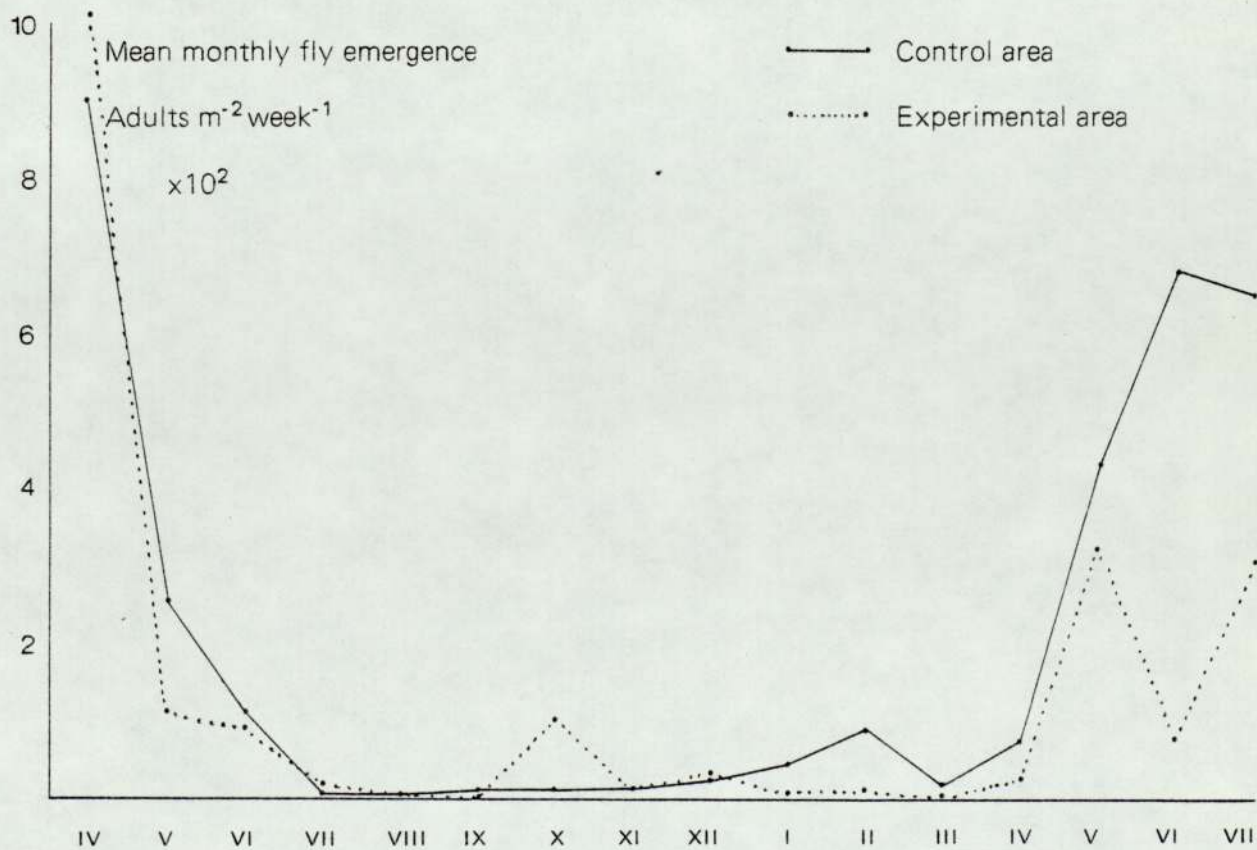


Fig. 69. Effects of splash plate on *M.hygropetricus* emergence (secondary filters)

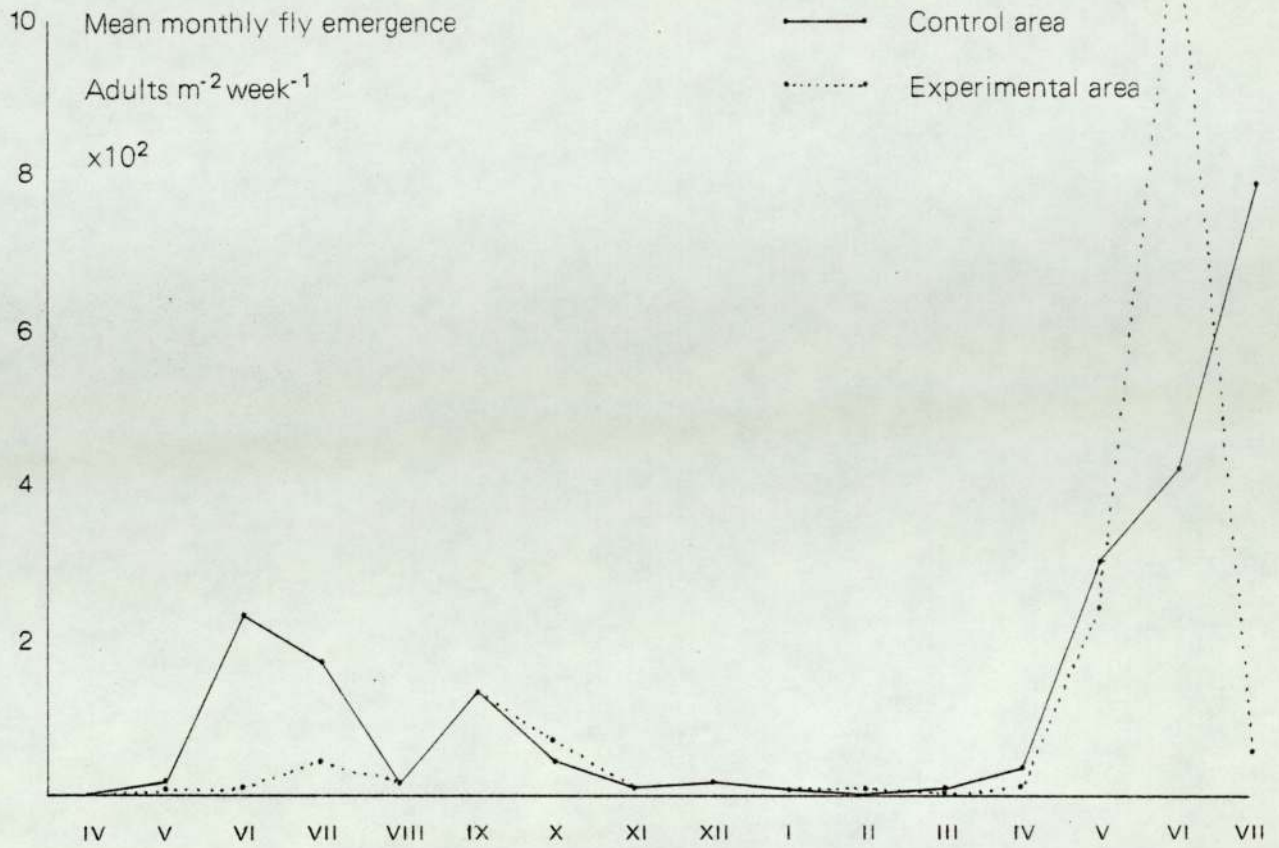


Fig. 70. Effects of splash plate on *P.severini* emergence (secondary filters)

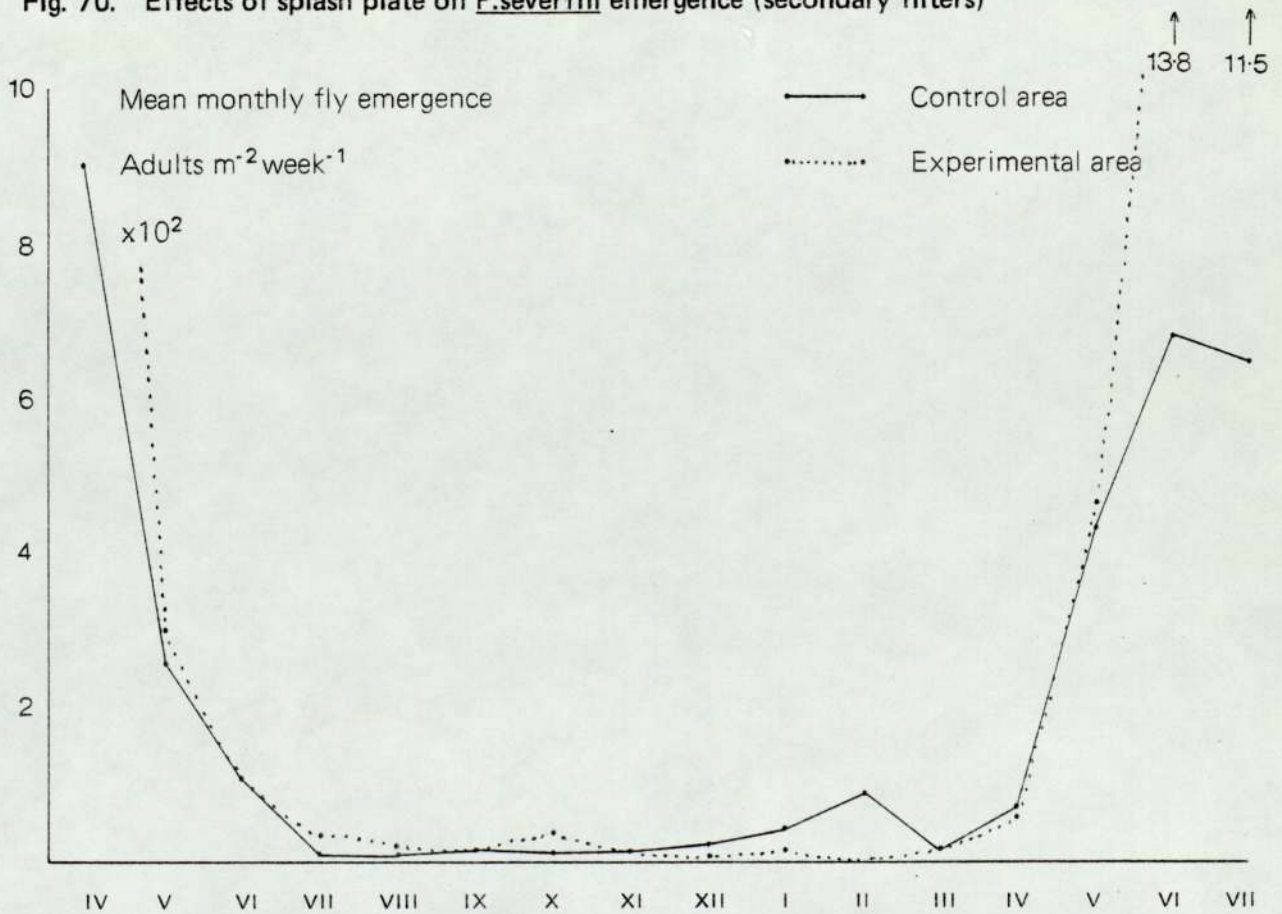


Fig. 71. Enchytraeid worm levels March 1976 — February 1977

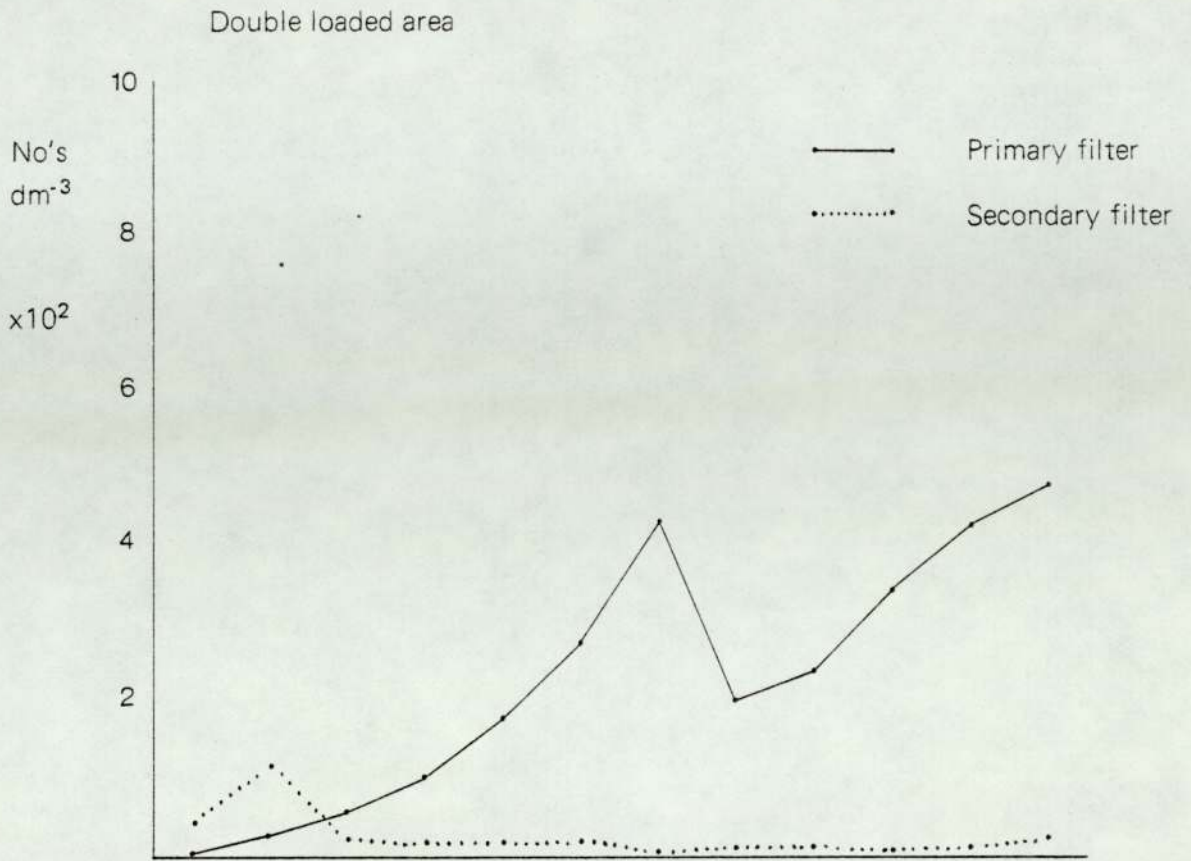


Fig. 72.

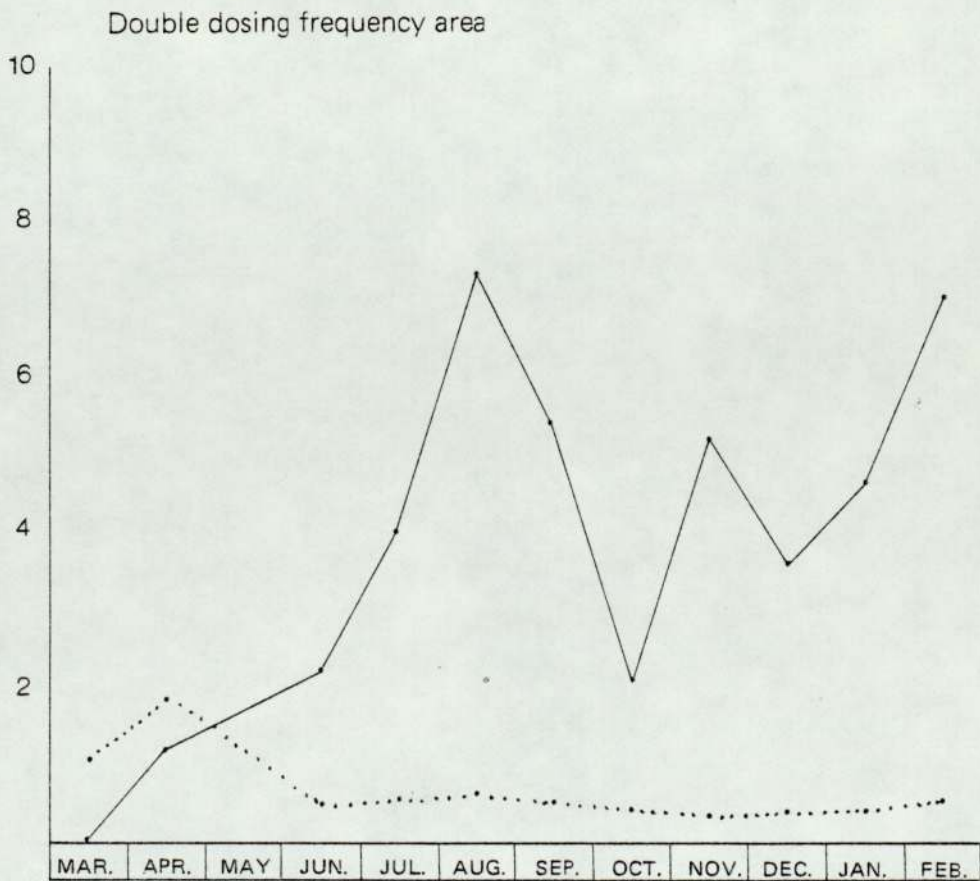


Fig. 73. Enchytraeid worm levels March 1976 – February 1977

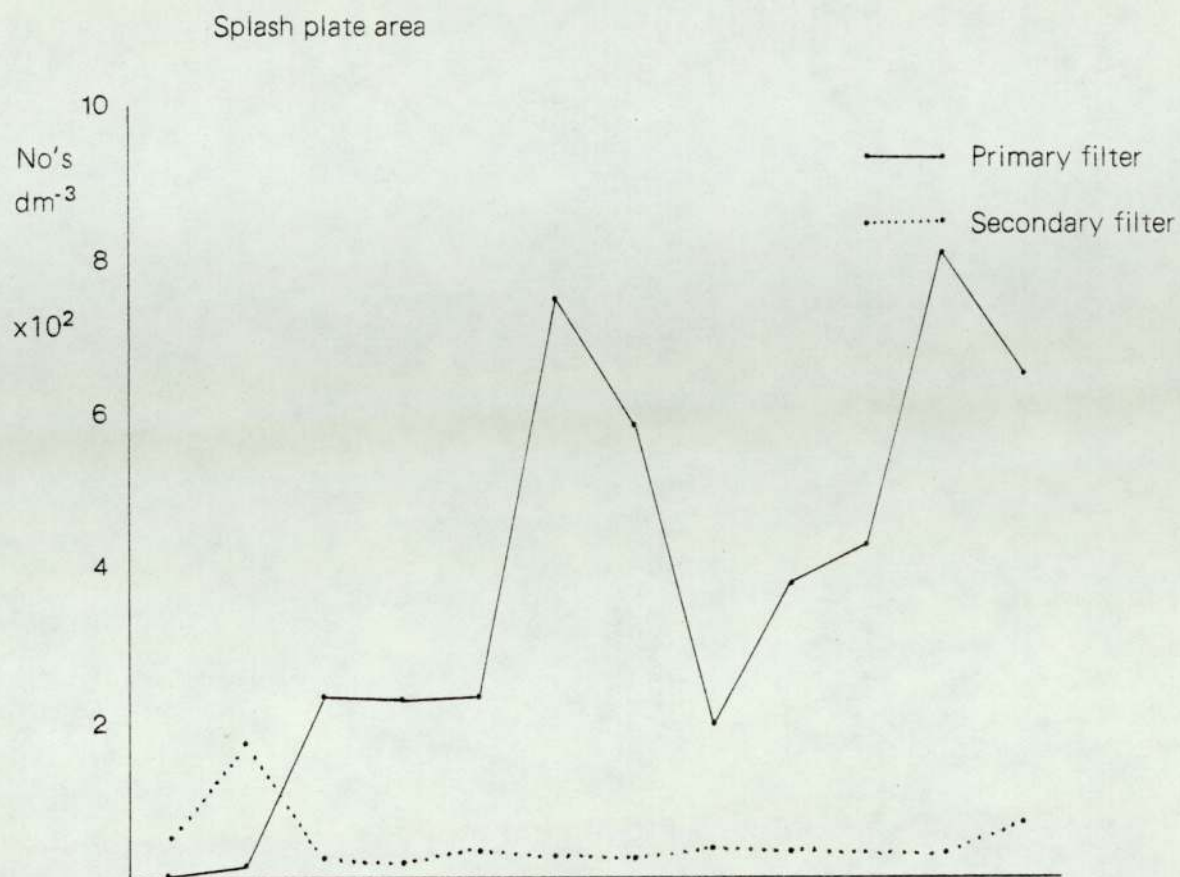


Fig. 74.

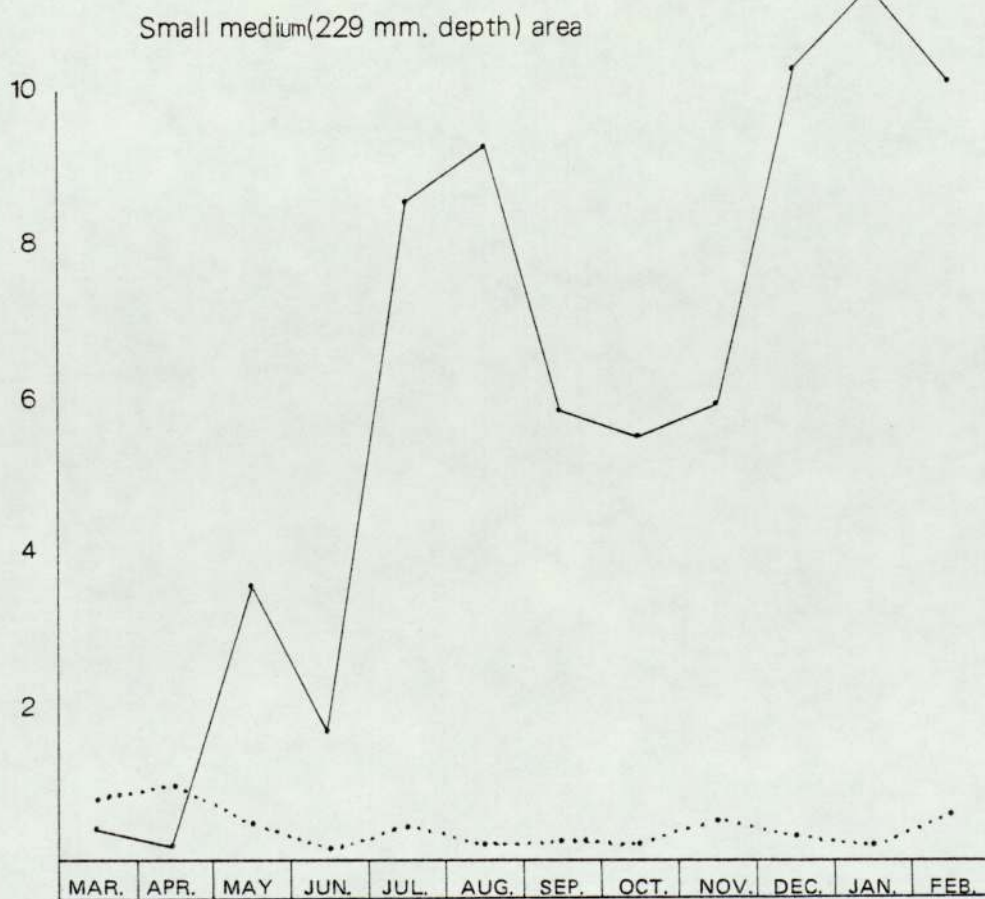


Fig. 75. Enchytraeid worm levels March 1976 — February 1977

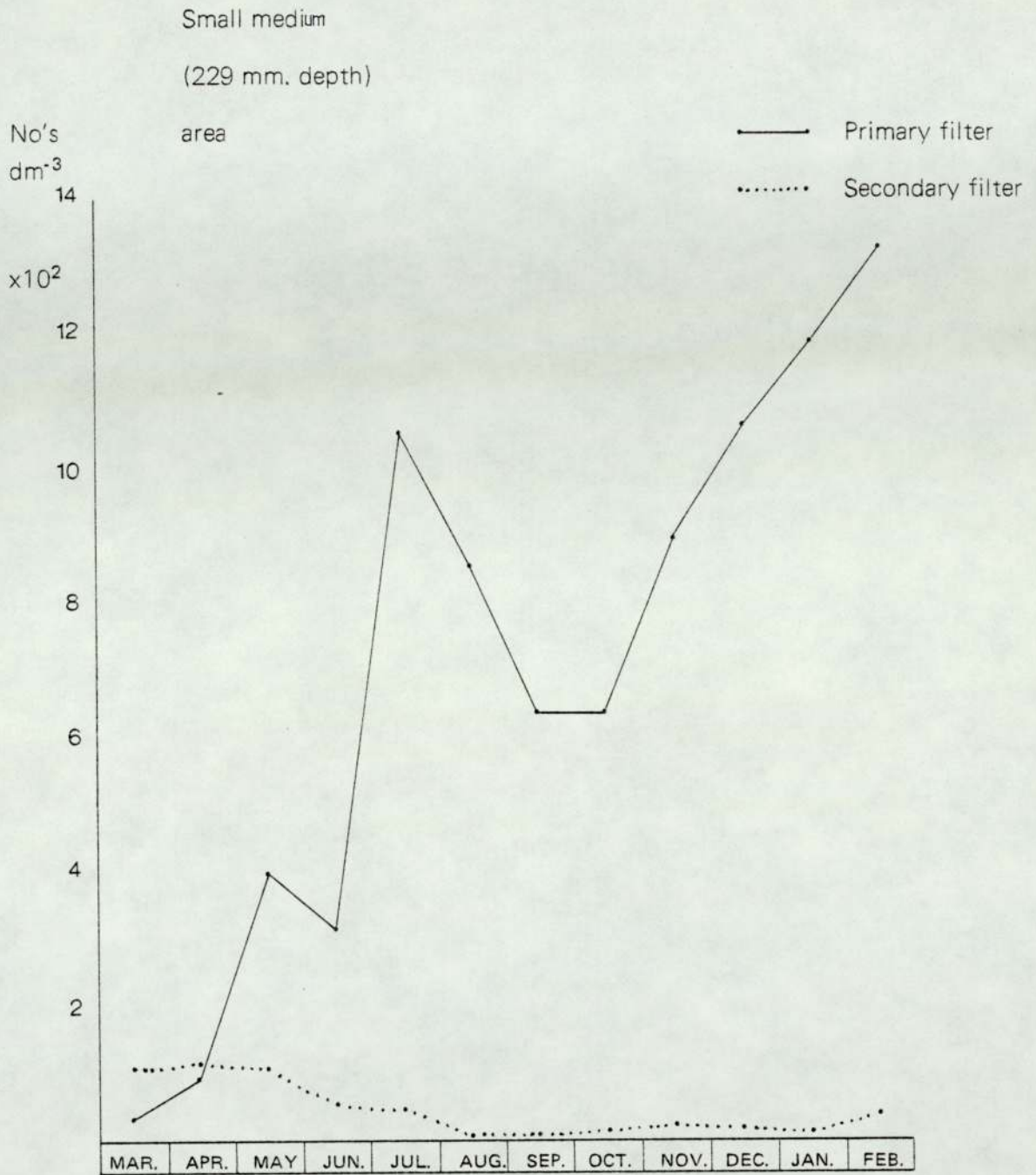


Fig. 76. Mean monthly volatile solids March 1977 — February 1978

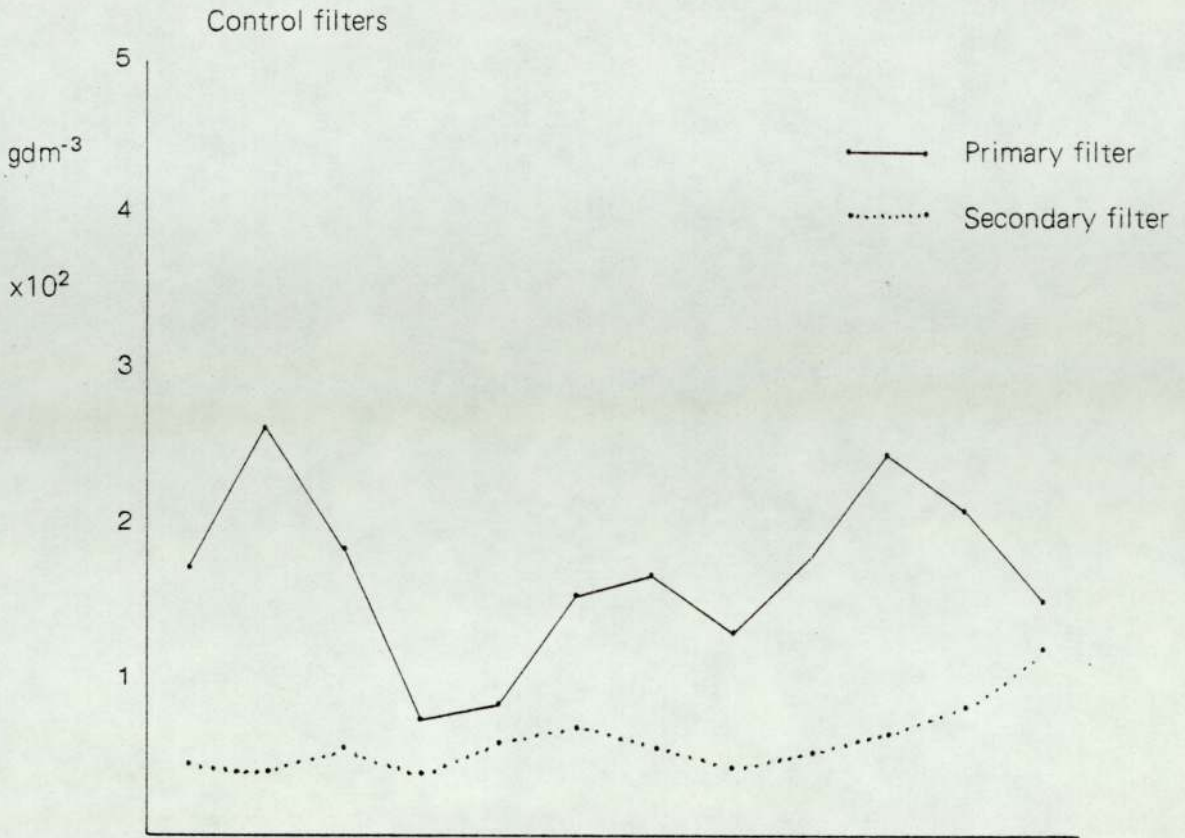


Fig. 77.

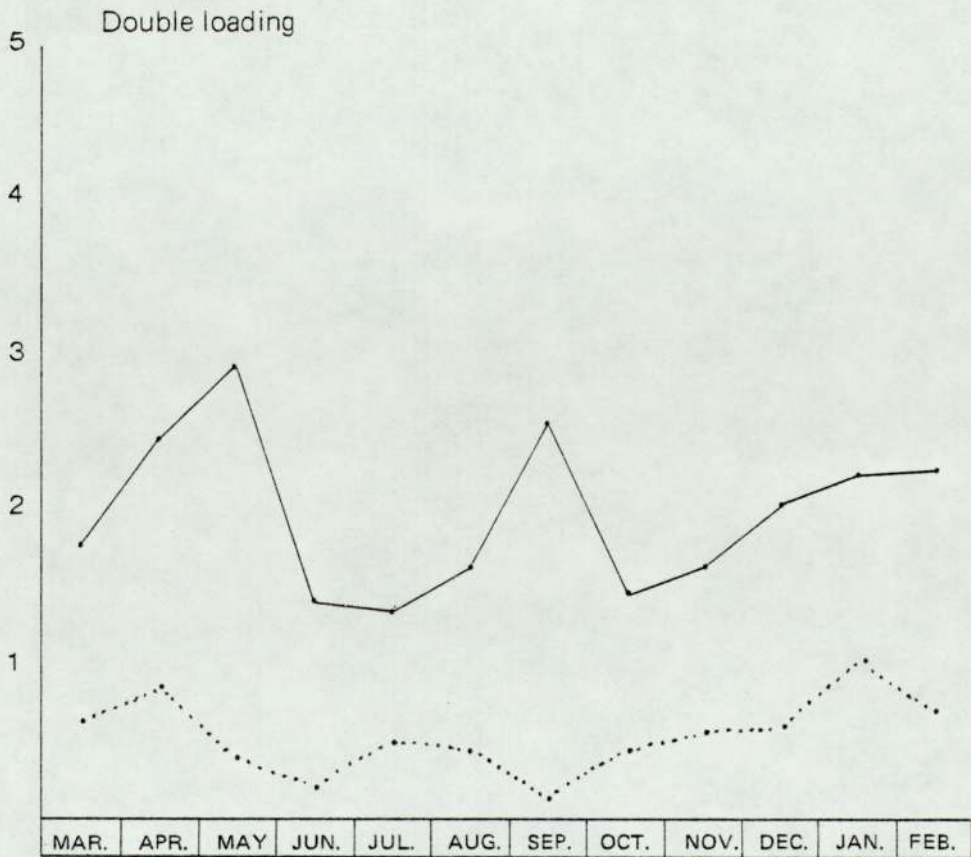


Fig. 78. Mean monthly volatile solids March 1977 – February 1978

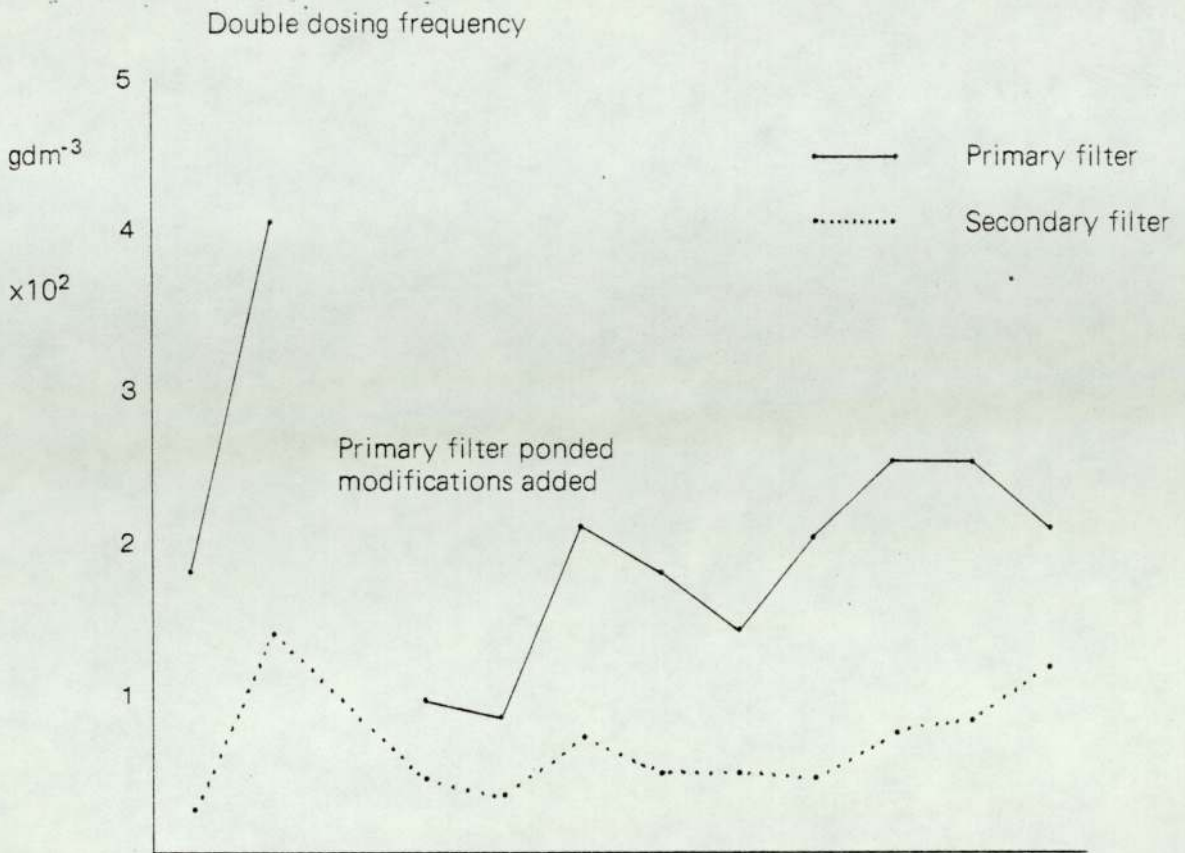


Fig. 79.

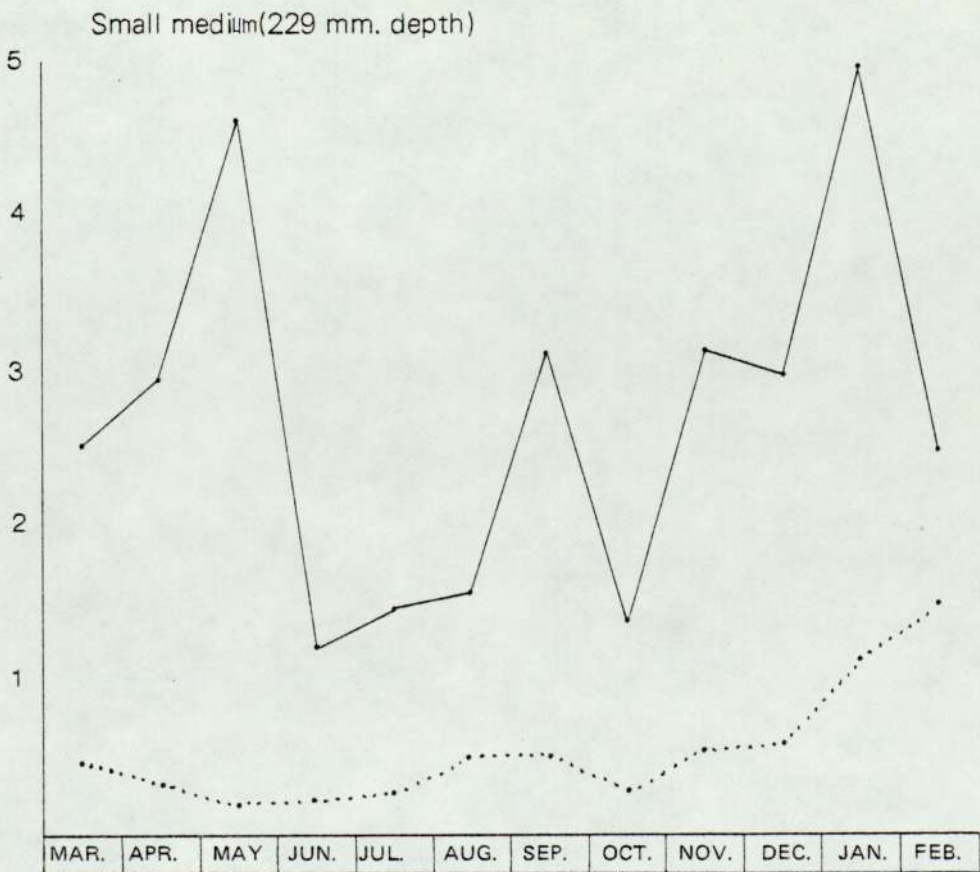


Fig. 80. Mean monthly volatile solids March 1977 — February 1978

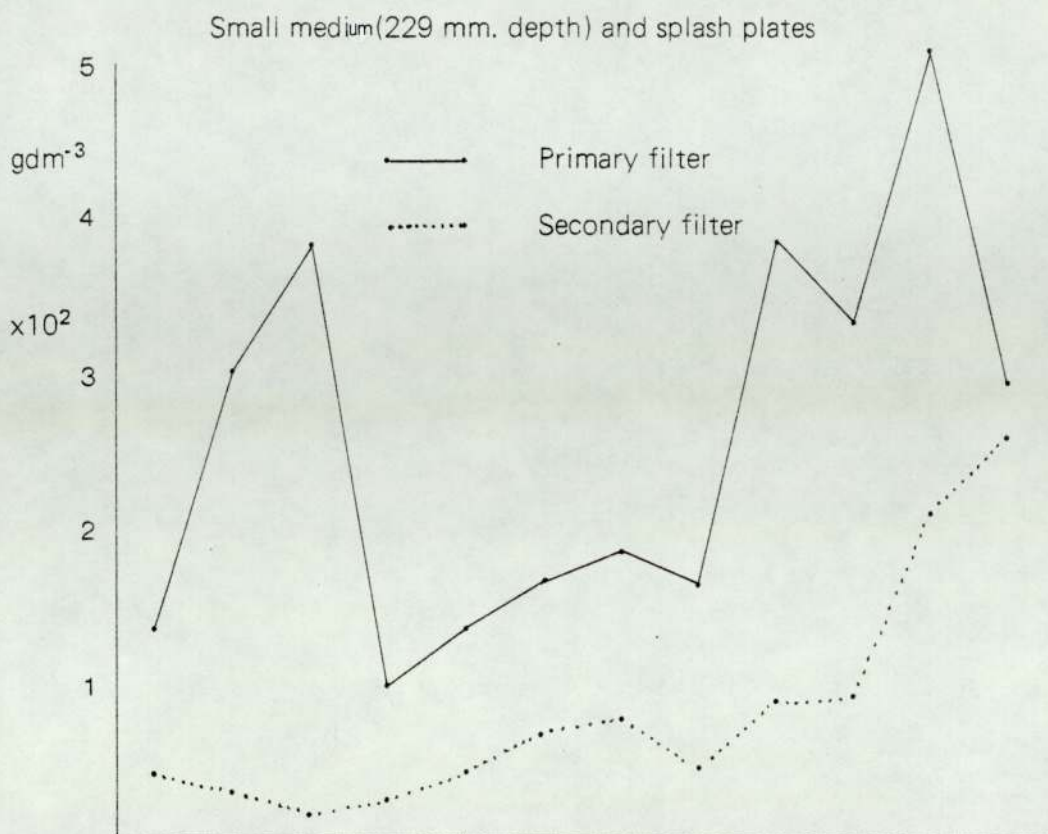
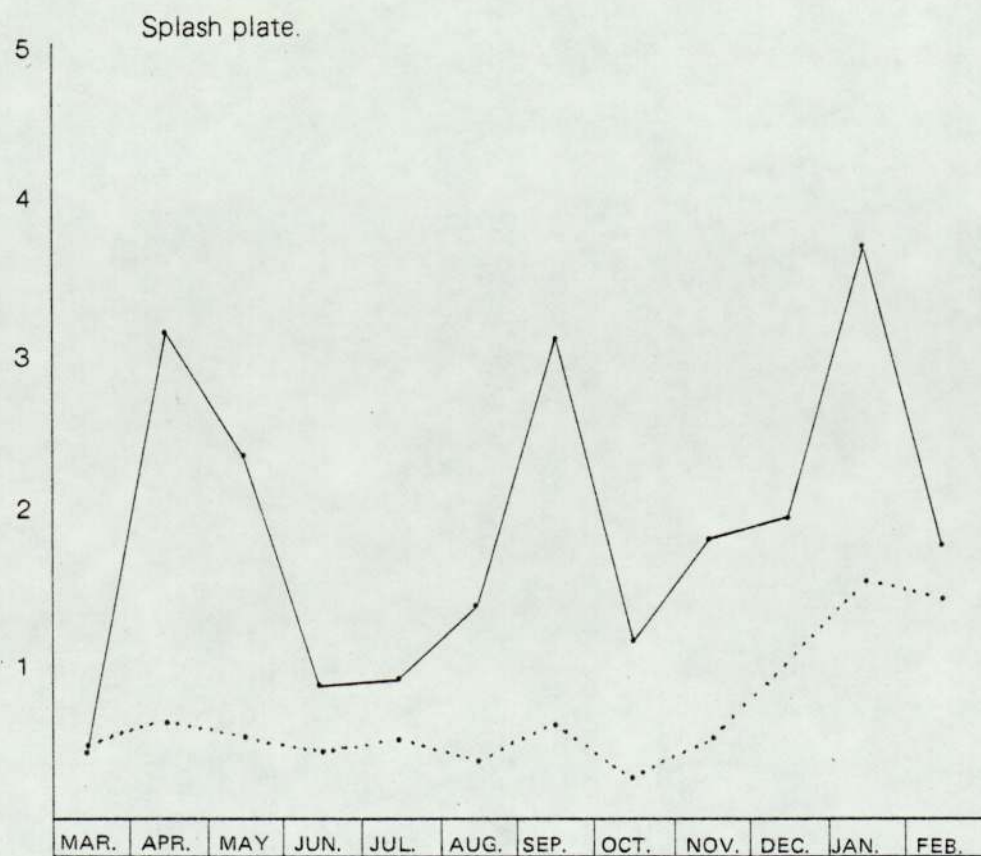


Fig. 81.



D. General discussion and summary of relative
advantages and disadvantages of operational
compared to insecticidal methods for fly control

i) Insecticidal control

This form of control does not seem too attractive when comparing its advantages and disadvantages. Its advantages are easy to appreciate in that when it is applied results are almost instantaneous, i.e. larval kills of 90 – 100% are often obtained giving a false impression that the problem is completely solved and equally drastic reductions will be obtained with each application. Other advantages with this system are that insecticides are easy to apply and can be applied at the specific time when they are needed, also only a low initial capital outlay (for a pump, tubes and containers) is needed, i.e. about £150 to treat a filter area the size of Tamworth Works (25135m²). This excludes the costs of insecticide which are £1100 per annum and are considered as ongoing costs.

When the disadvantages are considered however this form of control does not seem so attractive. Obviously insecticide treatment brings about an unnatural situation in the filters where one component of the grazing fauna is removed by treatment which may cause an increase in solids. During the period of investigation at Tamworth no significant solids increases were noted suggesting that the other grazers were compensating for the removal of the target species therefore it could be argued that insecticidal control is not undesirable on this account, however when other factors are investigated such as resistance build up and ongoing costs, this form of control seems to be less advantageous.

The phenomenon of resistance has already been discussed in the literature review section and even though reports of resistance occurring specifically to organophosphates are few (Wichmand 1953, Gjullin and Isaak 1957 and Quarterman

and Schoof 1958), these must be considered otherwise a situation may build up whereby a resistant strain is selected for by removing non-resistant individuals with each treatment. This then would have the effect of increasing the resistant gene pool which is obviously undesirable. The biochemical mechanisms of resistance have been described by Plapp (1976). Although resistance in the sewage works venue has not been well documented it is probably more important than is usually appreciated. For example it can be seen in the toxicity test section, the results of which will be described later, that M.hydropetricus larvae when collected from Tamworth filters had a reduced susceptibility to insecticide treatment compared to similar larvae collected from another works. The only difference between the two works to explain the reduced control was that Tamworth had been receiving an intensive insecticide treatment programme for three years whilst the other works had not experienced insecticide treatment during that time. Other explanations could be put forward for the reduced susceptibility, for example it could be argued that a different sub species of larvae was collected from the other works as this particular species is difficult to identify in that stage, however considering the results described previously when it was found that control of M.hydropetricus gradually declined over the three years of sampling from the treated filters at Tamworth this seems to suggest that resistance was building up.

Other results found from toxicity testing (to be described in detail later) suggest that other macrograzers are in fact affected by the insecticide in question albeit in higher concentrations than M.hydropetricus. This shows the importance of applying the minimum concentration possible to kill the target species and not to apply high concentrations or non-target species may be affected which would probably cause film accumulation problems.

Quite apart from the above effects, when the costs of a regular insecticide treatment programme are investigated this form of control seems even less attractive. It has

been found as described previously that the timing of the insecticide dose is critical in achieving maximum success with any one treatment. Therefore in order to estimate at which life cycle stage a population is to time the dose correctly it is necessary to carry out intensive sampling of media or monitor fly emergence. This sampling is very time consuming and laborious and therefore extra staff would be needed to carry this out. Also the cost of the insecticide used is quite considerable. At Tamworth 30 litres are used at each treatment to dose a total filter area of 25,135 m². Considering the cost of Actellic M20 which is approximately £18 per five litre container it is possible to calculate that each treatment costs approximately £108 and that the cost per unit area is approximately 4p per m² which is quite expensive considering such a large area is treated. Therefore overall assuming 10 treatments per year £1100 has to be budgeted for chemicals alone quite apart from the cost of the labour in supervising and setting up the dosing apparatus at each treatment.

Apart from the above points it is obvious that insecticide application is ecologically undesirable as toxicants are being added to the environment. It has been proved that many insecticides including the one utilised here do break down readily but it is unsure of the fate and the long term effects of their breakdown products. Table 4 should be consulted to appreciate how widespread organochlorine residues become after a long period of almost indiscriminate usage.

Therefore considering the above arguments insecticidal control measures seem less attractive in the long term from effectiveness, financial and ecological points of view. However if short term control is required these should be most effective as costs and the effect on the environment of single doses would be negligible.

ii) Operational control

Operational control is obviously far more desirable from an ecological point of view

as with this form of control no unnatural agents are being utilised therefore giving no risk to the environment. Nuisance populations are simply being reduced by either increased competition with another species whose numbers are being allowed to rise in the altered conditions, or changing the environment in some way so to provide conditions unsuitable for the nuisance species. With this form of control an obvious advantage is that in many cases the increase found in competitive species serves to control film levels from rising to excess unlike insecticide control measures where although no film problems were encountered it was shown that the insecticide did affect other non-target fly larvae and enchytraeid worms.

Another advantage with this form of control is that it is unlikely that resistance build up would cause problems. The reason for this is that the actual mechanisms of control in these cases are physical in being increased competition and inhibition of respiration whereas the mechanisms in insecticide control measures are much more subtle in being slight biochemical changes probably affected by one gene. Thus it is easy to imagine how more likely it is to select for such subtle differences in the insecticide resistance case rather than the massive physiological changes needed to overcome the operational control mechanisms which are probably controlled by more than one gene. Therefore it is likely that this form of control would be much more permanent and there would be no gradual decrease in control due to resistance build up.

Regarding costs there are advantages and disadvantages with these systems depending on whether ongoing costs or the initial outlay is considered. A review of the cost of producing various standards of effluents by biological treatment techniques was given by Sidwick and Preston (1976) however considering the specific modifications used in this study Table 16 compares the costs of the most successful ones from the fly control angle compared to insecticide control measures.

Table 16

Theoretical relative costs of different control measures
over 3 years at Tamworth (filter area 25135 m²)

1 Insecticide treatment

(a) Initial outlay	- pumps, tubing, containers etc	£150
(b) Ongoing costs	- insecticide (per dose)	£108
	- labour (3 hr. at £2 per hour per dose)	£60
	- Sampling programme labour	£?

Therefore assuming 10 doses per year the total cost over a period of 3 years would be £5190 (excluding the cost of running a sampling programme).

2 Small media and splash plate modifications

(a) Initial outlay

	<u>Sprinkling</u>	<u>76 mm depth</u>	<u>229 mm depth</u>
19mm gravel at £2.81 per tonne	£1,223	£4,913	£14,740
Aluminium sheet (splash plates) at £1.35 per kilo	£504	£504	£504
Estimated construction costs at £2 per hour	£512	not economically viable	

	<u>Sprinkling</u>	<u>76 mm depth</u>	<u>229 mm depth</u>
Clips and bands—			
£1.50 per set	£24	£24	£24
TOTAL COST	£2,263	£5,441	£15,268
		+ labour cost	+ labour cost

(b) Ongoing costs - nil

Therefore the total cost over 3 years would be the same as the initial costs.

3 Dosing frequency changes (15 - 10.5 min.)

(a) Initial costs

Changing gearing on winch house motors

(4 in total)

£400

(b) Ongoing costs

Approximately 15% increase in power consumption

(assuming top prices £0.025 paid per unit)

£77 p.a.

Therefore the total cost over 3 years of this modification would be £631.

The initial observation from Table 16 is that generally the operational modifications have a disadvantage of high initial outlays whereas later they have the advantages of negligible ongoing costs compared with insecticide treatment.

Three small media modifications were investigated, surface sprinkling, 76 and

229 mm depth and from Table 16 it can be seen that the 76 and 229 mm depths needed such a large volume of gravel it was considered economically nonviable to utilise such measures on a large scale. However the surface sprinkling of small medium proved quite attractive in that a moderate initial outlay was required (£2263) and ongoing costs were nil, with this system the modification would pay for itself in 1.3 years compared to the costs of insecticide treatment.

Concerning the dosing frequency changes it is planned in the future to change the system from 15 — 7.5 minutes on one filter. This can be attained simply by changing the gearing in the winch house motors. A similar experimental change was carried out towards the end of this project when the gearing on one of the motors was changed to alter the frequency from 15 — 10.5 minutes and no undue mechanical problems were reported over the 4 months since it was installed. Although no effects of this on fly emergence levels are known, according to theory it could only serve to decrease M.hygroptericus levels but obviously not so much as reducing the frequency to 7.5 mins. Therefore advantages with this system are that the costs are low (see Table 16) and that this system would pay for itself in 4-5 months compared to the costs of insecticide control measures.

In comparing the above modifications although the sprinkling of small medium and splash plate modification has been proved to be most successful in controlling M.hygroptericus it is suggested that either small scale trials i.e. one complete filter block or other small works trials utilising small filters with the same media and dosing frequency are carried out before this modification is applied on a larger scale, as it is appreciated that the absence of problems obtained with this modification on both main and pilot filters cannot always be guaranteed when applied on a larger scale. Also with a works the size of Tamworth the dosing frequency modification (assuming it works from a fly control point of view) would obviously be the most attractive as the initial outlay is so low and the operational problems often associated

with small media would not be found on such a system. However with such a system it is likely that along with any decrease in M.hydropetricus, an increase in Psychoda numbers may occur which although being non-migratory may be a slight inconvenience to plant operatives.

With a works such as Tamworth where the scale discourages certain operational changes there is no reason why integrated control systems cannot be used as long as the insecticide measure is used wisely. Such a system could entail changing the dosing frequency as described (which can be carried out quite cheaply - see Table 16) and integrating this with one or two treatments of insecticide early in the year to prevent the initial large build up in nuisance fly populations. This however must be combined with intensive sampling in order to time the insecticide doses correctly. Thus with such a system as small amounts of insecticide would be used the hazard to the environment would be negligible and the likelihood of resistance build up would be much reduced. Similarly no undue problems should be encountered with the operational change.

It is apparent that more thought should go into the design, operation and siting of new plants. Obviously when a new plant has to be commissioned near to dwellings certain operational codes need to be followed to prevent fly nuisance from occurring. Considering the plant at Tamworth it is obvious (to a biologist) that the low dosing frequency causing the high scouring action combined with the large media size will give ideal conditions for M.hydropetricus to survive. Therefore it is hardly surprising that a problem was found, however it must be appreciated that no problem would have appeared if residential development had not been allowed so close to the works, so planners must be at fault here. In such a situation in future there should be a close liaison between engineers and biologists concerning such factors as the dosing frequency and medium size. Therefore if more consultation had occurred between the engineers, biologists and planners it is likely that the fly problem at Tamworth would

have never arisen.

II Pilot filters

Comparison of operational modifications on filter ecology

i) Description of modifications and introduction to the filters

This section deals with the results from the 12 pilot filters as were described in the methods section. The reason for commissioning these filters was to investigate the effects on filter efficiency of the modifications used on the main filters which were most successful in controlling M.hygroscopicus. It was impossible to sample the effluent from the modified areas on the main filters, therefore separate filters had to be constructed for the investigation. These filters were filled with the same media, received the same sewage, at the same dosing frequency, and experienced similar average loadings as the main filters.

The modifications applied to these filters have already been described but to briefly reiterate they were:-

- (a) Small medium(19mm dia.) 229mm (9in) depth
- (b) Small medium(19mm dia.) 229mm (9 in) depth and splash apparatus
- (c) Small medium(19mm dia.) 76mm (3 in) depth
- (d) Small medium(19mm dia. surface sprinkling) and splash apparatus
- (e) Covering of entire filter surface with a rubber disc

All of the modifications were investigated in duplicate in the pilot filters together with 2 control filters giving 12 filters in all.

The small medium modifications were investigated because of their success in controlling M.hygroscopicus emergence on the main filters. It was found that on these filters fly emergence was reduced on all small medium areas with and without splash plates. Although no film accumulation problems were noted with these

modifications on the main filters, it was considered necessary to investigate the modifications as no specific modification could be recommended without evidence of its effects on filter efficiency. A range of small medium depths were therefore studied, some with and some without the splash apparatus. The splash apparatus used in these cases were plastic pots perforated along the walls and bottom which acted in a similar fashion to a watering can "rose" spreading the sewage out over the whole of the filter surface. As in the case of the main filters the sprinkling of small medium and splash apparatus modification was initially used with the splash apparatus alone without the small medium which was added in August 1977.

Another modification investigated on the pilot filters which was not studied on the main filters was the physical covering of the surface media with a rubber disc with a central aperture to allow the sewage feed to pass through. The reasoning behind this modification was to investigate the role of light and the addition of a physical barrier on fly emergence. Although this modification was not studied on the main filters, investigations are proceeding at the present time into a similar concept in providing a physical barrier to easy fly emergence by covering portions of the filters with "Netlon" thick nylon mesh (6mm aperture). The mesh when covered in film gives a reduced aperture size which could control fly emergence. The advantages of such a system is that it is easy to install and solids build up is unlikely because of high scouring action experienced at the surface, also any rags that may accumulate could simply be hosed or raked off. However there are disadvantages when considering the cost (approx. £1 per m²), thus it would not be feasible to cover an area the size of Tamworth main filters (22920 m²) but this system may be feasible on smaller filters.

Apart from investigating the effects of the modifications on filter efficiency it was found possible to trap flies from these filters thereby giving a check that modifications successful on a main filter scale continued to be successful on a pilot filter scale.

ii) Fly emergence from the pilot filters with reference to the main filters

By comparing fly emergence from control filters on the pilot group (Figs. 83 and 84) with that from the main filters (Figs. 16 and 17) it is apparent that M.hygroptericus levels were lower on the pilot filters (a weekly average of 123 adults per m² compared to 387 per m²) than on the main filters, and Psychoda emergence levels were higher on the pilot filters than on the main filters, (a weekly average of 377 adults per m² compared to 81 per m²). This then suggests that conditions were more favourable for Psychoda on the pilot filters allowing increased levels and M.hygroptericus numbers were reduced accordingly. The reasons for this difference probably lie in the application system used with the pilot filters. It has already been mentioned in the methods section that rubber bungs containing small glass tubes (5 mm diameter) were initially used to balance the flow to these filters and these were in place for the first 15 weeks from the time of commissioning (9th Feb. 1977). The effect of these reduction nozzles was to lengthen the period of dosage in the dosing cycle which had the desired effect of balancing the flow to each filter but also had the effect of "softening" the flow drastically thus reducing the scouring action. By consulting Psychoda emergence results in 1977 (Figs. 83, 85, 87, 89, 91 and 93) it is apparent that these insects were early to colonise these filters and that their numbers soon rose to a peak in all filters in May/June. At that time it was realised that Psychoda levels were far in excess of main filter levels therefore it was decided to remove the reduction nozzles and retain the 19 mm straight through pipes for the application system. Even though the bungs were removed it can be seen from the results that Psychoda levels continued at a much higher level on the main filters which suggests that either Psychoda had become well enough established initially to discourage large numbers of competitors, or that other conditions were continuing to favour their presence.

A reason for their initial abundance along with the reduced scouring action may be the season in the year in which the filters were commissioned. It was the view of

Lloyd (1937), Lloyd et al. (1940) and Solbe et al. (1967) that the maximum period of abundance of Psychoda was Feb./March - June (see Table 2); the maximum period for M.hydropetricus was later in the year. Other conditions which may have continued to favour Psychoda can be identified as follows. Although the reduction nozzles were removed it was still apparent that the scouring action from the feed nozzles was less in the pilot filter system than on the main filters, as the time required for gravity emptying of the feed trough was considerably more than the time taken for dosing a similar area on the main filter. Also due to the position of the pilot filters extremes of flow which were occasionally encountered on the main filters, when the flow was high, were not experienced on the pilot filters, thus providing the fauna with a slightly more sheltered environment. Another factor which may have favoured Psychoda is the temperature found in these filters. It should be mentioned here that P.alternata which were only very rarely caught from the main filters, constituted a sizeable proportion of the Psychoda population on the pilot filters. P.alternata can withstand much higher temperatures than M.hydropetricus, according to Lloyd et al. (1940) the temperature range at which there is no mortality is 5-18°C in the case of M.hydropetricus, and 15-28°C in the case of P.alternata. As great diurnal variations were found in the pilot filter temperature; as much as 15°C on a sunny day when the filter temperature often reached 25-30°C, it is not unreasonable to assume that these extremes of temperature favoured the P.alternata population and discouraged M.hydropetricus both physically and by increased competition with the increased numbers of P.alternata. Such an example of the pilot filter temperature variation diurnally can be seen in Fig. 101b. The mean weekly temperatures of these filters during the period of study was recorded by means of a probe inserted into a filter connected to a "Rototherm" chart recorder (see Fig. 82). If this is compared with the main filter effluent temperature (see Fig. 50) it is apparent that the pilot filter temperature generally reached greater extremes.

Therefore considering the above affects of generally reduced scouring and extremes

of temperature due to solar radiation it is not surprising that M.hygroptericus numbers were low and Psychoda numbers higher on the pilot filters than on the main filters as it has been shown that reduced scouring and increased temperature favour P.alternata and in such a situation an increase in P.alternata would bring about a decrease in M.hygroptericus due to competition.

The effects of temperature on fly emergence over the winter of 1977/78 can be seen in Figs. 84, 86, 88, 90, 92 and 94 where fly emergence was very low over that period from all filters. Even with insulation these filters on many occasions were colder than the main filters and in some cases iced up, temperatures as low as -2.5°C were recorded when the main filter effluent temperature was 9°C .

Although as mentioned pilot filter Psychoda levels were higher than main filter levels it can be seen from Figs. 83, 85, 87, 89, 91 and 93 that moderate numbers of M.hygroptericus were found during 1977 thus allowing a comparison of fly emergence from the separate modifications with control. During 1978 however no such comparison could be made as moderately large populations of M.hygroptericus did not build up. Similarly M.hygroptericus populations were late to build up on the main filters in that year and did not rise until late June, therefore with the large numbers of Psychoda found in that year on the pilot filters (see Figs. 84, 86, 88, 90, 92 and 94) it is likely that the M.hygroptericus build up would have been reduced or delayed.

iii) Effects of modifications on fly emergence

The effects of the modifications on M.hygroptericus, Psychoda and O.minimus emergence from May 1977 - June 1978 can be seen in the following figures.

	<u>1977</u>	<u>1978</u>
Control	Fig.83	Fig.84
Small medium (229 mm)	" 85	" 86
Small medium (229 mm) and splash apparatus	" 87	" 88
Small medium (76 mm)	" 89	" 90
Small medium (sprinkling) and splash apparatus	" 91	" 92
Surface covering	" 93	" 94

The effects of the modifications compared to control were investigated further by applying "t" tests. The changes in emergence levels and results of "t" tests between each modification and control for 3 species can be seen in Table 17.

Table 17 Unpaired 't' tests. Effects of modifications on fly emergence compared to control

	<u>M.hygropetricus</u>			<u>P.severini</u>		
	"t" value 118Df	'P' value	Mean weekly emergence per (X10 ²) m ²	"t" value 118Df	'P' value	Mean weekly emergence per (X10 ²) m ²
Small medium(229mm)	1.337	>10%	0.4 ± 0.2	1.179	>10%	3.3 ± 0.5
Small medium(229mm) and splash apparatus	1.768	5-10%	0.2 ± 0.1	0.354	>10%	4.1 ± 0.3
Small medium(76mm)	1.534	>10%	0.2 ± 0.1	0.217	>10%	4.0 ± 0.9
* Small medium sprinkling and splash apparatus	1.430	>10%	0.3 ± 0.2	0.793	>10%	5.6 ± 0.9
Surface covering	0.950	>10%	0.6 ± 0.3	0.308	>10%	4.1 ± 0.8
Control			1.2 ± 0.6			4.1 ± 0.7

* Result of Aug. 1977 - June 1978, all other results are May 1977 - June 1978.

± = SEM No observations = 60

Table 17 cont.

	"t" value 118 Df	<u>O.minimus</u> 'P' value	Mean weekly emergence per (X10 ²) m ²
Small medium(229mm)	1.130	>10%	0.5 ± 0.1
Small medium(229mm) and splash plate	1.301	>10%	0.4 ± 0.1
Small medium(76mm)	1.300	>10%	0.4 ± 0.1
Small medium(sprinkling) and splash plate	1.182	>10%	0.4 ± 0.1
Surface covering	1.200	>10%	0.4 ± 0.1
Control			0.9 ± 0.3

It can therefore be seen that in all cases a reduction in M.hygropetricus emergence was obtained compared to control. However unlike the results from the main filters the reductions were not so significant. In fact in only one case (small medium 229 mm, and splash apparatus) was the reduction slightly significant to the 5-10% level.

When the percentage reductions are compared from the main and pilot filters it can be seen that they were in fact quite similar (see Table 18).

Table 18

Percentage reductions in M.hygropetricus
emergence below control

	<u>Main filters</u>	<u>Pilot filters</u>
Small medium(229mm)	70.88	69.27
Small medium(229mm) and splash apparatus	79.04	86.89
Small medium(sprinkling) and splash apparatus	81.09	75.43

Therefore the lack of significance showing in the results may be due to the shorter sampling period, 14 months with the pilot filters compared to 27 months with the main filters. If observations were continued with the pilot filters it is likely that significant reductions would have appeared after another year.

(a) Small media sections

As in the case of the main filters it can be seen by comparing Figs. 85-92 with control filter fly emergence in Figs. 83 and 84 that M.hydropetricus emergence was considerably reduced on all small media sections. As before the highest reductions were obtained with the small medium incorporating the splash apparatus modification (see Table 18) showing the effectiveness of the apparatus in spreading the flow pattern out and giving an even spread of film.

Concerning Psychoda both on the small media 229 and 76 mm modifications decreased emergence levels were recorded (see Table 17). This reflects the sub-jet film conditions in these filters. In the case of the fixed straight through jet on the small medium there would have been a conical sub-surface, sub-jet film zone and very little film in the surrounding medium at the surface. Thus the possible area available for grazing would be less than in the splash apparatus case giving a decreased fly emergence. The splash apparatus modifications would obviously give a more even distribution of film and therefore a greater area available for grazing and a larger fly emergence. Both of the splash apparatus modifications produced increased numbers of Psychoda compared to control however a large increase was recorded in the surface sprinkling of small medium case suggesting that although larval and pupal numbers may have been greater in the 229 mm depth case due to a larger available grazing area, some physical hindrance to Psychoda emergence may have been occurring. Similarly with M.hydropetricus a greater reduction was obtained with the 229 mm depth of small medium and splash apparatus than with the surface sprinkling and splash apparatus again suggesting that the physical size of the medium was

impeding fly emergence. It could be argued that ventilation was an important factor here i.e. being restricted more in the case of the 229 mm depth of medium thus causing retarded development due to depletion of oxygen levels (Learner 1975), however as the scouring action on these filters was reduced compared to the main filters, the main accumulation of film was probably on the surface rather than sub-surface in the main filters, so it is likely that there were no great differences in ventilation facilities in the two cases. This then suggests that the size of the medium produced a physical barrier to fly emergence.

As with the main filters the effects of adding a surface sprinkling of small medium to the original splash apparatus only filter can be seen in Fig. 91. By comparing this figure with control in Fig. 85 it can be seen that M.hygroptetricus emergence levels were checked and prevented from rising by the addition of small medium showing the effectiveness of this alone in reducing M.hygroptetricus emergence.

Concerning O.minimus, these were found only sporadically and never in great numbers on the main filters allowing no comparisons to be drawn, however numbers from the pilot filters were greater but they still only accounted for a mean of 18.34% of the total fly emergence on the control filters. When the effects of the modifications on O.minimus emergence were analysed it was found that in all cases an approximate reduction of 50% was obtained (see Table 17). This reduction is not easy to explain as it is unlikely that O.minimus being such a small fly would be physically hampered from emerging by small medium. A factor which may be operating here is film levels per unit volume and therefore ventilation. It was found by Tomlinson and Stride (1945) that this species was found in great numbers on low loaded filters but none at all were found above the loading of $0.11 \text{ kg BOD m}^{-3} \text{ day}^{-1}$. The reason for this may be that high film levels associated with these loadings may have impeded ventilation and controlled O.minimus in the manner described by Learner (1975). It was found by Tomlinson and Stride (1945) that filters having a high output of Psychoda and

therefore probably containing high film levels produced few O.minimus.

(b) Rubber disc covering modification

This modification was applied simply as an exercise to investigate the effect of light and physical barriers on fly emergence. From Figs. 93 and 94 it can be seen that Psychoda emergence was similar however M.hydropetricus emergence was slightly decreased compared to control in Figs. 83 and 84. With this modification there should have been no change in film levels or scouring action as in the other modifications so the effects of light and physical barriers could be studied without interference from other factors. The reason for the decrease in M.hydropetricus but not in Psychoda is not immediately clear. Obviously if a barrier prevents fly emergence from 99% of the filter surface it would be expected that a decrease would occur in the emergence of both species. However this is not so as it has been shown that Psychoda levels were similar to control, therefore it could be postulated that Psychoda can easily migrate towards the light source and emerge through the aperture. With M.hydropetricus it could be argued that this should also occur so there should be no decrease in emergence levels. It is doubtful that M.hydropetricus is less able to migrate towards a light source so other factors must be looked at. Other factors that would be affected by covering the filter surface would be ventilation which would also indirectly affect filter temperature as heat loss would be lessened. A decrease in ventilation would lead to depleted oxygen levels which according to Learner (1975) would inhibit development. However this would probably affect both M.hydropetricus and Psychoda resulting in a decrease in both species emergence levels. However when the temperature is investigated it is clear that increases favour Psychoda and discourage M.hydropetricus therefore the total effect of this modification could be summarised as follows:

1. Decreased ventilation caused a decrease in Psychoda and M.hydropetricus.
2. Increased temperature caused a decrease in M.hydropetricus but an increase in

Psychoda.

3. The total effect would be a decrease in M.hygropetricus and not much change in Psychoda.

As far as can be determined no specific work has been carried out on the ecology of covered filters except for the work of Murray (1939) and Lundie (1940) where enclosed filters were used, but no fly species structure changes were mentioned. Enclosed filters were also discussed by Reynoldson (1942).

iv) Effects of modifications on effluent flow patterns from filters and chloride ion retention times

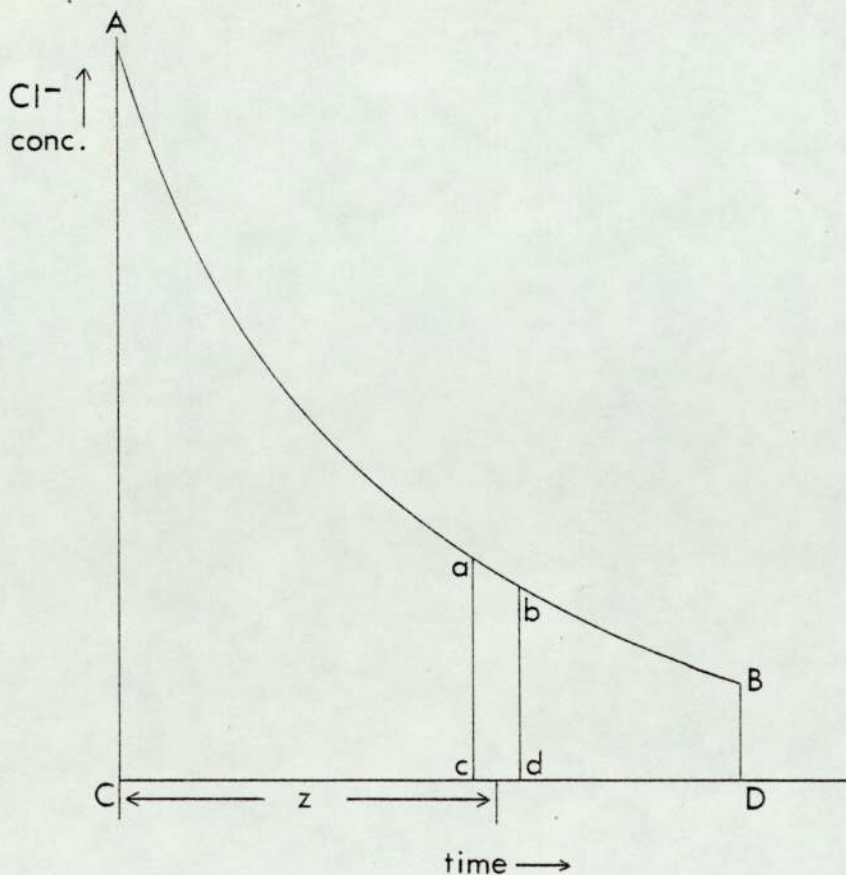
Various views are held on the significance of retention time on filter efficiency. Galler and Cotaas (1964) and Craft and Ingols (1973) were of the opinion that changes in retention time found with high instantaneous dosing did not affect filter performance. However other authors such as Balakrishnan (1969) and Tariq (1975) suggested that retention time has a significant effect on filter performance. The situation was clarified by Cook and Katzburger (1977) who stated that filter efficiency was hardly affected by retention time in low rate filters but was affected in high rate filters.

However apart from keeping a record of BOD (Biochemical oxygen demand) and TON (Total oxidized nitrogen) levels of the effluent to indicate the actual purification efficiency the calculation of retention time, although not being a good indicator of efficiency, serves to give a better understanding of filter hydraulics. Therefore it was decided to carry out determinations of retention times for the modifications applied to the pilot filters to investigate if they had any drastic effects on retention times.

The method used for determining the mean retention time of the pilot filters was that

described by McNicholas and Kirkwood (1953) and is described in full in the methods section.

The results obtained for the recovery of Cl^- (Chloride ion) along with the hydraulic flow patterns from the pilot filters can be seen in Figs. 102a - 107b. The actual method of calculation of the retention time from the results was to plot a graph of the concentration of Cl^- recovered in the effluent from its time of addition at specific times of 1, 2 and 3 minutes and then on at 5 minute intervals for a total of 60 mins. This gave a curve the centre of gravity of which could be projected onto the horizontal time axis which represented the mean time of contact or mean retention time of Cl^- . A useful way of achieving this according to Thompson (1942) and McNicholas and Kirkwood (1953) was to divide the area under the curve into a number of small units and by taking moments about the origin the centre of gravity could be determined. The procedure can be summarised as follows.



In the above graph the area a b c d is a small number of units (E) and z units distant from the origin (C).

Therefore the moment of a b c d is Exz .

The total area under the curve can be split into columns of such units and the total area $(Y) = \sum Exz$.

Therefore the centre of gravity for the curve ABCD would lie at $\frac{\sum Exz}{y}$ units from C along the time axis.

This result would then give the mean time of contact and therefore mean retention time of Cl^- in the filter. The results of the determination for each modification can be seen in detail in Figs. 102 — 107 (a's) and can be summarised as follows:

<u>Modification</u>	<u>Mean Cl^- retention time (min.)</u>
Control	25.1
Small medium (229mm)	25.8
Small medium (229mm) and splash apparatus	27.3
Small medium (76mm)	27.3
Small medium (sprinkling) and splash apparatus	29.6
Surface covering	23.8

From the results it can be seen that the variation was not very great and most of the times were found to be around 25 min. Few reports have been given on the retention times of specific media when mature. Taylor (1942) described retention times of a similar depth of mature 25 — 100 mm (1-4 in.) media at a hydraulic load of 1.14 m^3

$\text{m}^{-3} \text{day}^{-1}$ to be 27.5 min. which compares with the results obtained here on a hydraulic load of $0.8 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$, however no mention was made of dosing frequency which affects retention time greatly (Hawkes 1961b and Galler and Cotaas 1964). Other factors apart from the dosing frequency have been shown to affect retention times. Tariq (1975) showed that it varied directly with filter depth and inversely with the 0.646 power of the hydraulic surface loading rate and was also affected by film levels. Cook and Katzburger (1977) were of a similar opinion that it was affected by the biomass within the filter i.e. film levels. As regards the filters utilised in this study, the depth, hydraulic loads, dosing frequency and media, except for the surface toppings were identical in all of the filters, therefore it is not surprising that no great differences were found in retention times. The differences obtained however do show trends reflected in the modifications applied. For example all of the small medium modifications gave increased retention times in relation to control. This is as expected as obviously smaller medium gives higher film levels per unit volume as well as more physical resistance to liquid flow. Also the two splash apparatus modifications gave high retention times, results which again reflect the affect of spreading the flow out and giving higher film levels.

The differences in some cases are not those expected from theoretical considerations. For example it would be expected that the 229 mm depth of small medium with splash apparatus would give a higher retention time than the surface sprinkling of small medium and splash apparatus modification. Similarly there is no reason why the retention time obtained from the small medium (76 mm depth) filter should be the same as that obtained from the filter containing small medium (229 mm depth) and splash apparatus. The control and surface covering results according to theory should be identical. In explaining these results it must be appreciated that the filters were mature and contained film in varying amounts and different film levels have been shown to affect retention time (Cook and Katzburger 1977). Gameson et al. (1961) showed that identical filters were inherently variable and large numbers of replicates

were needed to show any trends, also Meltzer (1962) expressed disadvantages of the centre of gravity method of retention time determination. Thus it is likely that either variations in film levels caused the actual differences found in the retention times of these filters or the determination system failed to detect differences due to inherent disadvantages in the system and the low number of replicates used. Since retention times did not vary much, purification efficiency should not be greatly affected by the modifications.

By consulting Figs. 102a - 107a the effect of subsequent doses after the initial application of Cl^- can be seen. It is apparent that peaks of Cl^- were being obtained after the first and second doses in the small medium cases (Figs. 103a, 104a, 105a and 106a), whereas on the control filter (Fig. 102a) a peak appeared after the first dose but after this time the Cl^- levels continued to fall. Similarly a peak appeared after the third dose in the case of small medium (sprinkling) and splash apparatus (Fig. 106a). The results from this modification were also unusual in the Cl^- recovery being low initially and increasing to a peak level with the first dose of sewage. It could be attempted to explain these results by considering film levels in filters containing small medium which are likely to be higher than on control filters. Such high film levels could explain low initial Cl^- recovery and the peaks of Cl^- which were still being obtained after the third dose. As regards the surface covering modification initial recovery of Cl^- was in this case (Fig. 107a) higher than control (Fig. 102a), also the peak after the first dose was much reduced compared to control indicating that film levels in this filter were lower than on control. These results suggest therefore that the slight differences in retention times obtained may have been due to varying film levels and not directly to the physical effects of the modifications.

As regards the hydraulic flow patterns from the above filters the effects of the modifications on these can be seen in Figs. 102b - 107b. In these cases the initial dose i.e. the first three columns of the histogram should be ignored as the results here

are probably subject to interference from the dose previous to the start of the experiment. It can be seen from Figs. 103b, 104b, 105b and 106b that the second and third column of the histogram after each dose were higher in all the small medium filters than on control (Fig. 102b). Also higher columns were obtained in the case of small medium (sprinkling) and splash apparatus (Fig. 106b). In the case of surface covering however the results (Fig. 107b) are slightly lower than those obtained for control. Therefore these results seem to follow those obtained for the Cl^- retention times and are probably greatly influenced by film levels as these would be expected to control the volume of liquid held in the filter and its drainage rate.

v) Purification efficiency of the pilot filters in relation to the main filters

It was apparent that the control filter of the pilot system (Fig. 96) did not perform so efficiently as the control filter from the main filter system (Fig. 95). In fact the mean effluent BOD level from the main filters was 45% less than that from the pilot filters, also the mean effluent TON level was 44% higher in the case of the main filters. The reasons for this could be various. For example at the start of the sampling period the pilot filters were commissioned from new whereas the main filters had been in operation for one year and could be considered mature from the efficiency angle. Other reasons for the reduced efficiency could be the large variations in temperature, as shown in Fig. 82 and 101b or wall interference.

Dealing with temperature, Painter (1970) described its influence on nitrification and was of the opinion that it had a threshold below 10°C , however at less than 10°C heat had a disproportionate effect on nitrification. Bruce et al. (1975) found disappointing nitrification results from small scale filters which he attributed to the high loss of temperature in pilot plants. Other workers have shown how temperature influences filter efficiency including Thompson and Watson (1944), Hawkes and Shephard (1975) and Graaf (1976).

Considering wall interference it is generally accepted that small scale filters can never be as efficient as large filters even in identical operating conditions. This is mainly due to wall interference where a certain portion of the feed takes the shortest route down the filters length, i.e. down the inside wall. This interference is inversely related to filter diameter.

In the control pilot filter (Fig. 96) it can be seen that decreased nitrification occurred with increasing effluent BOD levels. A similar relation between carbonaceous oxidation and nitrification has been described by other workers including Fry (1955) and Stover et al. (1976) who suggested that actively metabolising carbonaceous micro-organisms produced metabolites which detrimentally affected the nitrifiers. Thus higher film levels in the pilot filters (due to reduced scouring) may have caused an increase in carbonaceous oxidation by extending the upper zone of these organisms, thus reducing the lower zone of nitrification. This theory however is only tentative as other workers such as Hockenbury et al. (1977) were of the opinion that carbonaceous micro-organisms did not affect nitrifiers. However there is no reason why a competition system such as found with higher organisms should not apply here. It is possible that there is competition for oxygen supply and space between carbonaceous microbes and nitrifiers in filters and an increase in one would probably cause a decrease in the other.

From Figs. 96 - 101a it is possible to see that on all of the pilot filters BOD removal was initially good but then rapidly deteriorated and recovery followed in May (3 months from commissioning). Similarly moderate levels of TON were recorded in the effluent initially but this then dropped off in all filters and did not recover until July. The possible explanation for this was that carbonaceous oxidation was initially good as there was only a thin layer of film, however it is likely that film levels increased rapidly due to the absence of grazers causing a drop in carbonaceous oxidation, as it has been shown by Hawkes (1957) that an excessive accumulation of film can

* Details of analysis of variance test.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	'F'ratio	'P'value	Significance
(a) Effluent BOD						
Between modifications	5	208	41.7	0.1963	>10%	NS
Within modifications	90	19121	212.5			
Total	95	19329				
(b) Effluent TON						
Between modifications	5	36	7.3	0.4065	>10%	NS
Within modifications	90	1624	18.1			
Total	95	1661				
(c) Effluent suspended solids						
Between modifications	5	28659	5731.9	0.4525	>10%	NS
Within modifications	90	1139810	12664.6			
Total	95	1168469				

Table 19 cont.

	Mean eff. suspended solids levels mgdm ⁻³	% change compared to control
Control	156.84	-
Small medium(229mm)	135.47	-13.63
Small medium(229mm) and splash apparatus	166.25	+6.00
Small medium(76mm)	179.87	+14.68
Small medium(sprinkling) and splash apparatus	166.44	+7.40
Surface covering	189.95	+21.11

These results were then analysed statistically in an analysis of variance tests to see if the variations were significant.*

<u>Parameter</u>	<u>Value of F (95/5 °F)</u>	<u>Result</u>
Effluent BOD	0.196	No significant variations
Effluent TON	0.407	" " "
Effluent suspended solids	0.452	" " "

Therefore from these results it can be seen that the modifications did not have a detrimental effect on effluent quality, in fact from Table 19 it can be seen that all of the modifications gave a marginally better performance than control as regards BOD and TON levels.

As the changes were so slight no significance can be attributed to the separate modifications. However in the case of the small media modifications a slight increase in efficiency would be expected as the medium surface area to volume ratio would increase allowing a greater area for microbial growth. It is a fairly general view that efficiency increases with decreasing media particle size assuming sufficient voidage remains to allow oxidation (Hawkes and Jenkins 1955). Cook and Fleming

(1974) showed that an increase was apparent up to a maximum surface area to volume of $87 \text{ m}^2 \text{ m}^{-3}$ and beyond that it decreased. Obviously this level would be controlled by loading and other factors determining film levels. The medium utilised in this study was 19 mm gravel which according to the method described in Truesdale et al. (1961) has a surface area to volume relationship of $214 \text{ m}^2 \text{ m}^{-3}$ and this gave no adverse effects. It can only be assumed that higher film levels were apparent in the case of Cook and Fleming (1974).

Other work concerning the efficiency of different mineral media was carried out by Truesdale and Wilkinson (1962) who found that small medium 25 mm (1 in), produced a good purification efficiency but this was seasonal and dropped off in winter presumably due to excessive film accumulation and reduced ventilation, however Krige (1962) dealing with different grades of small media found no decrease in efficiency in winter with his smallest medium of 38 mm (1.5 in). As can be seen from Figs. 100 - 104 no purification efficiency problems were encountered with small medium during the winter in this investigation suggesting that film accumulation was not excessive.

The effect on the efficiency of enclosing a filter has been discussed by Dekema and Murray (1942) and they were of the opinion that up to 2 fold increases in efficiency were found due to the fairly constant temperatures and constant ventilation. No such increase in efficiency was noted with the surface covering modification in this study however the two systems do not compare as forced artificial ventilation was used in the former case. Therefore it was not expected that efficiency from this modification should deviate much from control.

As regards solids levels, from Table 19 it can be seen that slight changes were recorded between the modifications however on an analysis of variance test no significant variations were found. When investigating the results on a seasonal basis

it can be seen from Figs. 108 - 113 that large peaks in solids were found on most filters in May 1977, December 1977 and March 1978. In the case of the first peak the solids discharges were higher from all of the modifications than from control, however in the second and third peaks the levels were similar from all filters. It is likely that the first peak can be attributed to the effects of the colonisation of film laden filters with grazers combined with sloughing. In all cases a drop in purification efficiency (Figs. 96 - 101a) preceded this discharge of solids suggesting that the excessive film build up prior to sloughing detrimentally affected efficiency. This phenomenon was discussed by Howell and Atkinson (1976). The reason why the solids discharges from the modifications were larger than that experienced on the control filter is probably due to the greater film content of the modified filters. For example higher film levels would be experienced using small medium or splash apparatus. However this does not explain the equally large discharge from the surface covering modification, it is likely that reduced heat loss and reduced ventilation caused higher film levels and higher effluent solids levels.

By the time of the second and third peaks in solids discharge similar levels were being experienced from all filters suggesting that grazing activity had equilibrated and that solids levels had been controlled on these filters. The third peak can be attributed to sloughing as it occurred in March/April, however the second peak is probably due to an unusual rise in temperature in early December (see Fig. 37) causing an increase in grazing activity and thereby an increase in discharged solids.

vii) General discussion on pilot filter results in relation to main filter results

The extrapolation of pilot filter results to a main filter situation may seem difficult due to the above mentioned interferences from temperature and wall effects, also it is appreciated that the distribution system to these filters could have affected the results. However considering all of the possible inaccuracies, results from these filters as regards fly emergence and effluent quality are remarkably similar between

the separate filters if not between these filters and the main filters.

Considering effluent quality, the experiment set out to find if any differences in purification efficiency could be attributed to the modifications utilised and none were found except slight increases in efficiency from the modifications in relation to control. The variability in efficiency between the modifications was slight and when this is compared with the variability found in other pilot filter studies, which is considered an inherent disadvantage (Gameson et al. 1961), this seems to suggest that the results obtained from this study are not variable. Also it seems not unreasonable to extrapolate these results to the main filter situation, since it is most likely that similar, if not better efficiency would be obtained with these modifications in the more constant environment of the main filters. The only problem as regards effluent quality was the massive discharge of solids found soon after commissioning these filters which would present a problem if found in winter due to decreased particle settlement rates (Shriver 1975). It is assumed that this discharge was aggravated due to these filters being commissioned just prior to the sloughing season therefore it is recommended that if any modifications are investigated on a large scale the optimum period to carry these out would be in late spring/early summer when the film levels would be low. Also it is important that no modifications are initially applied to filters in autumn/winter since the effects of these modifications are to produce potentially higher film levels and film accumulation problems may be found in the winter due to grazers being slow to build up.

Regarding fly emergence, the fact that sufficient numbers of M.hygroptericus built up in 1977 on these filters provided an opportunity to compare emergence between the modifications which was useful as it cross checked the effectiveness of these modifications as apparent from the main filters. Therefore the fact that similar reductions in M.hygroptericus emergence were obtained from both filter systems proved that the modifications could be considered effective in different filter

situations and that the effectiveness is not specifically confined to Tamworth main filters.

Therefore these pilot filter results suggest that when applying operational modifications to works filters to control fly nuisance no undue problems as regards efficiency should be experienced, assuming some thought is given to the season in which the modifications are applied.

Fig. 82. Mean weekly pilot filter temperatures August 1977 - July 1978

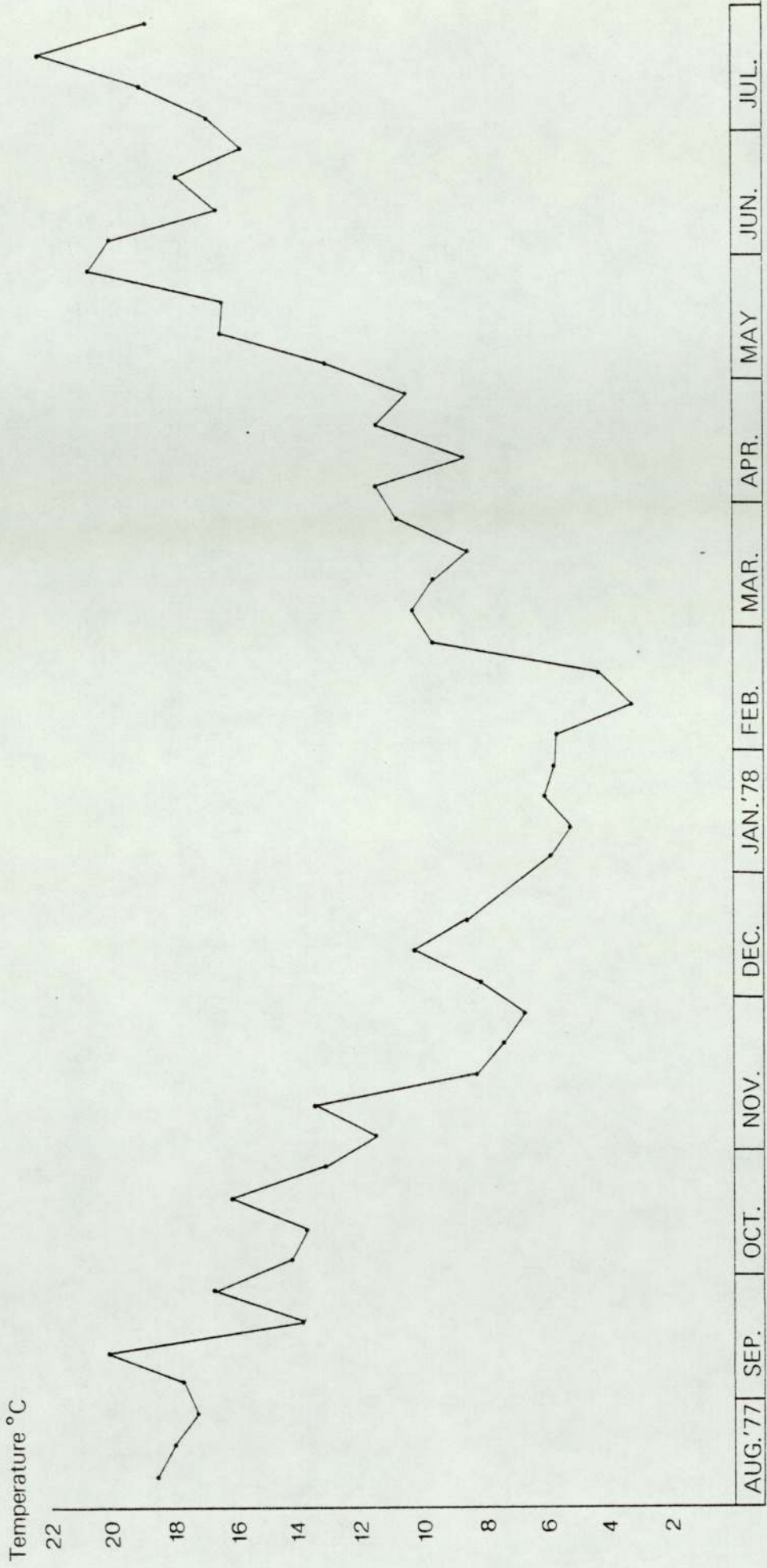


Fig. 83. Adult fly emergence from pilot filters May – November 1977

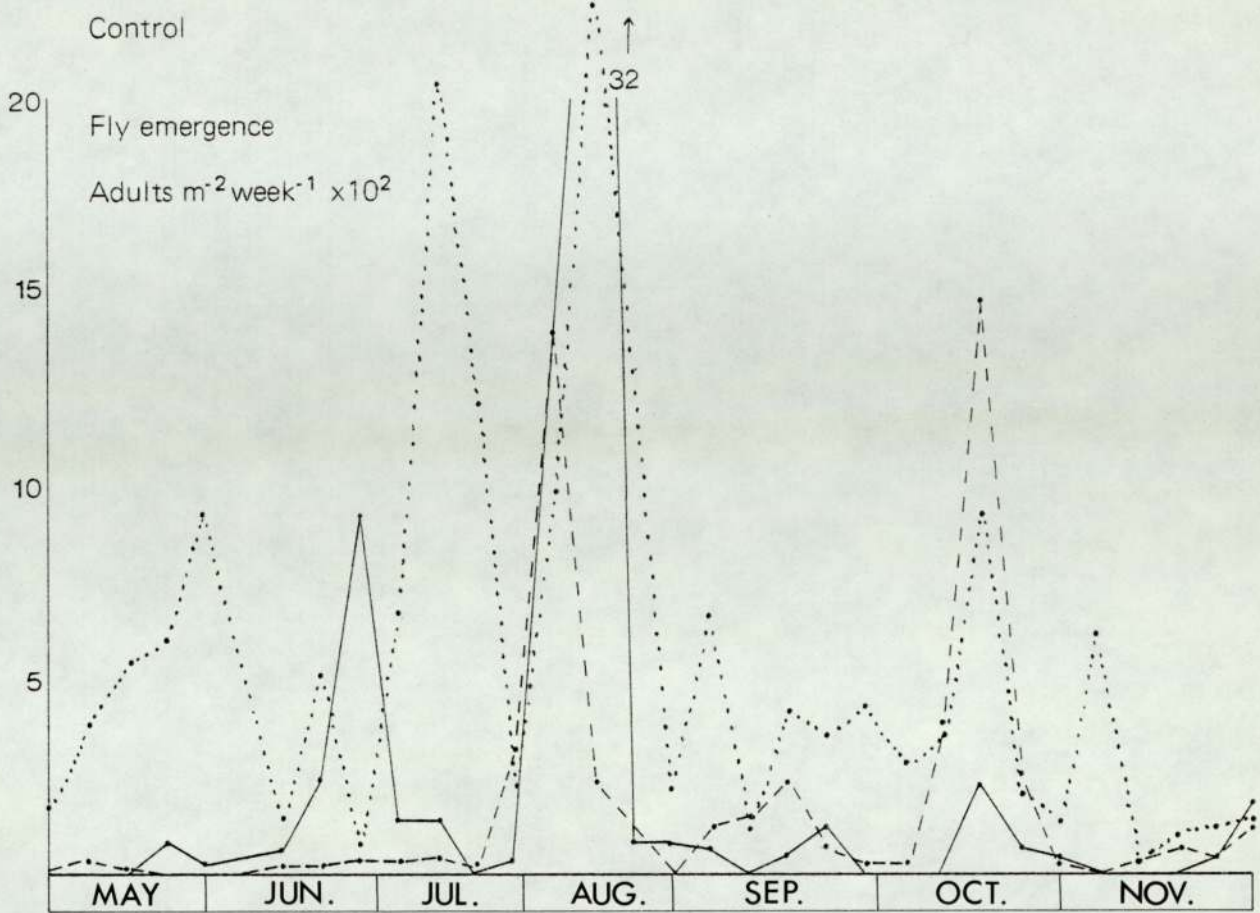


Fig. 84. December 1977 – July 1978

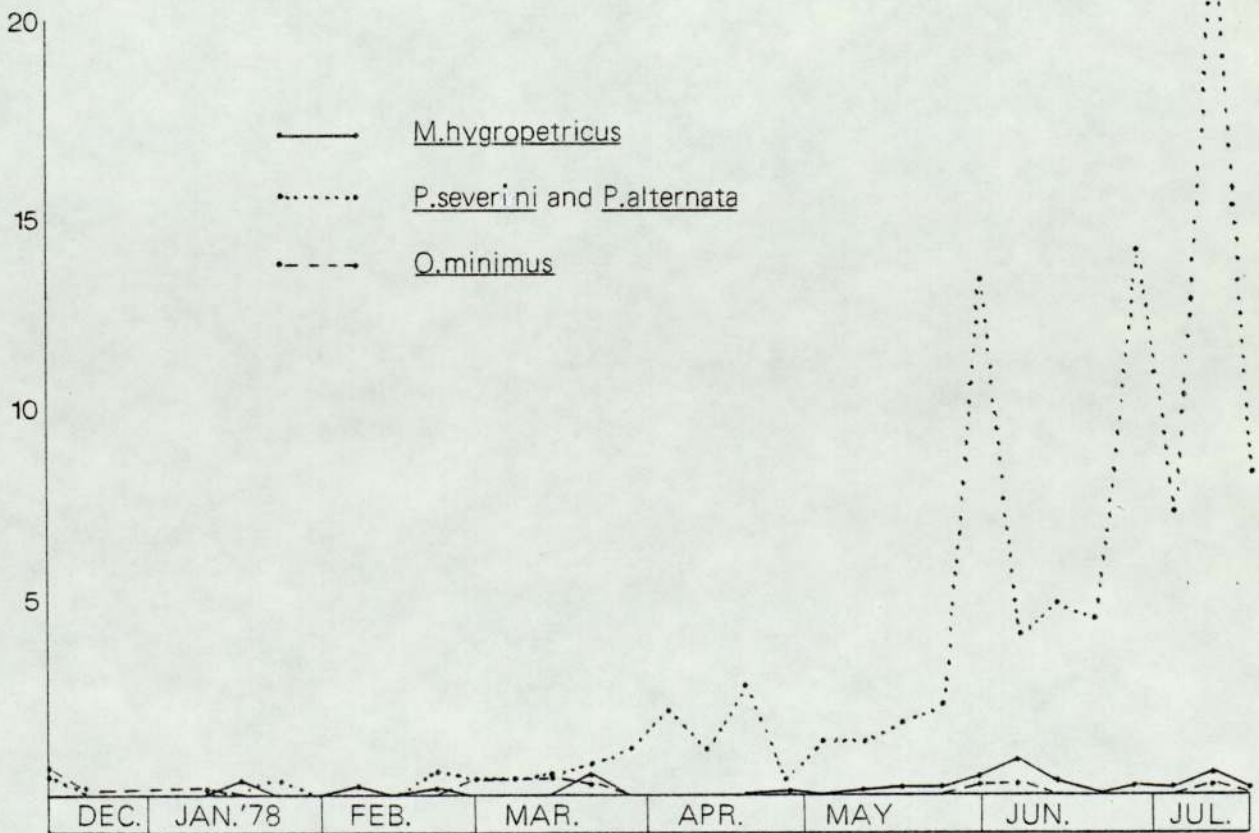


Fig. 85. Adult fly emergence from pilot filters May – November 1977

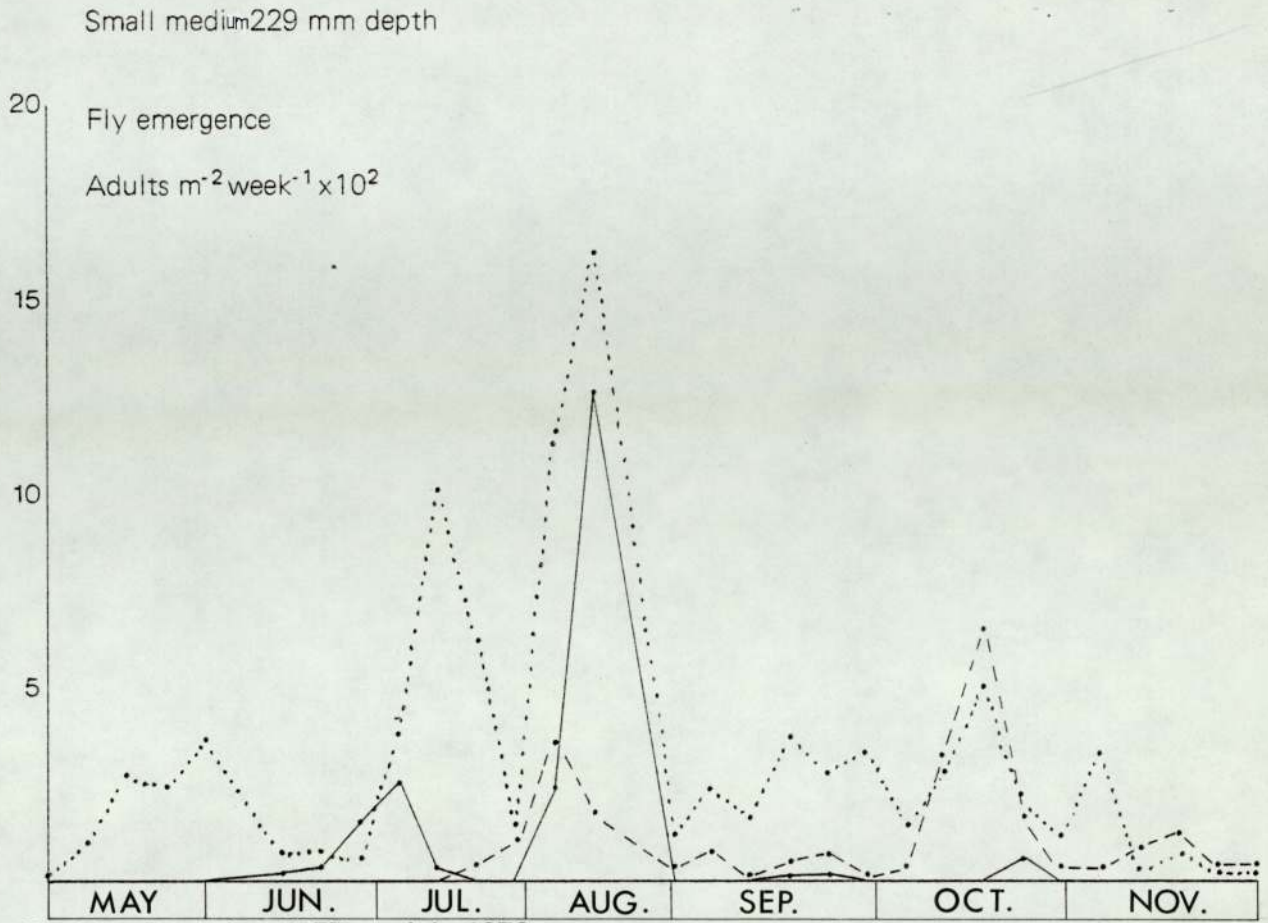


Fig. 86. December 1977 – July 1978

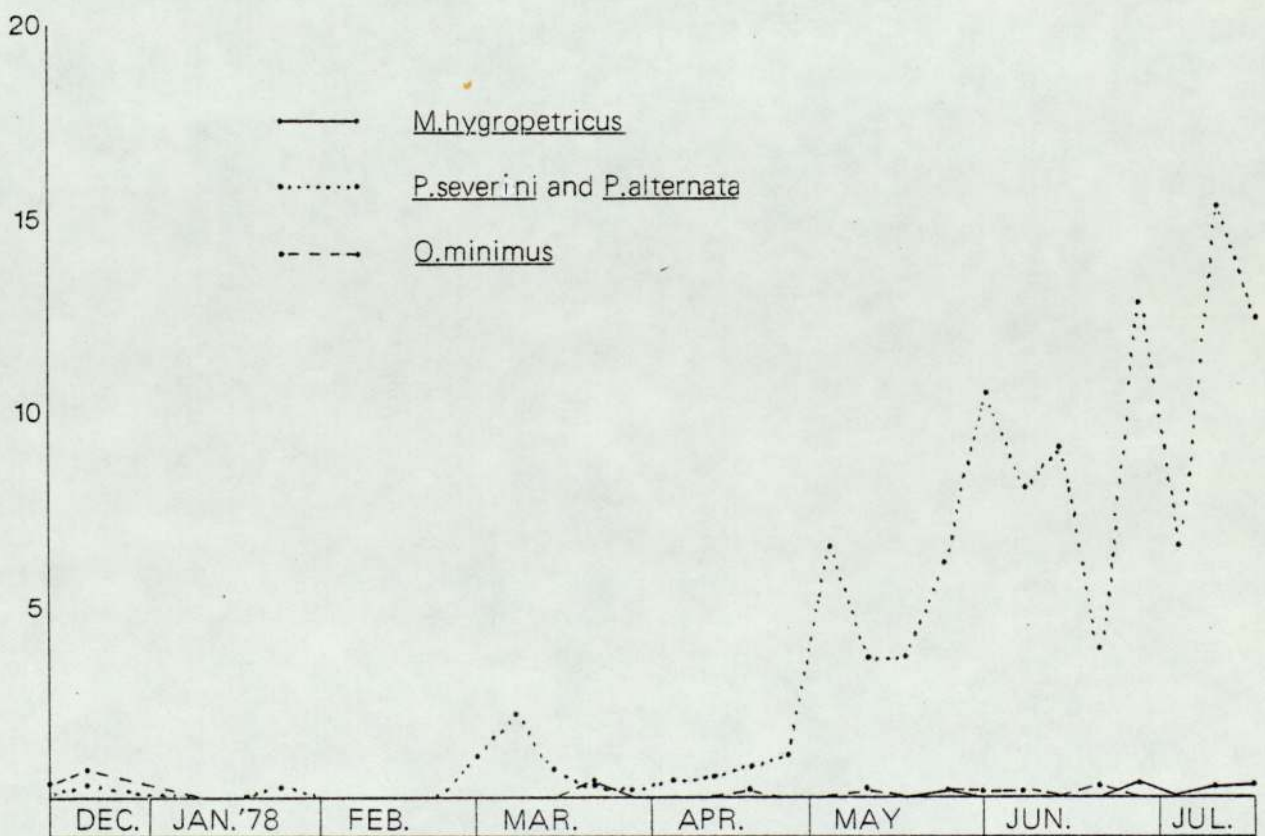


Fig. 87. Adult fly emergence from pilot filters May – November 1977

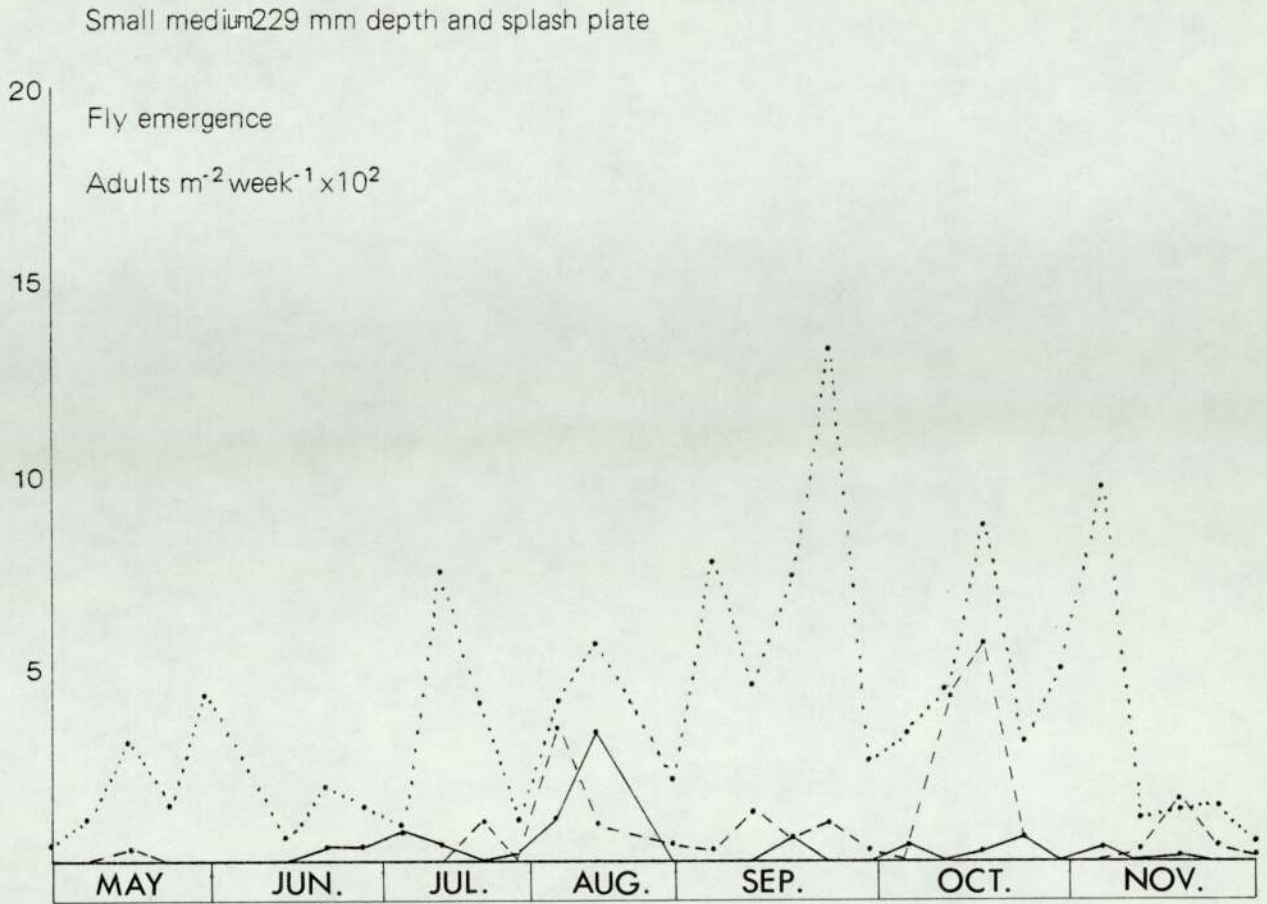


Fig. 88. December 1977 – July 1978

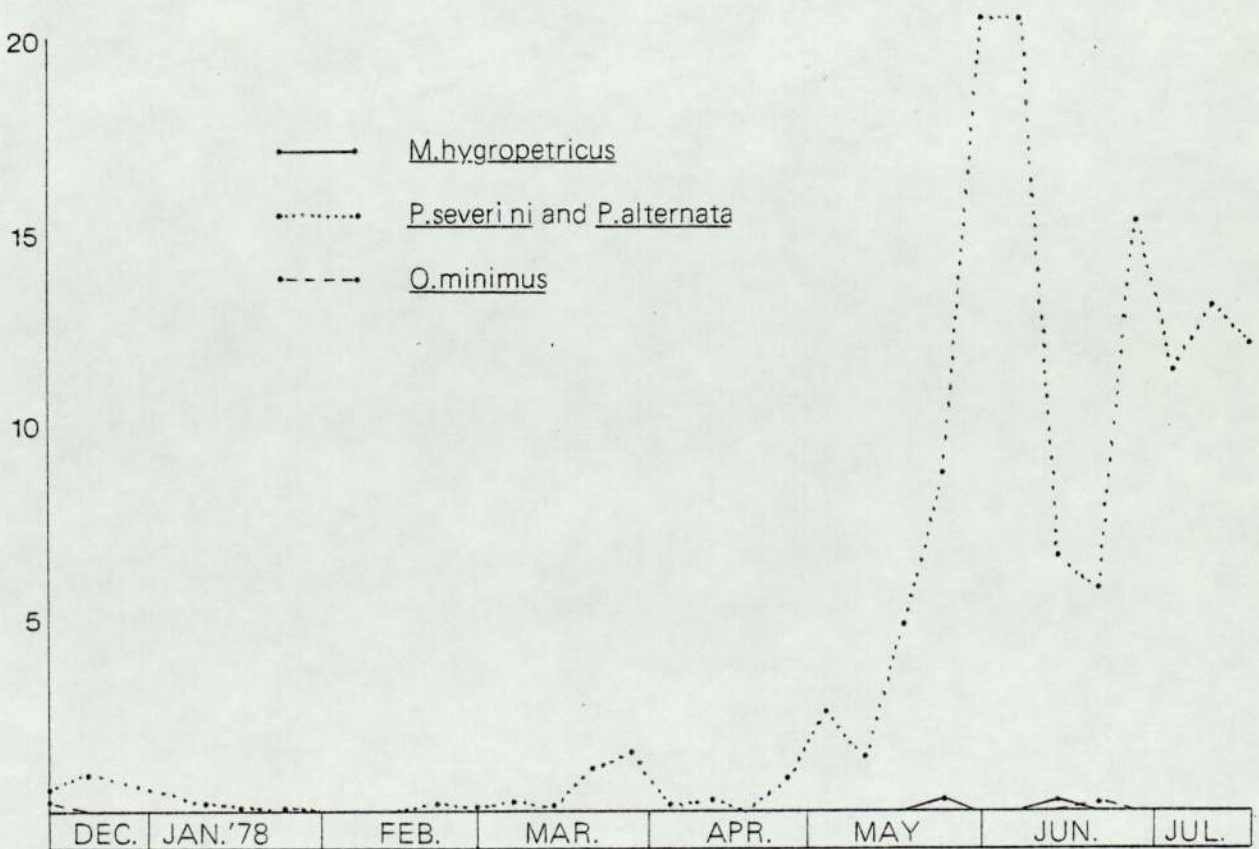


Fig. 89. Adult fly emergence from pilot filters May — November 1977

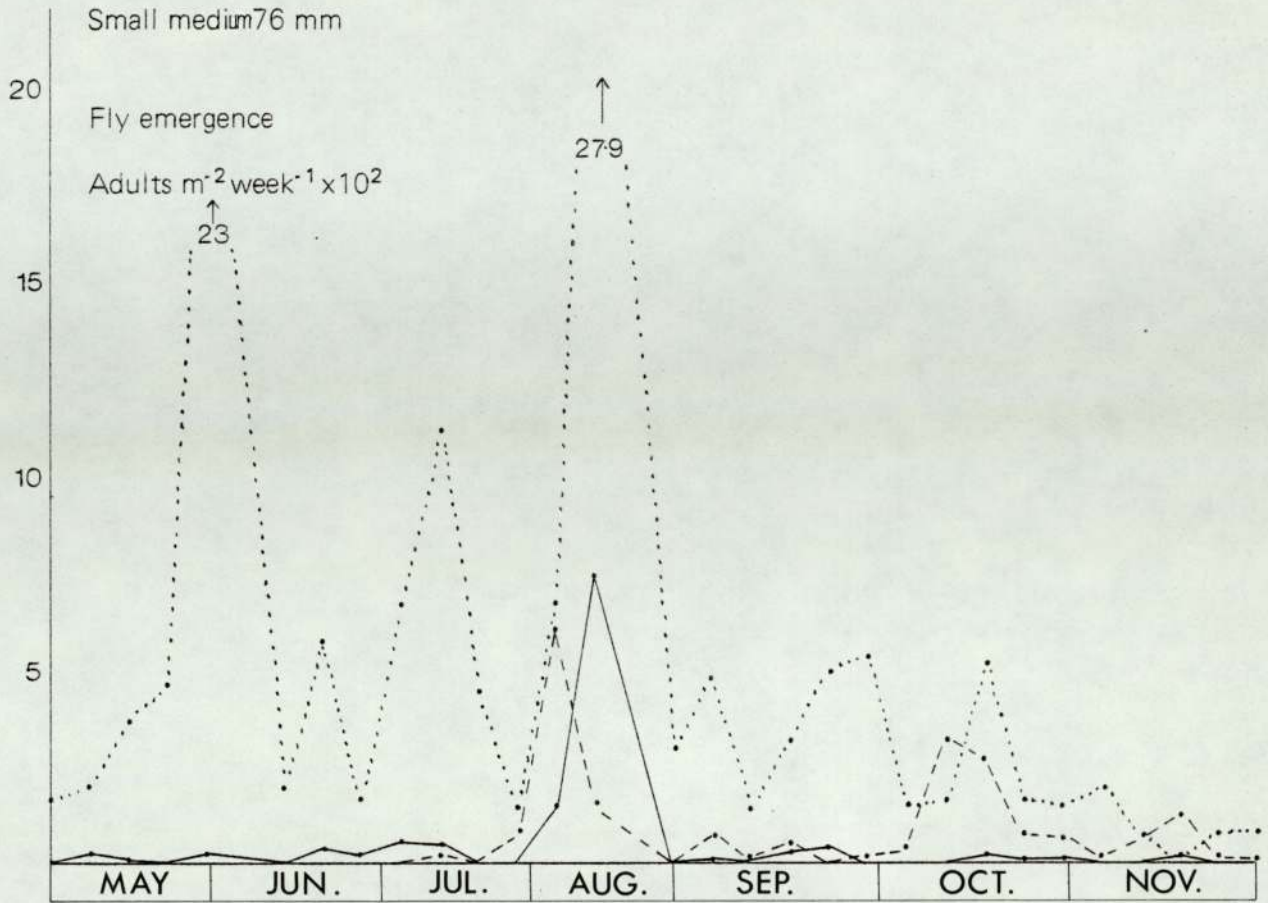


Fig. 90. December 1977 — July 1978

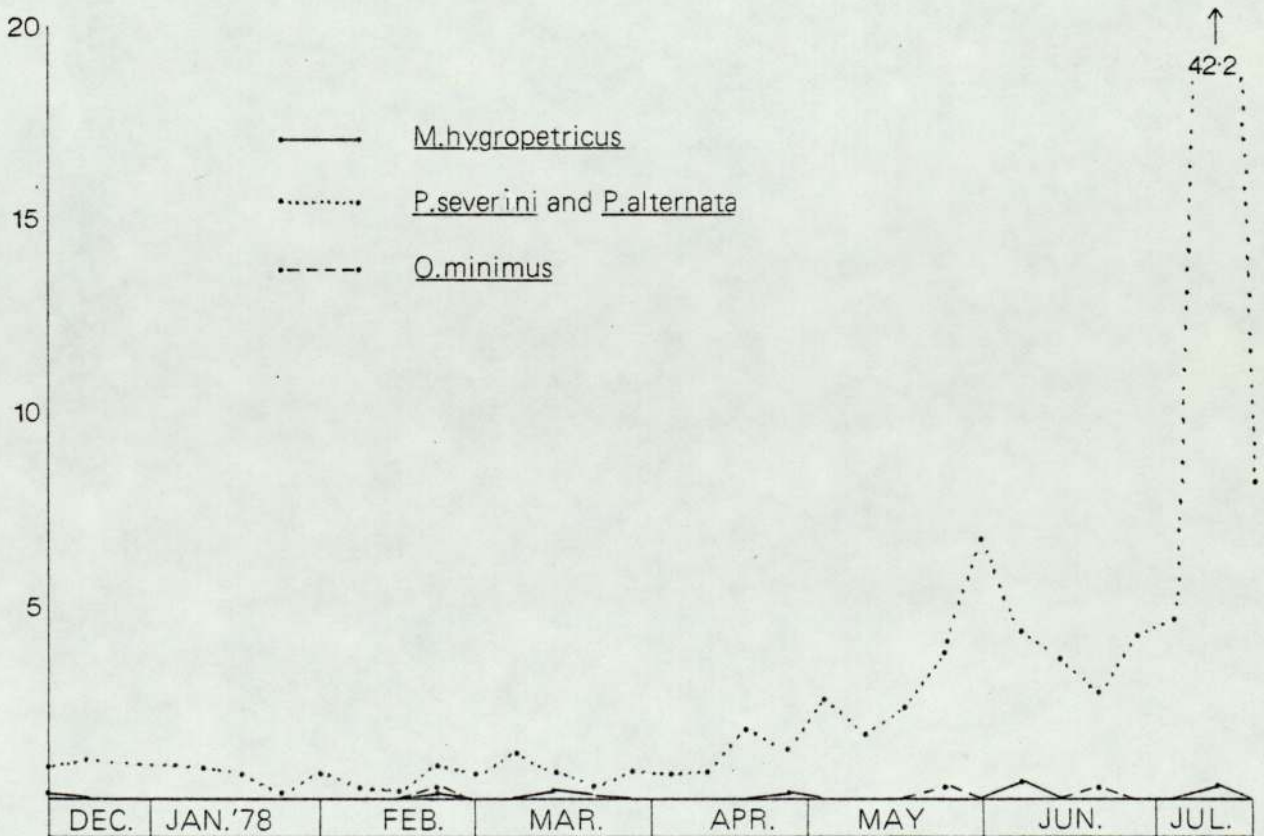


Fig. 91. Adult fly emergence from pilot filters May — November 1977

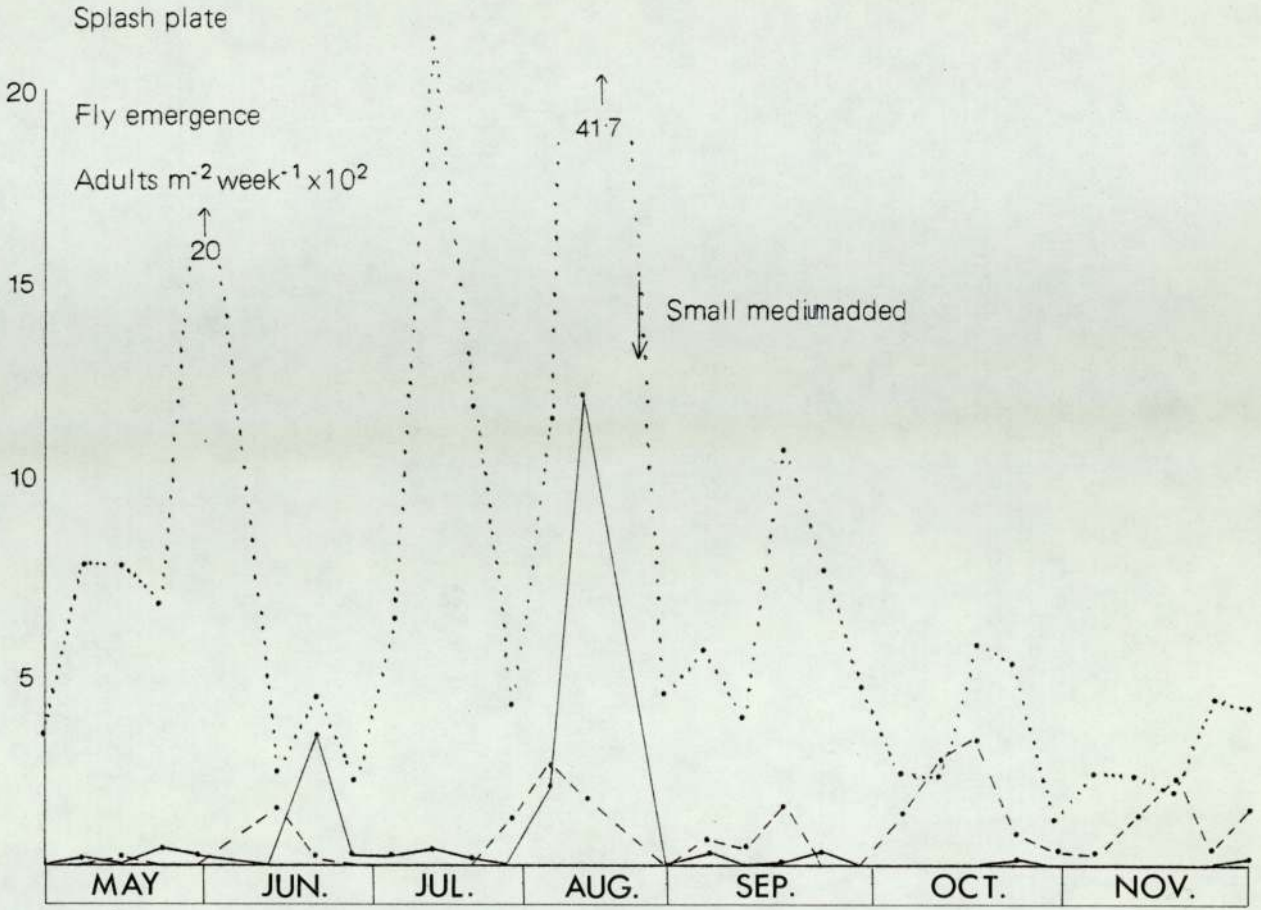


Fig. 92. December 1977 — July 1978

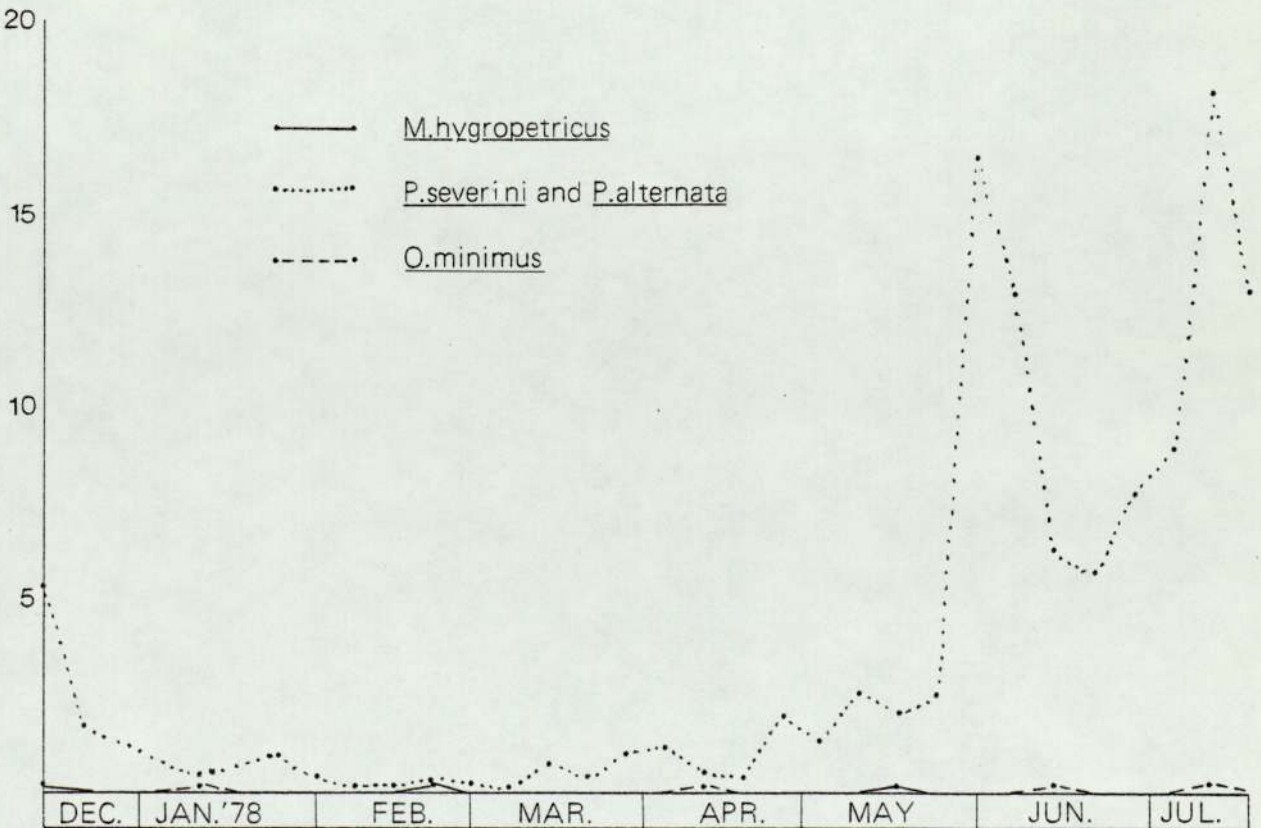


Fig. 93. Adult fly emergence from pilot filters May – November 1977

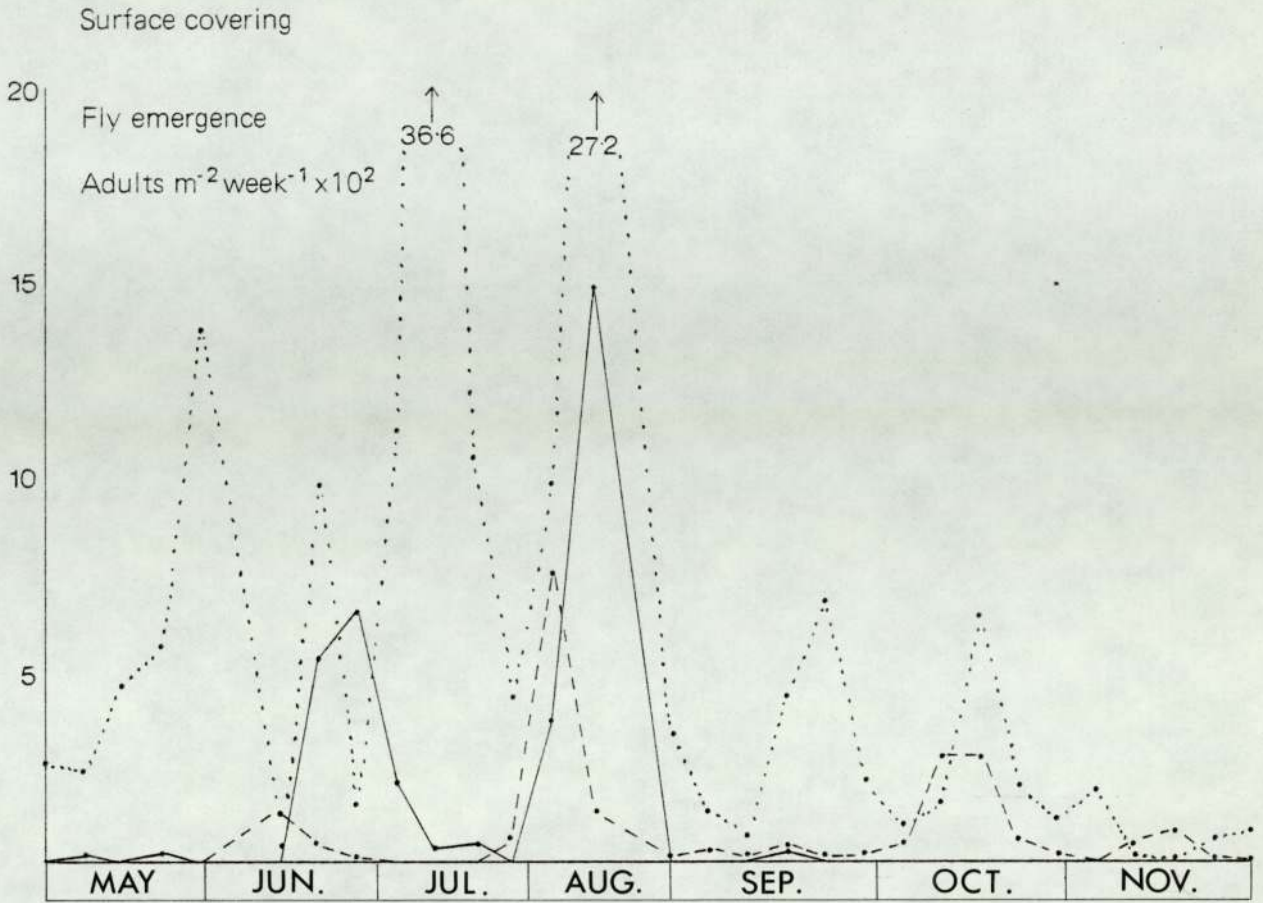


Fig. 94. December 1977 – July 1978

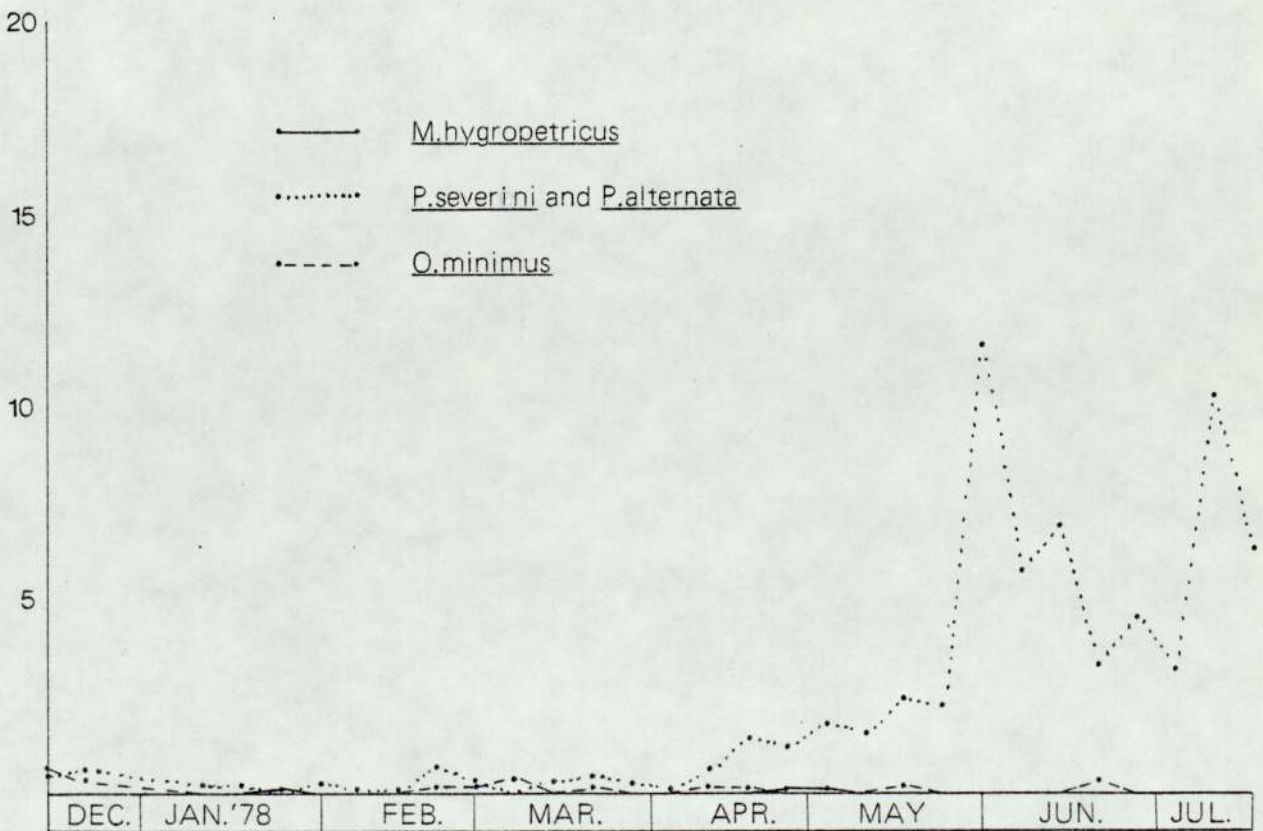


Fig. 95. Mean monthly effluent characteristics

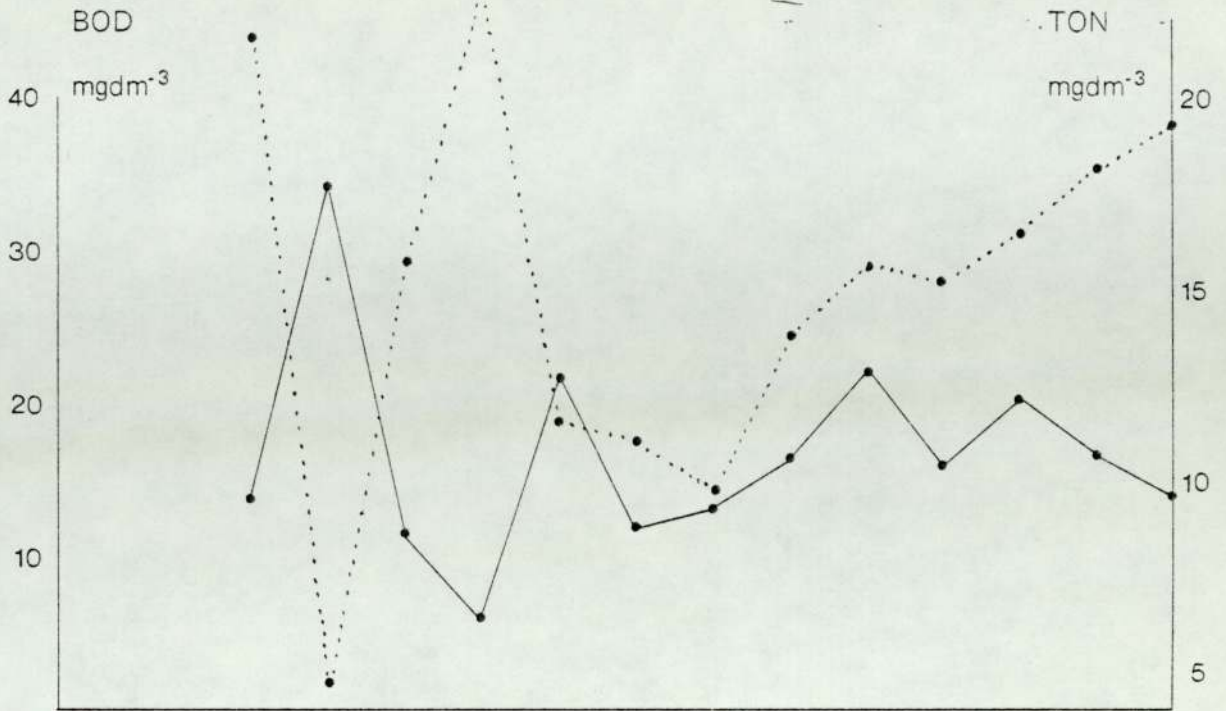


Fig. 96. Mean monthly effluent characteristics

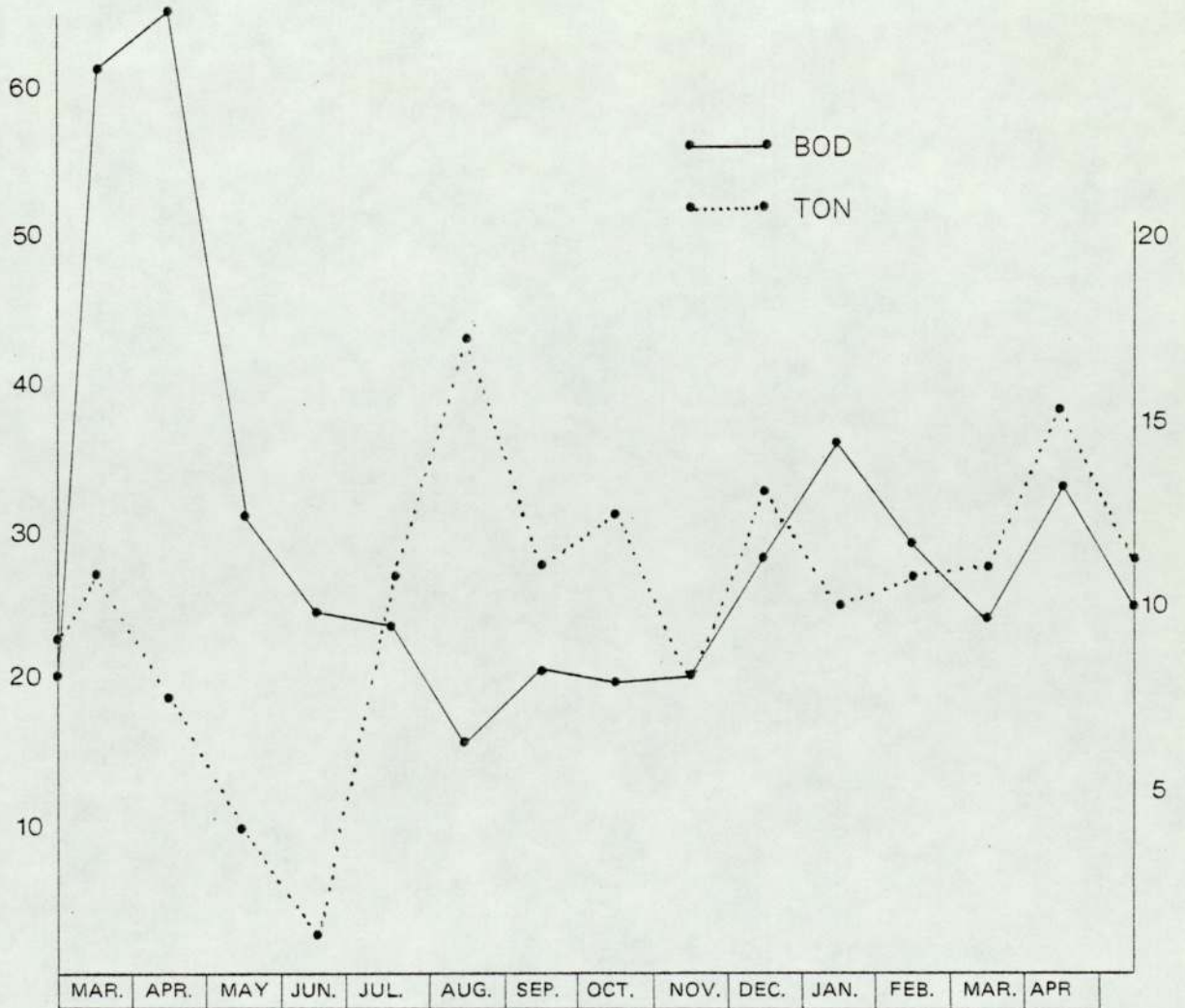


Fig. 97. Mean monthly effluent characteristics

Pilot filters
Small media 229 mm

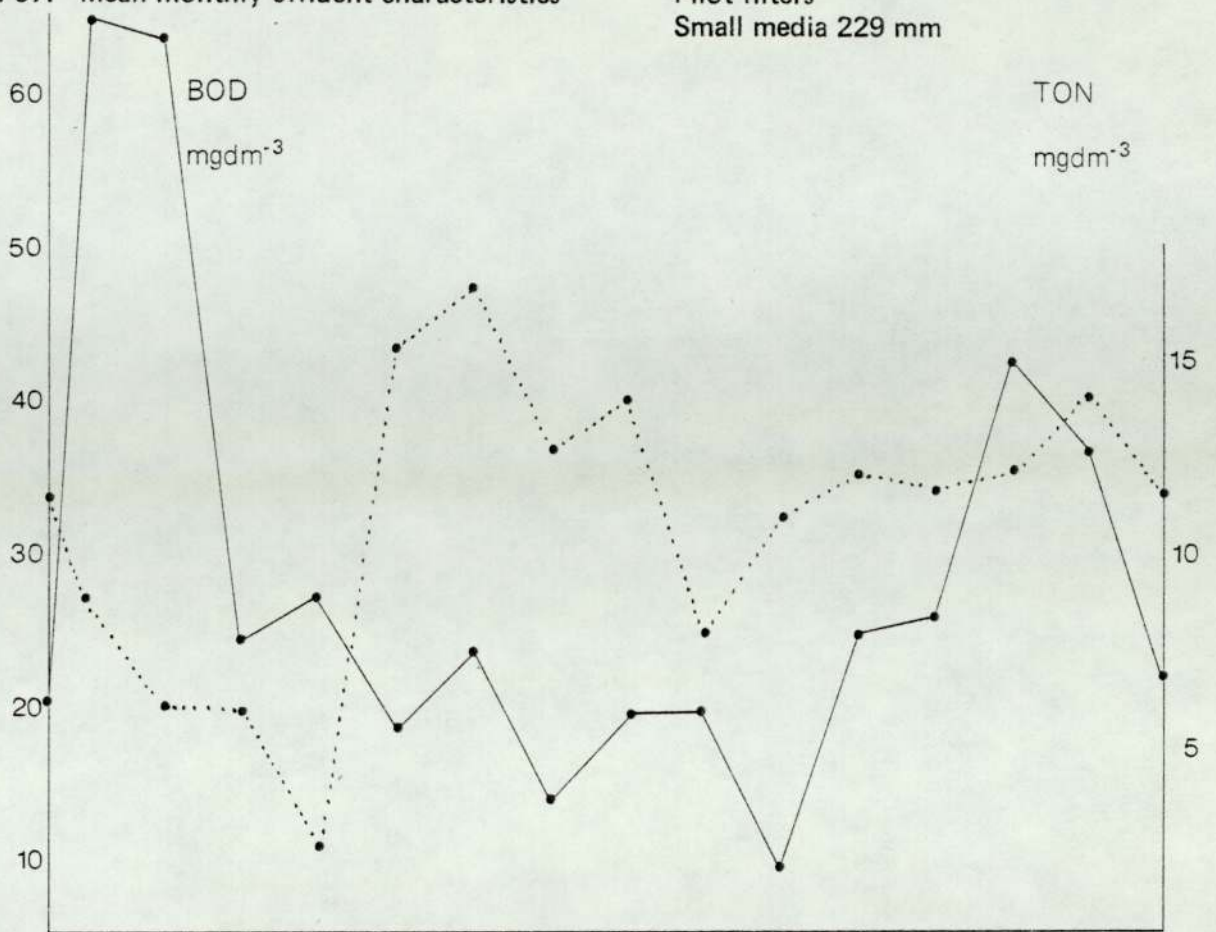


Fig. 98. Mean monthly effluent characteristics

Pilot filters
Small media 229 mm and splash plate

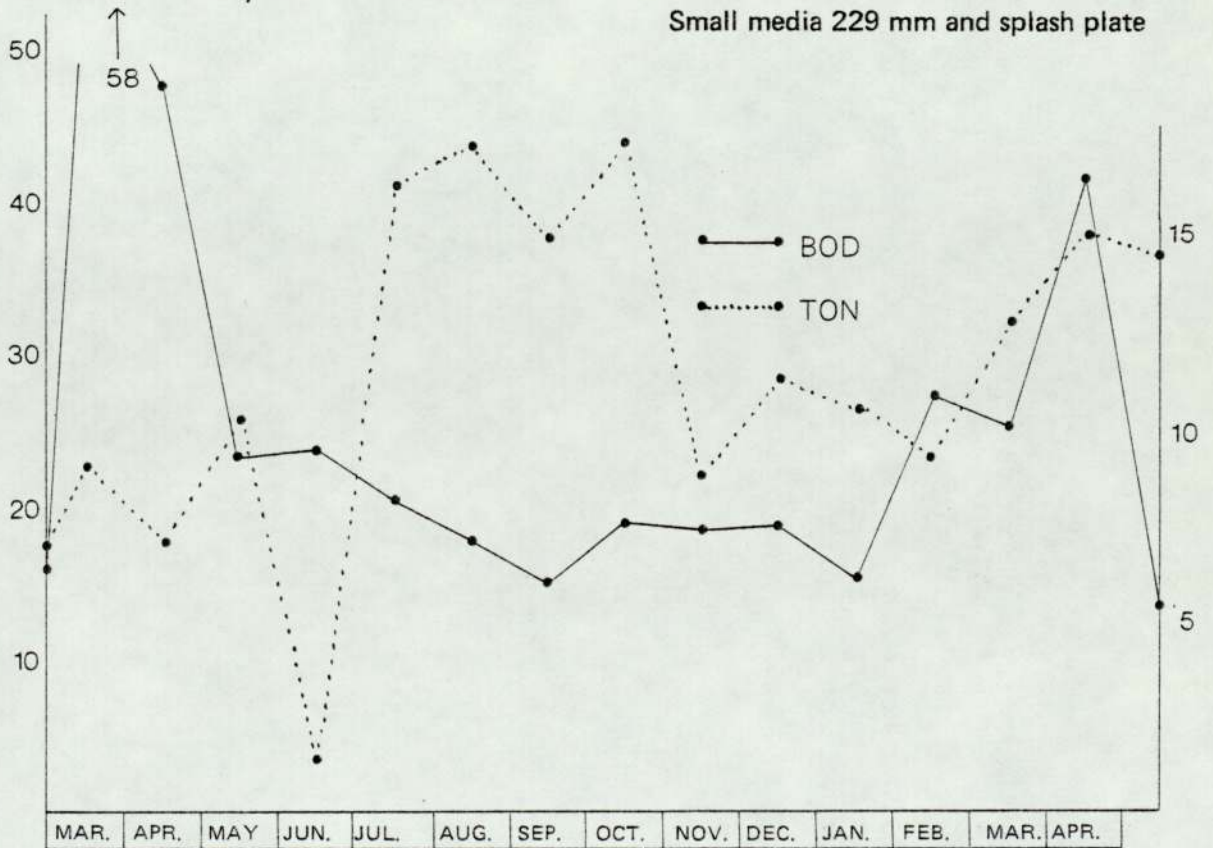


Fig. 99. Mean monthly effluent characteristics

Pilot filters surface covering

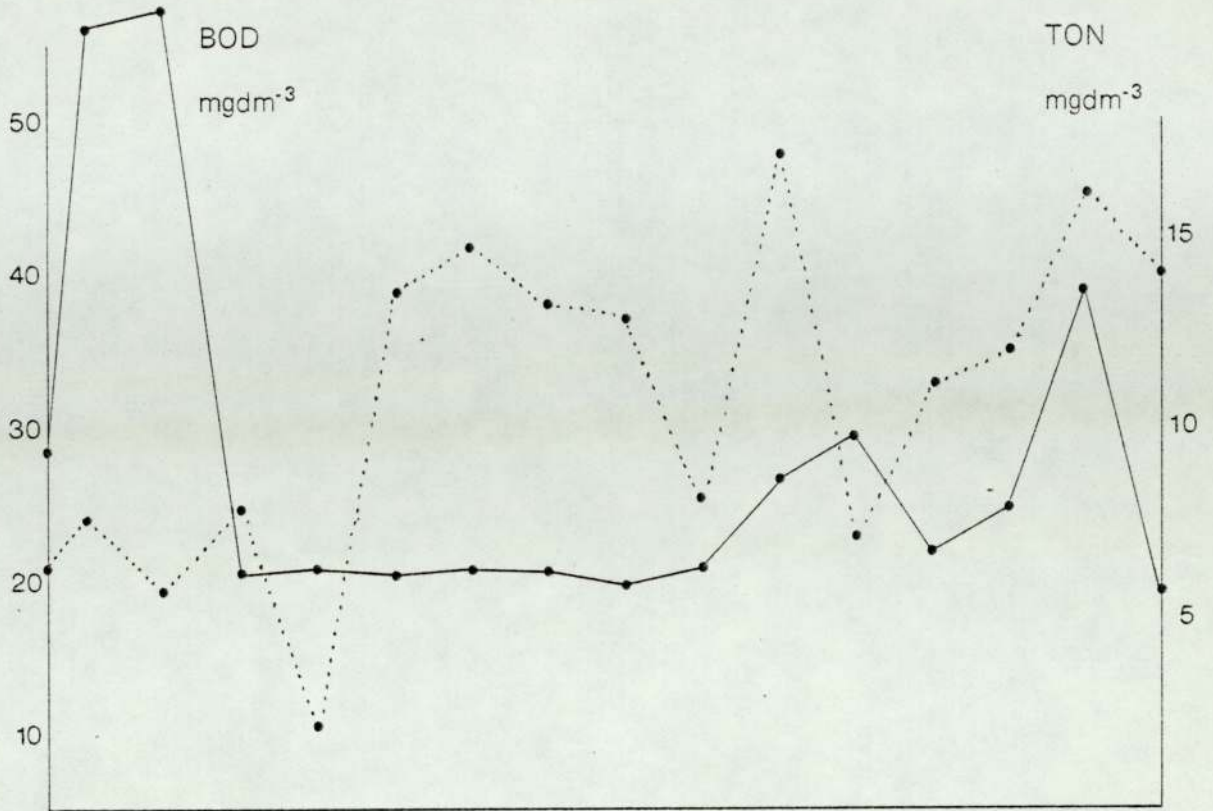


Fig. 100. Mean monthly effluent characteristics

Pilot filters small media (surface sprinkling) and splash plate

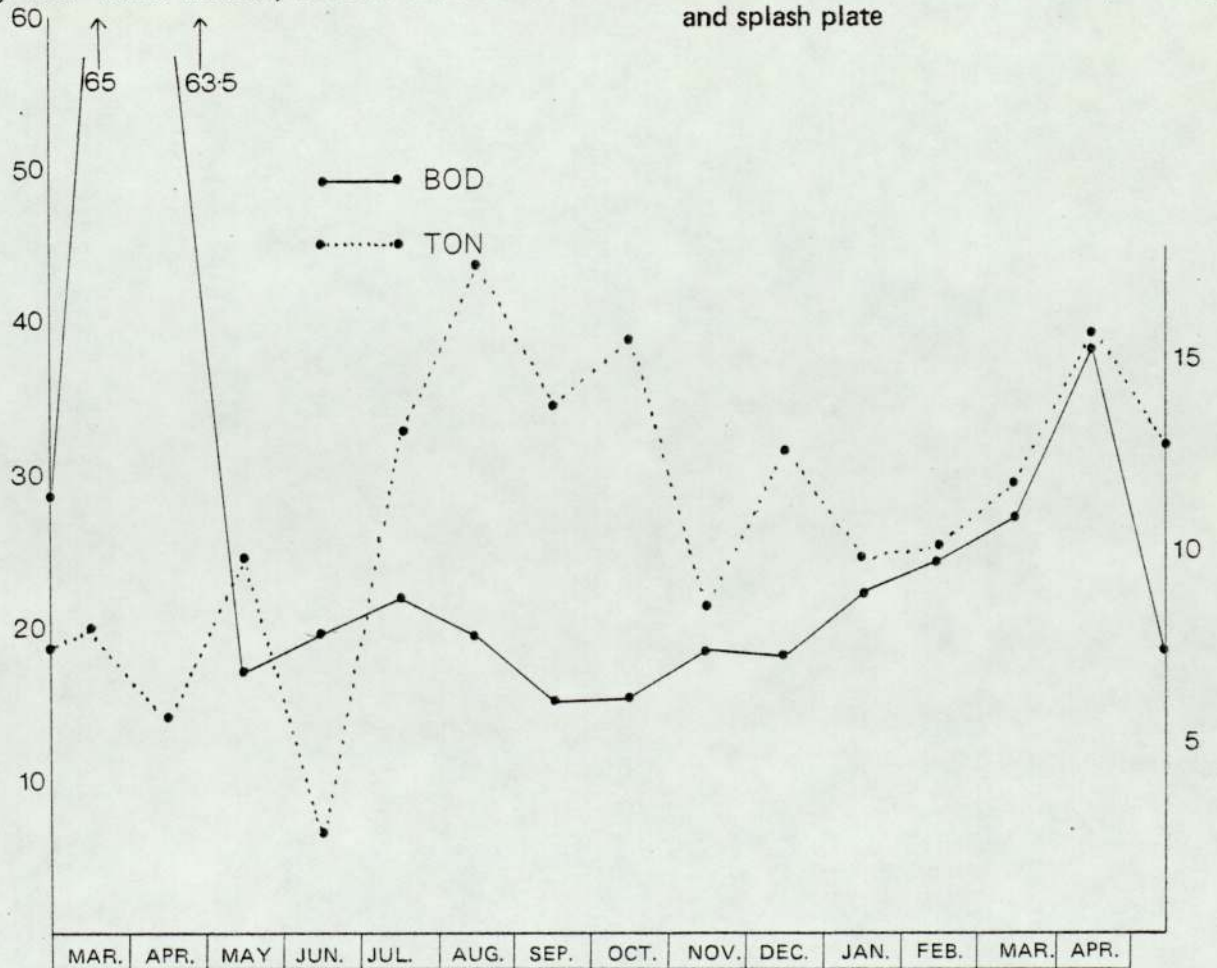


Fig. 101a. Mean monthly effluent characteristics

Pilot filters small media 76 mm

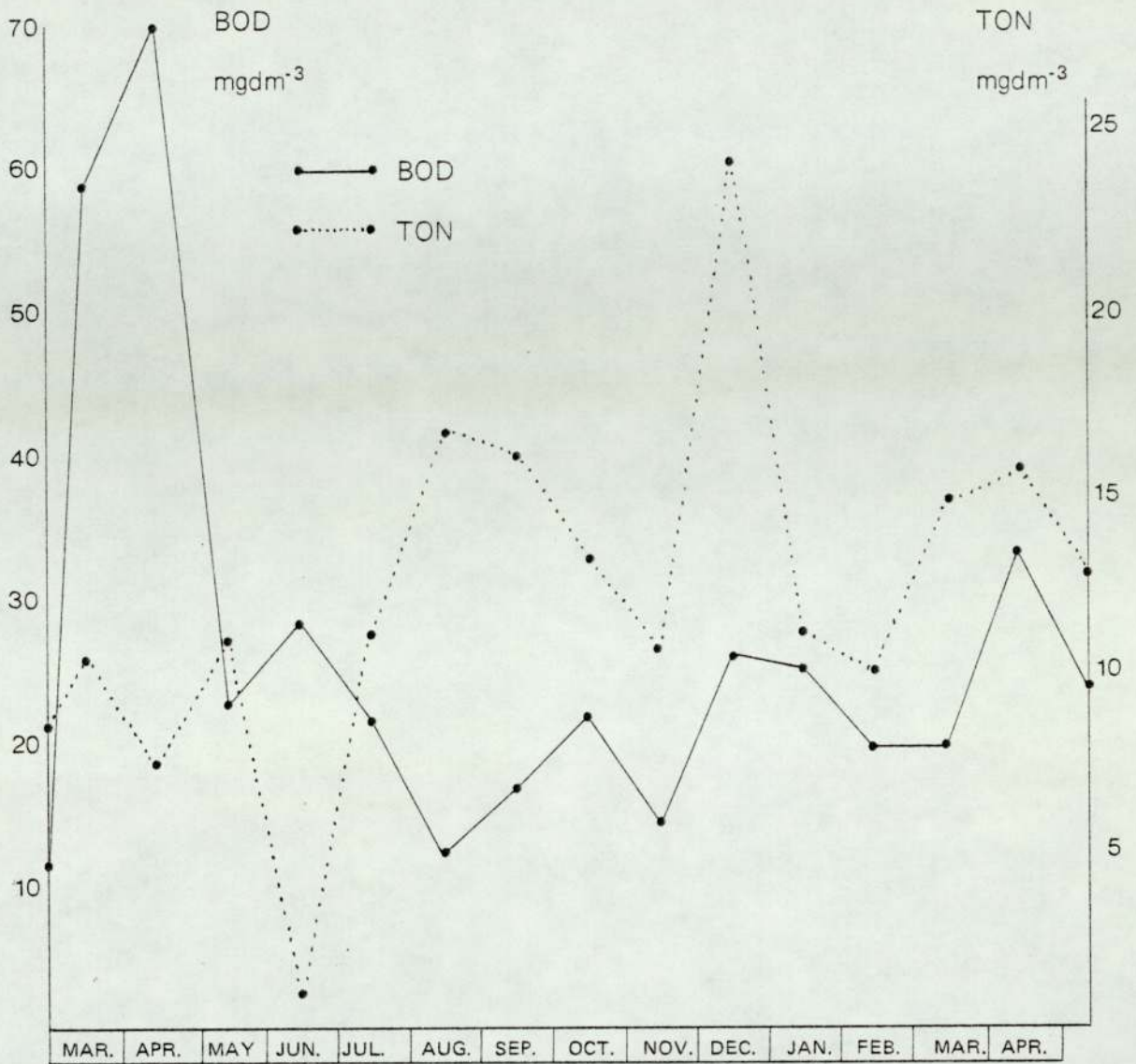


Fig. 101b. Diurnal variations in pilot filter temperatures 19th – 22nd May 1978

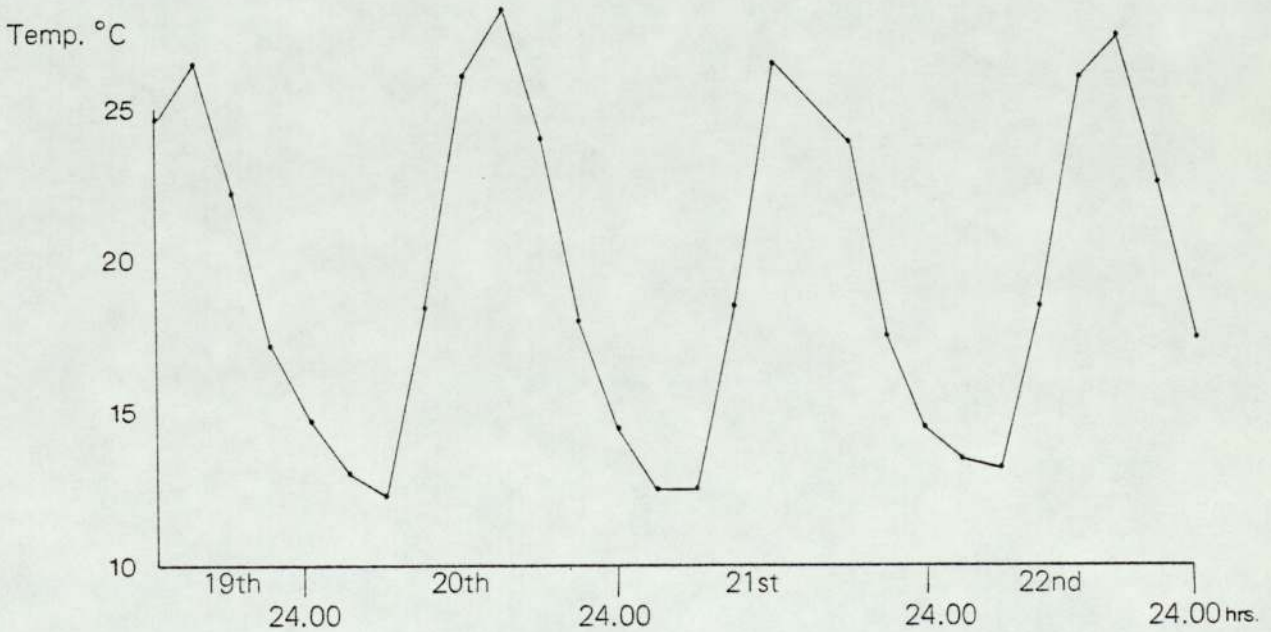


Fig. 102a. The discharge of Cl⁻ from a pilot filter following a single application of Cl⁻ at 0 min.

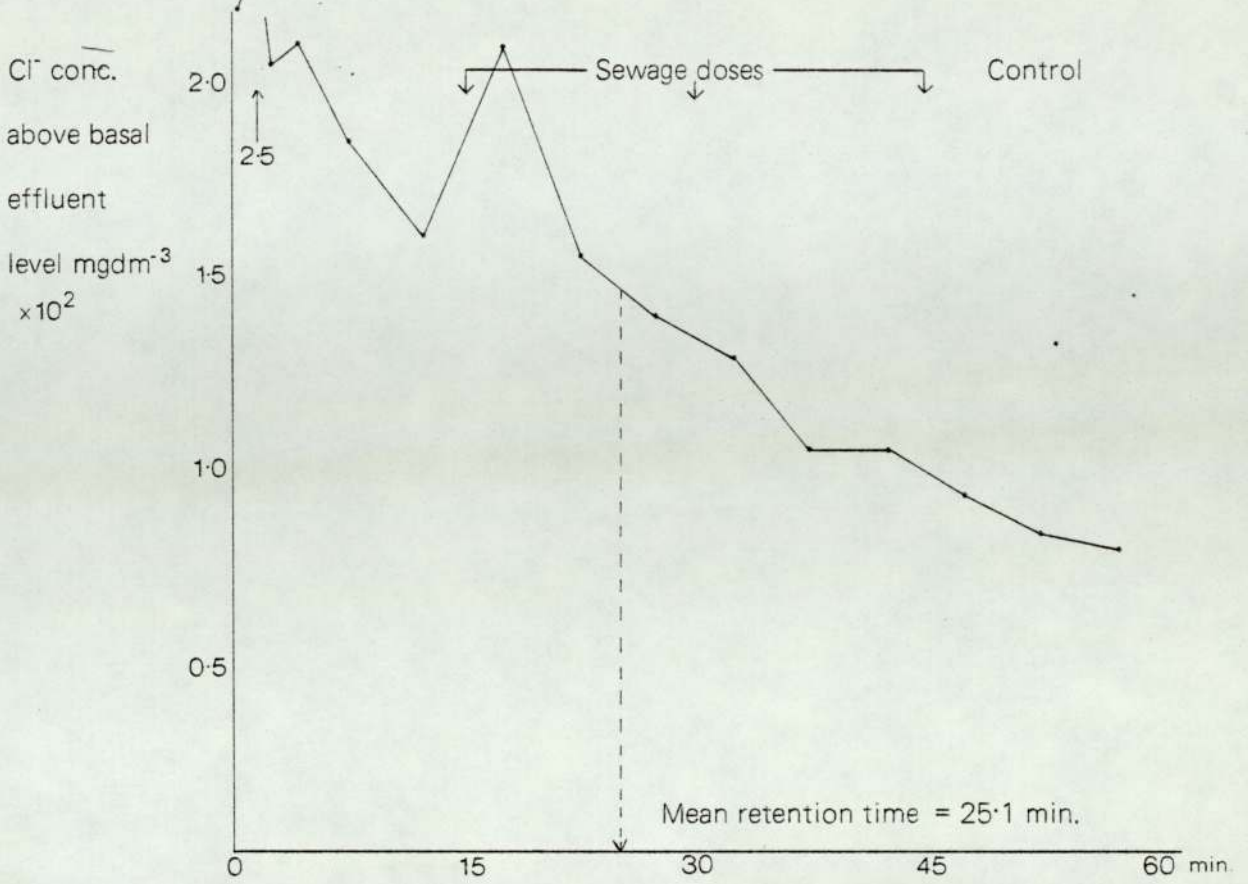


Fig. 102b. The hydraulic flow pattern from the above filter

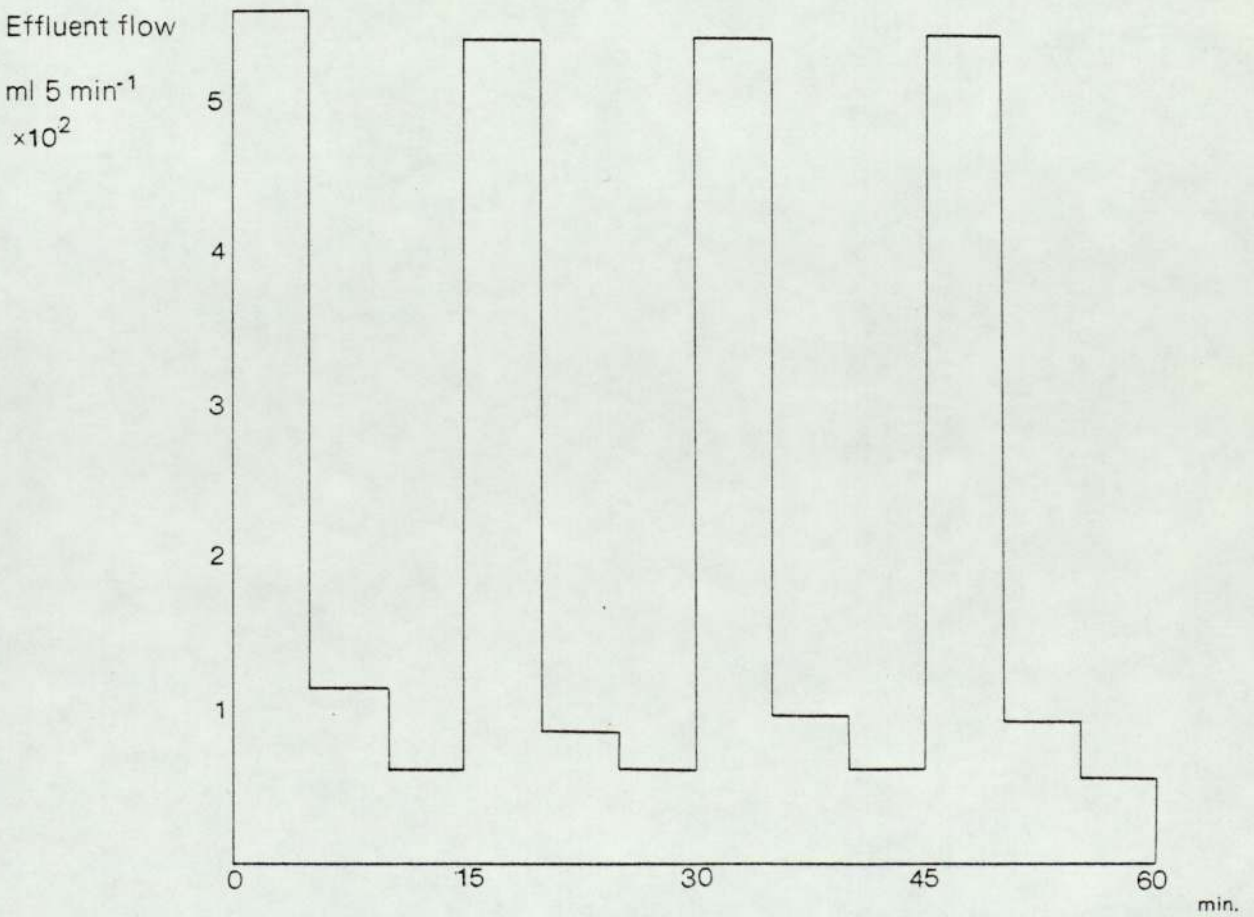


Fig. 103a. The discharge of Cl^- from a pilot filter following a single application of Cl^- at 0 min.

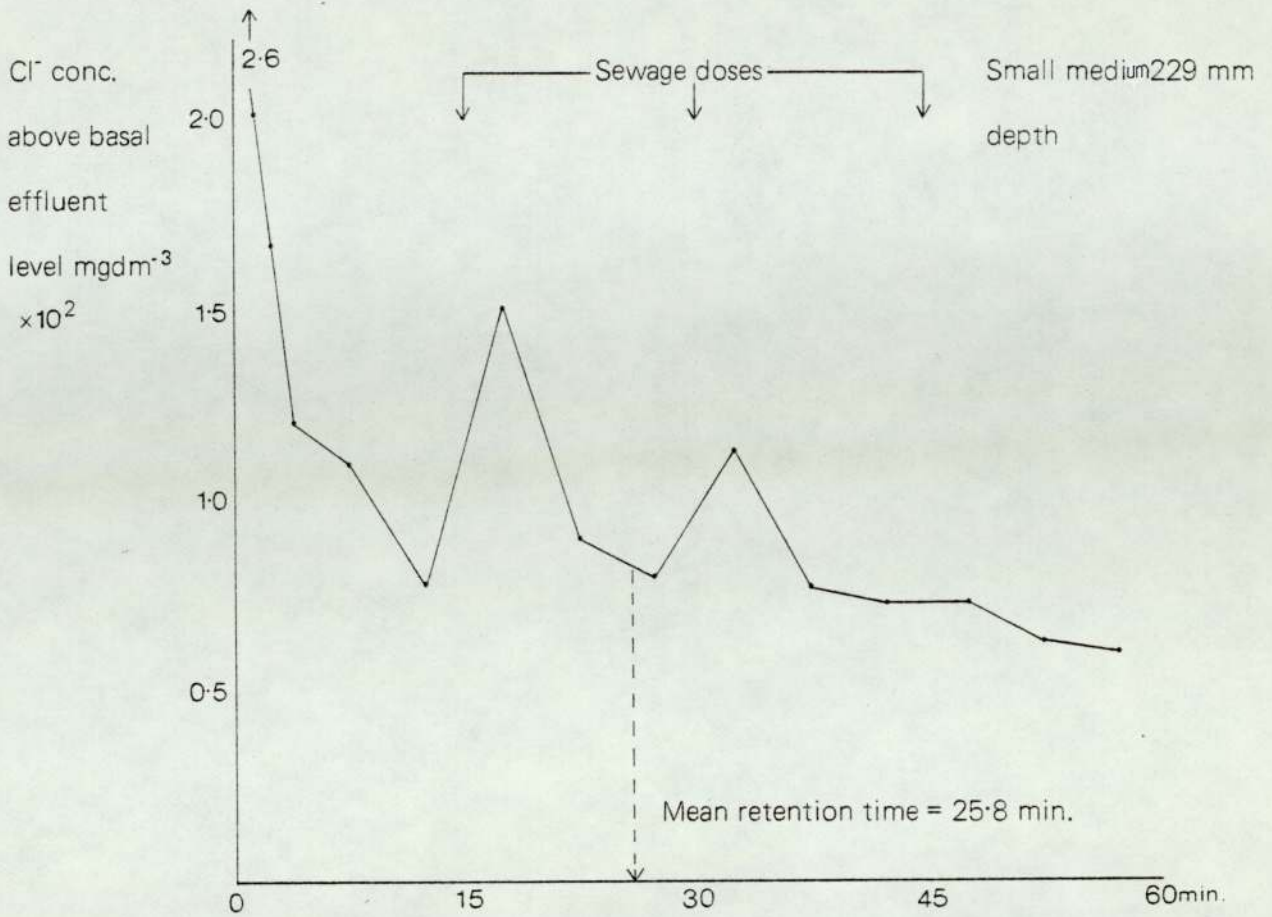


Fig. 103b. The hydraulic flow pattern from the above filter

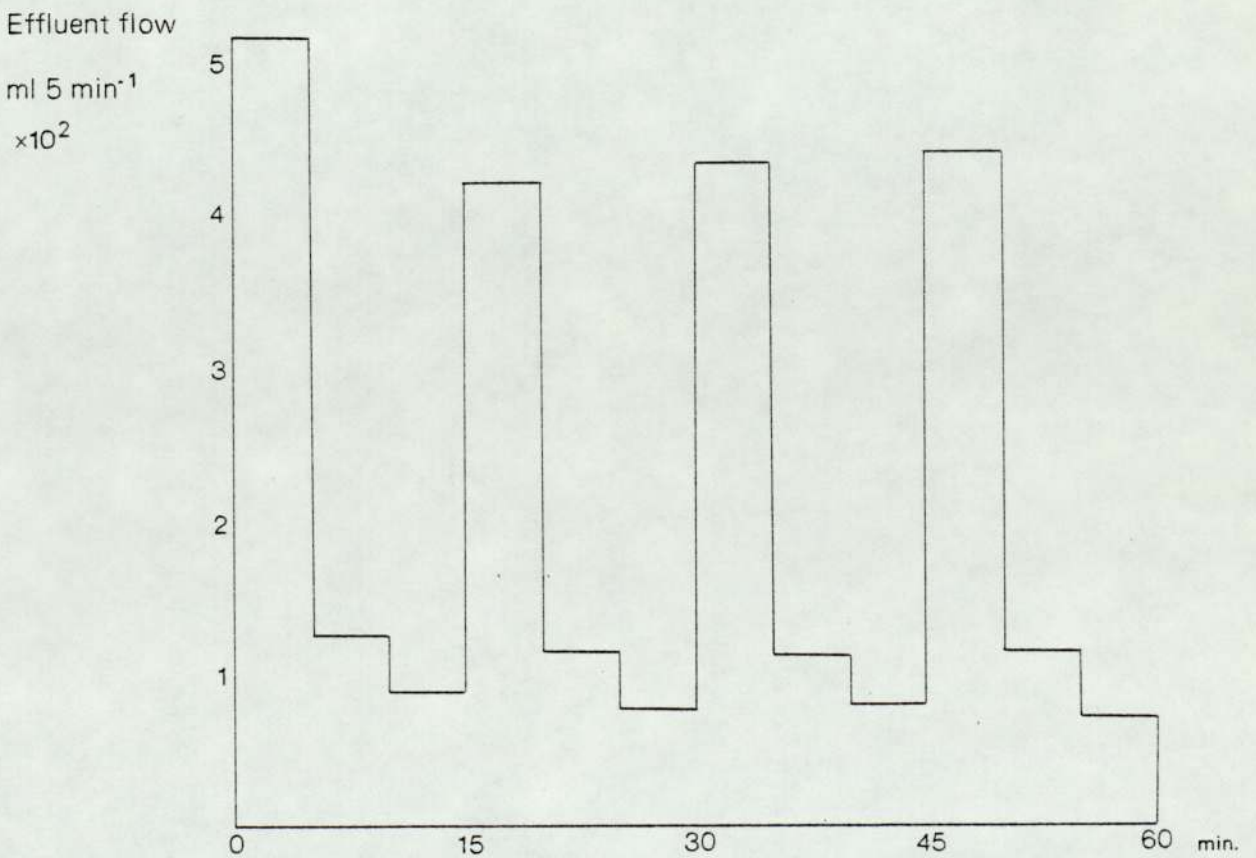


Fig. 104a. The discharge of Cl^- from a pilot filter following a single application of Cl^- at 0 min.

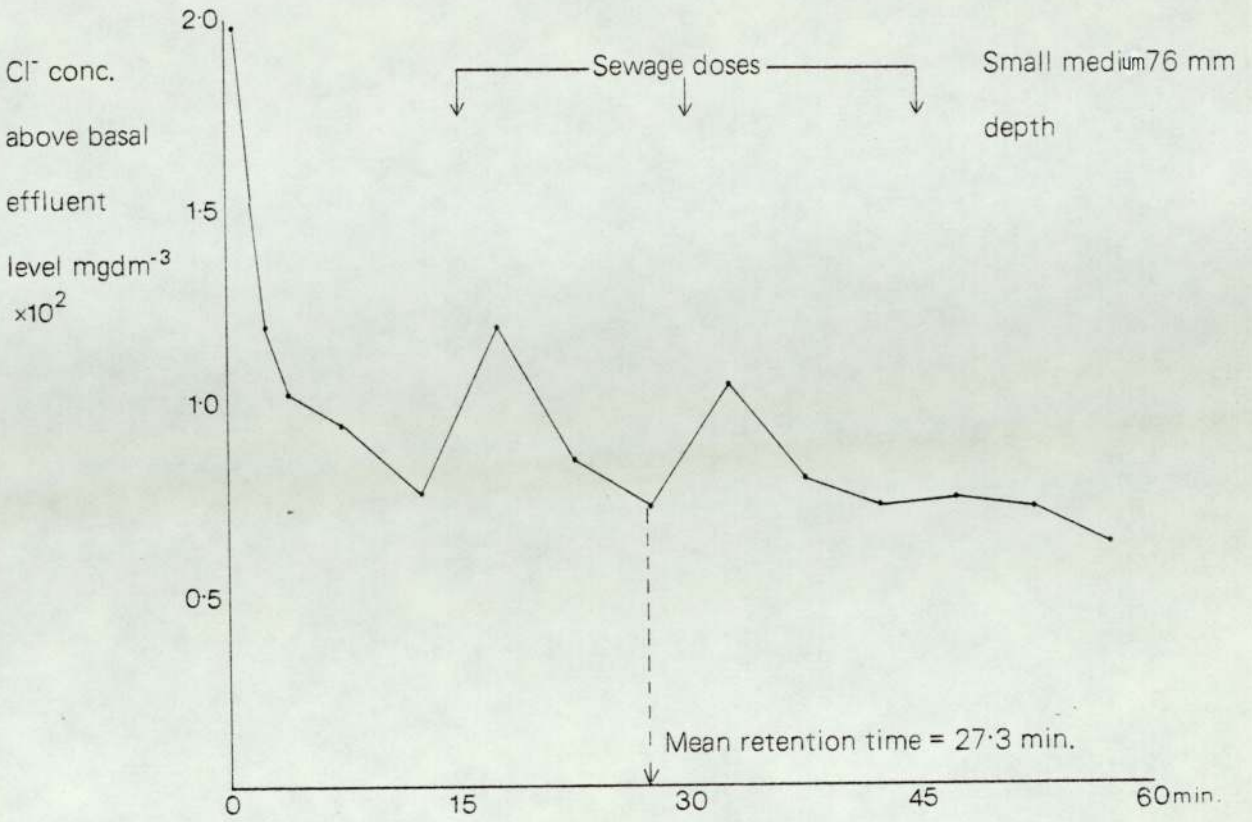


Fig. 104b. The hydraulic flow pattern from the above filter

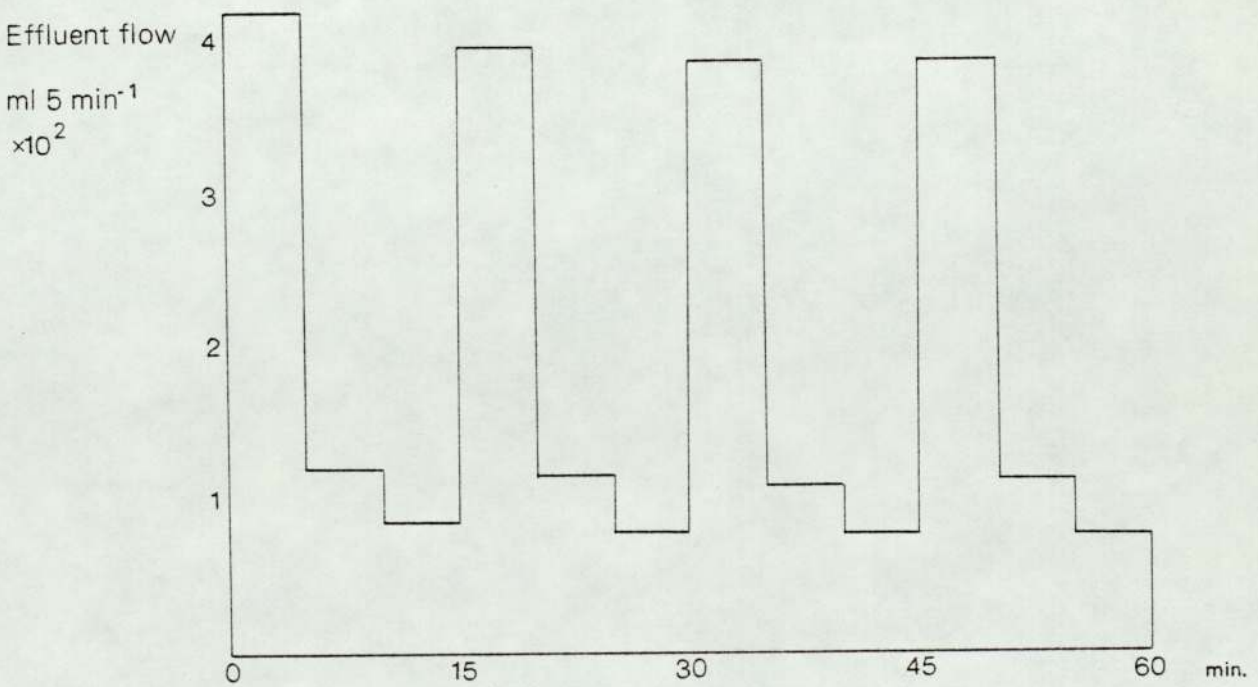


Fig. 105a. The discharge of Cl^- from a pilot filter following a single application of Cl^- at 0 min.

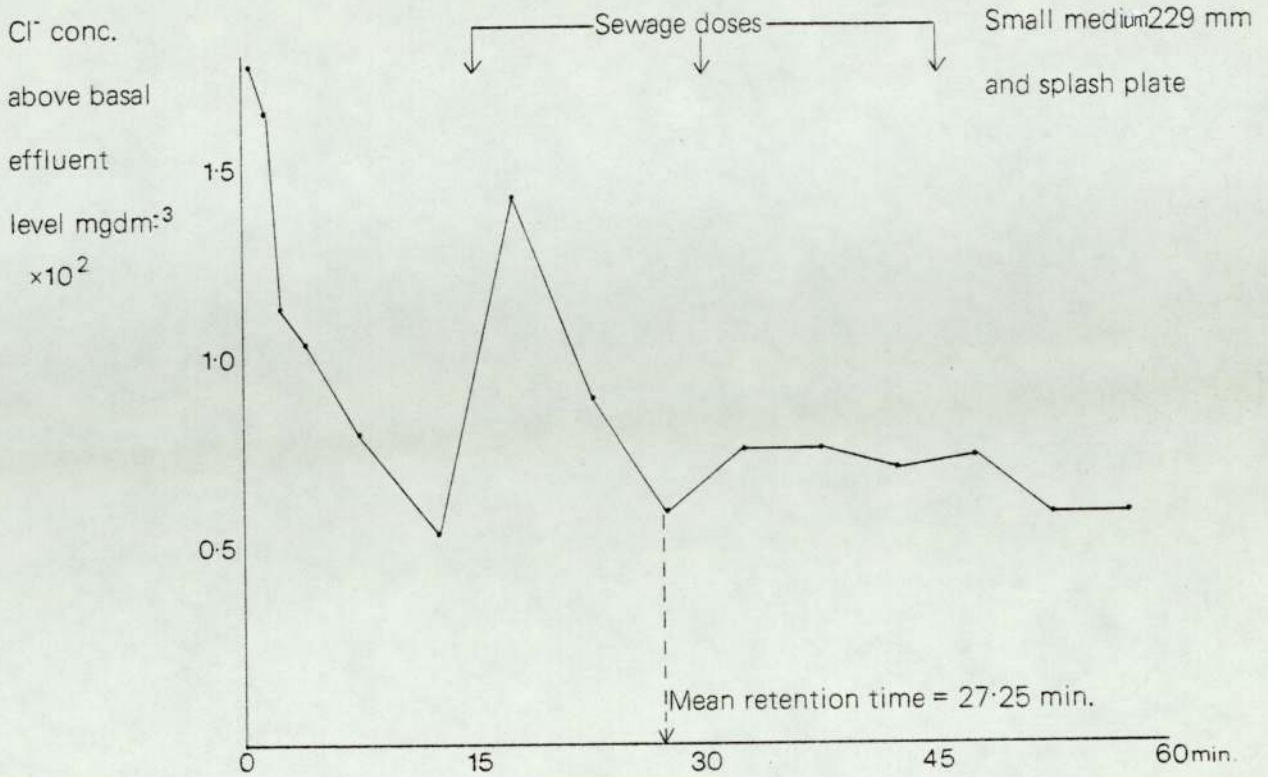


Fig. 105b. The hydraulic flow pattern from the above filter

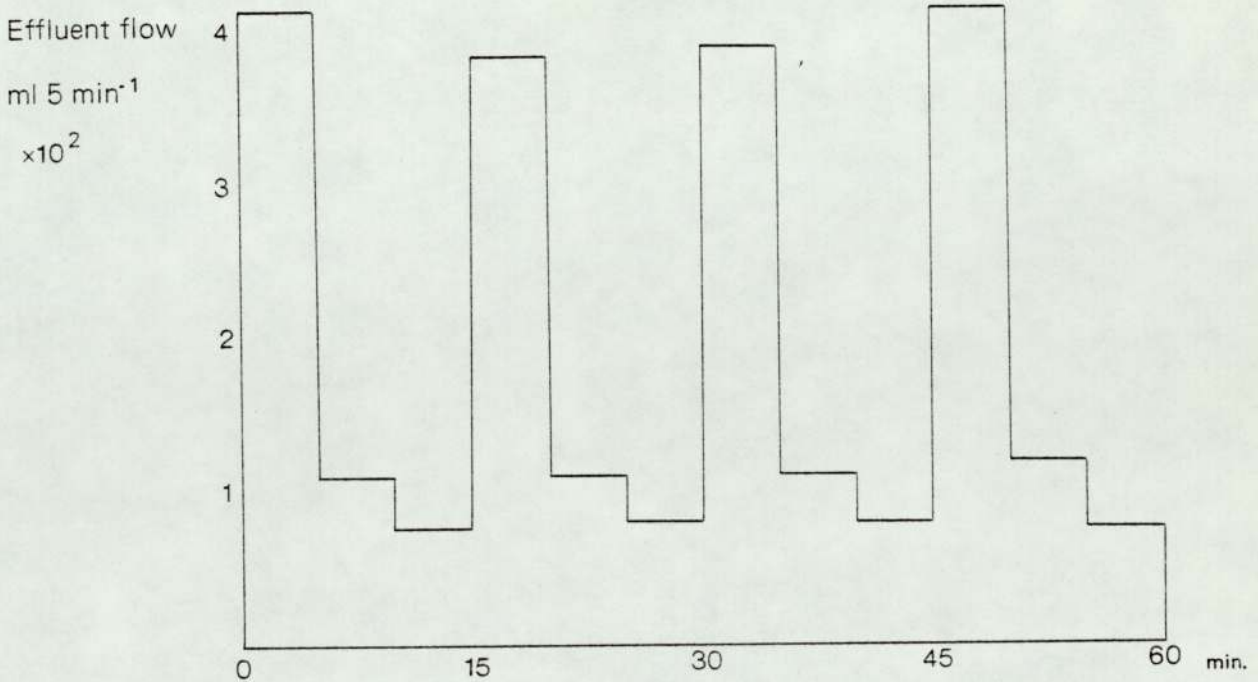


Fig. 106a. The discharge of Cl^- from a pilot filter following a single application of Cl^- at 0 min.

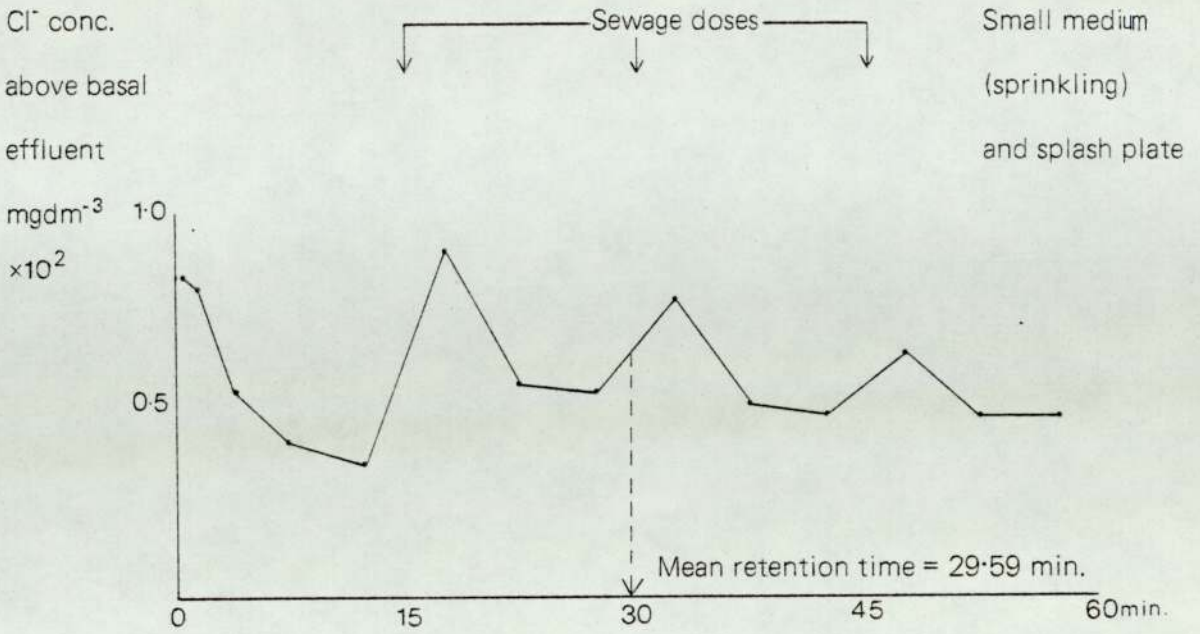


Fig. 106b. The hydraulic flow pattern from the above filter

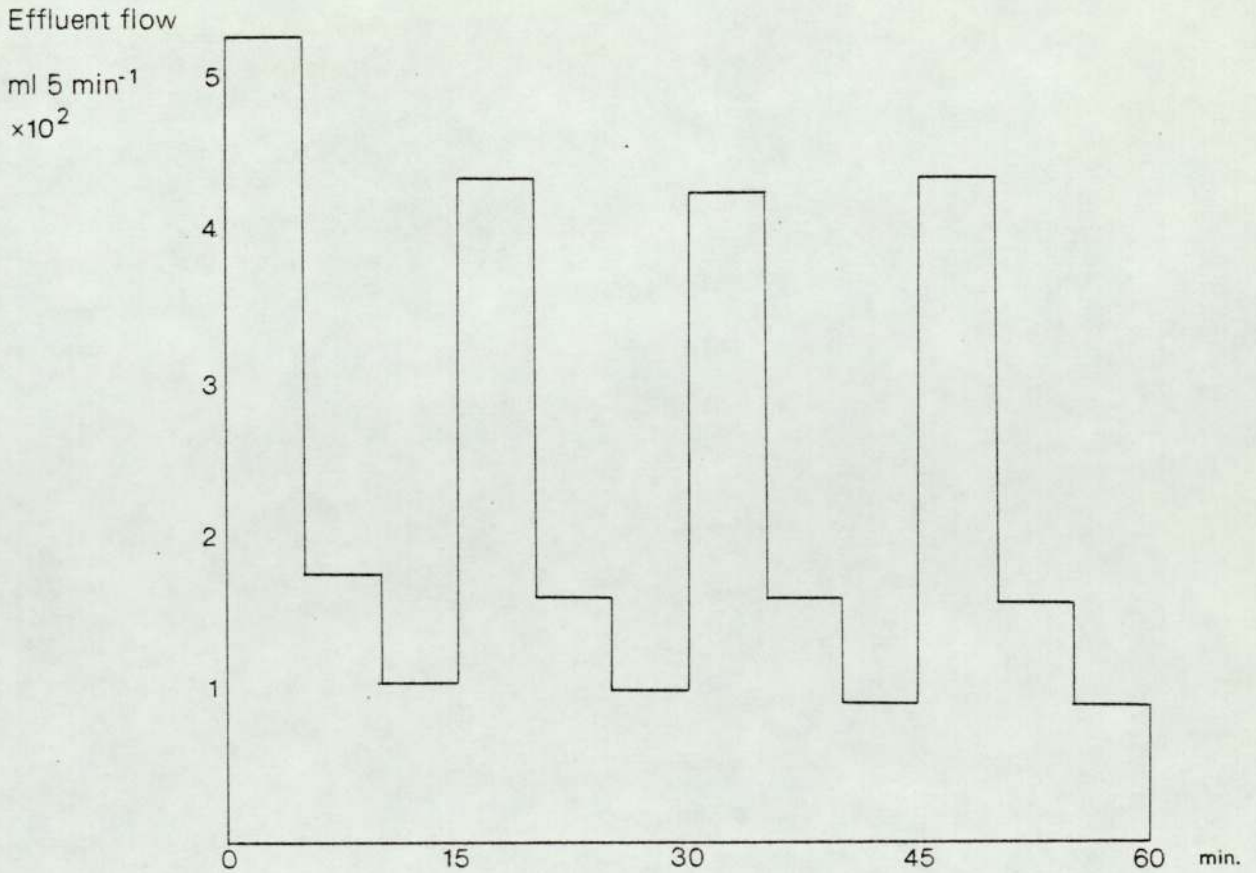


Fig. 107a. The discharge of Cl^- from a pilot filter following a single application of Cl^- at 0 min.

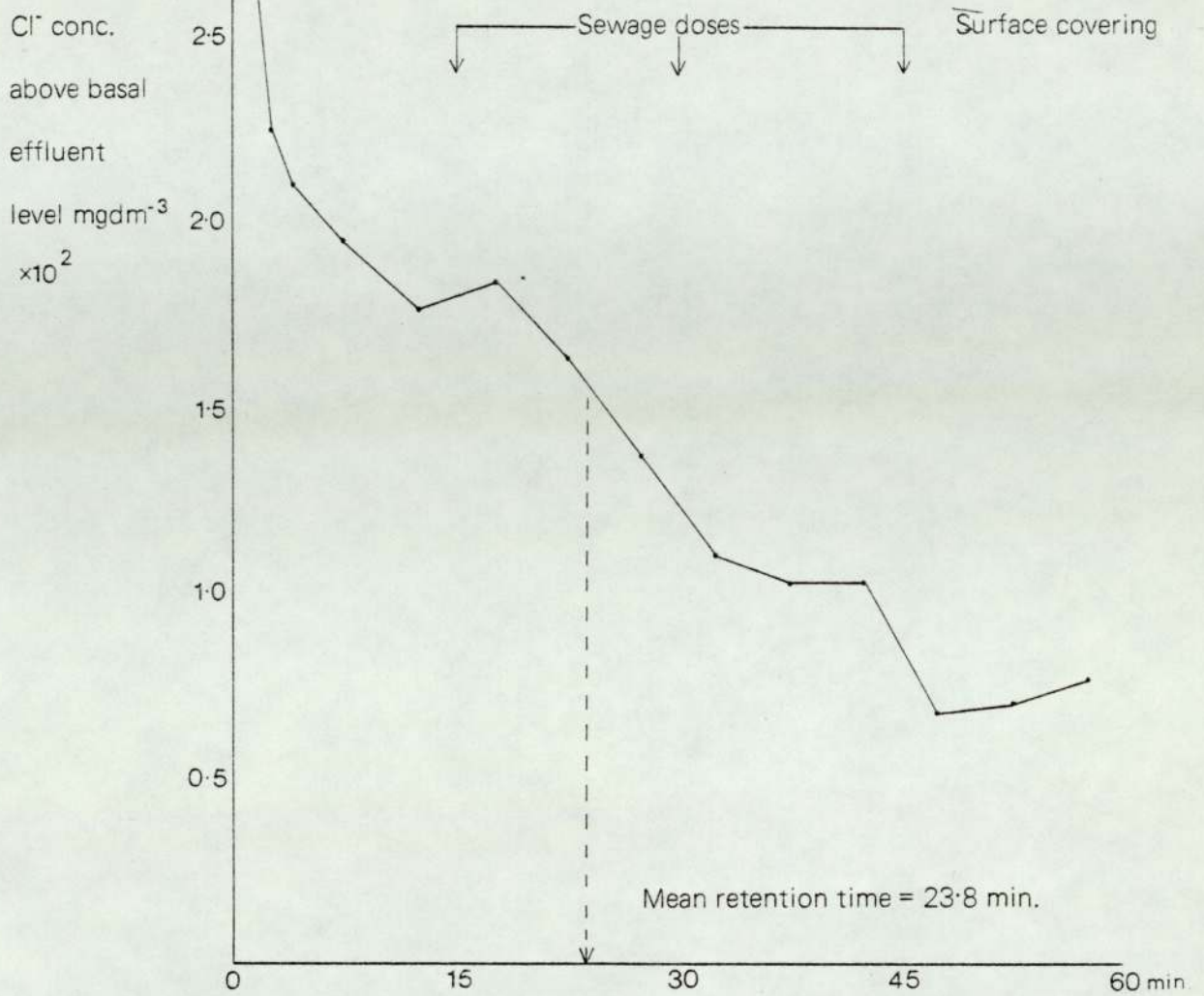


Fig. 107b. The hydraulic flow pattern from the above filter

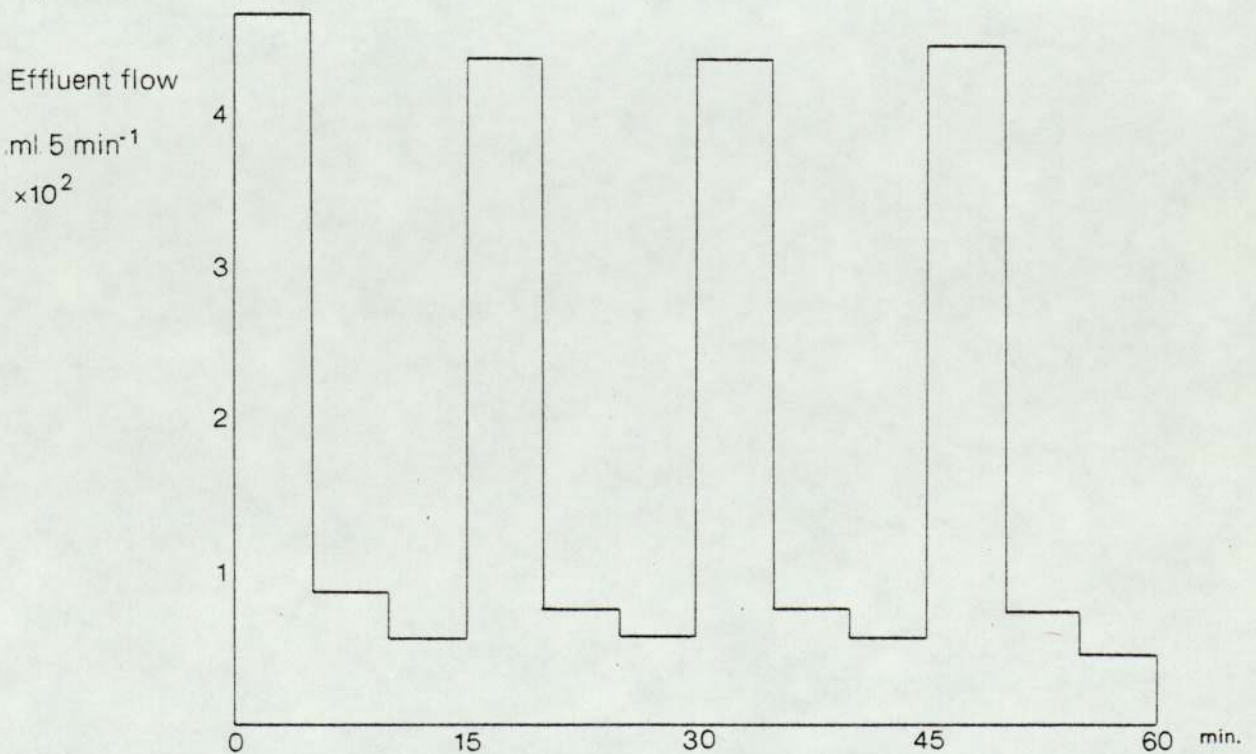


Fig. 108. Mean monthly effluent suspended solids Pilot filters

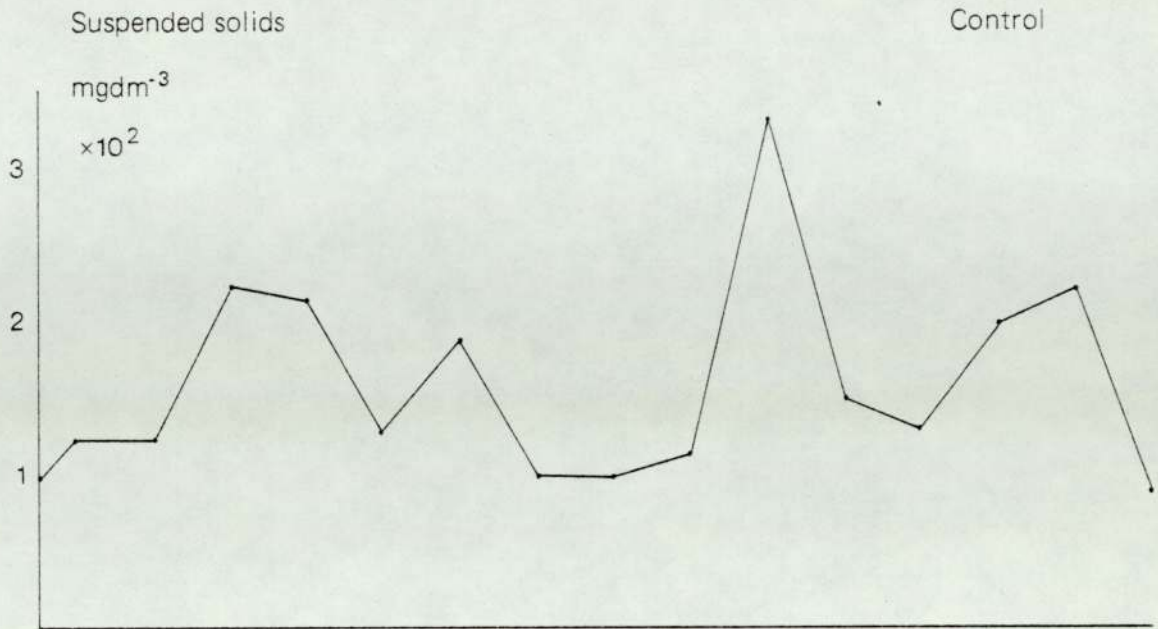


Fig. 109. Mean monthly effluent suspended solids Pilot filters

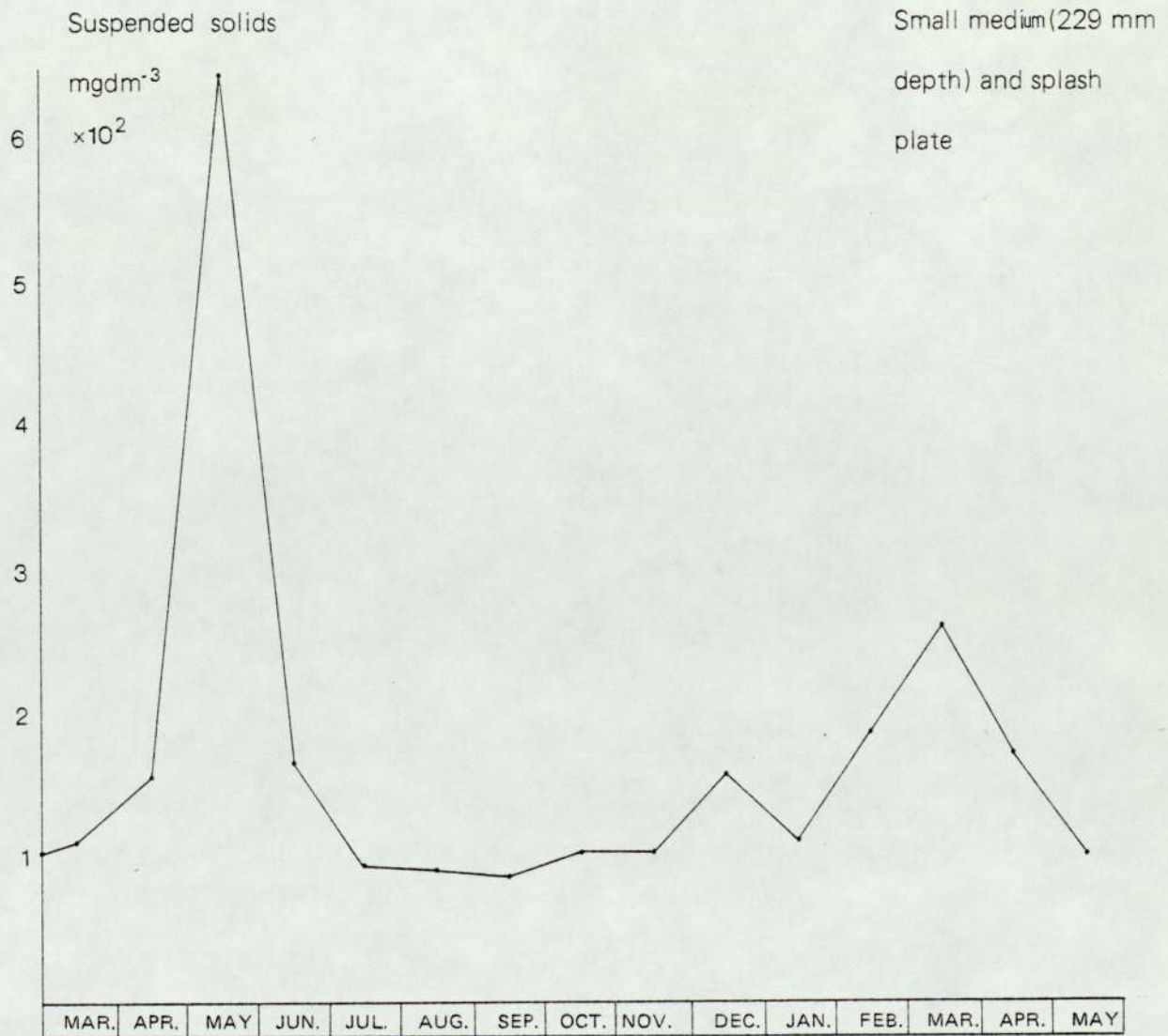


Fig. 110. Mean monthly effluent suspended solids Pilot filters

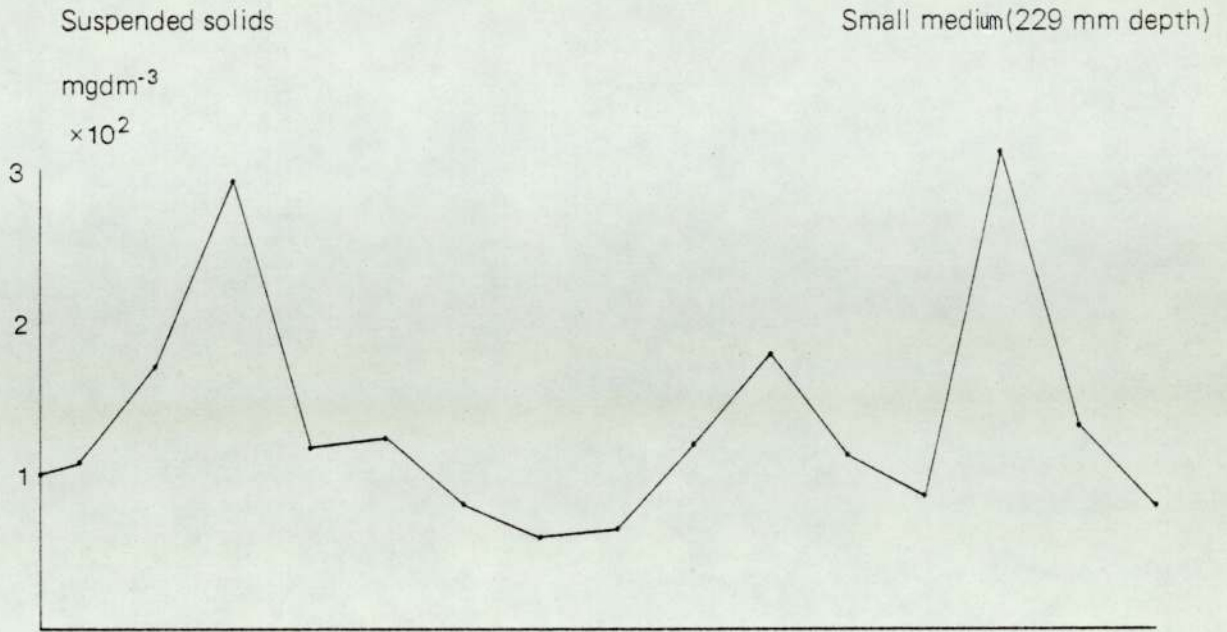


Fig. 111. Mean monthly effluent suspended solids Pilot filters

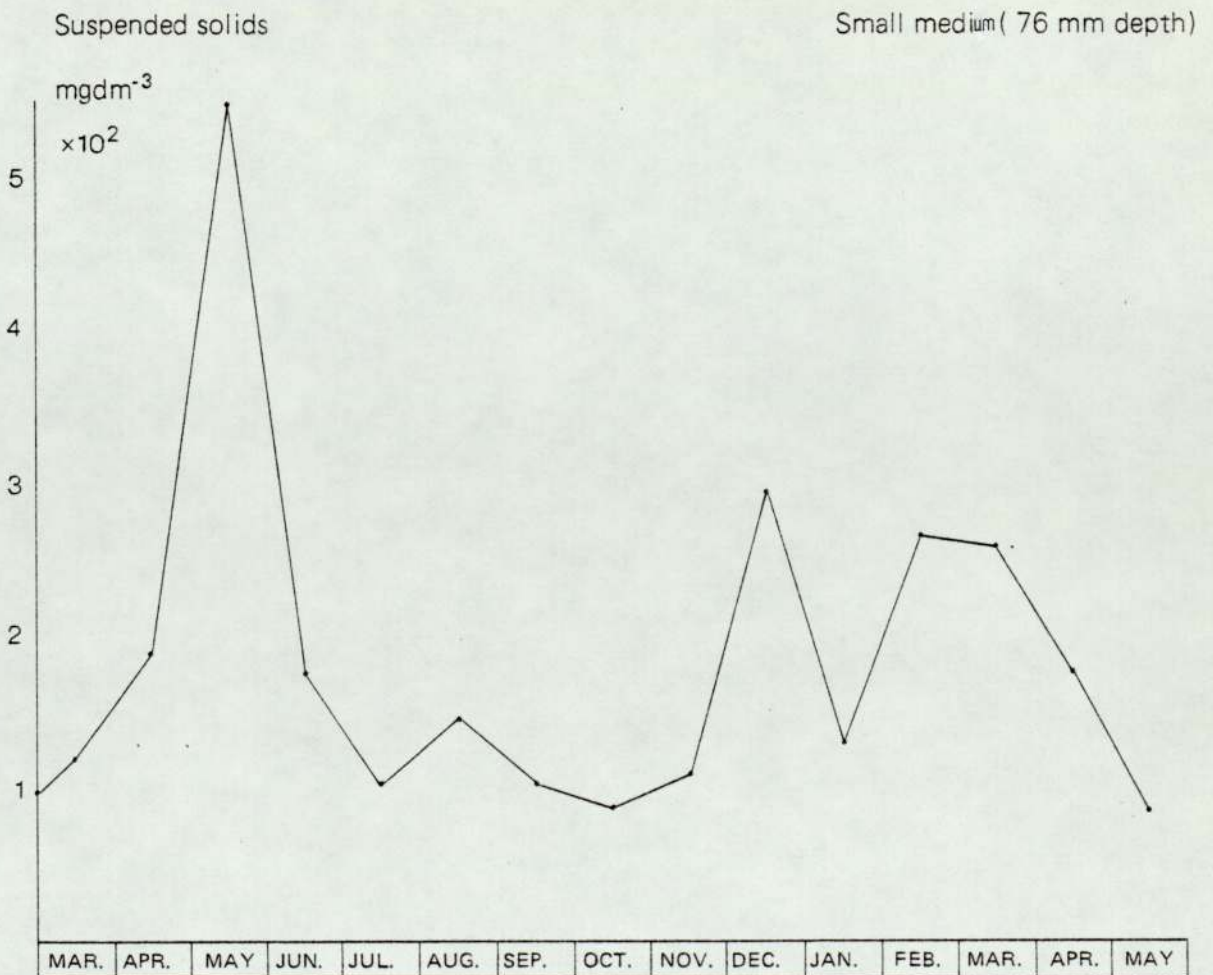


Fig. 112. Mean monthly effluent suspended solids Pilot filters

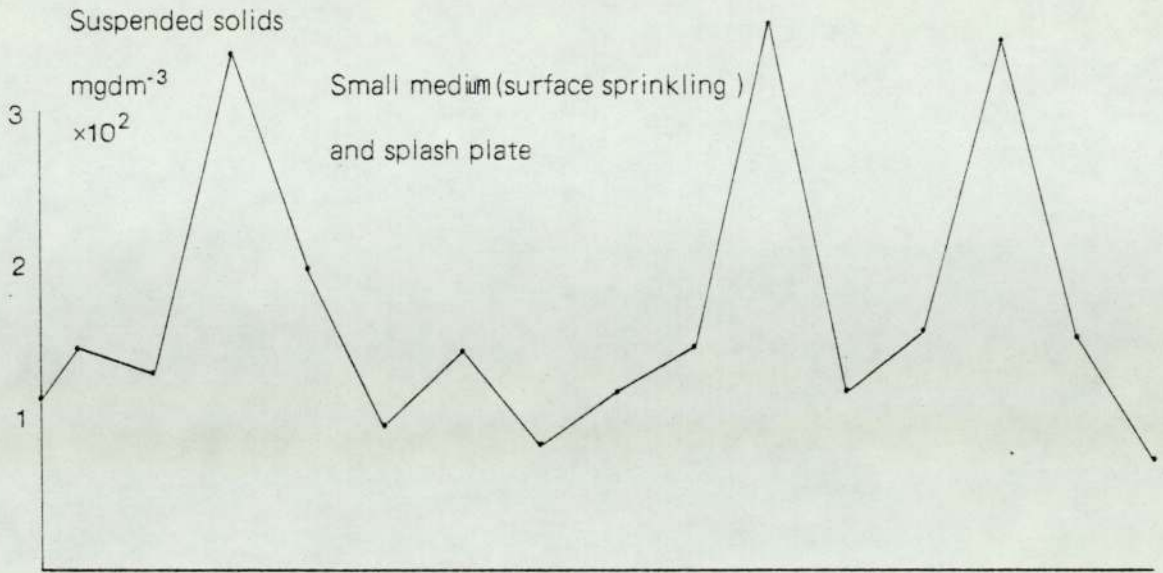
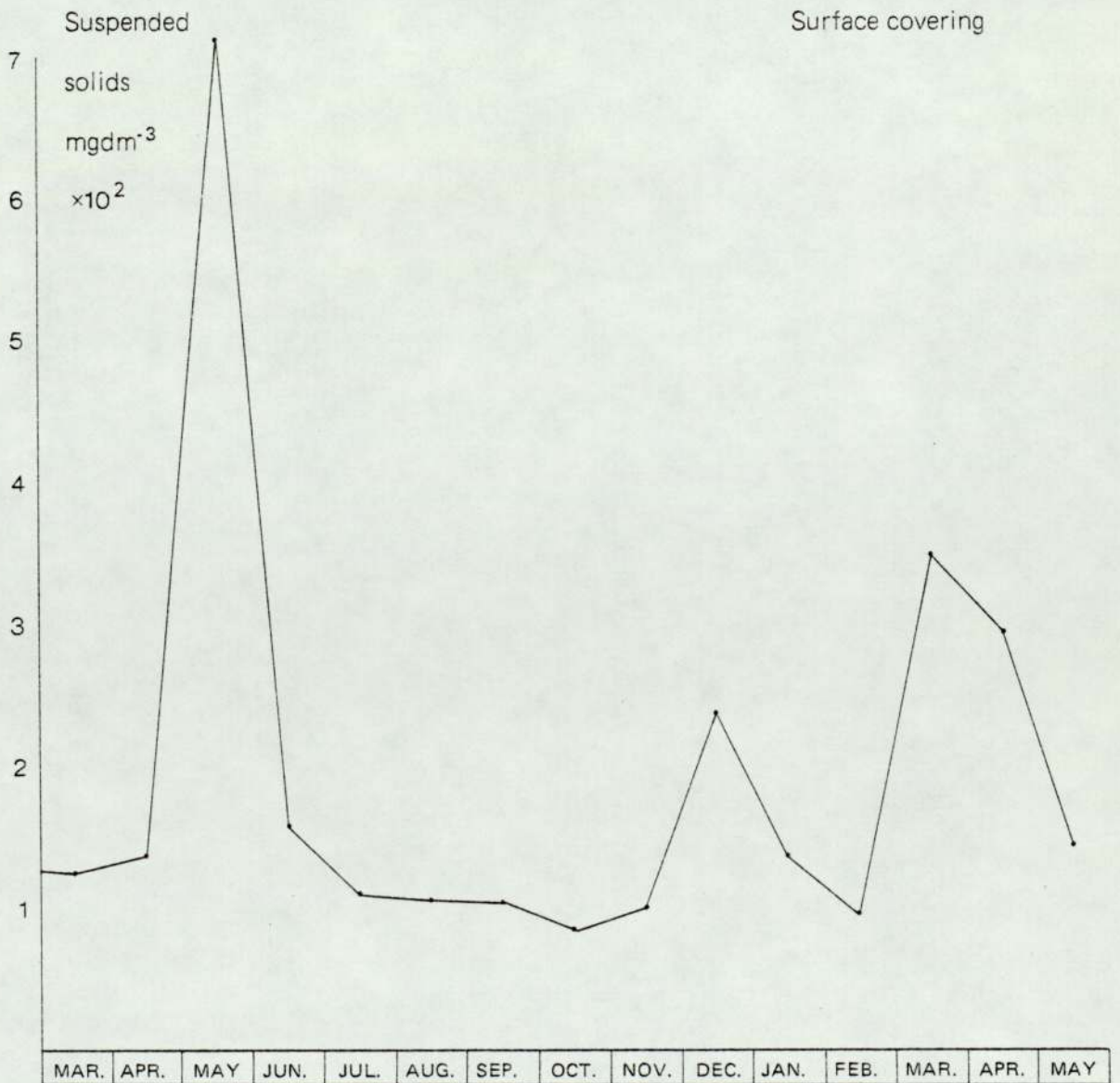


Fig. 113. Mean monthly effluent suspended solids Pilot filters



III Laboratory filter experimental work

(a) Phase I - Initial experimental work, Sept. - Dec. 1977

Introduction

The subject of competition has been discussed fully in the literature review section and the main purpose of the laboratory filter experiments was to investigate any possible competition between M.hygroscopicus and P.alternata. It was not clear whether these two species did compete for space and food in filters as some of the control measures found to give increased levels of one of the species in the main filters did not always produce a decrease in the other species. Obviously other factors may have accounted for the results experienced. For example subtle changes in other grazer populations e.g. enchytraeid worms, may have been caused by changes in fly population levels, or it could be suggested that direct competition between M.hygroscopicus and P.alternata for food and space would be unlikely as they have been shown to have different vertical distributions in filters by many workers including Fair (1934), Dyson and Lloyd (1935), Lloyd (1943a), Tomlinson and Hall (1950) and Solbé et al. (1967). It was the view of Fair (1934) that P.alternata was found in greatest numbers 76 - 305 mm (3-12 in.) below the filter surface and Lloyd (1943a) showed that chironomids were found nearer the surface. As the actual distribution of these organisms depends on film levels, the magnitude of which are affected by many factors including dosing frequency, loading and medium size, each filter would therefore achieve different vertical film distributions and it is likely that degrees of competition would be different in each case. In the case of experimental work carried out on the main and pilot filters at Tamworth, the main factor controlling film levels would be the low dosing frequency which would tend to produce a sub-surface band of film (Hawkes and Shephard 1972). Therefore it is possible to theorise that P.alternata may inhabit a lower level of the filters than M.hygroscopicus and that the two species would not interfere with each other except at the interface

between their two zones. This may account for the apparent unchanged P.alternata populations when M.hygroptericus populations were decreased. However in other situations where film distribution is more even it would be interesting to know if the two species were in direct competition with each other and this batch of experimental work set out to clarify that point.

Phase I of the experimental work consisted of 3 separate experiments. The methods used for these were described in detail in the methods section but to briefly repeat they consisted of seeding the filters with larvae of two species, M.hygroptericus and P.alternata, in pure and mixed culture and then investigating the subsequent adult fly emergence from these filters by trapping the flies on sticky plates situated above the filters. The intention was then to investigate any differences found in emergence between pure and mixed cultures in order to clarify competition theories. During this phase samples of effluent were taken from these filters before and after seeding with larvae to investigate the effects of introducing grazers on effluent suspended solids levels.

Experiment 1

The 18 filters were commissioned on two loadings on 3rd Oct. using Tamworth settled sewage and secondary main filter feed giving the same organic loadings as the primary and secondary filters on the main works (0.15 and 0.02 Kg BOD m⁻³ day⁻¹). The mean effluent suspended solids levels at the time of seeding each filter with 25 larvae and then 3 days after can be seen as follows.

<u>Initial suspended solids</u>		<u>Suspended solids 3 days</u>		
<u>mgdm⁻³</u>		<u>after seeding mgdm⁻³</u>		
<u>High loading</u>	<u>Low loading</u>	<u>High loading</u>	<u>Low loading</u>	
60.67 ± 0.94	8.44 ± 1.29	70.88 ± 3.07	18.22 ± 0.89	(± = S.E.M.)

Therefore it is apparent that a mean increase in suspended solids of 10.21 mgdm^{-3} was found on the high loaded filters and 9.78 mgdm^{-3} on the low loaded filters. Both of the increases can be seen to be significant by the small standard error of the means.

Flies were first noted to emerge on 12th Oct. and by 17th Oct. it was considered that the maximum number of flies had emerged, therefore the sticky plates were removed and counted, see Table 20.

Table 20 Fly emergence results - Expt. 1

<u>Filter no.</u>	<u>Original seeding</u>	<u>Loading</u>	<u>Flies recorded</u>	
			<u>M.hygropetricus</u>	<u>P.alternata</u>
A1	<u>P.alternata</u>	Low	-	-
A2	"	"	-	-
A3	"	"	-	-
A4	<u>P.alternata & M.hygropetricus</u>	"	3	-
A5	" "	"	1	-
A6	" "	"	-	-
B1	<u>M.hygropetricus</u>	"	-	-
B2	"	"	1	-
B3	"	"	-	-
B4	<u>P.alternata</u>	High	-	1
B5	"	"	-	1
B6	"	"	-	3
C1	<u>P.alternata & M.hygropetricus</u>	"	-	5
C2	" "	"	-	2
C3	" "	"	-	3
C4	<u>M.hygropetricus</u>	"	-	-
C5	"	"	-	-
C6	"	"	-	-

The results from Table 20 obviously are not satisfactory as emergence numbers were too low to show any specific trends. It is interesting to note however that P.alternata were only caught from the high loaded filters and similarly with M.hygropetricus from the low loaded filters, possibly suggesting that film conditions were unfavourable for the survival of these species in the filters from which they were absent. This suggests that the loadings applied were too extreme and in subsequent experiments the loadings were adjusted. Although the loadings applied were the same as those applied to the main filters, it must be realised that especially in the case of the high loading, other factors are operating on the main filters to keep film levels down including the presence of other fauna, e.g. enchytraeid worms, and high scouring action which is difficult to imitate with a small scale pumped flow.

According to data published by Lloyd et al. (1940), assuming the larvae used for seeding were in the mid stage of their life cycle, the peak adult emergence should have occurred on 17th Oct. in the case of M.hygropetricus, and 12th Oct. in the case of P.alternata at a temperature of 20°C. It was clear that the maximum possible emergence had occurred by 17th Oct. and the reasons for this emergence being so low could be varied. For example it may have been caused by sudden temperature shock causing a large mortality of the larvae. When the larvae were collected they were taken from an environment where the mean air temperature was 10.5°C (October) and transferred to an environment where the temperature was 20°C, so this may have detrimentally affected them. Other reasons for the low emergence may have been larval mortality due to rough handling in the transfer process from media to the filters. The media was scrubbed with a stiff brush in water to dislodge the larvae and on reflection this method may have damaged the larvae physically. The most important reason is that film conditions were too extreme to allow successful survival of the larvae introduced. For example film levels may have been too high on the high loaded filters and too low on the low loaded filters giving rise to starvation conditions.

Quite apart from the above mentioned disadvantages it was later realised that seeding with 3rd or 4th instar larvae, as was carried out in this experiment, would not always give true indications of possible competition as this may occur at other stages of the life cycle i.e. at a younger larval stage. Therefore considering all of the disadvantages apparent in this experiment the methods in the 2nd experiment were changed slightly.

Experiment 2

The filters were commissioned on the same loadings as in the previous experiment however certain factors and techniques were changed in the light of the poor results obtained in the previous experiment. It was decided to run this experiment at 15°C, a temperature more related to the external temperature, and to seed with a larger number of larvae (50 per filter). Also the method in which the larvae were transferred from the media to the filters was altered so as to minimise physical contact with the larvae. The method used was simply to immerse the media into water which was then agitated and the resultant washings were sieved. The larvae were transferred to the filters by pipette. Unlike the previous experiment it was attempted to choose only very young larvae (approx. 2nd/3rd instar) for this experiment in order to study any possible competition through as much of the life cycle as possible, however the task of finding 900 very young larvae was difficult quite apart from transferring them to the filters.

As in the previous experiment effluent suspended solids levels were investigated at the time of seeding (25th Oct.) and then after 14 days. The following results were obtained.

<u>Initial suspended solids</u>		<u>Suspended solids 14 days</u>	
<u>mgdm⁻³</u>		<u>after seeding mgdm⁻³</u>	
<u>High load</u>	<u>Low load</u>	<u>High load</u>	<u>Low load</u>
122.22 ± 2.88	13.67 ± 0.75	124.44 ± 2.68	19.44 ± 0.96 (± = S.E.M.)

Therefore it is apparent that as before an increase appeared in the suspended solids levels after seeding (a mean of $1.89 \pm 3.53 \text{ mgdm}^{-3}$ on the high loaded and $5.78 \pm 0.67 \text{ mgdm}^{-3}$ on the low loaded filters) however this increase was obviously not so great and was not significant on the high loaded filters as the standard errors of the means do overlap slightly.

As regards fly emergence the fly plates were counted on 22nd Nov. (28 days from seeding). According to Lloyd et al. (1940) the total period for the life cycle at 15°C would be 38.5 days in the case of M.hygropetricus and 22 days for P.alternata. Therefore it was considered that the maximum emergence should have occurred by that time considering larvae were used for seeding. The results of fly emergence for this generation can be seen in Table 21.

Table 21 **Fly emergence results Expt. 2**

<u>Filter no.</u>	<u>Original seeding</u>	<u>Loading</u>	<u>Flies recorded</u>	
			<u>M.hygropetricus</u>	<u>P.alternata</u>
A1	<u>P.alternata</u>	Low	-	-
A2	"	"	-	-
A3	"	"	-	-
A4	<u>P.alternata & M.hygropetricus</u>	"	-	1
A5	" "	"	-	-
A6	" "	"	-	-
B1	<u>M.hygropetricus</u>	"	-	-
B2	" "	"	-	-
B3	" "	"	-	-
B4	<u>P.alternata</u>	High	-	23
B5	"	"	-	28
B6	"	"	-	28
C1	<u>P.alternata & M.hygropetricus</u>	"	-	7
C2	" "	"	-	7
C3	" "	"	-	2
C4	<u>M.hygropetricus</u>	"	-	-
C5	"	"	-	-
C6	"	"	-	-

Again these results were quite disappointing as not one M.hygropetricus survived to maturity. In fact only one fly emerged from the 9 low loaded filters whereas moderate numbers were recorded from the high loaded filters, so it is clear that starvation conditions existed on the low loaded filters discouraging build up of larval populations. The results from the high loaded filters are not as good as expected however they did show a promising trend. That is that although M.hygropetricus did not survive to maturity on these filters it is possible that they did survive long enough to have some effect on P.alternata emergence, as it is clear that the P.alternata emergence from all 3 pure culture filters (B4, 5 and 6) was far in excess of that obtained for the mixed culture filters (C1, 2 and 3). Therefore it is possible that film conditions on these filters discouraged M.hygropetricus however they did survive long enough to exert some competition pressure on P.alternata in the mixed culture filters. On these filters numerous dead M.hygropetricus larvae (approx. 3rd instar) were observed suggesting that they only survived for a short period from seeding. At this stage the fact that the loadings were not correct for successful survival of the two species together was made clear. Obviously starvation conditions were experienced on the low loading allowing neither species to survive and the film levels were too high on the high loading to allow M.hygropetricus to survive successfully to maturity. However it seemed that the problems of temperature change and handling were solved as when conditions were conducive to development, as shown on filters B4, 5 and 6, reasonable numbers of flies were caught. Therefore in the next experiment certain loading alterations were carried out.

Experiment 3

The loading changes carried out in this experiment consisted of increasing the loading on one batch of filters (A1 - B3) from 0.02 - 0.05 kg BOD m⁻³ day⁻¹ and decreasing the loading on the other batch of filters (B4 - C6) from 0.15 - 0.12 kg BOD m⁻³ day⁻¹. These changes were simply obtained by altering the proportions of settled sewage to secondary filter feed to 1:1 and 3:1 and these were put into effect on 23rd Nov. On

12th Dec. the filters were seeded each with 50 2nd/3rd instar larvae as before. Samples of effluent were taken for suspended solids analysis on 12th Dec. and then on 21st Dec. with the following results.

<u>Initial suspended solids</u> <u>mgdm⁻³</u>		<u>Suspended solids 9 days after</u> <u>seeding mdgm⁻³</u>	
<u>High load</u>	<u>Low load</u>	<u>High load</u>	<u>Low load</u>
31.11 ± 2.85	25.11 ± 2.81	64.22 ± 3.84	58.89 ± 3.49 (± = S.E.M.)

It can be seen from these results that an average increase in solids discharge of $33.11 \pm 4.95 \text{ mgdm}^{-3}$ on the high loaded and $33.78 \pm 6.90 \text{ mgdm}^{-3}$ on the low loaded filters was apparent which must reflect the effects of grazers on effluent suspended solids levels. This experiment was carried out at a reduced temperature (12°C) in order to relate to ambient temperatures. The fly plates were removed for counting on 9th Jan. 1978 (28 days from seeding in a 49 and 28 day total life cycle for M.hygropetricus and P.alternata respectively) and the results of the emergence can be seen in Table 22.

Table 22

Fly emergence - Expt. 3

<u>Filter no.</u>	<u>Original seeding</u>	<u>Loading</u>	<u>Flies recorded</u>	
			<u>M.hygroptericus</u>	<u>P.alternata</u>
A1	<u>P.alternata</u>	Low	-	10
A2	"	"	-	13
A3	"	"	-	11
A4	<u>P.alternata & M.hygroptericus</u>	"	6	6
A5	" "	"	5	9
A6	" "	"	10	8
B1	<u>M.hygroptericus</u>	"	4	-
B2	"	"	6	-
B3	"	"	13	-
B4	<u>P.alternata</u>	High	-	11
B5	"	"	-	10
B6	"	"	-	11
C1	<u>P.alternata & M.hygroptericus</u>	"	-	7
C2	" "	"	3	8
C3	" "	"	9	6
C4	<u>M.hygroptericus</u>	"	2	-
C5	"	"	8	-
C6	"	"	12	-

Table 22 summary

<u>Original seeding</u>	<u>Loading</u>	<u>Average emergences</u>	
		<u>M.hygroptericus</u>	<u>P.alternata</u>
<u>P.alternata</u>	Low	-	11.3
<u>P.alternata & M.hygroptericus</u>	"	7	7.7
<u>M.hygroptericus</u>	"	7.7	-
<u>P.alternata</u>	High	-	10.3
<u>P.alternata & M.hygroptericus</u>	"	4	7
<u>M.hygroptericus</u>	"	7.3	-

The results shown in Table 22 indicate that optimal loadings were finally achieved for the successful survival of both species as both survived to maturity albeit not in very large numbers. Due to the small emergences it was not possible to definitely show competition however certain trends were shown which may suggest this. For example on both loadings there were slight decreases in P.alternata numbers in the mixed cultures, similar decreases in M.hydropetricus were found. Very slight decreases were found in both species emergence levels in mixed culture on the low loaded filters.

This then tentatively suggests that competition may have occurred in the fairly high film conditions of the high loaded filters where M.hydropetricus was reduced to a greater extent than P.alternata in mixed culture. This also suggests that P.alternata is better adapted to survive and compete in high film conditions.

General discussion on Phase I experimental work results

From the results it is apparent that even after solving the problems of seeding with the right age larvae, handling the larvae carefully and then adjusting the temperature and loading correctly, still insufficient numbers of flies emerged to definitely postulate that competition was occurring. Obviously if greater numbers of larvae were used larger emergences would have occurred and certain trends may have been clearer, however it has already been stated that finding such large numbers of larvae when dealing with 18 filters was difficult. Therefore in phase II of the experimental work on these filters, it was decided to modify them to allow breeding within the filters thereby allowing eggs to be laid and climax population levels to arise as naturally as possible. This was achieved by fitting separate nets to the top of each filter allowing free movement of the flies, emergence estimation was carried out simply by counting the flies daily above the filters. The main advantage of this system over the system used in Phase I work would be that the organisms would be in contact with each other in all stages of their life cycles and therefore this system would show if competition occurred at any stage, not specifically from the mid-larval to adult stages as investigated previously. Other advantages apparent, which will be

discussed later, included the organisms being able to find their own maximum population densities according to the food supply and being able to study fly emergence continuously over the total life cycle.

Apart from the generally disappointing results shown in Phase I work some interesting trends were shown as regards fly emergence from mixed and pure cultures and the effects of grazing on effluent suspended solids levels. Also it should be appreciated that in order to develop the methods for Phase II work it was necessary to iron out the disadvantages found in Phase I work.

As regards fly emergence from pure and mixed cultures it has already been described that in experiment 3 both species emergence were reduced in mixed culture on both loadings compared to pure culture suggesting that competition was occurring.

The suspended solids results do show some interesting trends when analysed further. From experiments 1-3 it was found that certain suspended solids increases were apparent at various times after seeding. The increases were fairly similar from both high and low loaded filters suggesting that they were directly due to the addition of a constant number of grazers to these filters. This can be seen as follows:

<u>Experiment no.</u>	<u>Average increase in solids after seeding mgdm⁻³</u>		<u>Time sample taken after seeding</u>
	<u>High load</u>	<u>Low load</u>	
1	10.21	9.78	3 days
2	1.89	5.78	14 days
3	33.11	33.78	9 days

The magnitude of the increase according to the time each sample was taken from the initial seeding suggests some interesting points. For example it seems that solids

levels and therefore grazing activity increased up to a point between 9 and 14 days from the addition of the larvae after which time it decreased. This may be a reflection of the most common life cycle stage present at those times, i.e. it would be expected that moderate grazing activity would occur initially as young larvae were used for seeding, also that this would increase as the larvae increase in size. Likewise it would be expected that grazing activity and effluent suspended solids levels would fall as larvae pupated and this is probably what occurred in the 14th day samples. It is appreciated that more samples would be needed to fully substantiate this relationship however these results do show an interesting trend. A similar relationship between life cycle stage and solids discharge from small scale filters was described by Hawkes and Jenkins (1951) using Sylvicola and Psychoda.

It is doubtful whether direct conclusions can be drawn from this data as the filters were subjected to different temperatures throughout the period studied.

(b) Phase II Experimental work Jan. - Jun. 1978

Introduction

This phase of experimental work consisted of 2 experiments. The first experiment was simply an extension of the experiments carried out in Phase I, i.e. continuing studies into possible competition between M.hygropliticus and P.alternata. However during this experiment the dosing frequency was changed from 15 to 5 mins. which produced some interesting results. In addition to fly counting, effluent collecting was carried out and analysis was done to assess purification efficiency and suspended solids levels during that time. The second experiment in Phase II set out to investigate specifically the effects of Actellic M20 on filter efficiency. This was carried out by repeatedly subjecting the filter feed to small concentrations of the insecticide.

Experiment 1

The filters were recommissioned on 8th Feb. after the above mentioned modifications. The loadings were the same as used in Phase I except they were applied to opposite filters i.e. 0.05 kg BOD m⁻³ day⁻¹ on filters B4 - C6 and 0.12 kg BOD m⁻³ day⁻¹ on filters A1 - B3. It was considered that film levels had built up after approximately 3 weeks and on 24-26th Feb. the filters were seeded each with 25 pupae in the same species combination as used previously. Pupae were used for seeding, as they are generally resilient and can easily be handled. The temperature was set at 15°C. Effluent sampling for suspended solids commenced on 8th Mar. (10 days from seeding) and continued weekly for 4 weeks. Fly monitoring commenced on 20th Mar. and continued until 22nd June, during May and June samples of effluent were taken from all filters to investigate purification efficiency. The periodicity of dosing was changed on 25th Apr. from 15 to 5 mins. The results of these investigations are seen as follows.

i) Fly emergence

The fly emergence from the 18 filters split into groups of 3 and averaged can be seen in Figs. 114-119. A summary of the contents of these figures are as follows.

<u>Species present</u>	<u>Loading</u>	<u>Fig. no.</u>
<u>P.alternata</u>	High	114
<u>P.alternata & M.hygroptericus</u>	"	115
<u>M.hygroptericus</u>	"	116
<u>P.alternata</u>	Low	117
<u>P.alternata & M.hygroptericus</u>	"	118
<u>M.hygroptericus</u>	"	119

These results will be dealt with regarding competition, loading and dosing frequency effects and then life cycle analysis.

Dealing with competition alone it can be seen by comparing Figs. 114 and 116 with 115 and 117, and then Fig. 119 with 118 that in all cases decreased emergences of species were apparent from mixed cultures compared with pure cultures on both loadings. These results were then analysed further and treated statistically to show if the decreases were significant (see Table 23).

Table 23 Paired 't' tests. Fly emergence from pure and mixed cultures

<u>Species</u>		Change in means (pure-mixed culture)	't' value	'P' value level	Significance
<u>M.hygroptericus</u>	(low load)	12.3 ± 2.0 - 8.9 ± 1.4	3.20	0.1-1%	VHS
"	(high load)	10.9 ± 2.0 - 7.2 ± 1.2	3.90	<0.1%	VHS+
<u>P.alternata</u>	(low load)	11.7 ± 0.7 - 0.9 ± 0.1	14.72	<0.1%	VHS+
"	(high load)	9.5 ± 0.8 - 4.4 ± 0.4	6.59	<0.1%	VHS+

± = S.E.M. No. observations = 73

It can be seen from Table 23 that the reductions of P.alternata found in mixed cultures were highly significant compared to the high significance found on both of the loadings with M.hygroptericus. This immediately suggests that competition does occur between M.hygroptericus and P.alternata and that the presence of P.alternata does affect M.hygroptericus greatly, also the presence of M.hygroptericus has a drastic effect on P.alternata. The inference from these results is that M.hygroptericus exerts a greater competing force either by carnivorous tendencies against P.alternata, or by more active grazing reducing the food supply. The fact that there are reductions in M.hygroptericus emergence in mixed cultures shows that P.alternata exerts a competition pressure and it is likely that this is simply by reducing the total food supply and not by strong carnivorous tendencies, as is the case with M.hygroptericus. Therefore from these results alone competition is demonstrated and M.hygroptericus has been shown to exert a much greater force than P.alternata.

Concerning loading, the effects of this on P.alternata and M.hygropetricus emergence in pure and mixed cultures can be seen by comparing Figs. 114 with 117, 115 with 118 and 116 with 119. These results were analysed statistically, the results of which can be seen in Table 24.

Table 24 Paired 't' tests. Fly emergence from low and high loaded filters

<u>Species</u>	Change in means (Low - high load)	't' value	'P' value	Significance
<u>M.hygropetricus</u> (pure culture)	12.3 ± 2.0 - 10.9 ± 2.0	0.65	>10%	NS
" (mixed culture)	8.9 ± 1.4 - 7.2 ± 1.2	1.74	>10%	NS
<u>P.alternata</u> (pure culture)	11.7 ± 0.7 - 9.5 ± 0.8	—	—	—
" (mixed culture)	0.9 ± 0.1 - 4.4 ± 0.4	9.07	<0.1%	VHS+

± = S.E.M. No. observations = 73

From these results it is initially apparent by comparing loadings that the high one caused decreased numbers of M.hygropetricus in both pure and mixed cultures. This is as expected as it is well known that M.hygropetricus prefer lightly loaded filters. Regarding P.alternata however it would be expected that high loads would produce an increased emergence of that fly, a very highly significant increase was apparent in the case of the mixed culture, however against expectations a slight decrease was apparent from the pure cultures. The difference here may be due to extremely high film conditions building up in the P.alternata pure culture high loaded filters giving rise either to physical hindrance to fly emergence or decreased oxygen levels inhibiting the insect's development. In the mixed culture filters it is possible that the grazing action of the few M.hygropetricus prevented the film from rising to such excess levels. This again suggests that M.hygropetricus is a more effective grazer than P.alternata.

It can also be seen from the results that changes in loading affect individual species to a greater extent when they are in mixed cultures compared to when they are in pure cultures. For example in pure culture the increased load gave a slight decrease

in M.hygropetricus emergence but in mixed culture the decrease was larger. Similarly with P.alternata, although an increase was not apparent with increased load in pure culture, in mixed culture the increase was very highly significant. The possible conclusions from these results are that the different loadings gave different film levels which favoured one species more than the other i.e. allowing one species to compete more successfully with the other. Also, as before, these results suggest that either P.alternata exerts weaker competition forces, or M.hygropetricus exerts stronger forces, or a combination of both occurs as generally P.alternata are more drastically affected by loading changes both in pure and mixed cultures than M.hygropetricus.

When studying the dosing frequency changes its effects can be seen in Figs. 114-119. It can be seen that large reductions in M.hygropetricus were apparent, but there were no great effects on P.alternata emergence. The results were analysed statistically over the 30 days before the change from 15 to 5 mins. to 30 days after giving the results shown in Table 25.

Table 25 Effects of changing the dosing frequency on fly emergence
Unpaired 't' tests

<u>Species</u>	<u>Culture</u>	Change in mean (15 - 5 mins. d.f.)	't' value	'P' value	Significance
<u>M.hygropetricus</u> (low load)	Pure	21.2 ± 3.7 - 11.6 ± 1.6	1.15	>10%	NS
" (high load)	"	26.7 ± 3.3 - 7.8 ± 0.9	3.03	0.1-1%	VHS
<u>P.alternata</u> (low load)	"	12.6 ± 0.8 - 14.3 ± 0.6	0.97	>10%	NS
" (high load)	"	12.4 ± 0.9 - 9.4 ± 0.8	—	—	—
<u>M.hygropetricus</u> (low load)	Mixed	17.1 ± 2.5 - 8.4 ± 0.9	1.64	>10%	NS
" (high load)	"	17.4 ± 1.8 - 5.3 ± 0.7	3.31	0.1-1%	VHS
<u>P.alternata</u> (low load)	"	1.4 ± 0.1 - 0.5 ± 0.1	—	—	—
" (high load)	"	5.3 ± 0.4 - 5.5 ± 0.6	-1.17	>10%	NS

± = S.E.M. No. observations = 50

Firstly dealing with pure cultures it can be seen that the increase in dosing frequency gave a decrease in M.hygroptetricus emergence on both loadings (Figs. 116 and 119). As already discussed an increase in dosing frequency gives increased film levels which would be unfavourable to M.hygroptetricus. The difference in results between high and low loadings is probably explained by the increase in dosing frequency increasing the already high film levels on the high loaded filters thereby reducing M.hygroptetricus to a greater extent than with the smaller increase in film levels on the low loaded filters. It can be seen from Table 25 that the decrease in M.hygroptetricus emergence on the high loaded filters was highly significant, whereas the decrease on the low loaded filters was not significant. In the case of P.alternata the change in dosing frequency produced an increase in numbers only on the low loaded filters (Fig. 117), on the high loaded filters (Fig. 114) a decrease in emergence was noted. It is likely that the already high film levels on the high loaded filters in this case rose to excess as the dosing frequency was increased, thereby either inhibiting fly emergence or development as explained previously. As regards the low loaded filters, film levels were probably increased due to the increased dosing frequency but not to excess as the load was low, thus giving the expected rise in P.alternata emergence.

Secondly dealing with mixed cultures the results of the dosing frequency changes generally follow those described above i.e. decreases in M.hygroptetricus and increases in P.alternata however there are some effects that require explanation.

In the case of M.hygroptetricus it can be seen from Table 25 that both at high and low loads reductions in emergence were slightly greater in mixed culture than in pure culture. This may be explained by the presence of P.alternata initially in low numbers exerting a slight competition pressure on M.hygroptetricus whose numbers would be expected to be reduced to a greater extent as film levels increased, this would be due to these higher film levels favouring P.alternata and increasing the

competition pressure on M.hygroptetricus. As before greater M.hygroptetricus reductions were shown from the high loaded filters than the low loaded filters. Regarding P.alternata in mixed culture, on the high load an increase in emergence was apparent with the increased dosing frequency, unlike the decrease found in pure culture which was explained by excess film levels. In mixed culture it is possible that the M.hygroptetricus present were preventing film levels rising to excess and that the film increase apparent on changing the dosing frequency would favour P.alternata and increase competition pressure on M.hygroptetricus, thereby giving an increase in P.alternata emergence. However in the case of P.alternata on the low loaded mixed culture filters it is interesting to note that against initial expectations the increase in dosing frequency did not produce an increase in P.alternata emergence. It is likely that in this case film levels were so low that an increase was not sufficient to discourage M.hygroptetricus and favour P.alternata, in fact it probably had the opposite effect in increasing food available for the strongly competitive M.hygroptetricus allowing an increase in their numbers and thereby decreasing P.alternata numbers. This theory of very low film levels is backed up by the initial very low P.alternata levels apparent before the dosing frequency change (see Fig. 118).

Regarding life cycle analysis, if the periodicity of the adult emergence peaks is analysed from Figs. 114-119, the results obtained for those specific temperatures and insects can be compared with theoretical considerations from the work of Lloyd et al. (1940) and Uvarov 1931. According to these theories as the temperature of the experiment was 15⁰C, the time taken for development through the life cycle would be

$$(a) \text{ For } \underline{M.hygroptetricus} \quad \frac{540}{15-1} = 38.5 \text{ days}$$

$$(b) \text{ For } \underline{P.alternata} \quad \frac{315}{15-1} = 22 \text{ days}$$

From Figs. 114-119 it can be seen that generally the initial peak was well defined in the case of M.hygroscopicus however the second was not so well defined and by the third generation the pattern had all but disappeared in the case of the high load (Fig. 116), whereas a peak was appearing on the low loaded filters (Figs. 119). Also on the mixed culture filters (Figs. 115 and 118) the pattern showed up but was generally less well defined. As in the case of the pure culture filters the pattern disappeared from the high loaded filters before the low loaded ones but M.hygroscopicus had completely disappeared from these filters at the time of the third peak seen on the pure culture filters.

Regarding P.alternata, emergence peaks were only apparent from the pure cultures (Figs. 114 and 116) and these tended to gradually lose their cyclical pattern after the dosing frequency change. In mixed culture, only on the high load (Fig. 115) were P.alternata peaks observed and these were less well defined than in the pure culture situation, on the low loaded filters (Fig. 118) no such peaks were observed. The periodicity of the above mentioned peaks in relation to theoretical life cycle times can be seen in Table 26.

Table 26

<u>Species</u>	<u>Culture</u>	<u>Load</u>	<u>Mid-point of adult emergence peaks</u>	<u>Period between peaks (days)</u>
<u>M.hygroptericus</u>	Pure	High	1st generation 2nd Apr. 2nd generation 8th May 3rd generation none	36 - -
<u>M.hygroptericus</u>	Pure	Low	1st generation 9th Apr. 2nd generation 13th May 3rd generation 20th Jun.	34 38 -
<u>M.hygroptericus</u>	Mixed	High	1st generation 5th Apr. 2nd generation 11th May 3rd generation none	36 - -
<u>M.hygroptericus</u>	Mixed	Low	1st generation 7th Apr. 2nd generation 21st May 3rd generation none	44 - -
<u>P.alternata</u>	Pure	High	1st generation 30th Mar. 2nd generation 20th Apr. 3rd generation 13th May	21 23 -
<u>P.alternata</u>	Pure	Low	1st generation 29th Mar. 2nd generation 20th Apr. 3rd generation 11th May	22 21 -
<u>P.alternata</u>	Mixed	High	1st generation 27th Mar. 2nd generation 17th Apr. 3rd generation 7th May	21 20 -
<u>P.alternata</u>	Mixed	Low	none	-

Therefore from Table 26 it can be seen that the periodicity of the adult emergence peaks obtained fit in well with the expected periodicity of 38.5 days for M.hygroetricus and 22 days for P.alternata.

It should also be noted from these results that in all cases on the high loaded filters (Figs. 114, 115 and 116) the initial emergence of both species slightly preceded the initial emergence from the low loaded filters (Figs. 117, 118 and 119) suggesting that film build up was slower, as expected with a lower loading.

Generally from these results it can be seen that the peaks of emergence pattern was noticeable up to the point where the dosing frequency was changed, after which time some unusual results were noted. For example the pattern seemed to disappear with P.alternata (Figs. 114 and 117) whereas extended emergences and "daughter" peaks were obtained with M.hygroetricus (Figs. 118 and 119). It can only be assumed that these effects were caused by altering the film levels after the dosing frequency change but the actual physical reasons for these pattern changes are unclear. The production of "daughter" peaks was discussed by Lloyd (1941) and was considered to be due to temperature changes, however clearly as temperature was constant throughout the experiment the changes cannot be attributed to this. One possibility is that these cycles may only be apparent when film levels are low and than an increase in film and therefore food supply caused an increase in development of some individuals thus disguising the original pattern, or an increase in film may have caused a decrease in ventilation and oxygen supply causing a decrease in development of some individuals. Other reasons may be that the actual development time used in the calculations is an average and is not of course followed by 100% of individuals of a particular species. Therefore it would be expected that the cyclical pattern would be found initially but this would gradually get out of phase as individuals take different times to develop, so the changes found in the pattern may not specifically be due to film changes within the filters.

ii) Effluent analysis results

Regarding effluent suspended solids levels; samples were taken from the 11th to the 31st day after seeding on all filters to see if discharge levels could be related to the life cycle times of P.alternata and M.hygropetricus. The results of the suspended solids discharge from the pure cultures of these insects can be seen in Figs. 120-123 and it can be seen that these show some interesting trends. Samples were taken from the mixed culture filters but the results did not show specific trends which is probably due to the two different life cycles with different development times operating there.

In interpreting the suspended solids discharge results it should be realised that a cyclical solids pattern follows the cyclical pattern in adult fly emergence. The two patterns have the same frequency but are approximately half a cycle out of phase. This is explained by a peak in solids discharge being experienced at the period of the life cycle when larvae were most abundant i.e. at the time of maximum grazing activity (Hawkes and Jenkins 1951) and troughs in solids being experienced when grazing activity is low i.e. at adult emergence peaks. From Figs. 120 and 122 it can be seen that in the case of P.alternata there is a difference in the pattern of discharge on high and low loaded filters. On the low load (Fig. 122) the peak in solids appears approximately 18 days from seeding indicating maximum larval populations and the trough appears after approximately 31 days. However on the high load the trough appears after the 18th day indicating that this time was the period of peak adult emergence and that the peak in solids discharge and therefore larval peak occurs sometime before that. On these filters it can be seen that a second peak in solids was building up around the 31st day indicating that the second larval generation was building up. The difference in timing of the peaks in these cases can possibly be explained by the larvae being initially slower to develop in the low film conditions found on the low loaded filters.

Concerning M.hygropetricus a similar difference in peaks and troughs of solids

discharge can be seen on the high and low loaded filters however these do not match those found for P.alternata as it must be realised that the two insects have different development times. From Fig. 123 it can be clearly seen that a peak in solids occurred around the 23rd day indicating maximum larval populations. However from Fig. 121 on the high loaded filter the solids discharge gradually fell from the start of sampling indicating that the solids peak was around or before the 11th day. Again this shows a difference in timing between high and low loads probably for the same reasons described for P.alternata, however it can also be seen that the solids peaks were in both cases later for M.hydropetricus than for P.alternata showing the effects of different development times.

It is appreciated that a longer sampling period would have been necessary to give good conclusions from this part of the experiment however the results obtained do follow the expected pattern and indicate that life cycle patterns may show up simply by studying suspended solids levels in the filter effluent. It must be realised that in a large filter situation other factors would affect solids discharge such as temperature, also the discharge may be irregular due to different grazers inhabiting one filter, however a large peak in solids from a filter usually indicates a peak in grazing activity, which could be a method of predicting a future emergence without actually sampling the media.

Regarding the purification efficiency of the filters used in experiment 1, samples were taken on 6 occasions from 17th May - 22nd June from all 18 of the filters to investigate percentage BOD removal. These are averaged and expressed in Table 27.

* Details of analysis of variance test.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F'ratio	'P'value	Significance
Between filters	5	1165	233.1	1.5922	>10%	NS
Within filters	30	4392	146.4			
Total	35	5557				

Table 27

Purification efficiency of laboratory scale filters

<u>Original seeding</u>	<u>Load</u>	<u>Mean percentage BOD removal</u>						
		17 May	24 May	1 Jun.	8 Jun.	15 Jun.	22 Jun.	Average
<u>P.alternata</u>	Low	62	77.3	42.9	43.5	43.4	53.9	53.8
<u>P.alternata & M.hydropetricus</u>	"	64.5	64.5	50	43.5	53.9	66.2	52.2
<u>M.hydropetricus</u>	"	61	82.6	60	65.3	63.5	65.3	63.3
<u>P.alternata</u>	High	61.3	72.2	62.2	75.6	75.7	78.3	66.2
<u>P.alternata & M.hydropetricus</u>	"	52.1	63	65.3	37	77	73.7	56.2
<u>M.hydropetricus</u>	"	52.1	30.6	66.3	62.2	68.4	76	52.7

The results shown in Table 27 were then subjected to an analysis of variance test with the following results.*

$$F \text{ (variance ratio)} = 0.751 \text{ with } 35/5^0F$$

∴ P > 10% and no significance can be attributed to the variation in this set of results. Therefore it can be seen that no significant differences in filter efficiency were apparent during the time of sampling, none were expected as film levels never rose to excess causing no ventilation or drainage problems.

Experiment 2

At the end of the first experiment it was decided that the laboratory filter apparatus would be most useful to test the effects of Actellic M20 on filter efficiency. It was expected that a slight decrease in efficiency may arise due to a build up in film accumulation (Hawkes 1955c). Therefore 6 of the 18 filters were subjected to a feed dosed with a quantity of Actellic M20 to give a final concentration of 5 mgdm^{-3} in the fresh feed when the containers were filled. Samples of effluent were taken from

* Details of analysis of variance test.

Source of variatio	Degrees of freedom	Sum of squares	Mean squares	F'ratio	'P'value	Significance
Between filters	5	83	16.7	0.2693	>10%	NS
Within filters	18	1114	61.9			
Total	23	1197				

the 6 treated filters and the remaining 12 filters on 4 occasions and the percentage BOD removal results can be seen in Table 28.

Table 28 Effect of Actellic M20 on filter purification efficiency

<u>Date</u>	<u>Mean control % BOD removal</u>	<u>Mean insecticide treated % BOD removal</u>	<u>% change control treated</u>
28th Jun.	68.10 ± 2.38	67.35 ± 4.35	-1.10
21st Jul.	62.50 ± 2.40	54.65 ± 6.85	-12.56
27th Jul.	75.40 ± 2.65	74.60 ± 4.80	-1.06
10th Aug.	67.58 ± 0.84	66.05 ± 1.35	-2.26

(± = S.E.M.)

An analysis of variance test was carried out on the results shown in Table 28 with the following result*

$$F \text{ (variance ratio)} = 0.257 \text{ with } 23/5^{\circ}F$$

∴ P > 10% and no significance can be attributed to the differences found in these results.

Therefore the results suggest that Actellic M20 treatment does not have a significant effect on filter efficiency (see Table 28). However slight decreases in efficiency were noted on all of the sampling occasions which may be a reflection of slightly increased film levels. As for the effects of Actellic M20 on nitrification, unfortunately only negligible TON levels were obtained from these filters throughout the investigations and no specific trends were shown on the treated filters. When considering the reason why nitrification was not occurring on these filters it should be remembered that these filters represented only the top 0.356 m of a 1.83 m depth filter and many workers doubt whether nitrifying organisms are found in this top zone.

As an additional exercise the sewage feed containing Actellic M20 was sampled and subjected to analysis to determine the actual Actellic M20 concentration on 3 occasions with the following results.

<u>Time sample taken</u>	<u>Actellic conc. mgdm⁻³</u>
i) Immediately after initial addition and mixing	4.5
ii) One day after addition	0.1
iii) Two days after addition	0.1

These results therefore show that the initial dilution was fairly accurate and sufficient concentrations of insecticide were being administered to kill the larvae, however the results also show how rapidly Actellic M20 breaks down as after one day the concentrations recorded were less than 0.1 mgdm⁻³.

General discussion on Phase II experimental work results

This phase of experimental work has given some useful results which gives a better understanding of the mechanisms operating on the main filters. It has been shown definitely that competition exists between M.hydropetricus and P.alternata as suggested by Golightly (1940) and it has been proved that M.hydropetricus is the dominant organism in this relationship. According to Lloyd et al. (1940) this competition between chironomids and psychodids only takes place when food is scarce, i.e. when film levels are low, however the results of the above investigations suggest differently, in that in conditions known to be unfavourable to a particular species, the competition pressure experienced by that species is greater than in conditions favourable to that species. Regarding conditions favourable to species these have been identified as low film conditions for M.hydropetricus and the general view is that the opposite conditions favour P.alternata. On the other hand

P.alternata in pure culture here did not seem to be favoured by low or high film levels. However in mixed culture P.alternata was increased by increased film levels which suggests that an element of competition is removed allowing P.alternata to increase when M.hygropetricus numbers fall.

As M.hygropetricus has been found to compete with P.alternata in low and high film conditions this suggests that the competition may not always be for a limited food source i.e. film, M.hygropetricus may even attack P.alternata in preference to film. The carnivorous habits of these have been described by Lloyd (1945).

On the subject of dosing frequency changes, when these were investigated it was found that the increased film levels caused by this had a profound effect on M.hygropetricus but not much effect on P.alternata. As the effects of most of the modifications used on the main filters to control M.hygropetricus was to increase film levels, this suggests therefore that the increases in Psychoda found with the modifications were due not directly to an increase in film levels, but more importantly to a removal of a carnivorous competitor or even a predator in the shape of M.hygropetricus.

Regarding the cyclical pattern of fly emergence the alternation between small and large emergence peaks found by Lloyd (1943b) was not encountered suggesting that film levels were never reduced to become a limiting factor to development.

Other useful conclusions provided by these experiments are that the life cycle development times described by Lloyd et al. (1940) are followed very well in pure and mixed cultures also that effluent suspended solids levels may be used to predict future fly emergences both of which would have a great bearing in fly monitoring programmes and in the estimation of optimum dosing times. Apart from these advantages it seems that this filter system is very versatile and could be used for

investigations of many operational changes on filter ecology and efficiency e.g. loading, dosing frequency and media changes, also this system could be adapted for testing the effects of insecticides and trade wastes on filter ecology and efficiency giving ideas of the effects of these substances on a larger scale.

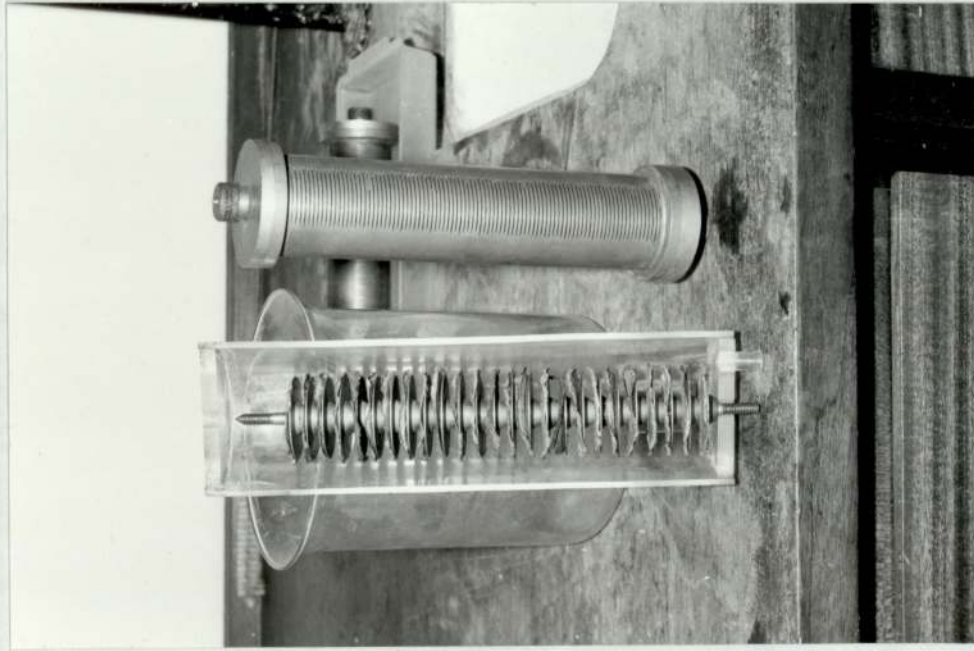


Plate 25. Suction fly trap miscellaneous equipment consisting of cloth covered discs on carrying spindle in insecticide solution bathing trough and disc collection chamber



Plate 26. *M. hygroplitricus* pupae on media in Laboratory scale filters



Plate 27. *M. hygropliticus* adults above laboratory scale filters

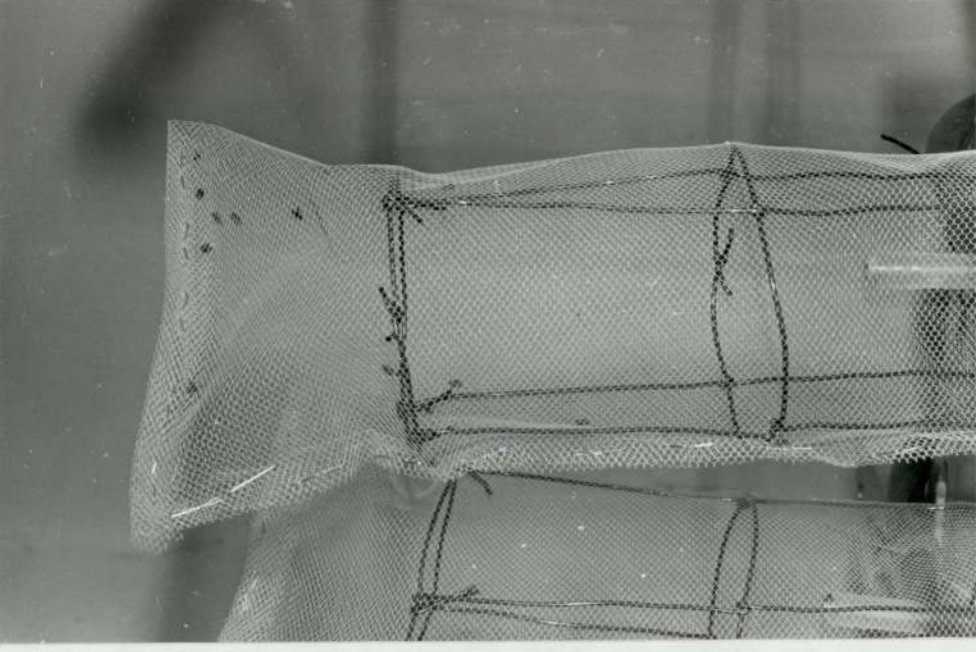


Plate 28. *P. alternata* adults above laboratory scale filters

Fig. 114. Average daily emergence of *P. alternata* from filters A1, A2 and A3 (high loading)

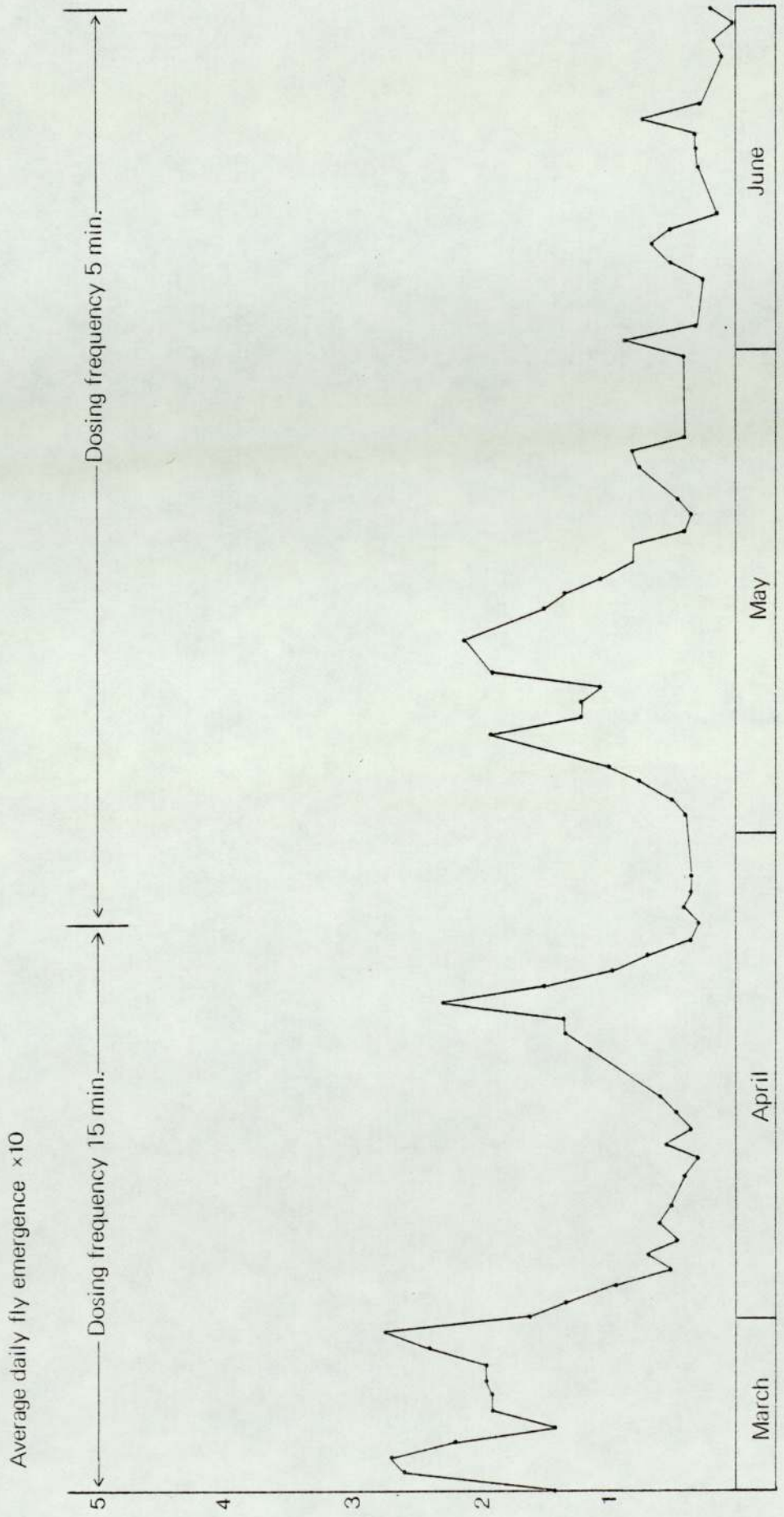


Fig. 115. Average daily emergence of flies from mixed culture filters A4, A5 and A6 (high loading)

--- *M. hygroplitricus*
— *P. alternata*

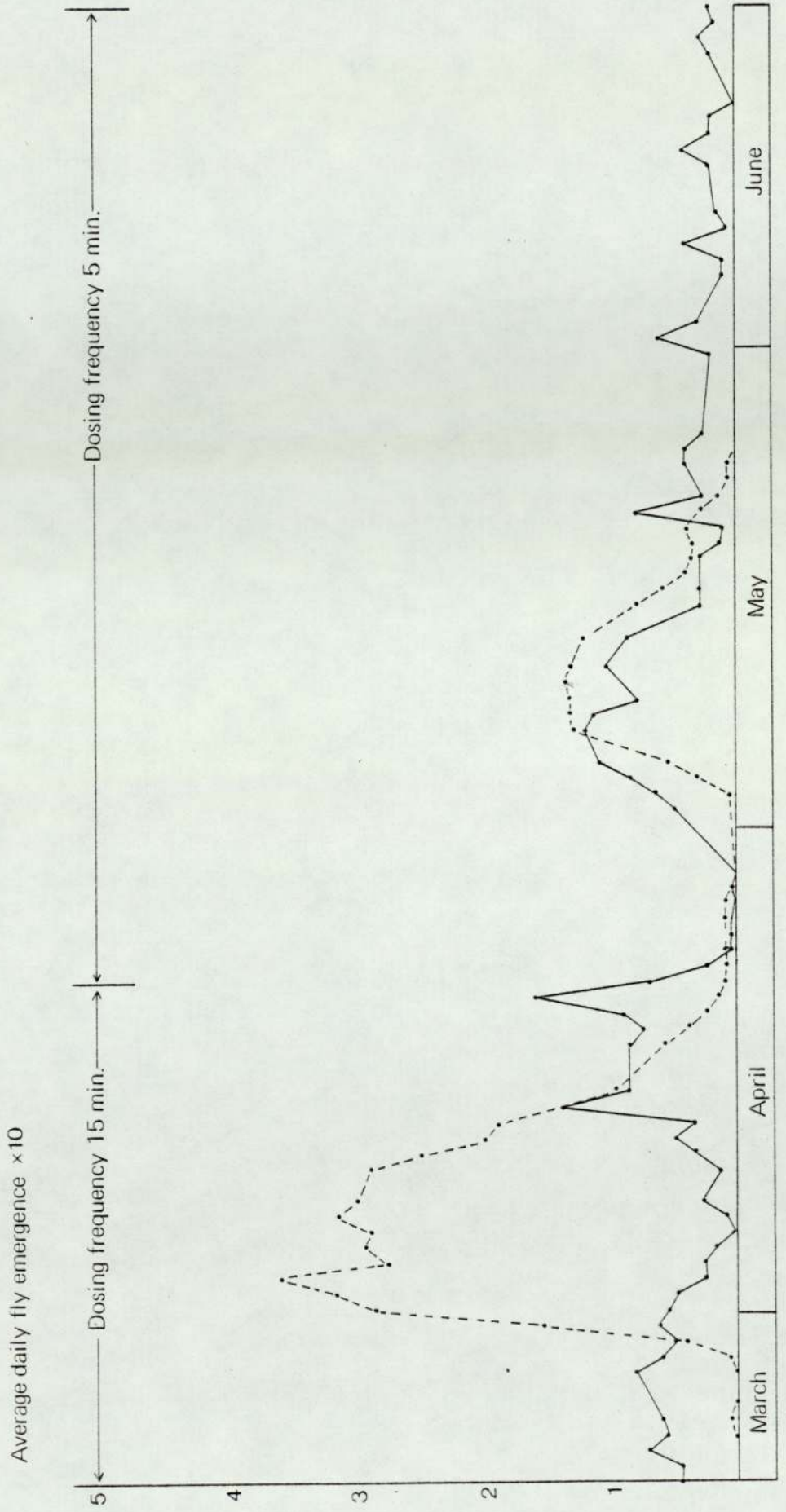


Fig. 116. Average daily emergence of *M. hygropliticus* from filters B1, B2 and B3 (high loading)

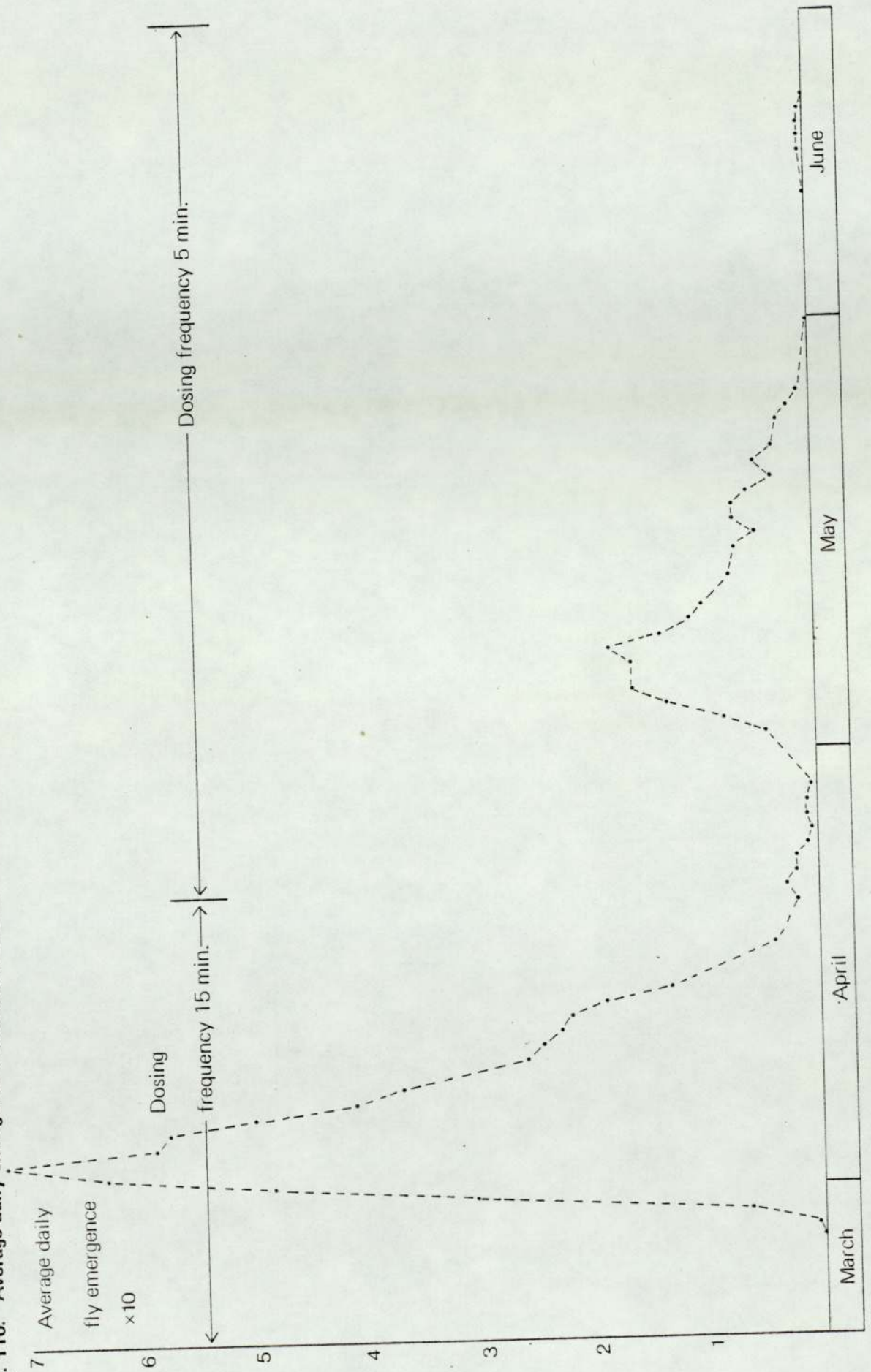


Fig. 117. Average daily emergence of *P. alternata* from filters B4, B5 and B6 (low loading)

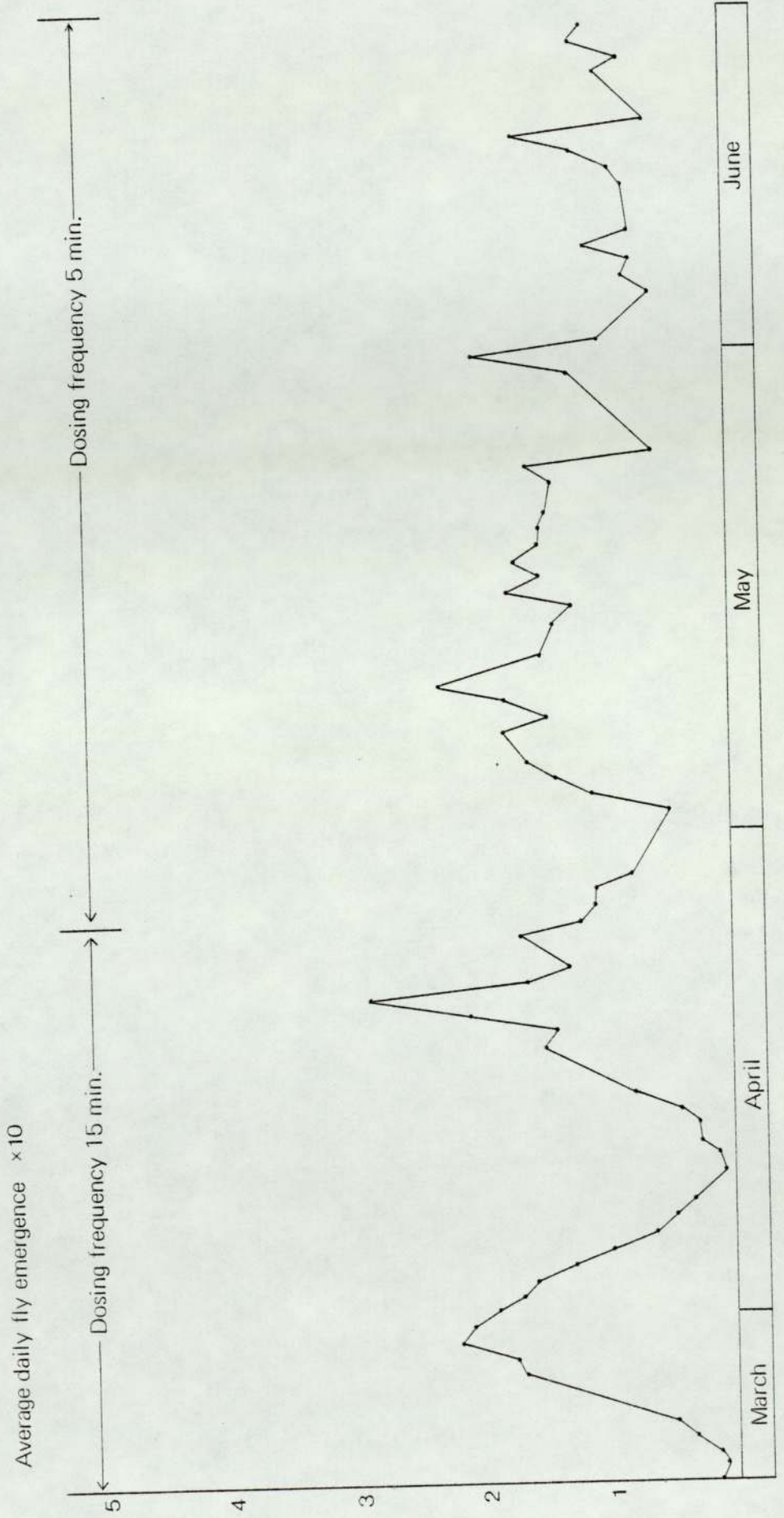


Fig. 118. Average daily emergence of flies from mixed culture filters C1, C2 and C3 (low loading)

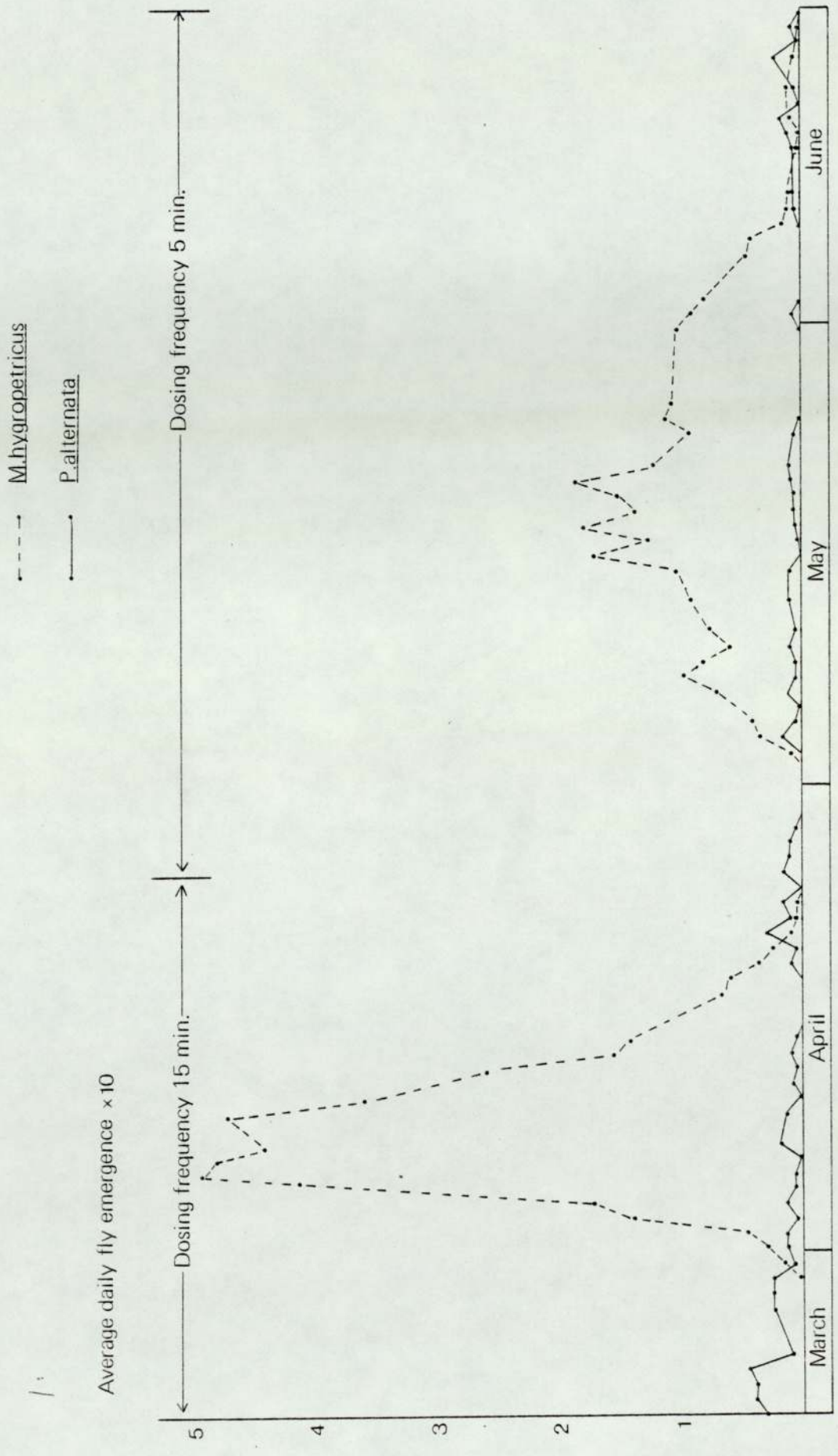
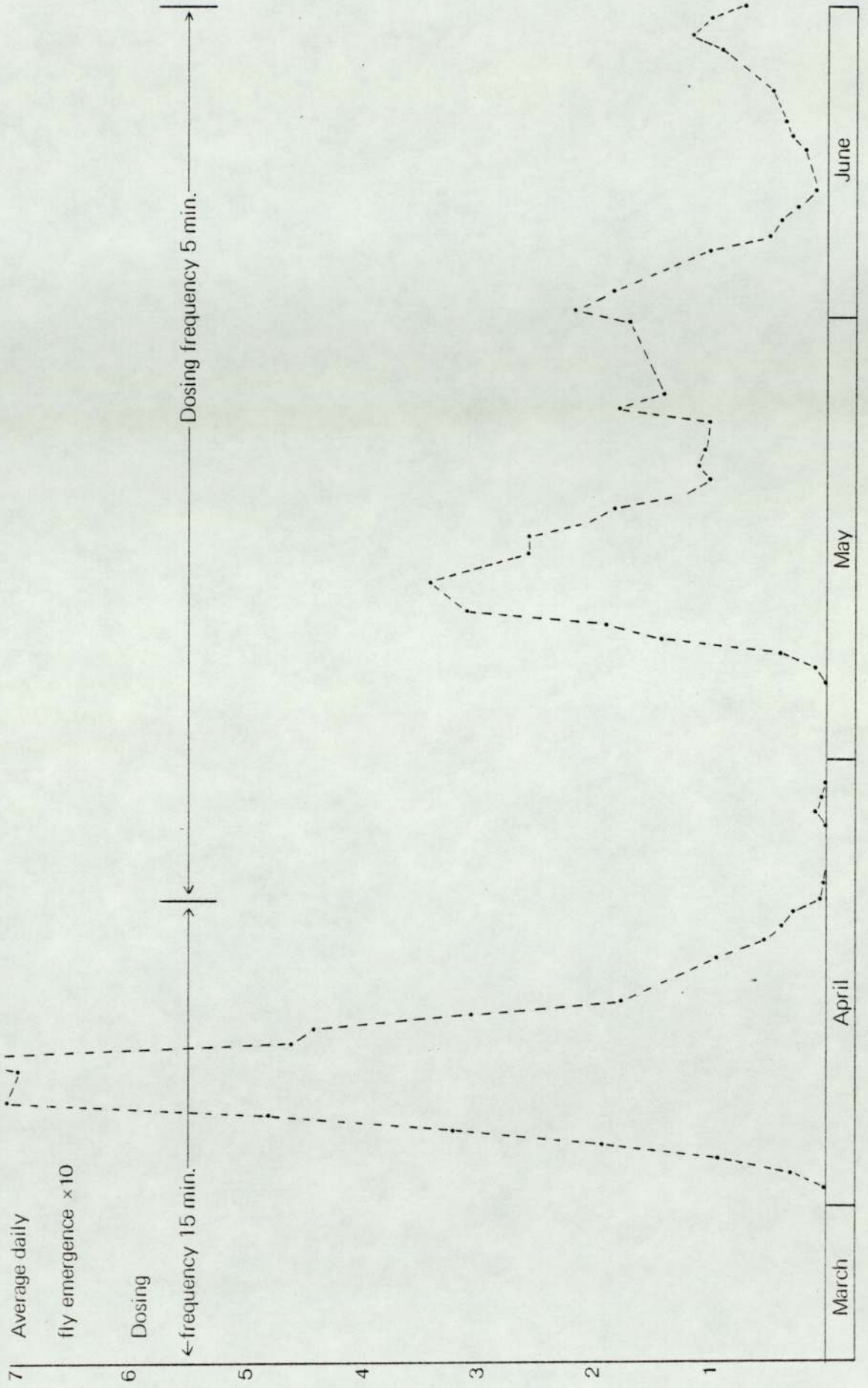


Fig. 119. Average daily emergence of *M. hygropliticus* from filters C4, C5 and C6 (low loading)



Graphs of mean effluent suspended solids from laboratory filters in relation to larval seeding time

Fig. 120. *P.alternata* (high load)

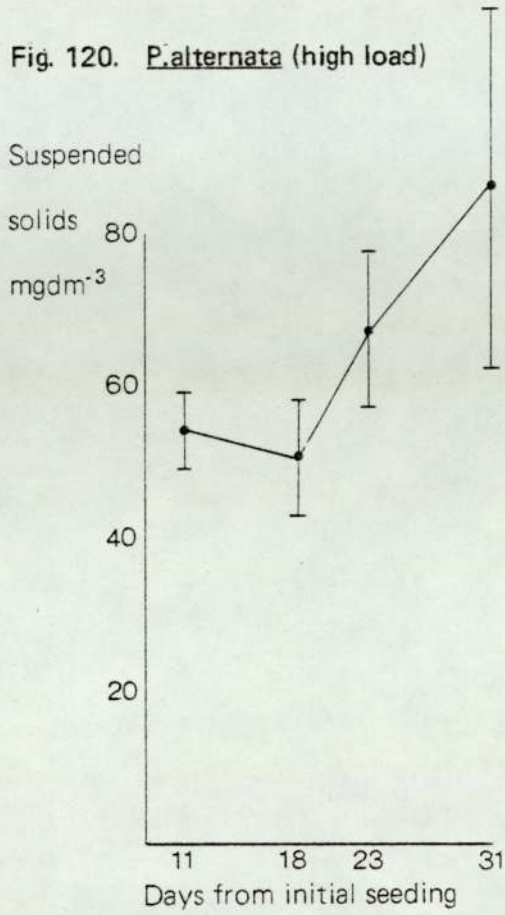


Fig. 121. *M.hydropetricus* (high load)

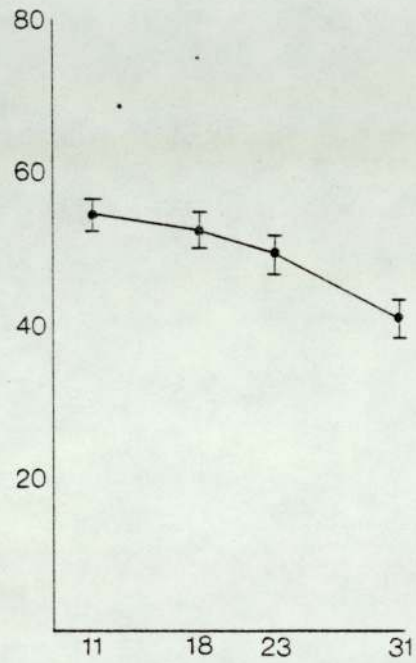


Fig. 122. *P.alternata* (low load)

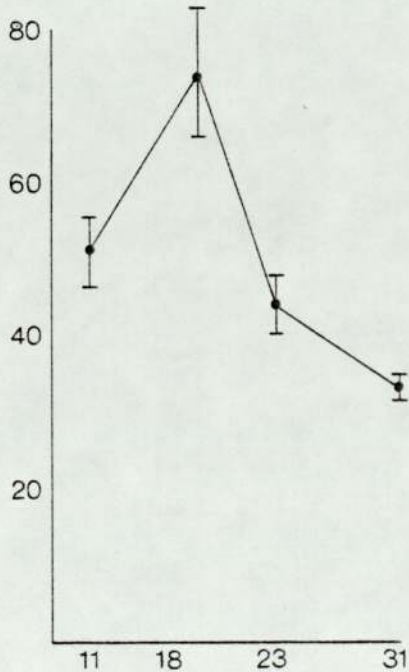
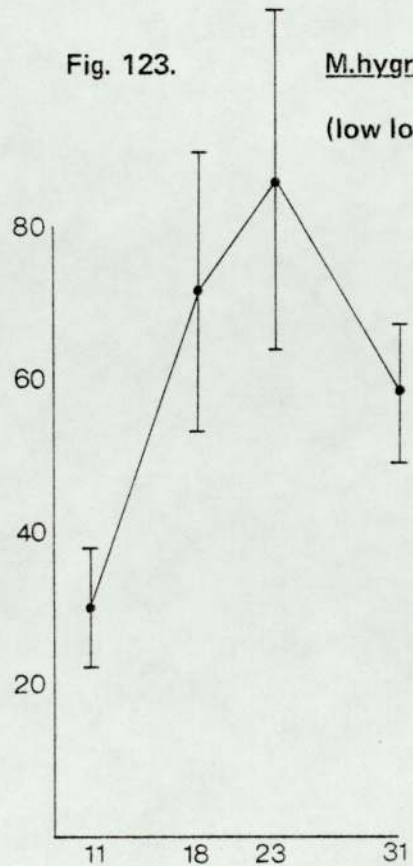


Fig. 123.

M.hydropetricus
(low load)



(\bar{I} = S.E.M.)

IV Toxicity Tests

Introduction

The need for testing a particular insecticide before its widespread use is important for many reasons. For example, although its effects on the target species may be known, its effects on non target species must be clarified as in the case of biological filters a complete removal of all grazing fauna by excessive treatment would obviously cause film accumulation and loss of efficiency problems. Other not so obvious reasons are that it is important that minimum quantities of insecticide are used, i.e. just sufficient to kill the target species and not in excess, as the ecological undesirability of insecticides and their breakdown products as regards environmental accumulation has already been stressed.

With these objectives in mind it was decided to fully investigate the toxic effects of Actellic M20 on a range of organisms including the particular target species (M.hygroscopicus) and other common grazers including P.alternata, P.severini, S.fenestralis and L.rivalis. At the time of carrying out the investigations it was intended to test the insecticide against another common grazer, O.minimus, however unfortunately this was not possible as sufficient numbers of their larvae could not be found.

The actual method of carrying out these tests has been fully described in the methods section but to briefly repeat it consisted of placing 60 larvae in petri-dishes containing filter paper pre-moistened with a hard water bathing solution containing 250 mg dm^{-3} of Calcium as CaCO_3 and containing the specific concentrations of insecticide being investigated. The larvae were then scored for mortality after specific time intervals. Control experiments using an equal number of larvae were carried out for each insecticide concentration and all of the experiments were carried out at a

temperature of 15^oC. Other workers (Green et al. 1975) used a temperature of 20^oC for worm toxicity tests and Bourton (private communication) used 10 and 20^oC for toxicity tests on P.alternata and P.severini. It was attempted to use 10^oC and 20^oC in these studies, however certain problems were encountered when using these temperatures. These were that at 20^oC grazers with a fairly short development time e.g. P.severini often pupated and sometimes emerged before the end of the experiment thus giving misleading results, also at 10^oC it was very difficult to detect movement in some of the sluggish grazers e.g. P.alternata and P.severini therefore giving problems when trying to determine positive responses to stimuli. Due to these problems it was decided to run all of the tests at 15^oC. It was also considered that this temperature was more closely related to actual filter temperatures than 10^oC and 20^oC.

One important point to mention here is that in order to obtain consistent results it was attempted to use equal age larvae for all of the tests i.e. 4th instar. The differential susceptibility of the different life cycle stages to insecticide treatment was proved in the main filter investigations, also Lloyd (in discussion of Tomlinson et al. 1949) has stated that differential susceptibilities to Gammexane were shown by S.fenestralis larvae of different ages.

Results and discussion for toxicity tests using Actellic M20

(a) The effects of film on the toxicity of Actellic M20 to M.hygroptericus

These investigations were split into two parts i.e. firstly the effects of Actellic M20 on this grazer were studied when they were in plain petri-dishes containing filter paper only, and secondly in dishes containing filter paper and film comprising 1g of dried film reconstituted with insecticide solution. The intention here was to see if film has any effect on the progression of mortality with the insecticide treated larvae. Control experiments where no insecticide was applied were carried out for

both filter paper only and filter paper and film investigations. The results of the percentage mortality during the sampling period corrected for any control deaths for 6 concentrations of Actellic M20 (0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 mgdm⁻³) can be seen in Figs. 124-129. It is apparent from these results that an inverse relationship existed between survival times and insecticide concentration as expected and the form of the mortality curves followed the expected semi-sigmoidal pattern in the first four concentrations used (Figs. 124-127). In the two lowest concentrations the sigmoidal pattern was not apparent and 100% mortality was not achieved during the experimental period (Figs. 128-129). In these cases the final reading was taken at 6000 minutes (approx. 4 days) from the start of the experiment and it should be appreciated that during such a long period it would be possible for certain factors to interfere with the accuracy of the results. For example, breakdown of the insecticide may be one source of inaccuracy, Actellic M20 was stated by Woods et al. (1978) to be extremely volatile. Other inaccuracies may be caused by the duration of the experiment approaching the time required for development through the particular life cycle stage of the insect studied and it has been shown that different life cycle stages show different susceptibilities. Another source of inaccuracy in long term tests which may lead to high mortality is that the larvae are devoid of any food source and therefore may be subject to stresses.

From Figs. 124-129 it can be seen that in all cases the larvae in the dishes containing the film gave a lower progression of mortality than the other larvae in the filter paper only dishes. The reason for investigating the effects of film on insecticidal action was to clarify which route the insecticide entered the larva's body i.e. cutaneous via body contact or via ingestion and contact. According to Thompson (1975) Actellic M20 is primarily a contact toxicant, however as insecticides have a great affinity for adsorption by humic substances e.g. film (Khan 1972) it would not be unreasonable to assume that Actellic M20 may be adsorbed by film and then ingested. Clearly these results* suggest that film in that case was not ingested as

* figs. 124 - 129

lower mortality rates were obtained in all cases in the film dishes.

The reasons for this are not clear, it could be that the larvae do not graze after the shock of transference or in the unnatural environment of the petri-dish, or more likely that the insecticide affected film is in some way unpalatable to the larvae. Although these results seem to suggest the entrance route of Actellic M20 is primarily by body contact they do show how the insecticide is readily adsorbed by film and this has important implications for example if treatment is carried out on filters with high film levels the associated high moisture levels would have the effect of diluting the insecticide feed, thus the larval kill may not be as large as expected. If the differences in mortality between larvae from dishes containing filter paper to larvae from dishes containing filter paper and film are studied further it is apparent that the mean differences in cumulative percentage mortalities between larvae from film and filter paper only generally increases with decreasing insecticide concentrations. For example at 10 mgdm^{-3} the mean cumulative percentage mortality over the last four readings in the film larvae was 45% of the values obtained for the non-film larvae. This percentage generally decreased with decreasing concentration i.e. at 5 mgdm^{-3} it was 39%, at 2.5 mgdm^{-3} it was 41%, at 1 mgdm^{-3} it was 37% and at 0.5 mgdm^{-3} it was 21%. Therefore it seems that the film has more effect on reducing the larval kill at lower concentrations of insecticide suggesting that at these low levels the film may disguise the effect of the insecticide altogether.

The actual mechanism by which the film absorbs the insecticide is not clear. Obviously it is not by absorption by living components of the film as the film used in this case was biologically "dead". The processes involved according to Khan (1972) concern mainly hydrogen bonding and ion exchange, however Khan states that other physical adsorption processes such as Van der Waals forces and hydrophobic bonding cannot be ruled out.

* Another method used for treating the results was that described by Litchfield and Wilcoxon (1949). This involved calculating the percentage mortality results in 24 hours (from the log/probability plots) and plotting these against their respective concentrations on log/probability graph paper. From these plots and the nomographs contained in Litchfield and Wilcoxon (1949) 24 hour LC50's with 95% confidence limits could be calculated.

The results shown in Figs. 124-129 need treating further to give an indication of the median lethal doses of insecticide required to kill the larvae. One method* used for treating these results was that of Bliss (1937) which has been fully described in the methods section. To summarise this involved taking the cumulative percentage mortality results and plotting them against time on Log/probability graph paper. For M.hydropetricus with and without film this produced a series of straight lines for each concentration used (see Figs. 130 and 131). From these graphs the median survival times for each concentration were plotted against dose on Log/log graph paper producing characteristic curves (see Figs. 132band 133b) from which the median lethal dose of insecticide required to kill 50% of the larvae (LC50) can be read off for specific times. For M.hydropetricus it can be seen from Figs. 132band 133bthat the 48 hour LC50 for Actellic M20 were 0.75 and 0.3 mgdm⁻³ for filter paper only and filter paper and film respectively. This shows how the film reduces the insecticide's effectiveness and this is even more important when it is considered that a 117% increase in insecticide concentration was needed to give the same kill as obtained without the film.

The curves obtained in Figs. 132band 133bfollow a characteristic pattern and certain information can be drawn from this. With toxic substances as the concentration decreases the curve generally becomes asymptotic to the median survival time axis as most organisms can develop a tolerance of low concentrations of these substances. Substances which produce curves like this are those which usually have a low background concentration in the environment e.g. ammonia, however with other substances which do not occur naturally in the organism's environment the curve does not become asymptotic to the median survival time axis. This situation can be seen in Figs. 132band 133band it is explained by the animal being unable to develop any tolerance to the substance or by the inability of the animal to break down or eliminate these substances. According to Jackson and Brown (1970) this form of curve is common with pesticides.

To summarise, these results indicate that film presence reduces the toxicity of Actellic M20 to M.hygropetricus as higher concentrations were needed to give certain percentage mortalities with the film treated larvae than the non-film larvae, also it is most likely that this insecticide is adsorbed onto the film by physical processes. In addition Actellic M20 seems to act as a contact toxicant and has no need to be injected as progression of mortality was more rapid when the larvae were in contact with the insecticide alone compared to the insecticide affected film.

(b) The effects of Actellic M20 on common filter grazers

Experiments similar to the ones described above were carried out on a range of common filter grazers. The results obtained from these were treated in a similar graphical manner as above and the information from this treatment together with the results can be seen as follows.

(1) M.hygropetricus

	<u>Concs. Investigated</u> (mgdm ⁻³)	<u>Correlation coefficient (r)</u>	<u>"P" value of straight line fit %</u>
<u>Probability plot (Fig. 130)</u>	0.25	0.961	P = 0.1-1
	0.5	0.993	P < 0.1
	1	0.997	P < 0.1
	2.5	0.982	P = 1-2
	5	0.985	P = 1-2
	10	0.992	P = 0.1-1

Litchfield and Wilcoxon plot Fig. 132a.
24 hr. LC50 = 0.52 (0.36 to 0.76) mgdm⁻³

Bliss plot Fig. 132b.
24 hr. LC50 = 0.36 mgdm⁻³

(2) P.alternata

	<u>Concs. Investigated</u> (mgdm ⁻³)	<u>Correlation coefficient (r)</u>	<u>"P" value of straight line fit %</u>
<u>Probability plot (Fig. 134)</u>	100	0.998	P = 2-5
	50	0.934	P > 10
	40	0.999	P < 0.1
	30	0.990	P < 0.1
	20	0.980	P = 0.1-1
	10	0.972	P = 0.1-1

Litchfield and Wilcoxon plot Fig. 135a.
24 hr. LC50 = 48 (36 to 67) mgdm⁻³

Bliss plot Fig. 135b.
24 hr. LC50 = 53 mgdm⁻³

(3) P.severini

	<u>Concs. Investigated</u> (mgdm ⁻³)	<u>Correlation</u> <u>coefficient (r)</u>	<u>"P" value of</u> <u>straight line fit %</u>
<u>Probability</u> <u>plot (Fig. 136)</u>	75	0.995	P < 0.1
	37.5	0.999	P < 0.1
	20	0.988	P = 1-2
	10	0.976	P = 1-0.1
	5	0.975	P = 1-0.1
	2.5	0.908	P = 2-5
	1.25	0.992	P = 5-10

Litchfield and Wilcoxon plot Fig. 137a.
24 hr. LC50 = 5.0 (2.9 to 8.5) mgdm⁻³

Bliss plot Fig. 137b.
24 hr. LC50 = 4.1 mgdm⁻³

(4) S.fenestralis

	<u>Concs. Investigated</u> (mgdm ⁻³)	<u>Correlation</u> <u>coefficient (r)</u>	<u>"P" value of</u> <u>straight line fit %</u>
<u>Probability</u> <u>plot (Fig. 138)</u>	40	0.989	P = 1-2
	20	0.970	P = 2-5
	10	0.987	P = 0.1-1
	5	0.995	P = 0.1-1
	2.5	0.977	P = 2-5
	1.25	0.991	P = 0.1

Litchfield and Wilcoxon plot Fig. 139a.
24 hr. LC50 = 4.8 (3.1 to 7.3) mgdm⁻³

Bliss plot Fig. 139b.
24 hr. LC50 = 3.45 mgdm⁻³

(5) L.rivalis

	<u>Concs. Investigated</u> (mgdm ⁻³)	<u>Correlation</u> <u>coefficient (r)</u>	<u>"P" value of</u> <u>straight line fit %</u>
<u>Probability</u> <u>plot (Fig. 140)</u>	250	0.993	P = 0.1-1
	100	0.995	P < 0.1
	50	0.985	P = 1-2
	25	0.998	P = 0.1-1
	10		(No values as only 2 points plotted)

Litchfield and Wilcoxon plot Fig. 141a.
24 hr. LC50 = 35 (24.3 to 50.4) mgdm⁻³

Bliss plot Fig. 141b.
24 hr. LC50 = 27.5 mgdm⁻³

It can easily be seen by comparing the Bliss method 24hr LC 50 that Actellic M20 is most effective against M.hydropetricus (0.36 mgdm⁻³) followed by S.fenestralis (3.45 mgdm⁻³), P.severini (4.1 mgdm⁻³), L.rivalis (27.5 mgdm⁻³) and P.alternata (53.0 mgdm⁻³). Similarly the straight line probability plots (Figs. 130, 134, 136, 138

and 140) when treated statistically most produced significant values for the correlation coefficient (corresponding to "P" values of 10% or less), therefore the lines plotted could be considered as an accurate representation.

Therefore it is apparent that Actellic M20 removes the target species M.hygropetricus effectively and could have uses against other flies i.e. P.severini and S.fenestralis which have both been reported to be nuisances in the past. However its effectiveness is put into perspective when it is considered that the 24hr LC50 of both of these species were approximately ten fold that of M.hygropetricus indicating that much higher concentrations of insecticide would be needed to control these species. Therefore it is doubtful whether control of P.severini and S.fenestralis by Actellic M20 would be viable on an economic basis.

As regards P.alternata it is clear that this species is not particularly susceptible to the insecticide and therefore control of this species would not be effected by its use. The reason for differing susceptibilities of the species studied is not clear. It was shown by Rosla t seva et al. (1975) that the permeability index of the cuticle (ratio of LD50's for topical v. injection application of organophosphates) was much higher for susceptible than resistant strains of M.domestica and this index was shown to be proportional to the amount of wax present on the cuticle of the strains studied. Therefore the differences found may be due to the particular cuticular permeabilities of the species studied. It is anticipated that this factor would be important as it was suggested in previous studies that the main route of entry to the larvae was via body contact and not orally.

As regards L.rivalis the results show that this organism is in fact slightly affected by Actellic M20 however the 24hr LC50 obtained for this species was approximately 100 times that found for M.hygropetricus so it is unlikely that this species would be affected at the concentrations used for M.hygropetricus control. Other workers have

used worms in toxicity tests. Green et al. (1975) used worms with industrial toxicants, however no mention was made of the mechanisms of these chemicals. Whitten and Goodnight (1966) used the organophosphates malathion and parathion on worms including Tubifex sp. and obtained 24hr LC₅₀ of 26.5 and 8.6 mgdm⁻³ for malathion and parathion respectively. They found classical acetylcholinesterase inhibition symptoms of hyperactive muscular spasms and convulsions as were found in the tests on L.rivialis suggesting that nervous transmitter substances are similar in worms and insects, however it is interesting to note that worms generally are less susceptible to organophosphates than insects. Possible reasons for this were outlined by Laverack (1963) and concern unusually large amounts of acetylcholinesterase present in the body wall of worms. Thus larger concentrations of organophosphate would be needed to tie up the large amount of enzyme present. Another possible reason, according to Laverack (1963), for the low susceptibility of worms is that they may lack a specific system for converting the thionophosphates in organophosphates to active acetylcholinesterase inhibitors which is the sequence of events that occurs in insect larvae.

Considering the above mechanisms it is possible that the differences in susceptibility found in insect larvae may be due to a combination of cuticular permeability, acetylcholinesterase presence in the body wall and thionophosphate conversion mechanisms. The actual mechanisms of organophosphate action apart from its acetylcholinesterase effects were outlined by Gerolt (1976) and concerned mainly water loss from the insect's cuticle. He suggested that after the insecticide has entered the cuticle it spreads to the intestinal and tracheal systems causing a decrease in respiration. The interference with water balance in the tracheal system affects respiratory gas exchange and control and this causes the insect to react with symptoms of excitation and paralysis. Thus it was suggested that the ultimate cause of death was degeneration of the vital tissues of the insect following a massive water loss.

As far as can be established there seems to be no published literature concerning Actellic M20 and L.rivalis therefore comparisons between this study and other peoples work cannot be given.

In conclusion the results suggest that the nuisance species (M.hydropetricus) is susceptible to low concentrations of Actellic M20 and therefore could be controlled by its application. It is apparent that two other flies both of which can cause problems (S.fenestralis and P.severini) could also be controlled but would need greater quantities of insecticide, however it is doubtful whether another nuisance species (P.alternata) could be controlled as much higher quantities of insecticide would be needed causing great expense. It is also shown from the results that L.rivalis are affected by Actellic M20 even in moderate concentrations suggesting that enchytraeid worm nervous systems utilise similar transmitter substances to the nervous systems of insects.

(c) Comparison of susceptibility of M.hydropetricus
to Actellic M20 from previously treated and untreated
sites with reference to possible resistance build up

At the start of the insecticide treatment programme the importance of resistance build up was not appreciated and on further research into the literature during the toxicity testing experiments it was found that resistance to organophosphatse was a far more common phenomenon than was originally expected. Therefore it was decided to subject M.hydropetricus larvae from different locations to a similar test as described previously, one location had been subjected to treatment with Actellic M20 for 3 years (Tamworth W.R.W.) and the other location had not received any treatment during that time (Airewas W.R.W.). As time was at a premium at this stage of the research project only one concentration of Actellic M20 was investigated, that being 1 mgdm^{-3} .

The method of testing was identical to that used previously and in this case to give more accurate results 120 larvae were used from each location i.e. 240 in total compared to 120 in total in previous tests. As before the cumulative percentage mortality was plotted against time on Log/probability graph paper giving the results shown in Fig. 142 (this graph depicts the results of the original test on Tamworth larvae in 1977 compared to the results of the tests in May 1978 on Tamworth and Alrewas larvae). It can be seen from these results that at the time of the test (May 1978) the Alrewas larvae showed a different susceptibility to the Tamworth larvae, the 50% mortality stage was reached in 115 mins. for the Alrewas larvae compared to 345 mins. for the Tamworth larvae. It is also noticeable that a slight difference in susceptibility can be seen between Tamworth larvae tested in November 1977 (50% mortality in 245 minutes) compared to those tested in May 1978 (50% mortality in 345 minutes).

The implications of these results are of practical significance in that a definite difference in susceptibility can be seen between larvae from filters which had never experienced treatment suggesting that resistance to Actellic M20 treatment was building up. The differences in susceptibility of Tamworth larvae between November 1977 and May 1978 also seems to suggest that resistance was building up. To prove resistance build up tests would need to be carried out using different concentrations of insecticide, as described by Tsukamoto (1963) and W.H.O. (1975) but unfortunately time would not allow this. On the other hand, as mentioned previously, the resistance phenomenon most especially to organophosphates has been well documented recently. For example Georghiou et al. (1973) found a decrease in the susceptibility after each treatment period and more importantly after each year of treatment of the mosquito Anopheles albimanus^{Wied.} to a whole range of organophosphates including parathion, methyl-parathion, malathion and fenitronthion all of which have a similar structure to pirimiphos-methyl (see table 3). Similar resistance build up to organophosphates has been reported in California by Gutierrez et al. 1976 studying the mosquito Aedes

nigromaculis^(Ludl.), Culex tarsalis^{Coq.} and Culex pipiens^{L.} and in Scotland by Devonshire et al. (1977) studying Myzus persicae^(Sulz.) on potato plants. More importantly resistance specifically to pirimiphos-methyl (the active ingredient of Actellic M20) was reported by Attia (1976) studying Cadra cautella^(Wik.) in New South Wales, Australia.

Although it is usually assumed that resistance only builds up to a single compound at any one time this is not always the case as reports of cross resistance have been found, i.e. the resistance of an organism to one compound being conferred to a whole range of compounds of similar structure. Such cases have been reported by W.H.O. (1976) and Micks and Rougeau (1977). An alarming case of this phenomenon was reported by Georghiou et al. (1975) studying the mosquito Culex pipiens quinquefasciatus^{auct.} which had become resistant to the organophosphate chloropyrifos. It was found on further testing that the larvae had developed a broad spectrum resistance to 5 other organophosphates including abate, parathion, malathion, fenitrothion and methyl-parathion. In this case extensive studies were undertaken to elucidate this problem and it was found that resistance could be overcome by the addition of certain compounds comprising the following:- the synergist DEF *, 2 synthetic pyrethroids - cismethrin * and biopermethrin *, 2 carbamates - CRC 11786 * and CRC 11783 *, 2 insect growth regulators - methoprene and dimilin and a certain biodegradable DDT analogue * (The chemical names of the compounds marked * can be seen in Table 29).

Table 29 - Chemical names of compounds used to overcome resistance

<u>Cismethrin</u>	(5 benzyl - 3 - furyl) methyl (1R - cis) chrysanthemate
<u>Biopermethrin</u>	3 - phenoxybenzyl - d - trans - 2, 2 - dimethyl - 3 - (2, 2 - dichlorovinyl) cyclopropane - carboxylate.
<u>CRC 11786</u>	2, 2 - dimethyl - 2, 3 - dihydro - 7 - benzofuranyl - methyl (4 - tert-butyl - 2 - methylphenylthio) - carbonate
<u>CRC 11783</u>	0 - isopropoxyphenyl methyl (4-tert - butyl - 2 - methyl - phenylthio) - carbamate

DDT analogue 2 - (p-tolyl) - 2 - (p-ethoxyphenyl)-1, 1, 1-trichloroethane

Thus it can be seen that resistance can be overcome by the addition of complex organic compounds but obviously this is ecologically undesirable as these compounds are not naturally occurring, also if resistance is caused by subtle genetic changes it is possible that given time the organisms may build up a resistance to these compounds as well.

The main resistance mechanisms seem to involve certain enzyme mutations or increases in their activities. It was the view of Devonshire (1975) that the development of an insensitive form of acetylcholinesterase (in a form which would not be blocked by the insecticide) was the main mechanism. He found that enzyme extracted from resistant Musca domestica was more slowly inhibited than that extracted from susceptible flies. Baker (1977) on studying resistance in the aphid Myzus persicae found an association between acetylcholinesterase activity and resistance to organophosphates, 3 times greater enzyme activity was found in populations subjected to 2 treatments of insecticide suggesting that the enzyme was produced in larger quantities as a result of treatment. Other workers however have suggested that insensitive acetylcholinesterase is not the major mechanism of resistance. Guzman-Varnon et al. (1974) extracted acetylcholinesterase from larvae from low, moderate and high organophosphate dosage areas and were of the opinion that the small differences in enzyme activity found were not great enough for this process to be the sole mechanism of resistance. It was the view of Sudderuddin and Tan (1973) that resistance mechanisms may involve other enzymes including Carboxylic ester hydrolases, similarly Motoyama and Dauterman mention differences in activity of these enzymes along with others including phosphatases and glutathione transferases extracted from resistant strains. It was also the view of the above workers that non specific esterases found in resistant strains may actually degrade organophosphates.

Much work has been carried out on the genetics of organophosphate resistant M.domestica (SKA strain) and Sawicki (1973) found the following chromosomes to be involved with resistance mechanisms. These were chromosome 2 controlling glutathione-s-ethyl transferase and phosphate production, chromosome 3 controlling a non-specific penetration delaying mechanism and chromosome 5 controlling microsomal detoxication of insecticide. The latest reports of resistance mechanisms appeared from Oppenoorth et al. (1977) whose findings seem to be quite similar to those described previously suggesting that resistance is due to insensitive acetylcholinesterase, high glutathione-s-transferase and insecticide degradation by certain hydrolytic esterases.

Therefore in conclusion it is apparent that resistance to Actellic M20 by M.hydropetricus was being suggested after 3 years of treatment. It is likely that resistance mechanisms are numerous and not completely understood but they seem to involve subtle changes in enzyme structure or activity. It is interesting to note that the physical characteristics of resistant M.domestica were studied by Rosla tseva et al. (1975) and simple changes such as thicker epicuticle wax on resistant strains were found, this was found by washing the wax off with a solvent and on analysis it was found that the wax from resistant and susceptible strains was identical in chemical composition, the only difference was that greater amounts were found from the resistant strains. In such a case it is doubtful whether insecticides are directly affecting the gene controlling wax production, the most likely explanation is that selection is occurring for individuals with thick wax coverings, therefore it seems the resistance mechanisms may concern such various factors as simple physical features to genetically controlled enzyme activities.

Fig. 124. Progression of mortality with time for *M.hygropertricus* subjected to Actellic M 20

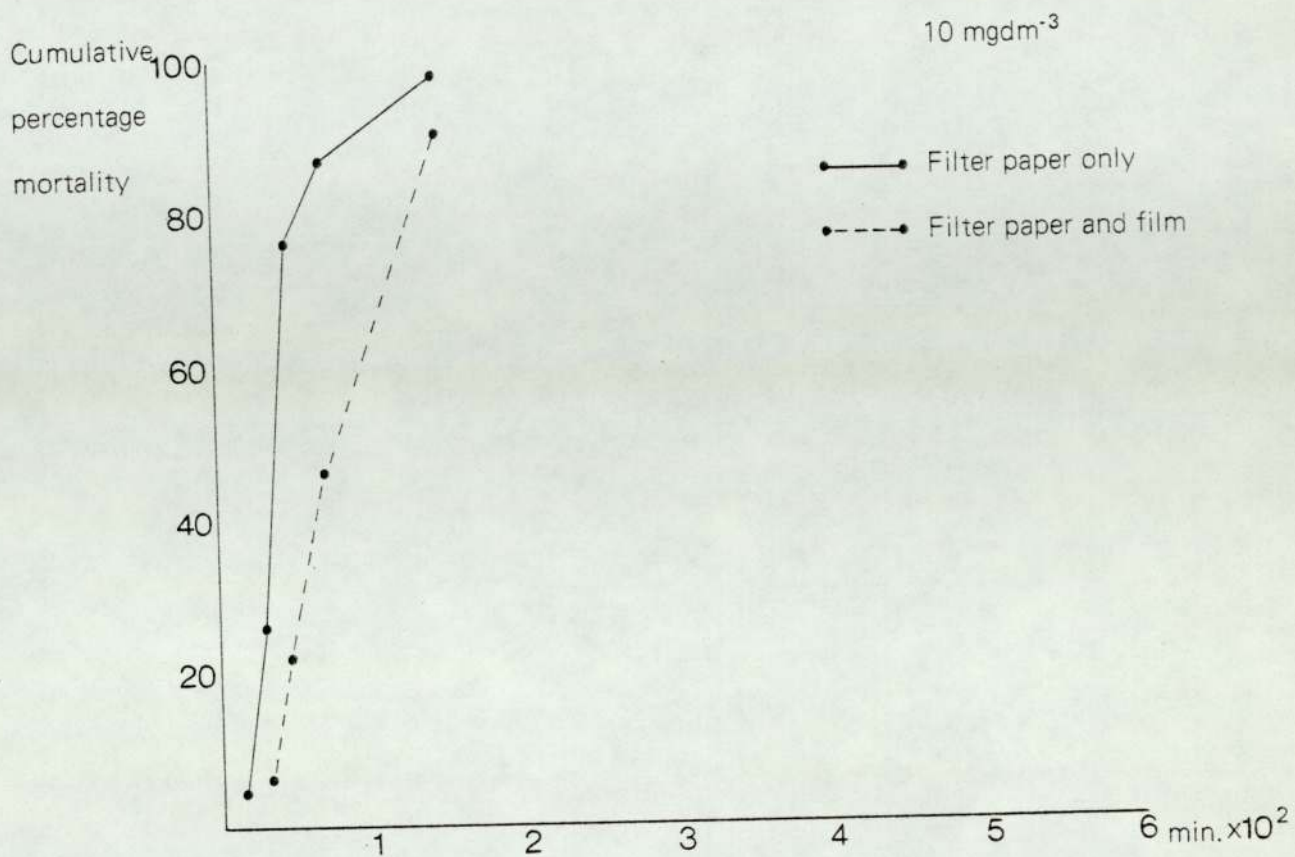


Fig. 125. Progression of mortality with time for *M.hygropertricus* subjected to Actellic M 20

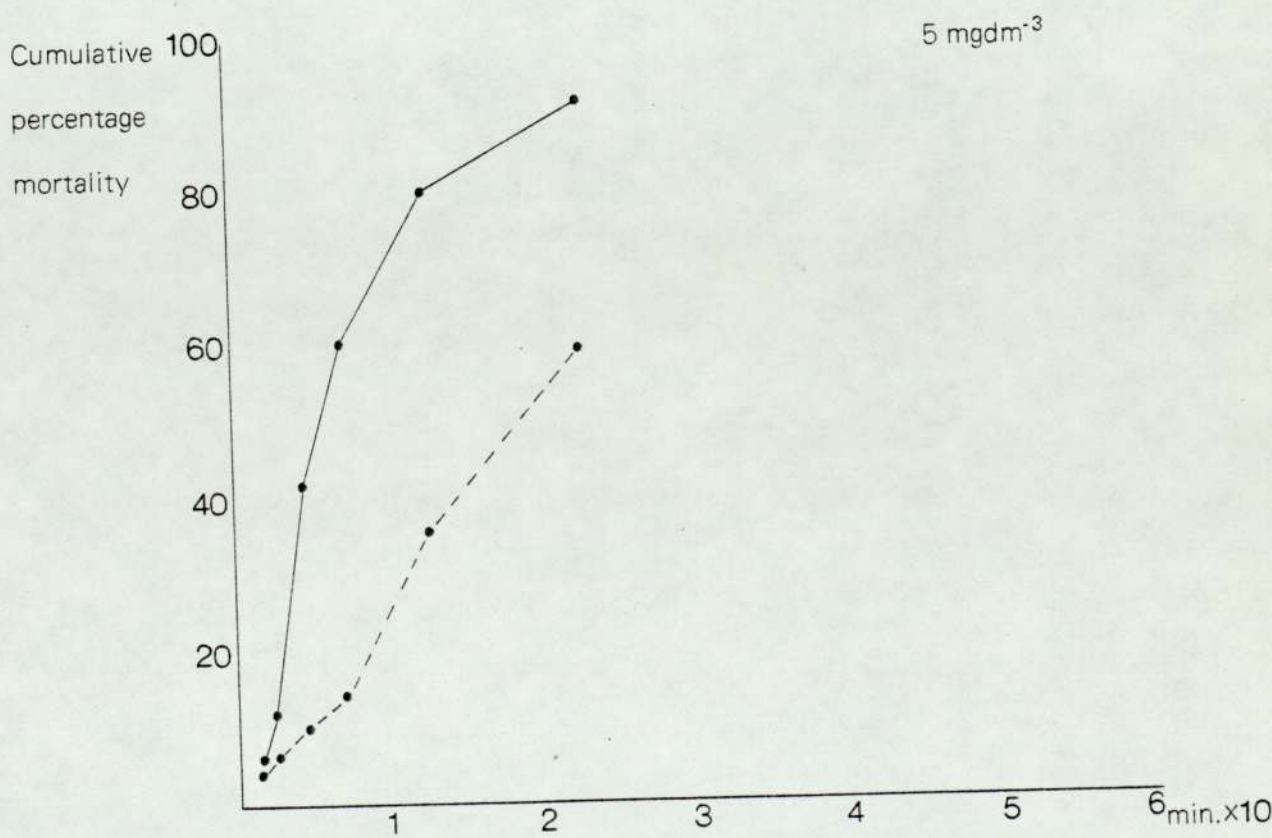


Fig. 126. Progression of mortality with time for *M.hygropetricus* subjected to Actellic M20

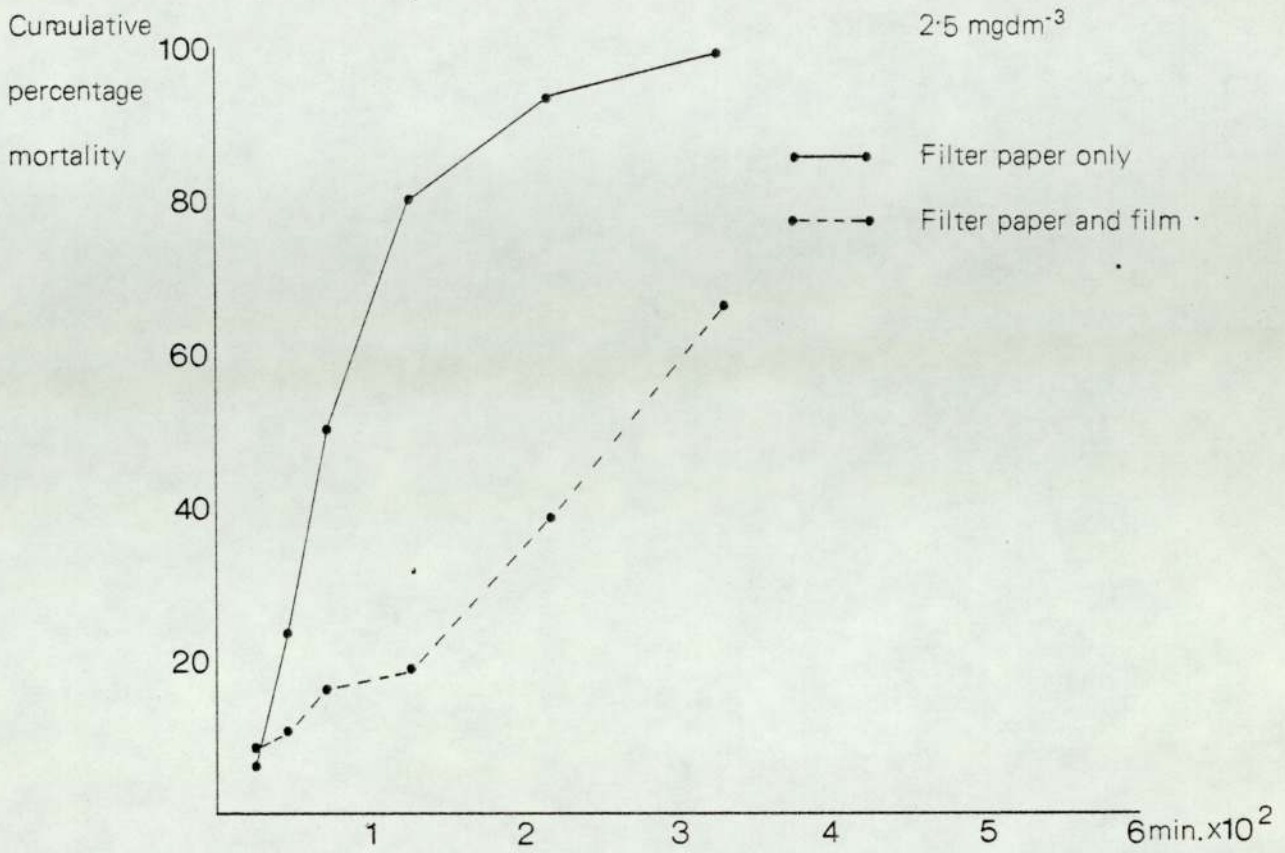


Fig. 127. Progression of mortality with time for *M.hygropetricus* subjected to Actellic M 20

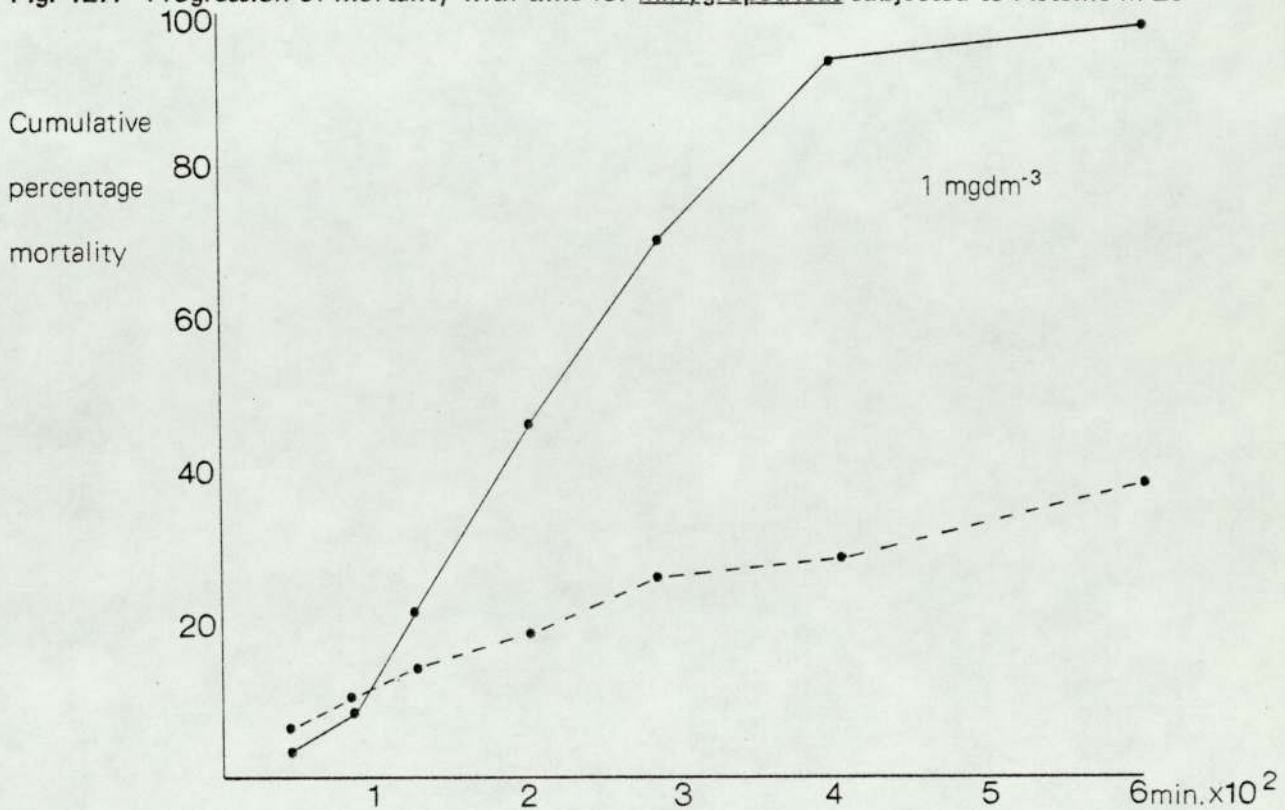


Fig. 128. Progression of mortality with time for *M.hygropetricus* subjected to Actellic M 20

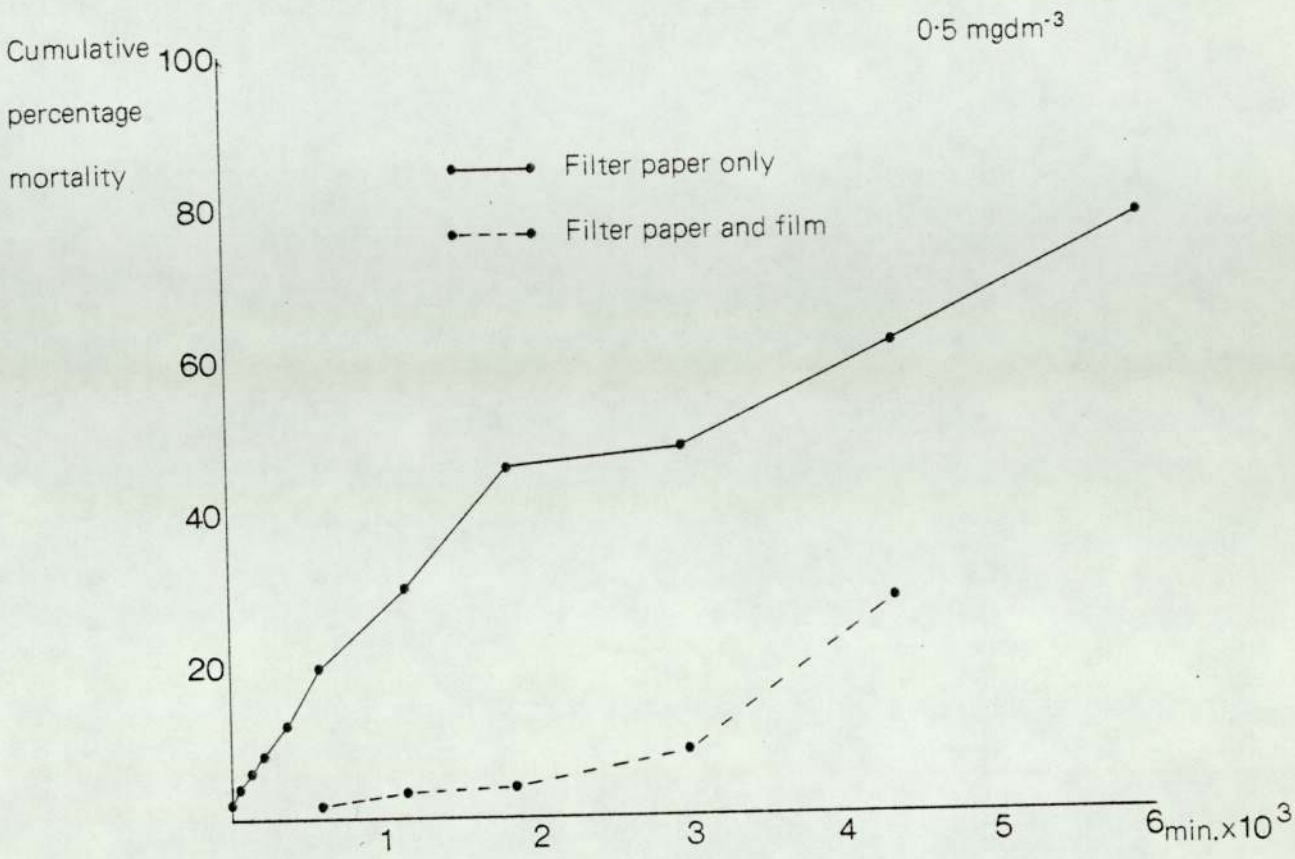


Fig. 129. Progression of mortality with time for *M.hygropetricus* subjected to Actellic M 20

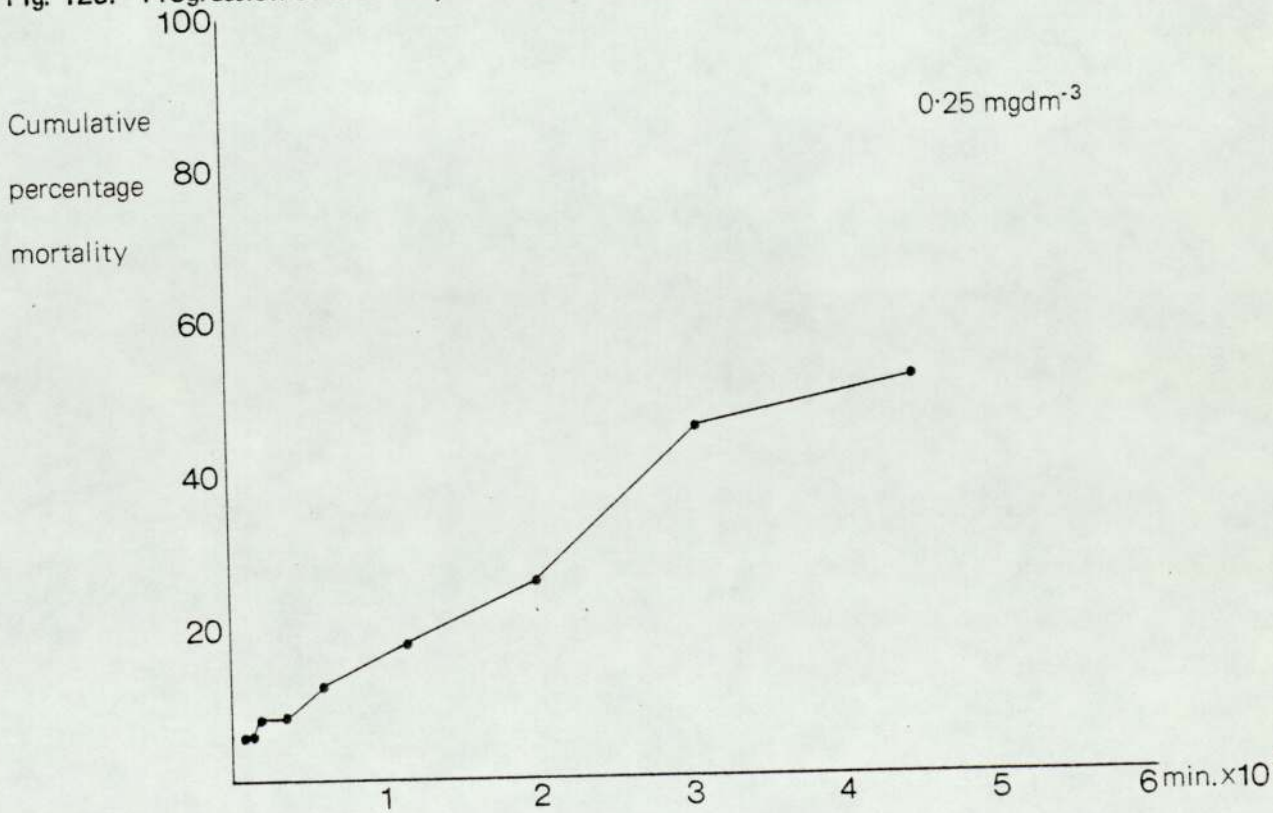


Fig. 130. Probability plot for *M. hygroscopicus* (filter paper substrate)

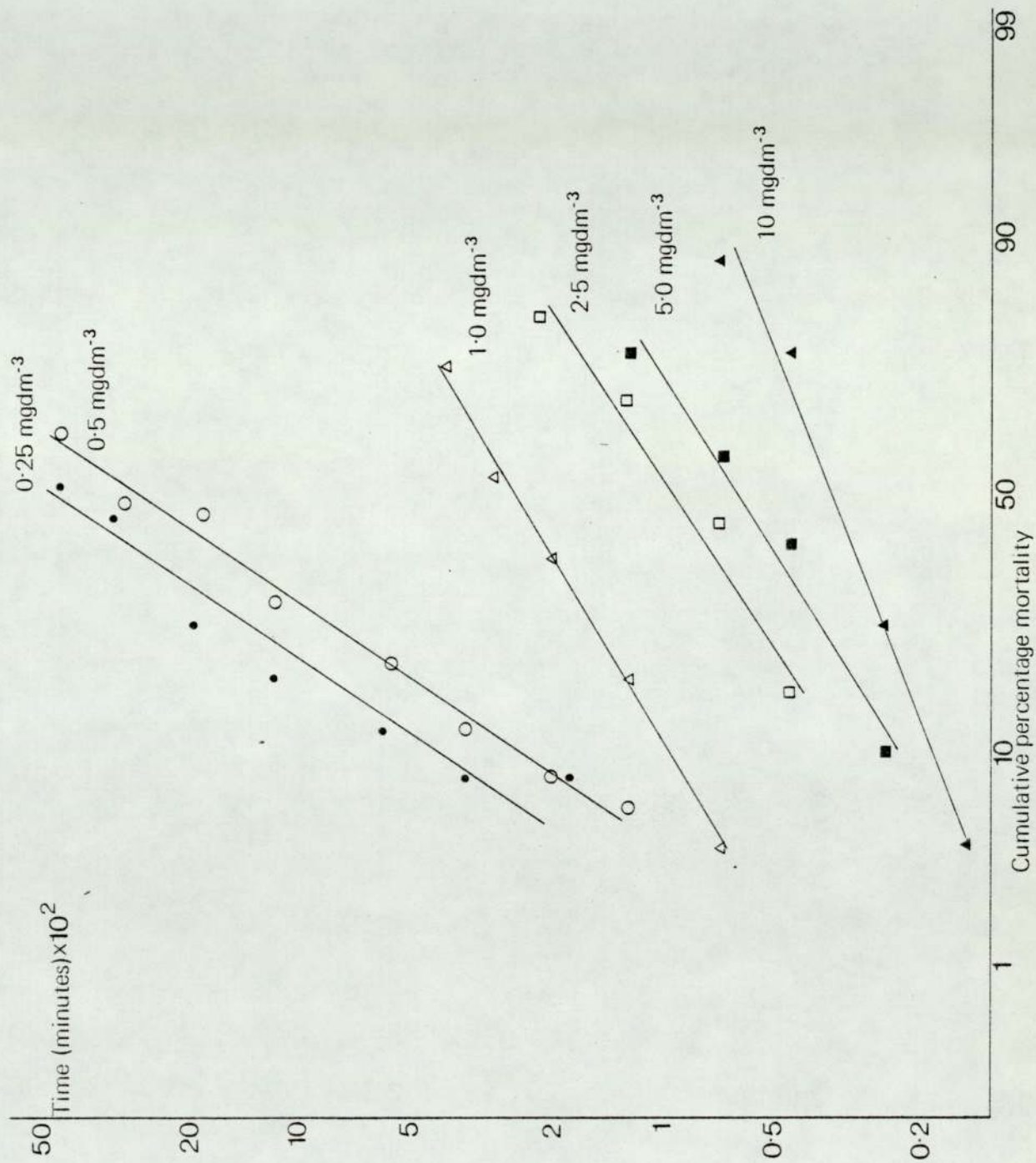


Fig. 131. Probability plot for *M. hygroscopicus* (filter paper and film substrate)

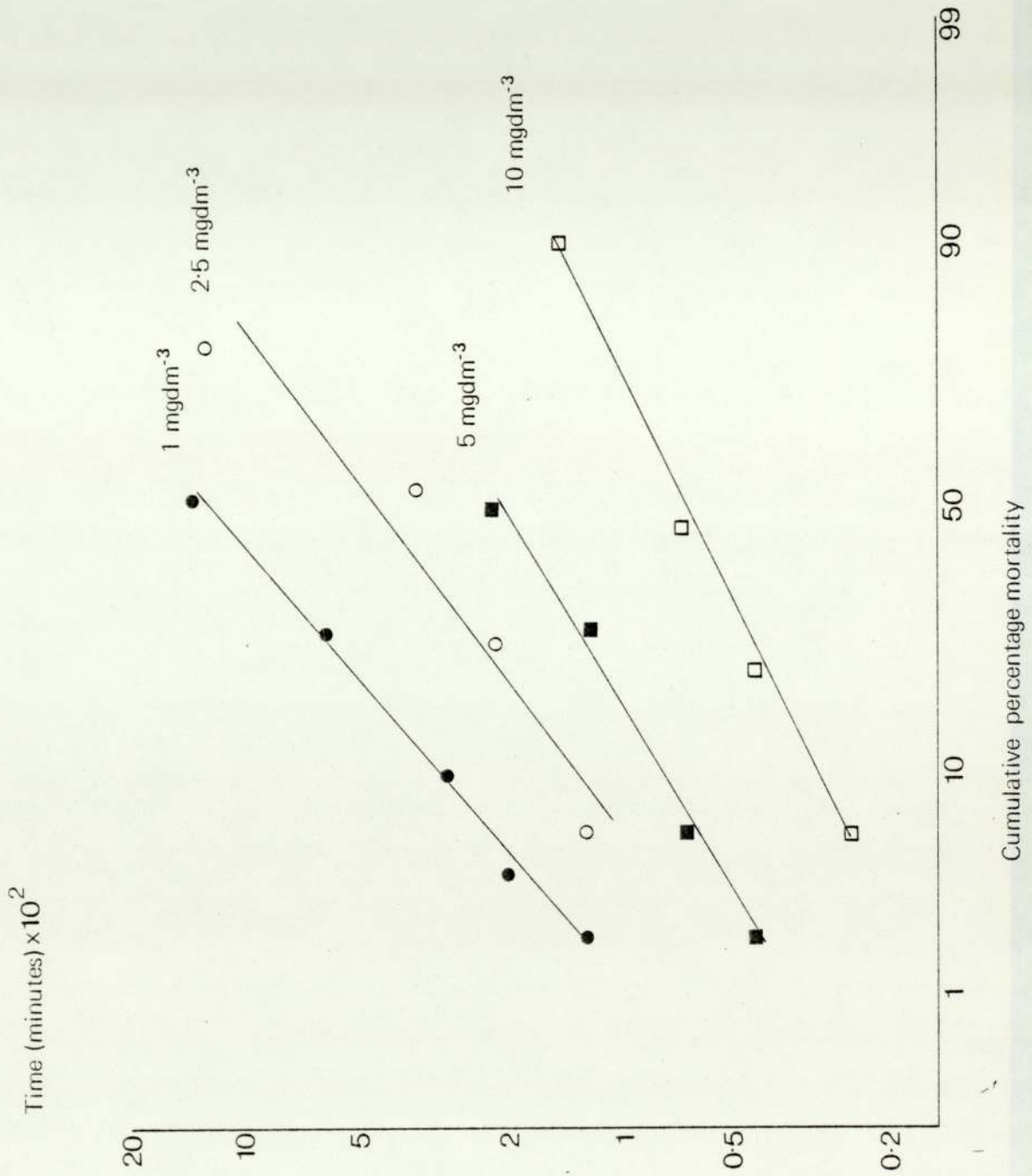


Fig. 132a. Litchfield and Wilcoxon (1949) LC50 determination - *M. hygropetricus*
(filter paper substrate)

(a) Statistics of dose effect plot

Dose mgdm ⁻³	Observed % mortality in 24 hr. (O)	Expected % mortality in 24 hr. (E)	O - E	Chi ²
2.5	100 (97.7)	93	4.7	0.035
1.0	100 (92.5)	73.5	19	0.16
0.5	37	49	12	0.054
0.25	22	25	3	0.0047

No. individuals per dose = 20 Degrees of freedom = 2

Chi² (for P = 5% with 2 degrees of freedom) = 5.99

Chi² = 0.2537 x 20 = 5.07 ∴ line is a good fit (heterogeneity not significant)

(b) Calculation of 24 hr. LC50 and line slope function with 95% confidence limits

(For explanation of symbols and details of method see Litchfield and Wilcoxon 1949)

$$LC_{84} = 1.45$$

$$R = 2.5/0.25 = 10$$

$$LC_{50} = 0.52$$

$$\therefore A = 1.65$$

$$LC_{16} = 0.18$$

$$\therefore fS = 1.62$$

$$S = (1.45/0.52 + 0.52/0.18) \div 2 = 2.84$$

$$\therefore \text{slope function} = 2.84 (1.75 \text{ to } 4.60)$$

$$K = 4 \quad N' = 60 \quad \sqrt{N'} = 7.75$$

$$\therefore fLC_{50} = 1.45$$

$$\therefore 24 \text{ hr. LC}_{50} = 0.52 (0.36 \text{ to } 0.76) \text{ mgdm}^{-3}$$

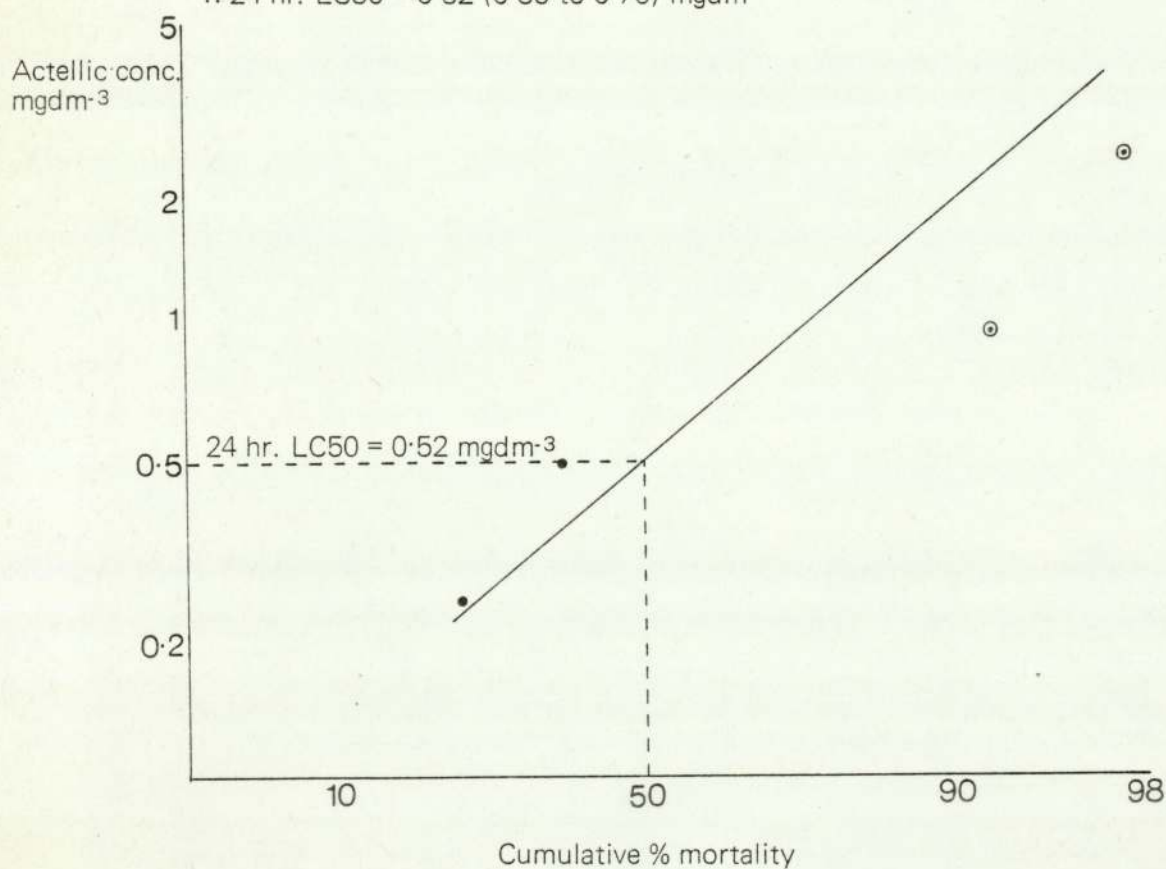


Fig. 132b. Bliss (1937) LC50 determination — *M. hygroscopicus* (filter paper substrate)

Median survival
time (mins.) $\times 10^2$

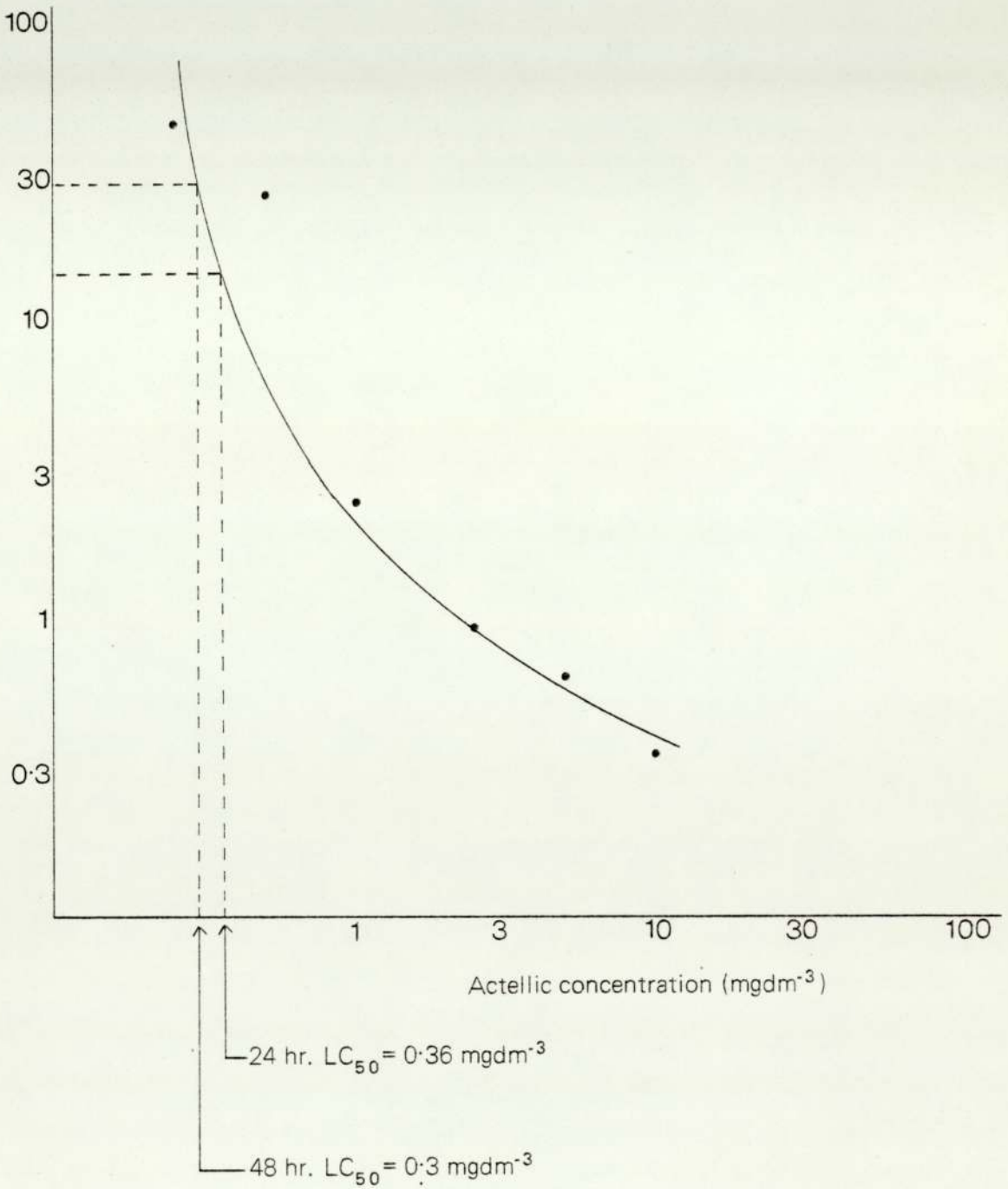


Fig. 133a. Litchfield and Wilcoxon (1949) LC50 determination - *M.hygropetricus*
(filter paper and film subst)

(a) Statistics of dose effect plot

Dose mgdm ⁻³	Observed % mortality in 24 hr. (O)	Expected % mortality in 24 hr. (E)	O - E	Chi ²
10	99.99	99.98	0.01	0.001
5	99.5	99.4	0.1	0.001
2.5	90	92.3	2.3	0.008
1	57	50.5	6.5	0.016

No. individuals per dose = 20 Degrees of freedom = 2

Chi² (for P = 5% with 2 degrees of freedom) = 5.99

Chi² = 0.026 x 20 = 0.52 ∴ line is a good fit (heterogeneity not significant)

(b) Calculation of 24 hr. LC50 and line slope function with 95% confidence limits

(For explanation of symbols and details of method see Litchfield and Wilcoxon 1949)

LC84 = 1.88

R = 10/1 = 10

LC50 = 0.98

∴ A = 1.22

LC16 = 0.52

∴ fS = 1.40

S = (1.88/0.98 + 0.98/0.52) ÷ 2 = 1.90

∴ slope function = 1.90 (1.36 to 2.66)

K = 4 N' = 20 √ N' = 4.47

∴ fLC50 = 1.475

∴ 24 hr. LC50 = 0.98 (0.66 to 1.45) mgdm⁻³

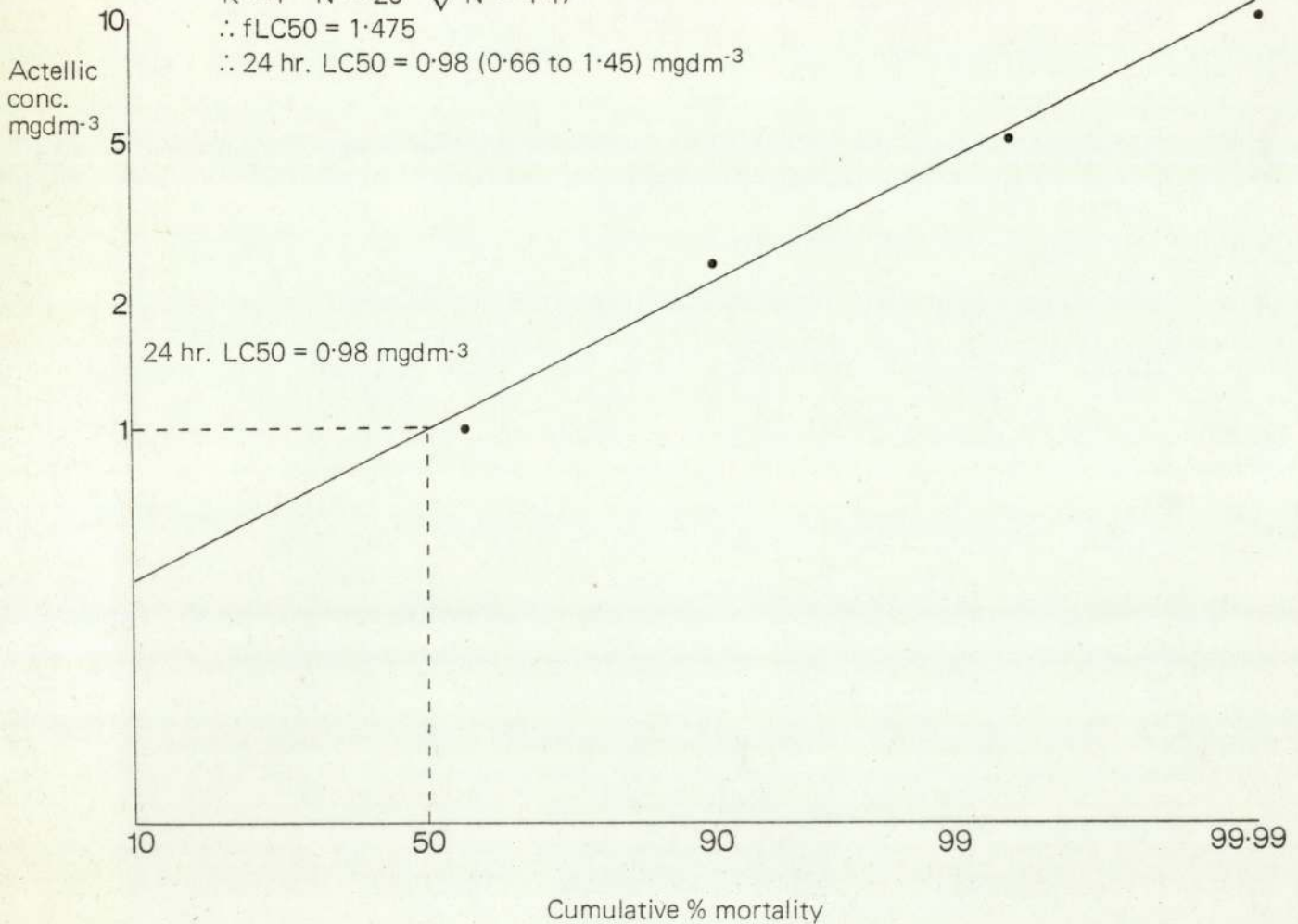


Fig. 133b. Bliss (1937) LC50 determination – *M.hygropetricus* (filter paper and film substrate)

Median survival
time (mins.) $\times 10^2$

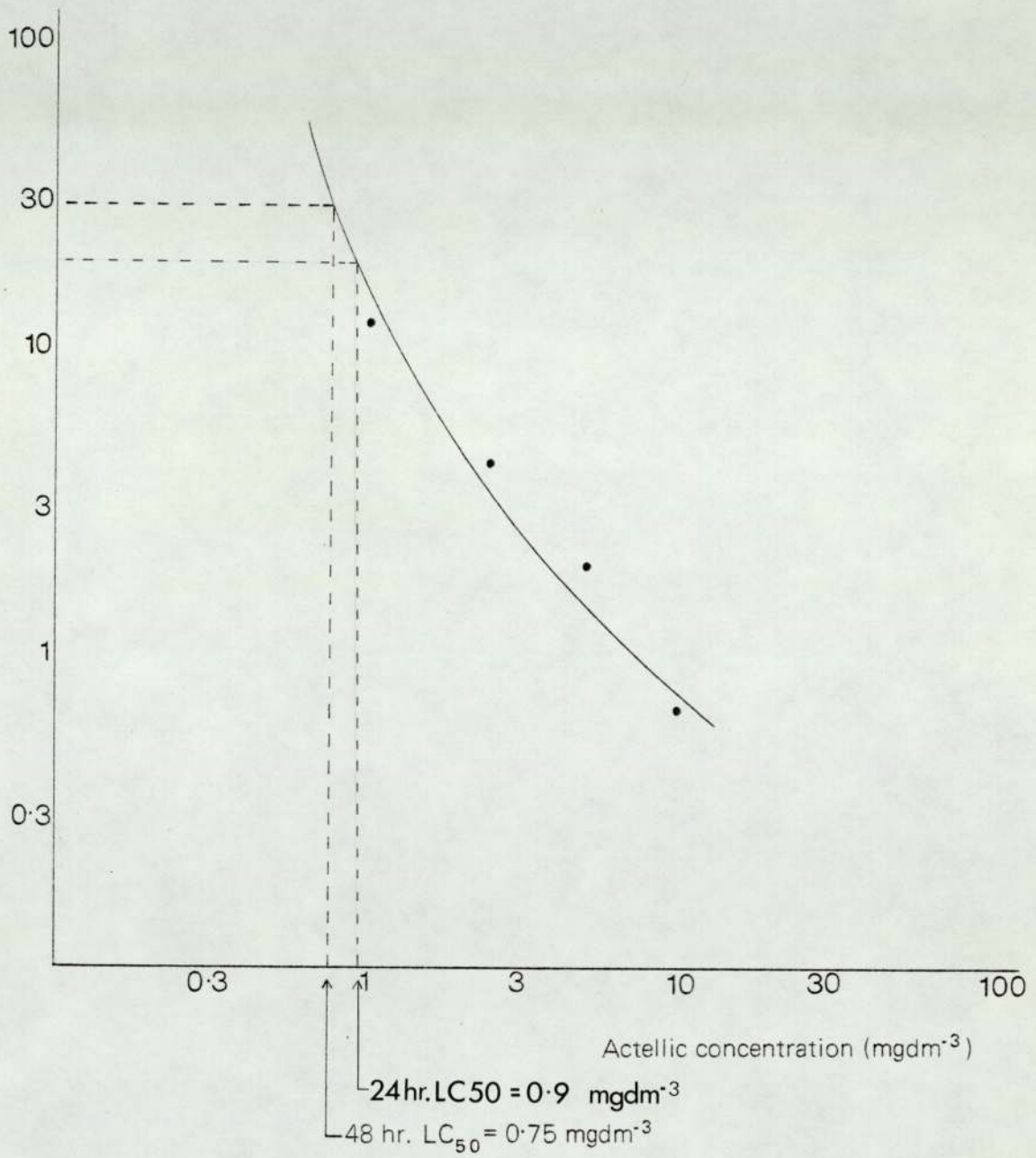


Fig. 134. Probability plot for *P.alternata*

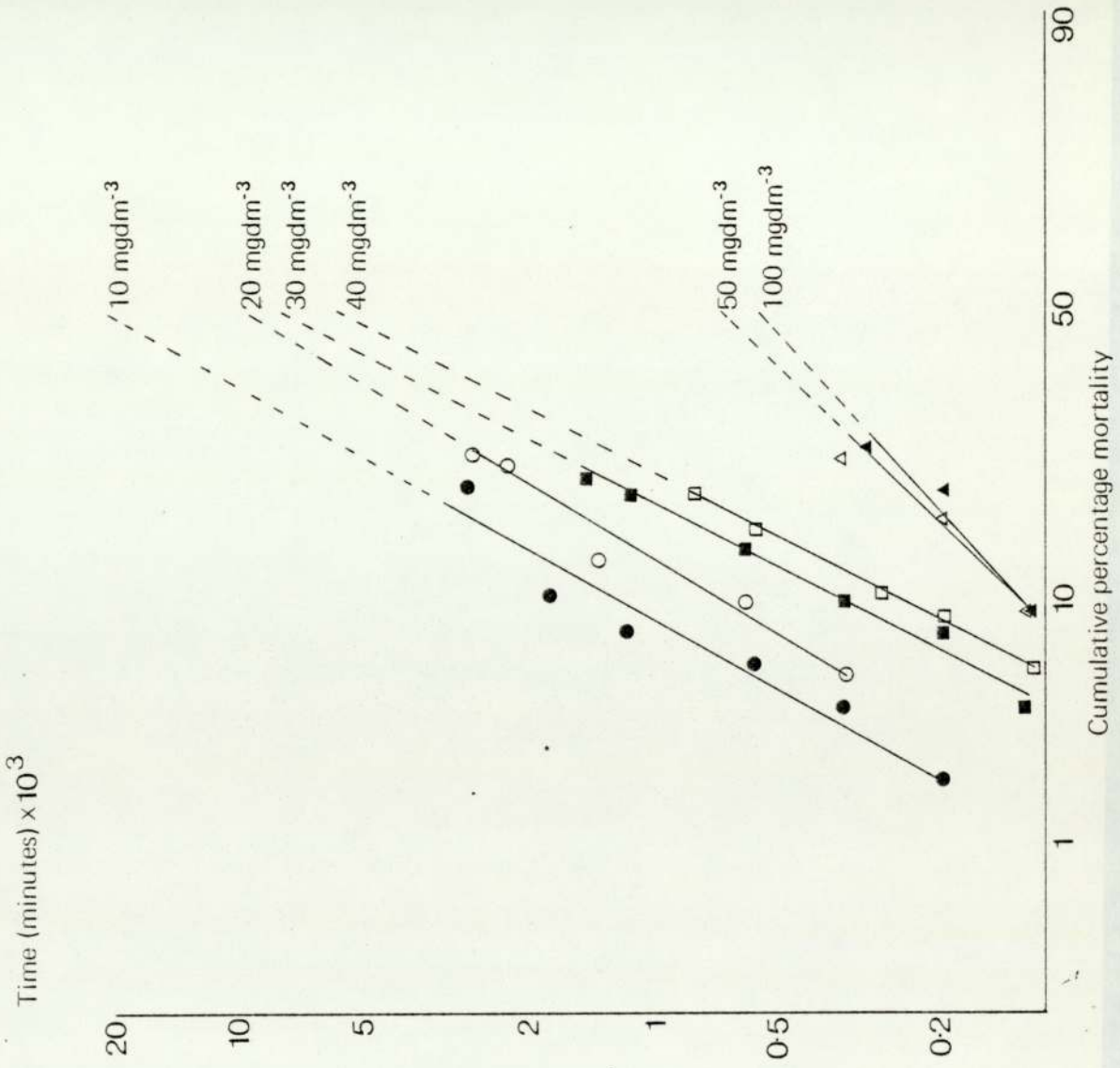


Fig. 135a. Litchfield and Wilcoxon (1949) LC50 determination - P.alternata

(a) Statistics of dose effect plot

Dose mgdm ⁻³	Observed % mortality in 24 hr. (O)	Expected % mortality in 24 hr. (E)	O - E	Chi ²
100	79	76	3	0.0047
50	71	52	19	0.14
40	29	42	13	0.068
30	24	32	8	0.027
20	18	20	2	0.0023
10	11.5	13	1.5	0.002

No. individuals per dose = 20 Degrees of freedom = 4

Chi² (for P = 5% with 4 degrees of freedom) = 9.49

Chi² = 0.244 x 20 = 4.88 ∴ line is a good fit (heterogeneity not significant)

(b) Calculation of 24 hr. LC50 and line slope function with 95% confidence limits

(For explanation of symbols and details of method see Litchfield and Wilcoxon 1949)

LC84 = 135

R = 100/10 = 10

LC50 = 48

∴ A = 1.63

LC16 = 17.3

∴ fS = 1.5

S = (135/48 + 48/17.3) ÷ 2 = 2.79

∴ slope function = 2.79 (1.86 to 4.19)

K = 6 N' = 100 √ N' = 10

∴ fLC50 = 1.325

∴ 24 hr. LC50 = 48 (36 to 67) mgdm⁻³

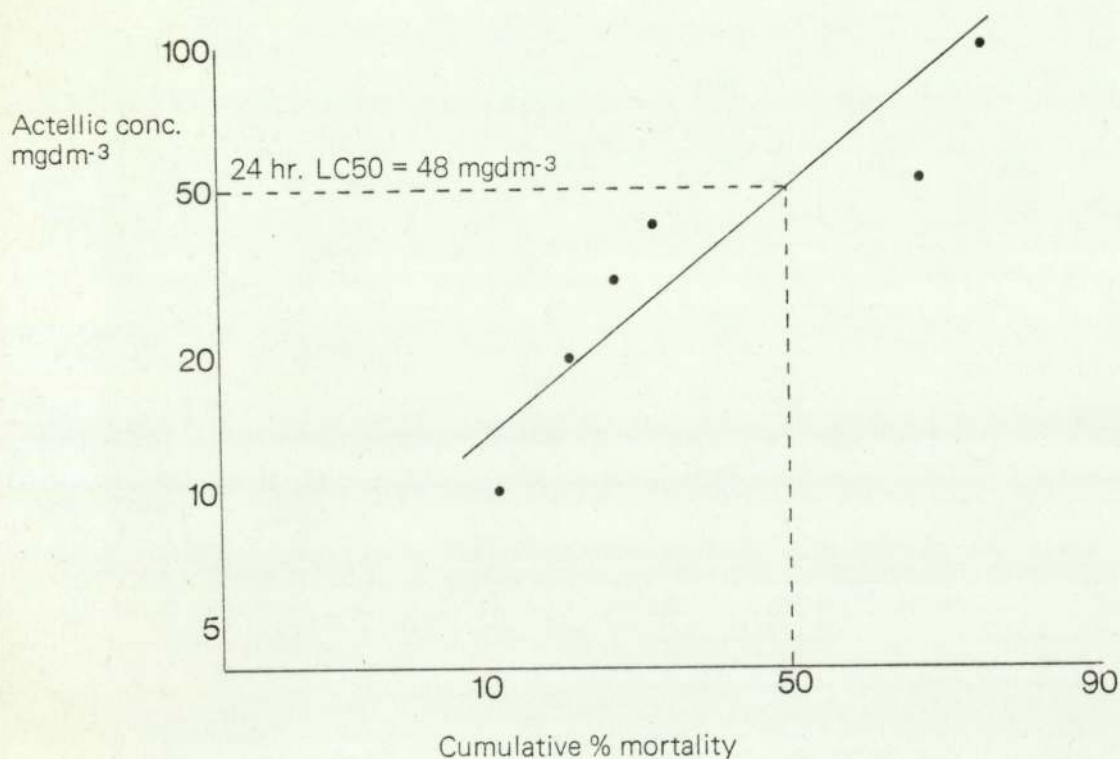


Fig. 135b. Bliss (1937) LC50 determination — *P.alternata*

Median survival
time (mins.) $\times 10^2$

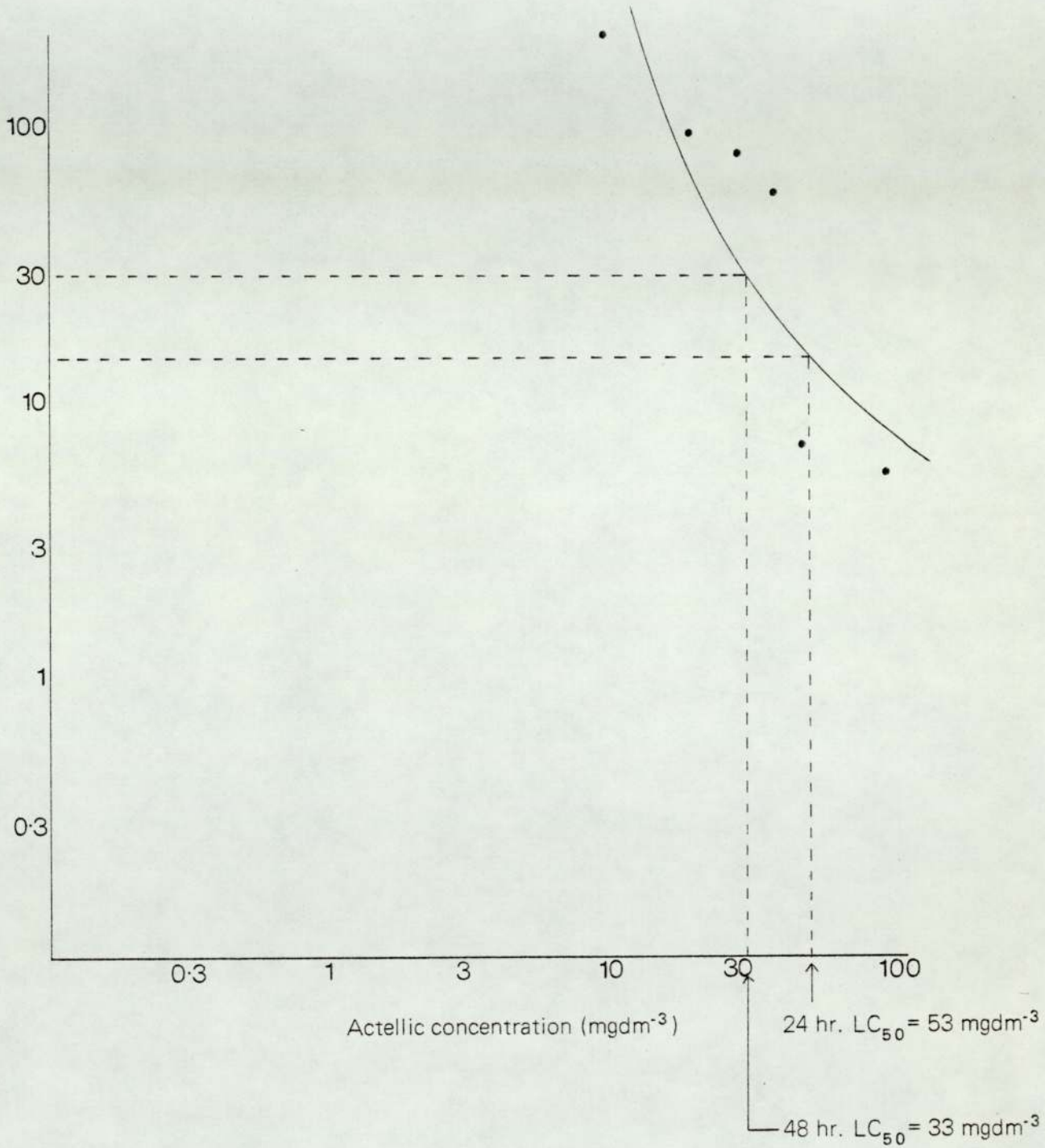


Fig. 136. Probability plot for *P.severini*

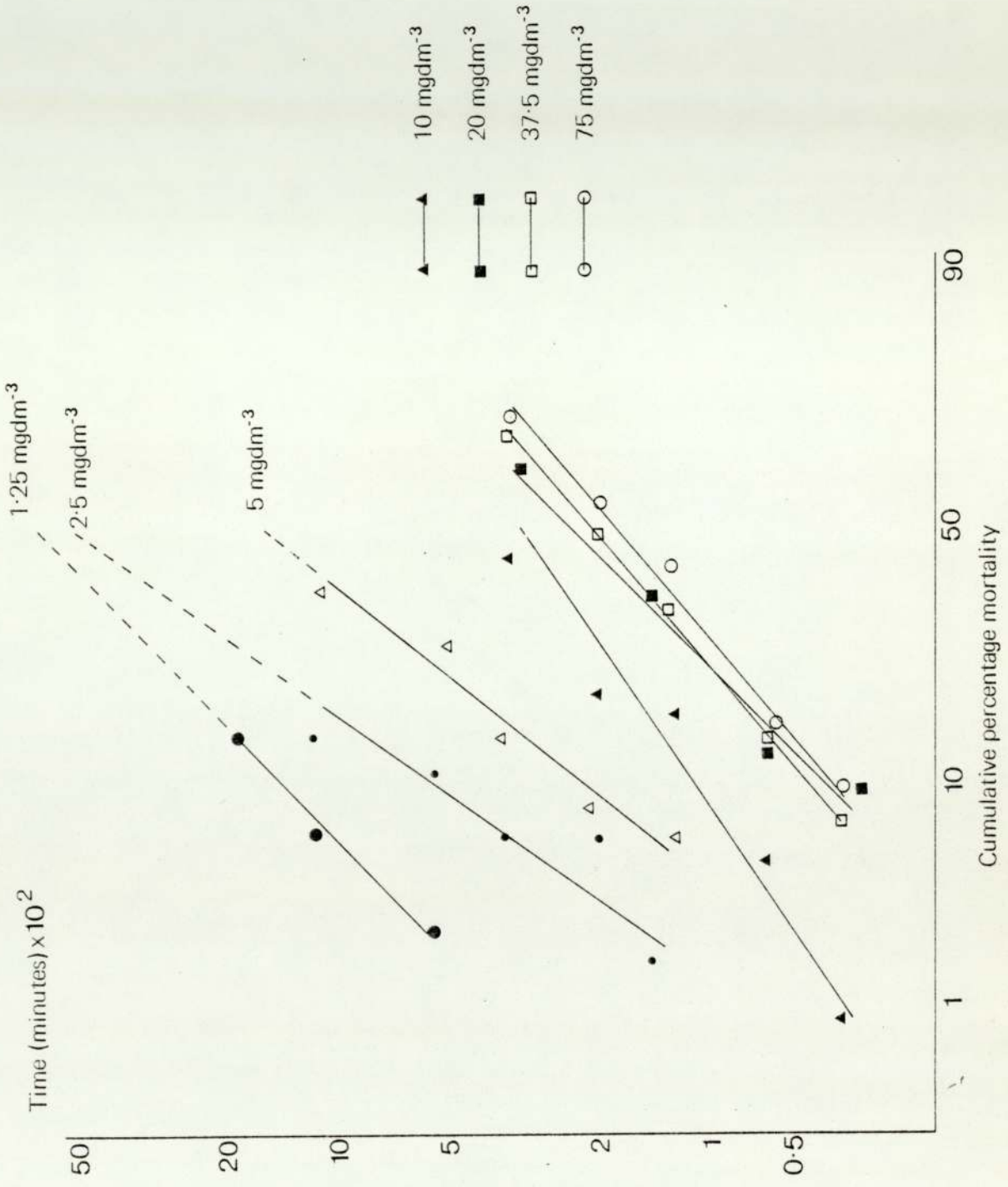


Fig. 137a. Litchfield and Wilcoxon (1949) LC50 determination - P.severini

(a) Statistics of dose effect plot

Dose mgdm ⁻³	Observed % mortality in 24 hr. (O)	Expected % mortality in 24 hr. (E)	O - E	Chi ²
75	97.8	98.6	0.8	0.0055
37.5	97	95	2	0.008
20	93	87	6	0.03
5	48	50	2	0.0015
2.5	25	29	4	0.007
1.25	11.5	13	1.5	0.002

No. individuals per dose = 20 Degrees of freedom = 4

Chi² (for P = 5% with 4 degrees of freedom) = 9.49

Chi² = 0.054 x 20 = 1.08 ∴ line is a good fit (heterogeneity not significant)

(b) Calculation of 24 hr. LC50 and line slope function with 95% confidence limits

(For explanation of symbols and details of method see Litchfield and Wilcoxon 1949)

LC84 = 17

$R = 75/1.25 = 60$

LC50 = 5

∴ A = 1.47

LC16 = 1.48

∴ fS = 1.66

$S = (17/5 + 5/1.48) \div 2 = 3.39$

∴ slope function = 3.39 (2.04 to 5.63)

K = 6 N' = 40 $\sqrt{N'} = 6.32$

∴ fLC50 = 1.70

∴ 24 hr. LC50 = 5.0 (2.9 to 8.5) mgdm⁻³

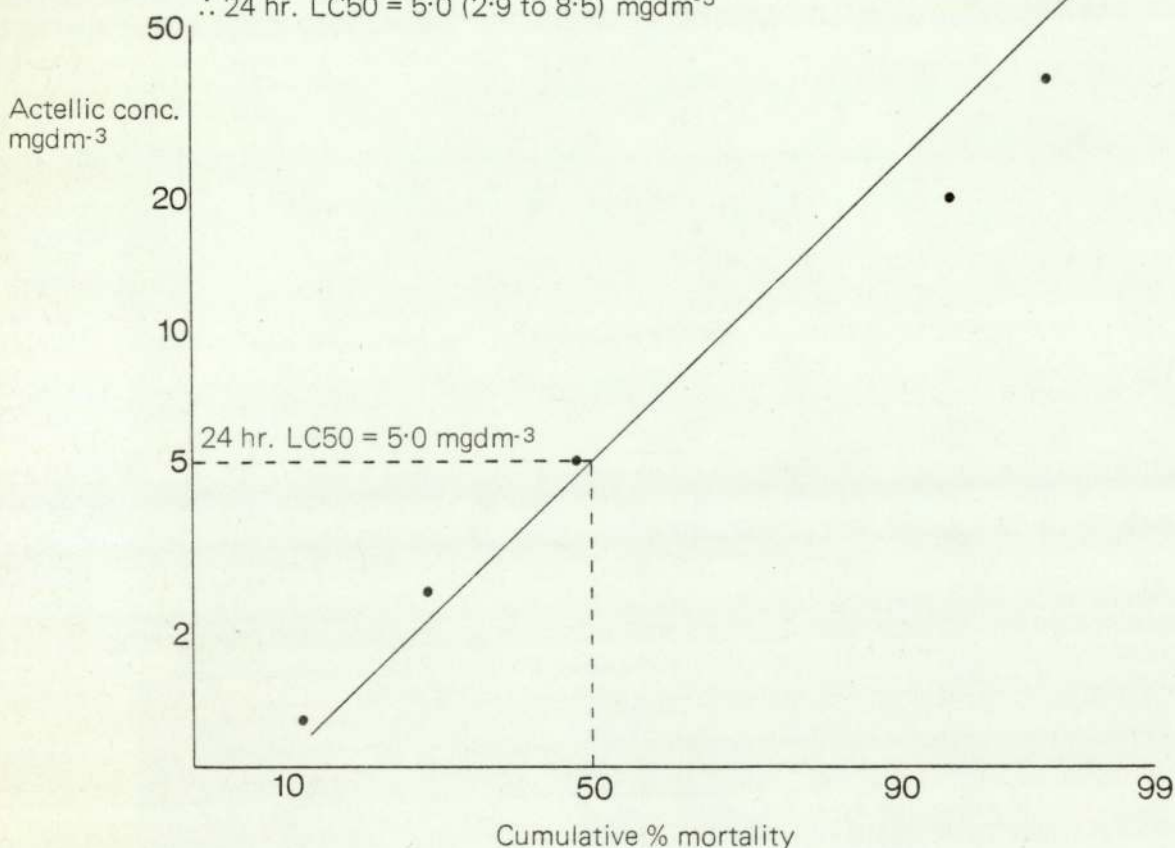


Fig. 137b. Bliss (1937) LC50 determination – *P.severini*

Median survival
time (mins.) $\times 10^2$

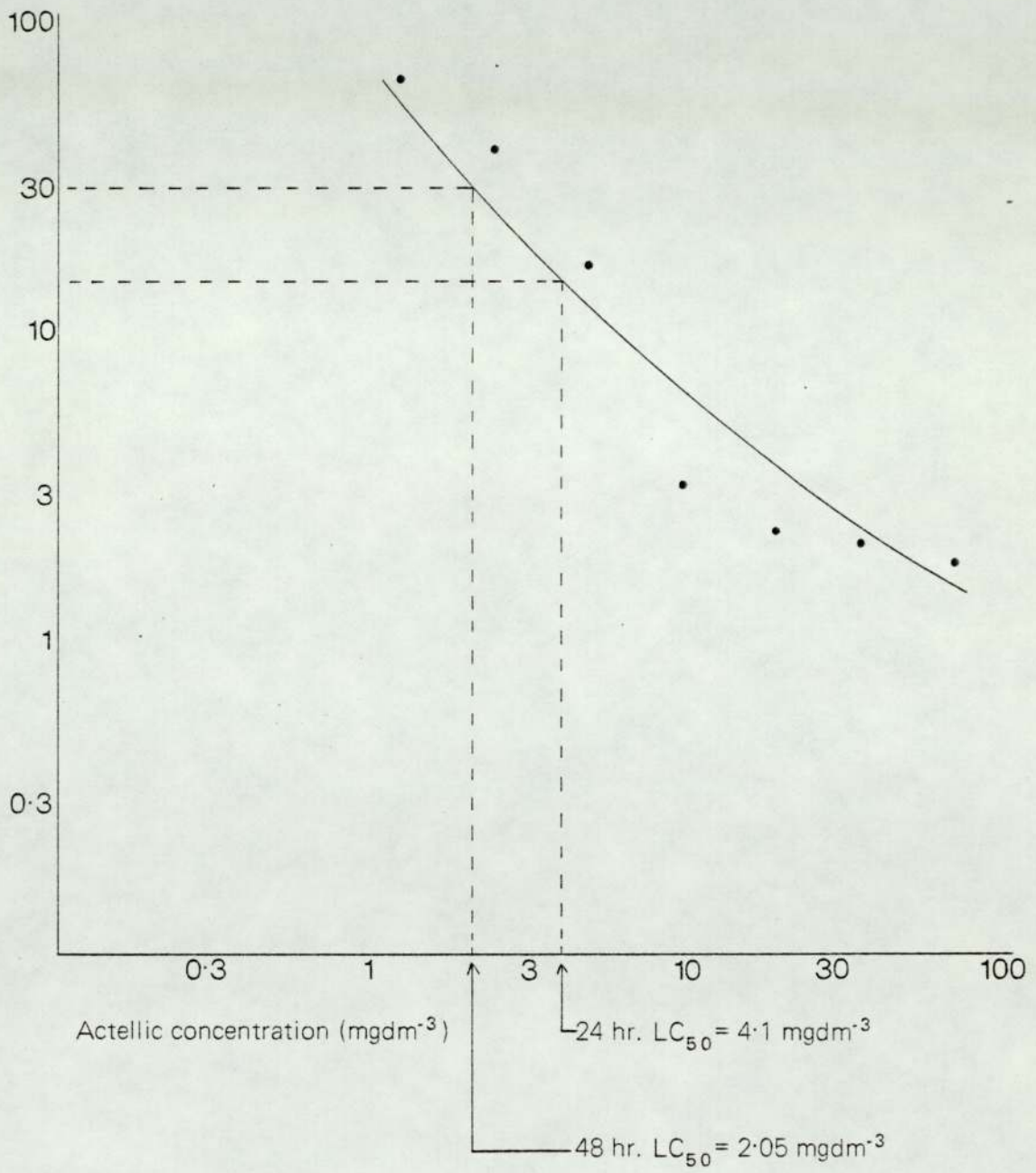


Fig. 138. Probability plot for *S. fenestralis*

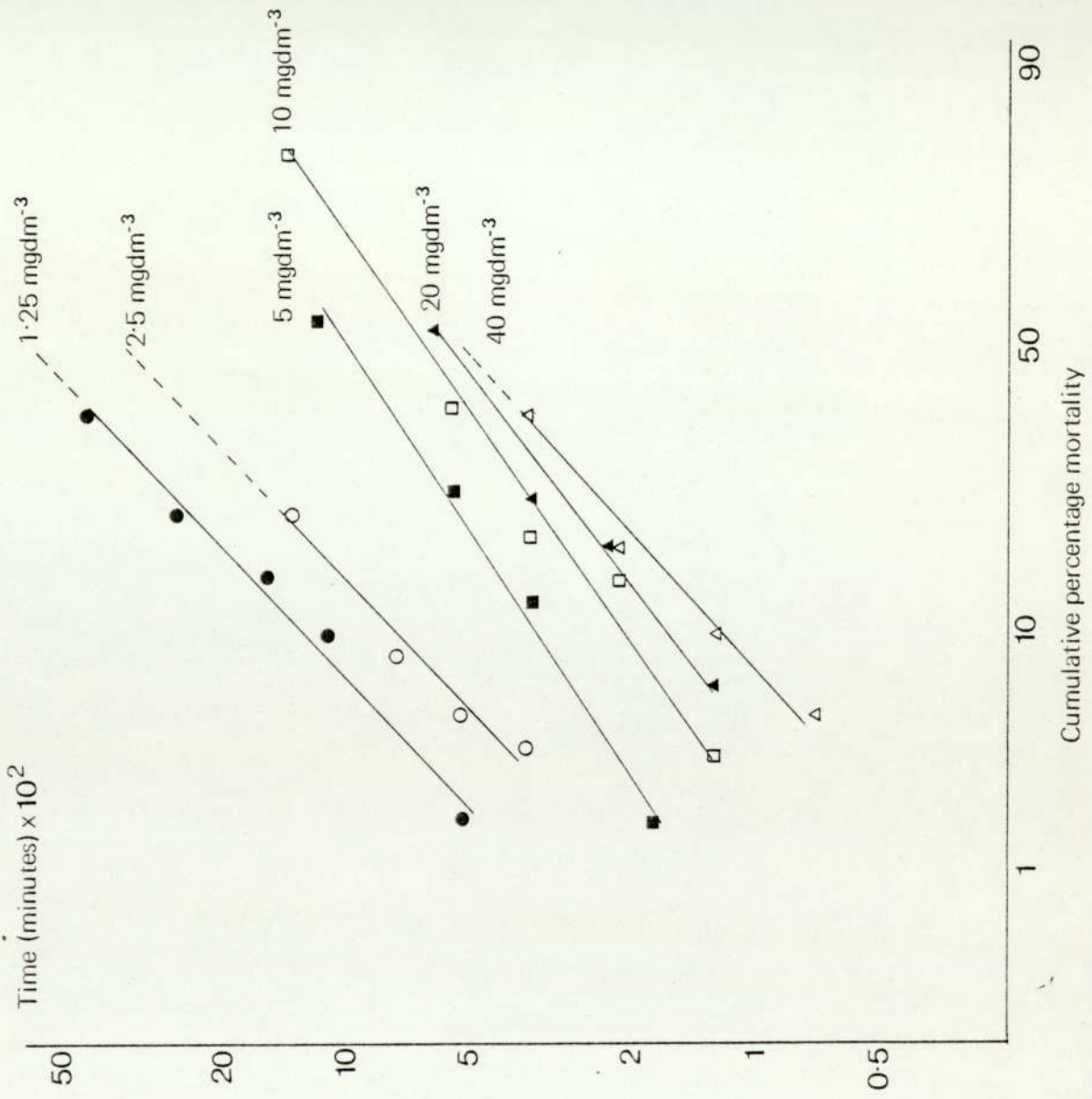


Fig. 139a. Litchfield and Wilcoxon (1949) LC50 determination - *S. fenestralis*

(a) Statistics of dose effects plot

Dose mgdm ⁻³	Observed % mortality in 24 hr. (O)	Expected % mortality in 24 hr. (E)	O - E	Chi ²
20	84.5	88	3.5	0.011
10	83.5	73	9.5	0.045
5	68	52	16	0.1
2.5	24	30	6	0.017
1.25	12	13	1	0.001

No. individuals per dose = 20 Degrees of freedom = 3

Chi² (for P = 5% with 3 degrees of freedom) = 7.82

Chi² = 0.174 x 20 = 3.48 ∴ line is a good fit (heterogeneity not significant)

(b) Calculation of 24 hr. LC50 and line slope function with 95% confidence limits

(For explanation of symbols and details of method see Litchfield and Wilcoxon 1949)

$$LC_{84} = 15.5$$

$$LC_{50} = 4.8$$

$$LC_{16} = 1.45$$

$$S = (15.5/4.8 + 4.8/1.45) \div 2 = 3.27$$

$$K = 5 \quad N' = 60 \quad \sqrt{N'} = 7.75$$

$$\therefore fLC_{50} = 1.53$$

$$\therefore 24 \text{ hr. LC}_{50} = 4.8 (3.1 \text{ to } 7.3) \text{ mgdm}^{-3}$$

$$R = 20/1.25 = 16$$

$$\therefore A = 1.72$$

$$\therefore fS = 1.80$$

$$\therefore \text{slope function} = 3.27 (1.82 \text{ to } 5.89)$$

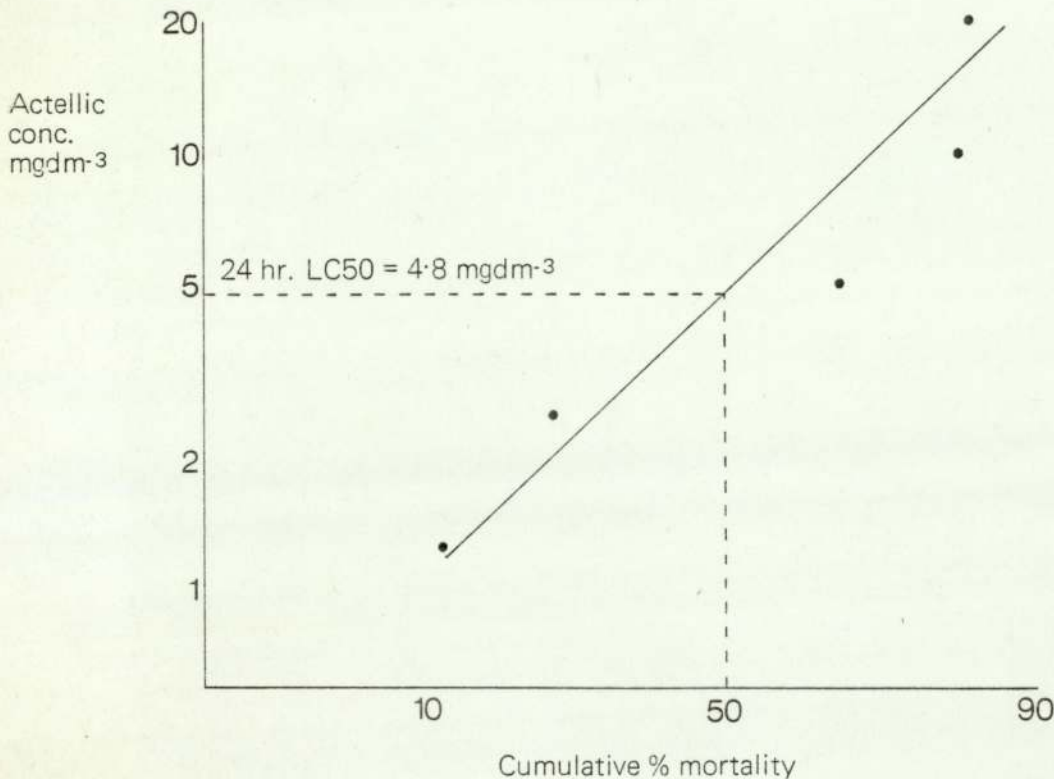


Fig. 139b. Bliss (1937) LC50 determination — *S. fenestralis*

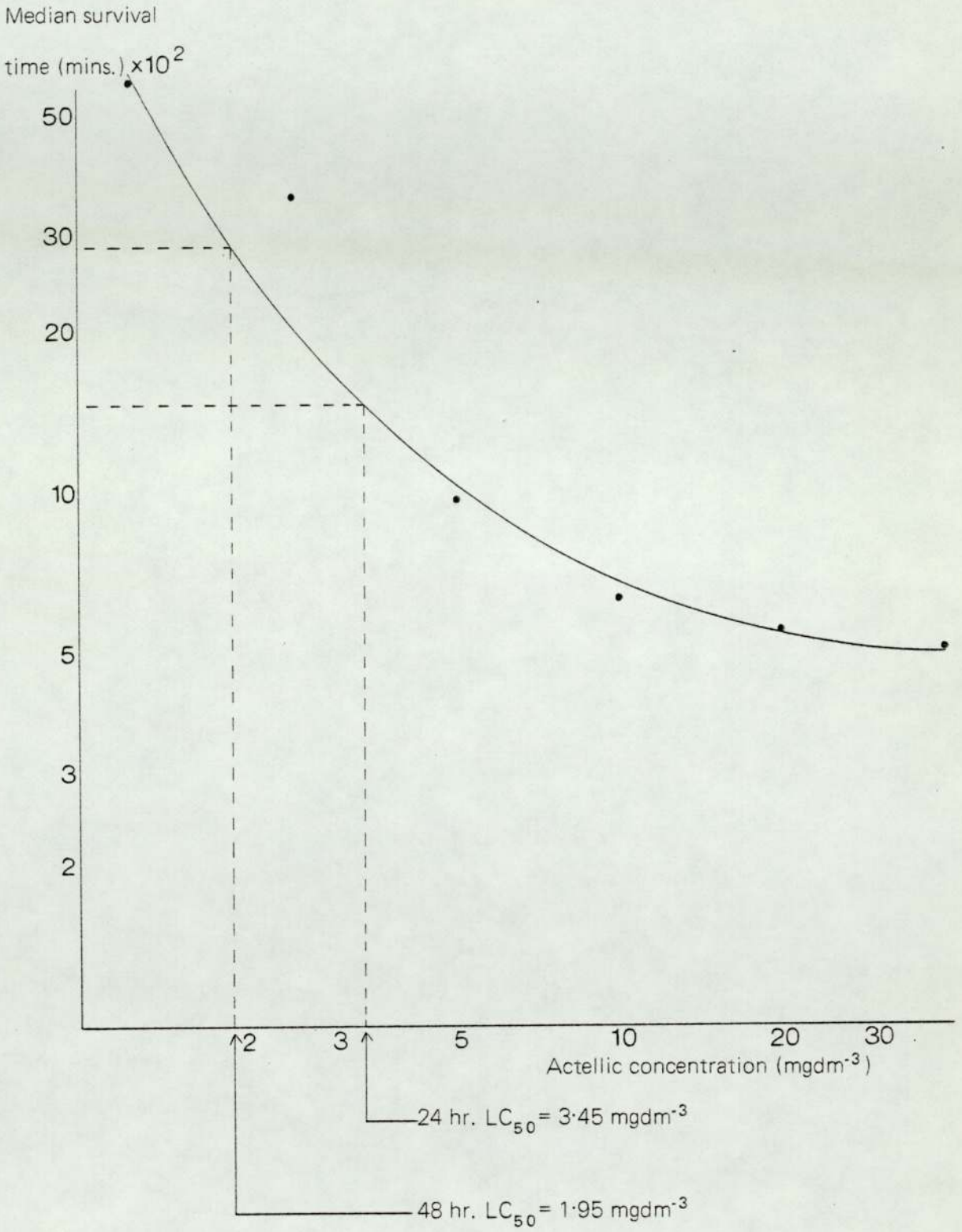


Fig. 140. Probability plot for *L. rivialis*

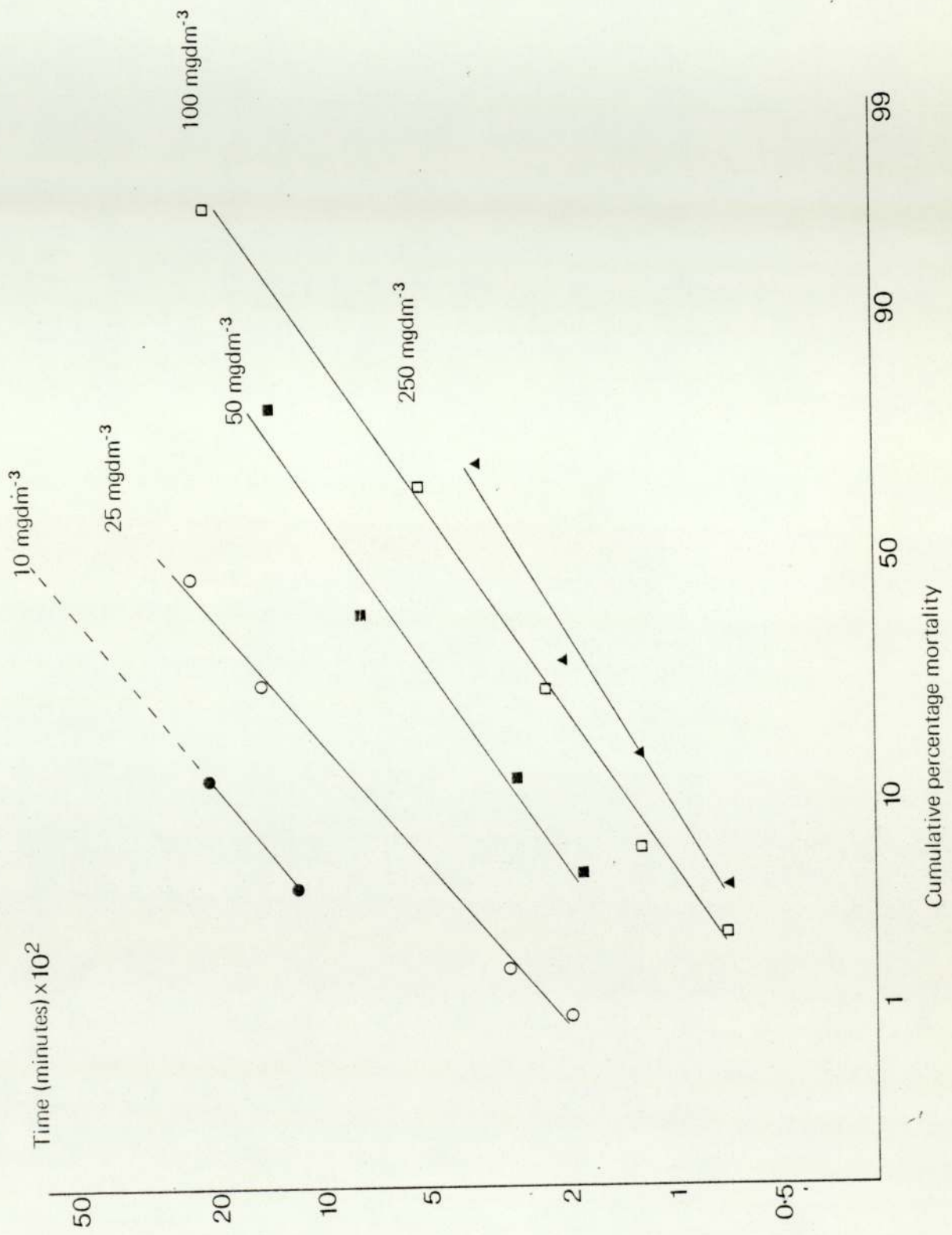


Fig. 141a. Litchfield and Wilcoxon (1949) LC50 determination - L.rivalis

(a) Statistics of dose effect plot

Dose mgdm ⁻³	Observed % mortality in 24 hr. (O)	Expected % mortality in 24 hr. (E)	O - E	Chi ²
250	98.6	98.9	0.3	0.001
100	93	89	4	0.016
50	75	67	8	0.027
25	27	35	8	0.032
10	7	7.5	0.5	0.001

No. individuals per dose = 20 Degrees of freedom = 3

Chi² (for P = 5% with 3 degrees of freedom) = 7.82

Chi² = 0.077 x 20 = 1.54 ∴ line is a good fit (heterogeneity not significant)

(b) Calculation of 24 hr. LC50 and line slope function with 95% confidence limits

(For explanation of symbols and details of method see Litchfield and Wilcoxon 1949)

LC84 = 80

$R = 250/10 = 25$

LC50 = 35

∴ $A = 1.27$

LC16 = 15

∴ $fS = 1.32$

$S = (80/35 + 35/15) \div 2 = 2.31$

∴ slope function = 2.31 (1.82 to 3.05)

$K = 5 \quad N' = 40 \quad \sqrt{N'} = 6.32$

∴ $fLC50 = 1.44$

∴ 24 hr. LC50 = 35 (24.3 to 50.4) mgdm⁻³

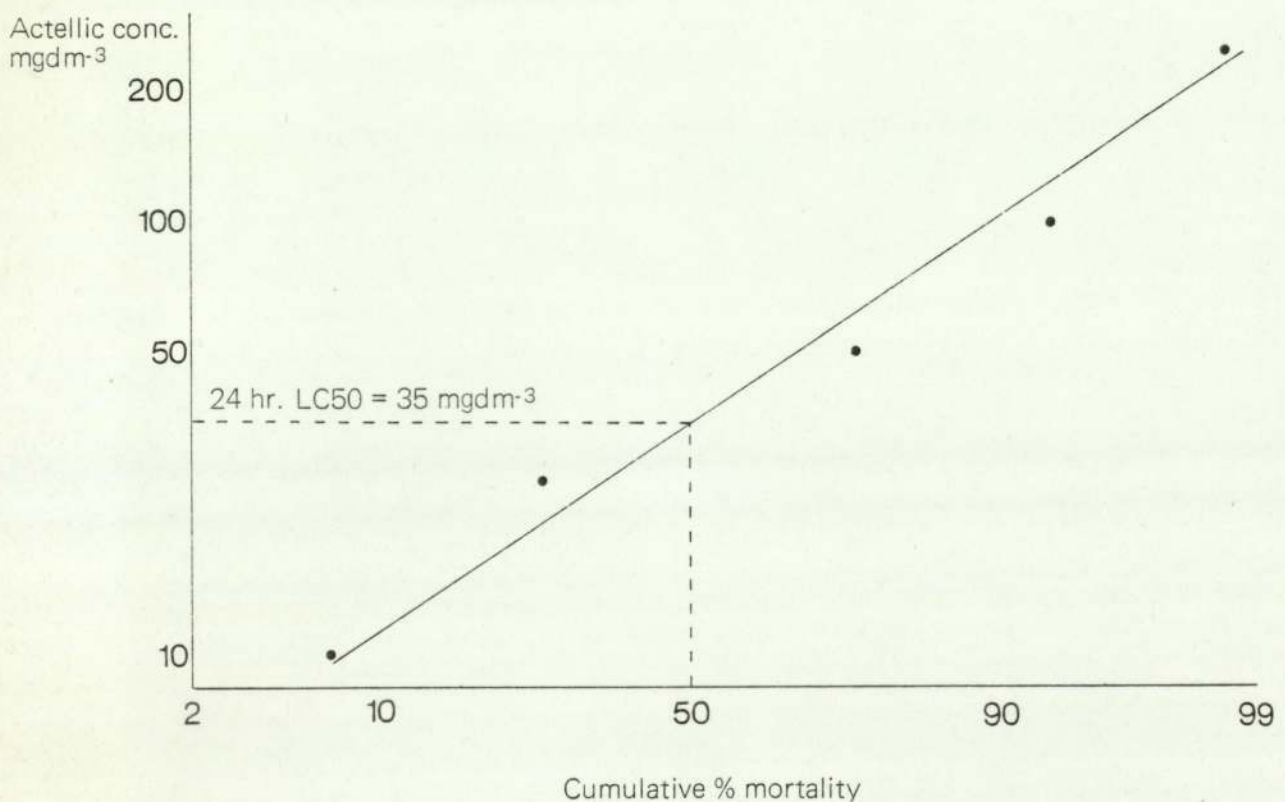


Fig. 141b. Bliss (1937) LC50 determination – *L.rivalis*

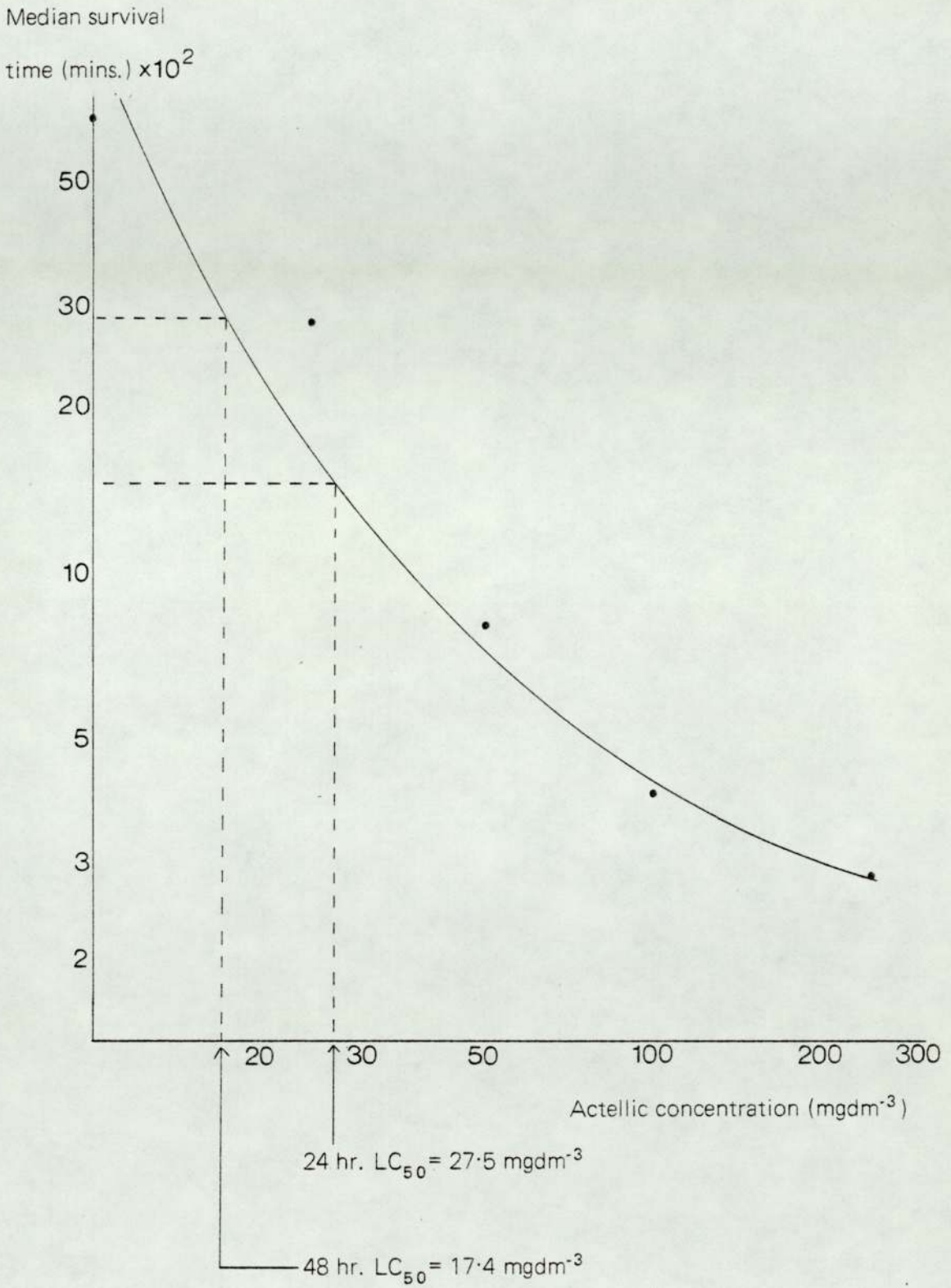
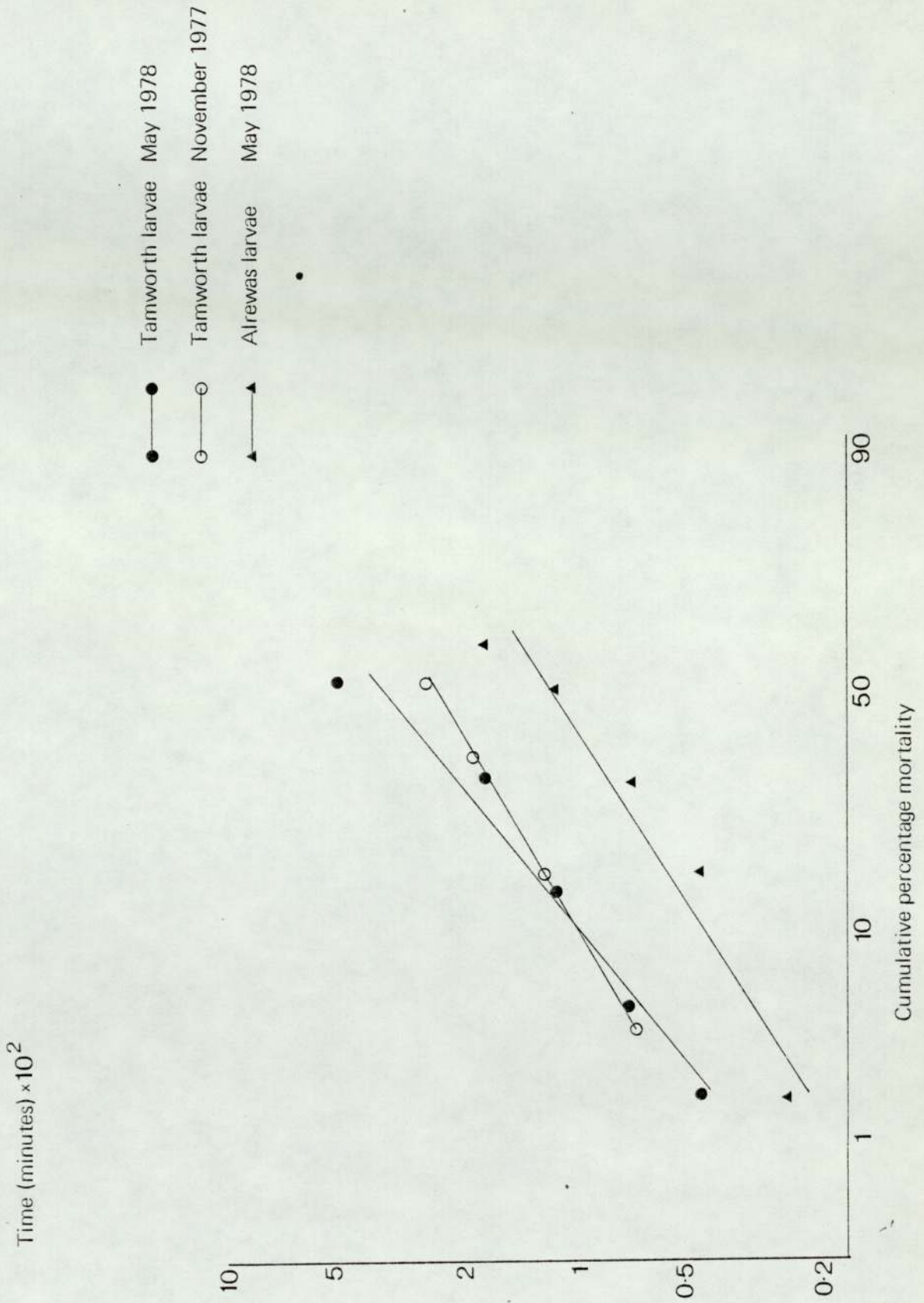


Fig. 142. Probability plot for *M. hygropliticus* (Actellic concentration 1 mgdm^{-3})



V Miscellaneous experimental work - results and discussion

(a) Investigations into the diurnal emergence patterns of filter flies

As equipment was available for this investigation in the form of a disc dropping suction fly trap as described by Taylor (1951) and used by Hawkes (1961a) it was thought that such an investigation would be a useful exercise and give a greater understanding of the habits of filter flies. The suction trap and its method of operation were described in full in the methods section; it can be seen in Plate 24 along with its associated equipment in Plate 25. The trap was used on 2 occasions (19th-20th May and 13th-14th Sept. 1977), both were days when flies were seen to be swarming on the works. On these days the traps were put out in mid morning and in the first case 22 segregated hourly catches were obtained whilst in the second case 16 hourly catches were obtained.

The results of the 2 catching periods can be seen in Tables 30a and 30b. These tables are presented in graphical form in Figs. 143 and 144 however the graphs omit the miscellaneous flies which may confuse any patterns evident. It is initially apparent from both sets of results that the filter insects caught do show certain diurnal periodicities. In both cases the peak catches of M.hygropetricus were found around mid day to early afternoon, this then declined throughout the afternoon and levelled out at a low level during the early evening and continued at this level through the hours of darkness. Concerning O.minimus, from Figs. 143 and 144 it can be seen that these were only caught in significant numbers in the May trapping period and from that investigation it is interesting to note that the maximum numbers were caught in a distinct dusk time period between 18.00 and 22.00 hrs. Unlike O.mimimus, P.severini were most abundant in the September trapping period and these showed an interesting trend that in both trapping periods maximum numbers were caught in a sharp peak around 18.00 hrs. followed by smaller peaks in the night. Leptocera spp. were only caught in significant numbers in the September trapping period and these

showed a peak periodicity at around 16.30 hrs.

It should be appreciated that the total number of flies caught was much greater in the September trapping period which can probably be attributed to the higher mean air temperature at this time (13°C compared to 8°C in the May trapping period). Many workers have noticed how increases in air temperatures can cause increases in total fly emergence including Gibson (1945) and Williams (1940).

As regards the actual diurnal periodicity of filter fly emergence, the only work discovered on this topic was that of Hawkes (1951a and 1961a) and this dealt only with Sylvicola; a fly which is known to have shade seeking habits and to emerge mainly in the hours of darkness. As far as can be established not^{much} work has been carried out on the diurnal periodicity of the flies found in this study therefore comparisons are difficult. However Gibson (1945) studying swarming habits found Metricnemus and O.minimus to swarm during the day. As constant observations on swarming behaviour were not carried out it is difficult to compare hourly insect catches to swarming habits therefore the results obtained in this study are not directly comparable to those from other people's work.

When explaining the results reference should be made to the wind velocity which according to Gibson (1945) is interrelated with light in determining swarming activity. Unfortunately wind velocity measurements were not taken during the investigation however it was noticed on both trapping occasions that the wind velocity had increased on the second day of trapping which may explain the low catch found in the early morning of the^{second} day of the trapping period. It is difficult to explain the reasons for the periodicities found as if wind is an important factor changes in velocity may have been reflected in the periodicity of the flies, for example there may have been a drop in the wind velocity around 18.00 hrs. allowing an increased catch of the lighter O.minimus and P.severini whilst the absence of these and presence of

M.hygropetricus earlier in the day may have corresponded to higher wind velocities. It is likely that wind was the controlling factor here as it is unlikely that light intensities would vary drastically between 16.00 and 18.00 hrs. causing a sudden increase in activity of P.severini (Fig. 144). Gibson (1945) noticed that a decrease in light intensity caused decreased activity of O.minimus, as the light intensity would be decreasing slightly between 18.00 and 20.00 hrs. in May (at which time sunset is 20.50 hrs.) this would not explain the sudden increase in O.minimus catch found at this time of day (Fig. 143) so other factors must be operating here and the most likely one is wind.

Therefore these results do give some idea of the diurnal periodicities of filter flies but these are specific to the wind conditions experienced during the trapping periods which are not accurately known. However looking at the results generally it is clear that M.hygropetricus emerge in greatest numbers in the hours of daylight unlike Sylvicola, P.severini and O.minimus which emerge mainly at dusk just preceding the hours of darkness.

(b) Observations made on life cycles during culture work

Certain techniques were used to induce egg laying and larval development in confinement for M.hygropetricus, P.alternata and P.severini. These techniques were fully described in the methods section and to summarise they involved placing adult flies caught from the main filters in petri dishes lined with moist, sterile filter paper to induce egg laying. These dishes were placed in an incubator and the resulting developing larvae were fed on sterile film which was prepared beforehand and frozen for storage. Frequent observations were made through the life cycles as follows.

(i) M.hygropetricus

Day 0 8 mating couples were collected from the ground beneath a male mating swarm. 2 pairs were placed in each petri dish in a 16⁰C incubator.

Day 1 Egg masses were noted in the petri dishes only round the side of the dish walls and not in the centre suggesting that some thigmotaxic response determines the site of egg deposition. The spherical eggs which were yellow in colour were laid in masses in a gelatinous matrix, 39 masses were noted in one dish and each mass contained 20-40 eggs.

Day 3 The eggs had changed in colour from yellow to yellow ochre/brown, also in shape from spherical to ovoidal. The most advanced eggs seemed to consist of 2 halves forming a case which could split open in a similar manner to a "mussel" shell to liberate the young larvae inside. In these larvae, which were elipsoidal, some physical organisation was apparent. The anterior end was the large diameter of the ellipse and showed a grey area forming the head capsule and darker mouthparts and eye primordia whilst the small diameter end of the ellipse seemed to be the posterior end with dark areas being the proleg primordia.

Day 6 The newly hatched larvae were approximately 0.75 mm in length. The larval body was almost colourless and the length could only be determined by locating the black spots at the posterior end and the black spots just before the head capsule which were developing anterior prolegs. The head capsule was grey with black eye spots. As this stage it was noticed that some eggs had not developed and these were found some distance away from the introduced food mass. The developing larvae had originated from egg masses in contact with or in close proximity to the food, this suggests that if the distance between the larvae and the food source exceeds a certain value development will not occur.

- Day 7 The larvae were developing visible segments. The first thoracic segment was large and clear and the others were smaller towards the posterior end. Each segment was tinged with a violet coloured band.
- Day 8 The larvae were 1 mm long with well developed prolegs.
- Day 9 The larvae were 1.25 mm long and the violet colouration on the segments was becoming darker tending towards purple.
- Day 15 The larvae were clearly visible with the naked eye for the first time and were strongly banded with purple, they were 1.75 mm in length. At this stage some differences were noted in one dish. In that dish the larvae were sluggish, not so well developed and had colourless head capsules, the reason for this was not known but it could have been a pigment mutation.
- Day 20 The larvae were 2.0 mm long and were quite sluggish unless disturbed.
- Day 22 The larvae were 3.5 mm in length, the whole of the body was strongly banded with purple and the bands extended approximately half of the segments width.
- Day 24 The larvae were 4.0 mm in length.
- Day 27 The larvae were 4.5 mm in length (3rd instar size, 4th instar = 6.5 mm length)
- Day 53 The larvae had all pupated and most had emerged as adults.

According to the development time formula of Uvarov (1931), at 16°C the total life cycle period should have been 36 days, in fact it was found to be 53 days. The reason for the delay is not clear but it should be appreciated that it is difficult to simulate natural conditions in the incubator environment. The larvae were in perpetual darkness and did not experience diurnal fluctuations in temperature, also certain factors such as oxygen or food may have been limiting both of which may have caused the retarded development.

(ii) P.alternata and P.severini

Day 0 9 individuals of P.severini and 9 of P.alternata were placed in petri dishes prepared as before.

Day 6 P.severini eggs had been laid in masses containing 70-80 white ovoidal eggs (0.5 mm max, diameter). These eggs on development did not form cases but developed directly into larvae of which various stages of development were observed. The most advanced larvae were up to 0.75 mm in length, ovoidal and were distinguishable into posterior and anterior ends. The anterior end was grey forming the head capsule and at the posterior a yellow area contained the remains of the yolk and the respiratory siphon was developing.

Day 8 The larvae had developed head capsules, mouthparts and respiratory siphons and were actively grazing in the film. The largest was 1 mm in length, these were found to be quite sluggish and many were buried in the film with just the posterior respiratory siphon projecting. Black striations were present over the entire dorsal body surface however the larvae were still not visible to the naked eye.

- Day 13 P.severini larvae were well developed (3 mm in length) and were visible to the naked eye. The P.alternata larvae were much thinner and not so sturdy looking as the P.severini. The black striations on P.alternata were only present on the posterior end and in reduced numbers (4-5). The P.alternata larvae were showing carnivorous tendencies by eating their mothers' bodies.
- Day 15 P.severini larvae were 3.5 mm in length and consisted of a head, 10/11 segments and a respiratory siphon tail. There were 3 dark striations running across the dorsal surface of each segment. The P.alternata larvae were 2.5 mm in length and were physically similar to P.severini except that they had only 6 dark striations at the posterior end only.
- Day 17 The P.severini larvae were 4.5 mm in length, the P.alternata were 3.0 mm.
- Day 20 An incubator fault developed and a temperature as high as 30⁰C was noted, then it cooled below 16⁰C. Most of the P.alternata larvae died along with approximately half of the P.severini larvae, probably as a direct result of the temperature fluctuations.
- Day 21 Half of the P.severini larvae and the remaining P.alternata had pupated.
- Day 26 Both P.severini and P.alternata adults had emerged, 26 days from time of egg laying. According to Lloyd et al. (1940) at 16⁰C the life cycle development time for P.severini is 32 days, whilst for P.alternata it is 21 days.

Insufficient P.alternata were left to allow accurate life cycle time determination also it is likely that the temperature fluctuations gave misleading results, therefore it is not possible to compare these results with other published results.

In conclusion, although the results for life cycle periods do not fit well with published results this exercise was useful in allowing a better understanding of the development of these organisms. On reflection a more accurate method would have been to introduce diurnal temperature and light rhythms or even better to use a laboratory scale filter system (described earlier) as the development site as such a system would provide regular flushes with sewage, a fresh source of food as film, and ventilation all of which are suspected to be important factors controlling filter insect development. An important finding from these results and from the mating experiments with the laboratory scale filters is that it is possible to culture these insects through their entire life cycles from eggs to adults and on to successive generations.

Table 30a Suction fly trapping results

Date 19 – 20 May 1977

Mean air temperature 8°C

Weather Sunny/Calm (19th), Sunny/Stiff breeze (20th)

Sunset 20.50 hrs.

Hourly catches	<u>M.hydropetricus</u>	<u>P.severini</u>	<u>O.minimus</u>	<u>Leptocera spp.</u>	Misc
10.00 – 11.00	5	–	1	2	131
11.00 – 12.00	25	2	3	–	151
12.00 – 13.00	–	–	10	–	60
13.00 – 14.00	10	–	2	–	33
14.00 – 15.00	20	–	6	–	39
15.00 – 16.00	10	–	1	–	6
16.00 – 17.00	14	–	1	–	10
17.00 – 18.00	11	–	3	–	6
18.00 – 19.00	6	8	14	–	10
19.00 – 20.00	4	1	26	–	2
20.00 – 21.00	5	3	12	–	3
21.00 – 22.00	2	3	10	–	–
22.00 – 23.00	–	–	2	–	1
23.00 – 00.00	3	1	4	–	–
00.00 – 01.00	1	–	2	–	1
01.00 – 02.00	1	–	2	–	1
02.00 – 03.00	3	1	1	–	–
03.00 – 04.00	–	–	–	–	–
04.00 – 05.00	–	–	1	–	–
05.00 – 06.00	1	–	–	–	–
06.00 – 07.00	1	–	–	–	–
07.00 – 08.00	–	–	–	–	–

Total insect emergence 10.00 – 04.00 (16 hours) = 693

Table 30b Suction fly trapping results

Date 13 – 14 Sept. 1977

Mean air temperature 13°C

Weather Sunny/Light breeze (13th), Sunny/Stiff breeze (14th).

Sunset 19.20 hrs.

Hourly catches	<u>M.hydropetricus</u>	<u>P.severini</u>	<u>O.minimus</u>	<u>Leptocera spp.</u>	Misc.
11.00 – 12.00	63	–	–	–	–
12.00 – 13.00	286	–	–	–	6
13.00 – 14.00	55	–	–	2	6
14.00 – 15.00	49	–	–	2	1
15.00 – 16.00	52	1	–	6	1
16.00 – 17.00	52	6	–	20	–
17.00 – 18.00	21	17	–	4	–
18.00 – 19.00	43	120	–	2	46
19.00 – 20;00	9	15	1	–	3
20.00 – 21.00	3	13	–	–	3
21.00 – 22.00	1	44	–	–	1
22.00 – 23.00	3	27	–	–	1
23.00 – 00.00	2	22	–	–	1
00.00 – 01.00	16	16	–	–	1
01.00 – 02.00	10	3	–	–	1
02.00 – 03.00	2	–	–	–	–

total insect emergence over 16 hours = 1059

Fig. 143. The diurnal catch of flies in a suction trap 19th – 20th May 1977

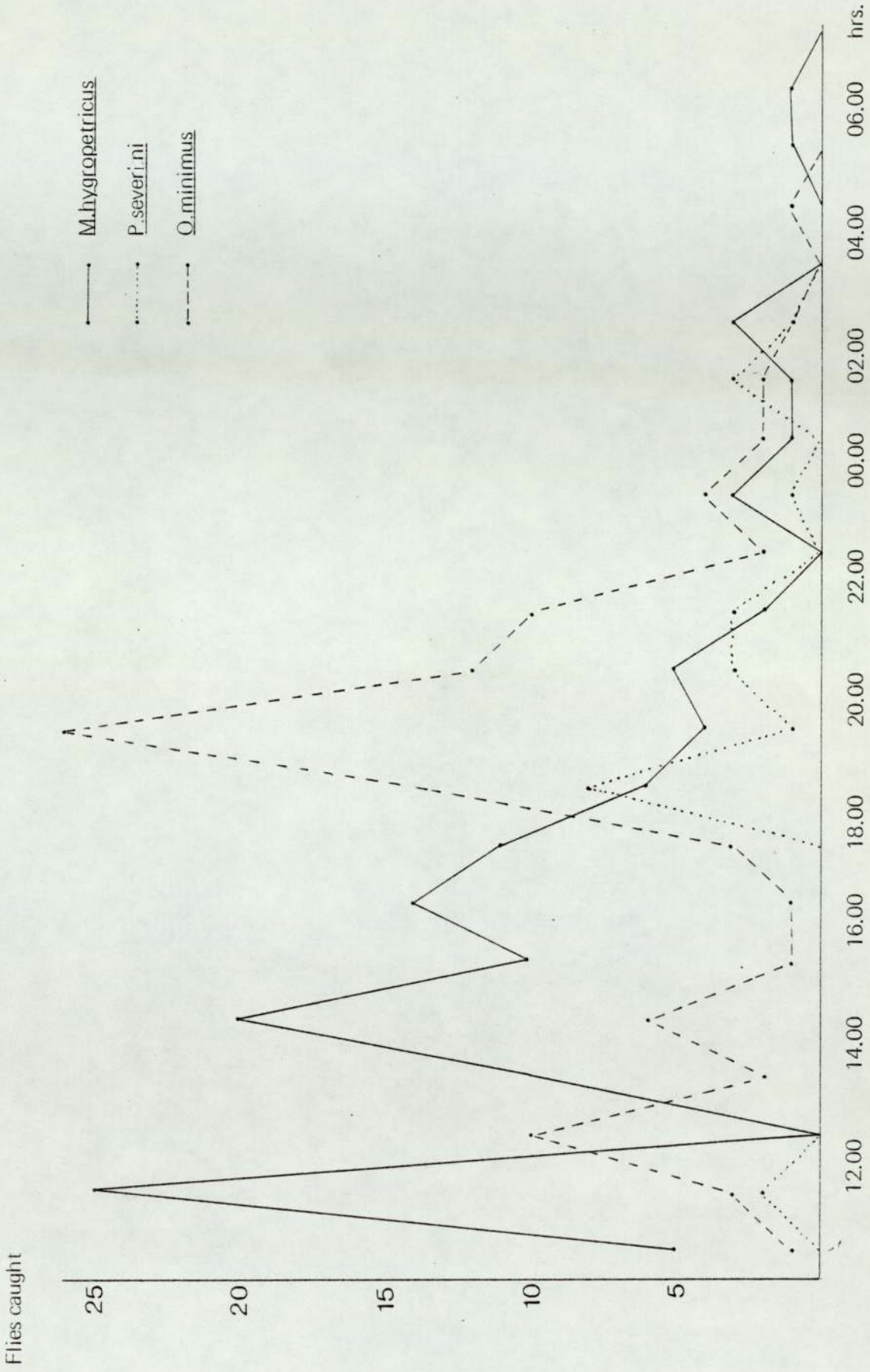
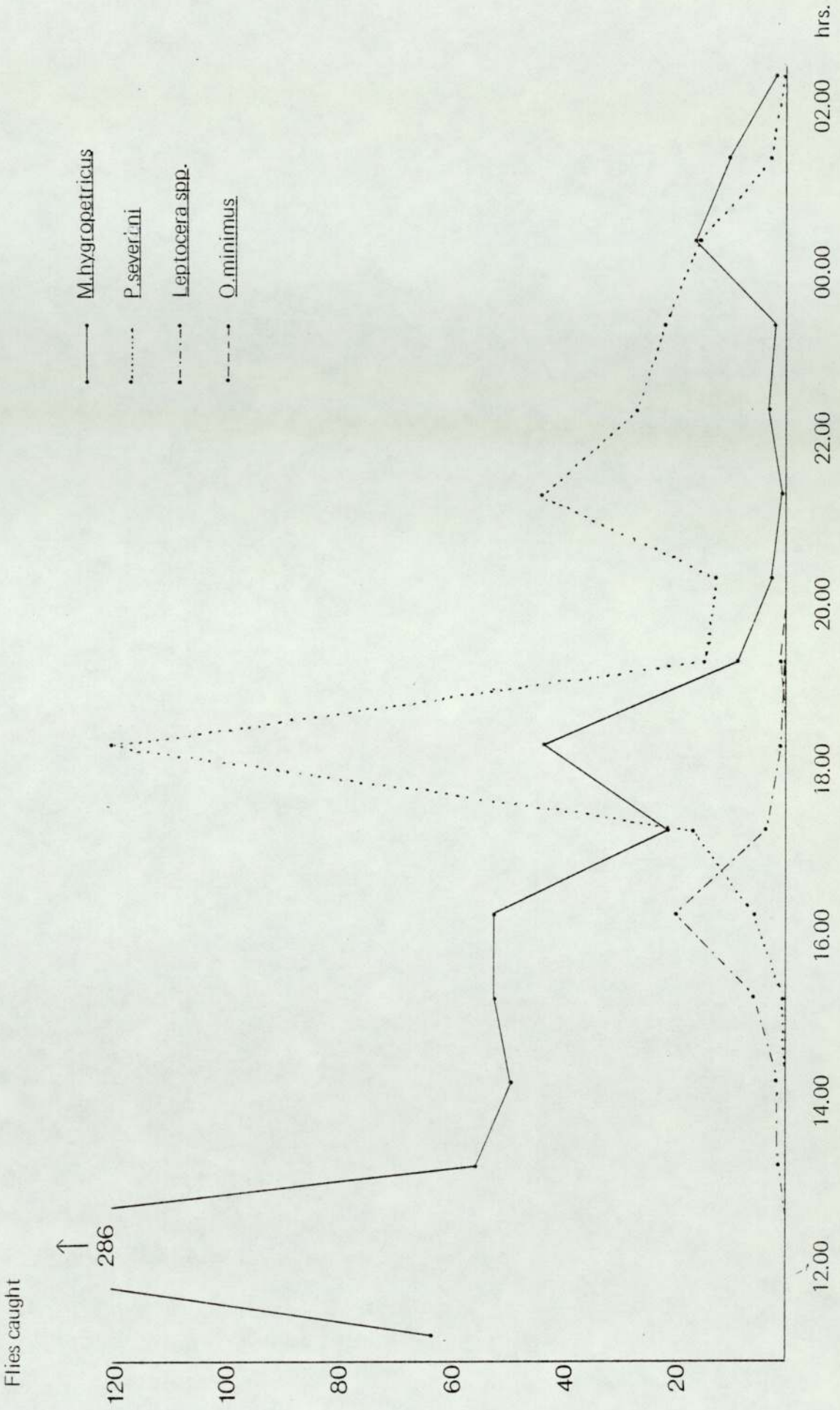


Fig. 144. The diurnal catch of flies in a suction trap 13th - 14th September 1977



Conclusions and recommendations

I Main filters

(a) Colonisation, succession and maturation of filter fauna

1. A fairly similar protozoan succession is apparent in newly commissioned filters to that found in activated sludge plants with initial flagellate domination of the microfauna, however in the initial seven weeks numbers of ciliate forms do not rise so sharply as found in the activated sludge situation.
2. Various factors affect the order of colonisation of filter macrofauna including operating conditions, the season of the year in which the filter is commissioned and the presence or not of already operational filters which can act as a source of inoculation.
3. It is clear that filter maturation, from the macrofauna population structure angle, is not achieved in a time period of 3 months from commissioning even though maturation from the filter efficiency angle may be achieved.

(b) Effects of Actellic M20 on filter ecology and factors to be considered on its usage

1. Actellic M20 can control M.hygroptericus if applied at a concentration of 5 mgdm^{-3} (active ingredient). P.severini numbers were not reduced at this concentration showing that higher concentrations are needed for their control, this has been borne out in toxicity testing work.
2. Competition in filters between M.hygroptericus and P.severini was suggested as P.severini numbers were found to rise when M.hygroptericus was controlled, this was substantiated in the laboratory scale filter experimental work.

3. Actellic M20 treatment caused enchytraeid worm levels to be erratic but not to be consistently different. This suggests that enchytraeids are in competition with M.hygroetricus as increases were apparent each time M.hygroetricus were removed by insecticide treatment.
4. No film accumulation problems were apparent on Actellic M20 treated filters.
5. The general view that the effectiveness of insecticide treatment decreases with increasing film levels does not always hold as it was found that the quality of the film and its water content had an effect. In extremely low film conditions, as found in the secondary filters of a double filtration system, control may not be so effective as not all larvae may be in contact with the insecticide affected film. This situation would not arise with higher film levels found in primary filters thereby allowing a more effective control.
6. The timing of an insecticide dose is important in relation to fly control effectiveness and film accumulation. The optimum time to dose M.hygroetricus is at the mid larval stage (283 day^{degrees} into a 540 day^{degrees} life cycle). Pupae and adults were not affected by the concentrations of Actellic M20 used on the filters.
7. It is important that excessive insecticide treatment is not applied in winter or prior to filter sloughing as film accumulation problems may occur. Operational conditions need to be studied before treatment as if film levels are potentially high, i.e. with small media or a high dosing frequency, this may cause film accumulation problems.
8. No problems with a decrease in filter efficiency on insecticide treated filters were found which was supported by laboratory scale filter tests.
9. A drop in effectiveness of Actellic M20 treatment was noticed over 3 years of usage and it is possible that this is due to resistance build up which has been

supported by the reduced susceptibilities found in treated compared to non-treated larvae in toxicity testing work.

10. Certain disadvantages are apparent with insecticide treatment which can be summarised as follows. Firstly concerning costs, although an insecticide treatment programme may have a low initial outlay, its ongoing costs are high for materials and for running a sampling programme which is necessary for correct dosage timing. Secondly supply problems can be encountered and the specific M20 formulation is not always stable so regular effective doses cannot always be guaranteed. Thirdly resistance problems may occur. Finally it is not environmentally desirable to add unnatural compounds such as insecticides to the environment when other methods of control could be used.

(c) Effects on filter ecology of modified operational procedures including filter loading, sewage dosing frequency, filter media size and sewage application method and factors to be considered when using operational methods for fly control

1. An increase in filter loading both hydraulic (0.8 to $1.6 \text{ m}^3 \text{ m}^{-3} \text{ day}^{-1}$) and organic (1.2 to $2.4 \text{ kg BOD m}^{-3} \text{ day}^{-1}$) caused a decrease in M.hygroptericus and P.severini emergence explained by lower surface film levels due to the increased scouring effect.
2. An increase in the dosing frequency from 15 to 7.5 mins. caused a drastic decrease in M.hygroptericus emergence and an increase in P.severini emergence. The dosing frequency change caused an increase in film levels due to lessened scouring action and bacterial growth effects and the fly population changes found can be explained by P.severini's physical features enabling it to respire in thicker film unlike M.hygroptericus.

3. A 229 mm (9 in.) depth of 19 mm ($\frac{3}{4}$ in.) dia. small medium caused a drastic decrease in M.hygropetricus emergence but did not have much effect on P.severini emergence. It is likely that the decreases in M.hygropetricus were caused by either the small void space found with this medium preventing emergence physically or increased film levels within the medium preventing their successful survival. The physical hindrance theory is likely as moderate numbers of M.hygropetricus larvae and pupae and large numbers of P.severini larvae and pupae were found in this medium which never manifested themselves in a large emergence.
4. Tracking problems, apparent with small medium alone, can be overcome by using a splash plate to spread the jets of sewage out to a continuous sheet. The advantages of small medium in fly control are retained.
5. Changing the application method from discrete jets to continuous sheet application of sewage on to the normal filter media (55 - 60 mm dia. slag) caused an increase in M.hygropetricus emergence but did not have much effect on P.severini emergence. The expected effect of this modification in increasing film levels was not apparent; more likely the effects of this were to spread the film out more evenly increasing the habitat for the already dominant M.hygropetricus.
6. The addition of a surface sprinkling of 19 mm ($\frac{3}{4}$ in.) dia. small medium to this modified area caused a rapid decline in M.hygropetricus emergence again pointing to the physical hindrance of emergence as the control mechanism.
7. On the secondary filters fly emergence levels were too low from modified operational and control areas for specific conclusions to be given. The main effects of the modifications on the secondary filters centre around slight increases and decreases in already very low film levels and the physical hindrance effects of small medium.

8. No film accumulation problems were experienced with the modifications described which is probably a reflection of the presence of large numbers of actively grazing enchytraeid worms controlling excessive film accumulation in conjunction with the scouring and nutritional effects of the low dosing frequency.
9. No decline in filter efficiency was noticed on the filters subjected to the modifications, these results were supported by pilot filter experiments.
10. It is considered that the advantages of operational methods of fly control outweigh the disadvantages. The only appreciable disadvantage is that use of some of the modifications would involve a high initial outlay, e.g. covering filters with 76 mm (3 in.) or 229 mm (9 in.) depth of small medium would not be economically viable however with other modifications this cost can be offset by the ongoing costs which are negligible. Another disadvantage is that operational methods may be slow to act whereas insecticidal methods give rapid fly control.
11. With operational forms of control no problems with toxic chemicals reaching the environment or loss of control due to resistance build up are found therefore they must be preferable over chemical control methods.
12. It is difficult to recommend a specific modification for the control of M.hydropetricus as different ones can be recommended depending on whether the filter is already operational or has yet to be built. With already operational filters a sprinkling of small medium in conjunction with splash plates could be applied, however the more preferred form of control would be to increase the dosing frequency as although no film accumulation problems with small medium were found they may arise in exceptional circumstances such as increased load or extreme weather conditions so making the filter less flexible.

13. When new filters are built it is recommended that the distributors are motor driven and provision for variable speed drive is given. The benefits of such a system could be twofold as the dosing frequency could be increased to control M.hydropetricus nuisance during the summer and then could be decreased to control film accumulation and retain efficiency in the winter.
14. Finally the best method of preventing fly nuisance is not to build dwellings near works sites, however as land space gets more scarce and dearer this problem can only increase. In such a situation where a works is surrounded by dwellings and operational methods have failed to remove a fly problem it is suggested that other forms of biological treatment are investigated e.g. activated sludge.

(d) Effects of climatic conditions on fly emergence and life cycle periods

1. Concerning weather conditions it has been found that large fly emergences are often found after a period of bad weather and high flows suggesting that these conditions retard development causing a larger than expected emergence when weather conditions improve.
2. The results obtained for the life cycle studies of M.hydropetricus, P.alternata and P.severini in relation to temperature have generally agreed with published work however it has been found that sudden extremes of temperature can cause inaccuracies in life cycle period predictions.
3. When predicting life cycle periods an accurate estimation of filter temperature is required. It has been the general view that the sewage temperature indicates the filter temperature however it is suggested that in certain operational conditions such as low dosing frequency, the sewage temperature does not indicate filter temperature. Obviously the most accurate means of measuring the filter temperature is to sink a thermocouple into the filter and measure the temperature directly. If this facility is not available the effluent temperature

can be used as it was found that this temperature was the most accurate for predicting life cycle periods when testing a range of others including the sewage temperature and the mean of the minimum and maximum air temperature. Therefore it is suggested that the effluent temperature closely resembles the filter temperature.

II Pilot filters

Effects of modified operational procedures including filter media size, sewage application method and filter surface covering on fly emergence and filter efficiency and factors to be considered in pilot filter experiments

1. Four of the five modifications investigated (small medium 229 mm depth with and without splash apparatus, small medium 76 mm depth, and splash apparatus with standard media and with a sprinkling of small medium) gave moderate decreases in M.hygropliticus emergence. One modification (physical covering of filter surface) gave only a slight reduction in M.hygropliticus emergence.
2. With the small medium modifications the greatest M.hygropliticus reductions were found when incorporating the splash apparatus suggesting similar control mechanisms as found in main filter investigations were operating. Similarly when a sprinkling of small medium was applied to the splash apparatus modification M.hygropliticus emergence was found to be drastically reduced suggesting that control mechanisms concern physical hindrance as found in the main filter investigations.
3. Covering the surface of a filter caused no change in Psychoda emergence but a slight decrease in M.hygropliticus emergence. The changes can be explained by the barrier causing a decrease in ventilation and consequently a higher filter temperature, the former tending to discourage both species and the latter

tending to favour Psychoda and discourage M.hygroptericus thus giving the observed effects.

4. As the physical barrier did not drastically decrease fly emergence it is deduced that flies can migrate towards a light source for emergence.
5. The control filter of the pilot filter system produced larger numbers of P.alternata (P.severini was the dominant Psychoda species on the main filters) and lower numbers of M.hygroptericus than the control filter on the main filter system. This could be explained by the dosage system on the pilot filters producing a much decreased scouring action. Another reason for the domination of the filters by P.alternata may be the higher temperatures experienced by these filters due to solar radiation favouring this species which thrives at higher temperatures than M.hygroptericus.
6. Generally large diurnal temperature variations (which are a common problem in pilot filter systems) and extremes of temperature were experienced by the pilot filters. This is in contrast to the more stable temperature environment found in full size filters.
7. The modifications applied to these filters had no great effects on hydraulic and chloride ion retention times. Very slight differences were noticed but these were most probably due to varying film levels in the different filters and not the physical effects of the modifications on the flow pattern through the filters.
8. Concerning purification efficiency, immediately after commissioning the expected pattern of initial good efficiency followed by a drop and then a recovery was followed. This phenomenon can be explained by the low film levels initially giving good efficiency and these film levels later increased causing efficiency to drop. The recovery was due to grazer populations building up so reducing the film levels.

9. No significant differences were found in purification efficiency (concerning BOD removal and TON production) between any of the modified filters and the control filters suggesting that no problems should be encountered if the modifications were applied on a larger scale.
10. Generally the pilot filters did not perform so well as the main filters regarding purification efficiency, this can be attributed to factors considered inherent disadvantages in pilot filter systems including wall interference and temperature effects. Such temperature effects mainly concern large diurnal variations as small scale filters are more susceptible to daily air temperature changes than full scale filters which have a high temperature buffering capacity.

III Laboratory scale filters

- (a) Effects of filter loading and sewage dosing frequency on fly emergence with reference to competition and investigations into filter solids discharge in connection with the activity of grazers
 1. M.hygroptericus can compete with and cause a reduction in P.alternata. This competition occurs at some time up to the third instar age as in initial experiments the larvae died at this age.
 2. P.alternata can compete with M.hygroptericus in mixed culture more successfully on high loads as P.alternata experienced a lower reduction in emergence (in mixed culture compared to pure culture) than M.hygroptericus experienced. Thus it can be concluded that P.alternata is better adapted to survive in high film conditions that are associated with high loadings.
 3. Although both species can exert competition forces on each other the forces exerted by M.hygroptericus are stronger than those exerted by P.alternata, as

P.alternata were always decreased to a greater extent than M.hygropetricus were when mixed and pure culture emergences were compared.

4. Increased loadings cause a decrease in M.hygropetricus emergence, also increased loadings cause an increase in P.alternata emergence assuming film levels do not rise to excess. In a mixed culture situation P.alternata seems to be affected by loading changes to a greater extent than M.hygropetricus again indicating that P.alternata exerts weaker competition forces than M.hygropetricus.
5. An increase in the dosing frequency causes a decrease in M.hygropetricus emergence. This is not specifically due to an increase in P.alternata favoured by high film levels exerting increased competition, but seems to be also due to the high film levels directly affecting M.hygropetricus. These effects are intensified when filters are on a high loading as high film levels are already present.
6. P.alternata is not always increased by high dosing frequencies as film levels, if already high, may rise to excess therefore causing ventilation, development and emergence problems. Also if starvation conditions exist in a mixed culture filter on a low loading an increase in dosing frequency giving a slight increase in already low film levels may allow a competitor of P.alternata (e.g. M.hygropetricus) to increase thereby checking P.alternata numbers so no increase would be noticed.
7. It was shown that the theoretical life cycle periods were followed both for M.hygropetricus and P.alternata when studied in constant temperature conditions. It is apparent that the pattern of emergence peaks can gradually lose their definition by the 3rd generation; an effect probably caused by not all of the members of an insect population developing at exactly identical rates, therefore this effect becomes increasingly apparent in subsequent generations.

These life cycle patterns seem to be most apparent when the insect concerned is in optimum conditions i.e. in the absence of other competitors and on an ideal loading, if not in optimum conditions these patterns are not so well defined.

8. It was found that the introduction of grazers to a filter caused an increase in effluent suspended solids levels. This increase was similar on filters experiencing different loadings and with different film levels therefore the increase must be due to the effects of grazing.
9. In pure culture a cycle of suspended solids discharge was apparent which was similar to the adult emergence pattern but was half a cycle out of phase with this. This cycle was found to have different lengths depending on which species was investigated as different species have different development times. Therefore the implications from these findings suggest that it should be possible, by studying changing suspended solids levels, to predict at which life cycle stage the bulk of an insect population is in, which is important when considering the time and effort taken by a sampling programme which is required for successful insecticide treatment.
10. Suspended solids peaks were slower to appear on low loaded than high loaded filters suggesting that loading and consequent film levels have an effect on insect development times.
11. No significant differences in purification efficiency were shown between filters containing M.hydropetricus and P.alternata in pure and mixed cultures and on different loadings.

(b) Effects of Actellic M20 on purification efficiency of laboratory scale filters

Actellic M20 does not have a significant effect on the purification efficiency of filters concerning carbonaceous oxidation. It is not possible to comment on the effects of Actellic M20 on nitrification as none of the laboratory filters nitrified properly. Actellic M20 was found to be very volatile and rapidly degraded to negligible levels in 24 hours when stored in settle sewage at an initial concentration of 4.5 mgdm^{-3} .

IV The toxicity of Actellic M20 to common filter grazers with reference to film effects and resistance build up

1. A relationship between cumulative percentage mortality and time when using Actellic M20 against M.hygroptericus larvae was apparent and this relationship followed a semi-sigmoidal pattern as expected with the cumulative percentage mortality being slow to increase initially, followed by a rapid increase and then a levelling off as 100% mortality was approached.
2. The presence of film in the test dishes reduced the susceptibility of M.hygroptericus larvae to Actellic M20. This suggests that the entry route of the insecticide is cutaneous and not via oral ingestion. This also shows that Actellic M20 is adsorbed by the film and that the adsorption process concerns physical attraction forces as the film used was biologically dead.
3. Film has a greater effect on reducing the susceptibility at low Actellic M20 concentrations suggesting that a unit volume of film can absorb a certain amount of insecticide.
4. A Log/log plot of median survival time against dose produces a curve which does not become asymptotic to the median survival time axis. Therefore

Actellic M20 is truly toxic to M.hydropetricus and no low level tolerance is shown.

5. Actellic M20 is also toxic against other filter grazers comprising S.fenestralis, P.severini, L.rivalis and P.alternata in decreasing order of susceptibility. The levels of susceptibility suggest that Actellic M20 could be used successfully against M.hydropetricus, fairly successfully against S.fenestralis and P.severini, however costs would be high, also it is doubtful whether it could be used against P.alternata as costs would be prohibitive.
6. The fact that the enchytraeid worm L.rivalis is susceptible to Actellic M20 treatment shows that these worms use similar nervous transmitter substances to those found in insects.
7. The quantity of Actellic M20 required to kill M.hydropetricus is much lower than that required to give the same kill of other species by at least a factor of 10 showing that Actellic M20 can be used on a filter in specific quantities to kill M.hydropetricus which would not detrimentally affect other grazers, therefore no film accumulation problems would arise in a mixed culture filter. Therefore Actellic M20 shows some degree of target specificity.
8. When the susceptibility of M.hydropetricus larvae from a site subjected to Actellic M20 treatment for 3 years is compared to the susceptibility of M.hydropetricus larvae from a site not subjected to treatment differences are shown which suggests that resistance could build up. Further work needs to be carried out to definitely state whether resistance is building up and when researching into the literature resistance build up to organophosphates, of which Actellic M20 is a member, is a far more common occurrence than is usually appreciated.

V Miscellaneous experimental work

(a) Diurnal emergence of filter flies

Certain filter insects do show a diurnal pattern to their emergence. M.hydropetricus are found in maximum numbers around midday/early afternoon whilst O.minimus is mainly caught in a dusk period between 18.00 and 22.00 hrs. Similarly most P.severini are found around 18.00 hrs. with smaller peaks into the hours of darkness and Leptocera spp. are found mainly in the afternoon around 16.30 hrs.

(b) Culture experiments

1. It has been found possible to culture M.hydropetricus, P.severini and P.alternata in petri-dishes through their life cycles from eggs to adults.
2. With M.hydropetricus it seems that a tactile stimulus is needed for egg laying as eggs were only laid around the side edges of the petri-dishes and not in the centre.
3. In culture experiments insect life cycle periods according to temperature were not followed well. With P.severini and P.alternata an incubator fault gave a shorter than usual life cycle. However with M.hydropetricus when the incubator was working normally the life cycle was much longer than expected at the specific temperature used. This effect can be explained by the stresses present in the petri-dish environment including the lack of fresh food, ventilation and photoperiodicity.

References

Agersborg H.P.K. Hatfield W.D. 1929

Sewage Wks. J. 1929 1 411-424

Allison 1940

"The control of flies on percolating filters"

J. Inst. Sew. Purif. 1940 212-218

Attia F.I. 1976

"Insecticide resistance in Cadra cautella in New South Wales"

J. Economic Ent. 1976 69 6 773-774

Baker J.P. 1977

"Assessment of the potential for and development of organophosphate resistance in field populations of Myzus persicae"

Ann. Appl. Biol. 1977 86 1 1-9

Balakrishnan S. 1969

"Organic removal by a selected trickling filter medium"

Water and Waste Eng. 1969 6 422

Barker A.N. 1942

"The seasonal incidence, occurrence and distribution of protozoa in the bacteria bed process of sewage disposal"

Ann. Appl. Biol. 1942 29 23-33

Barritt N.W. 1939

"Double filtration"

J. Inst. Sew. Purif. 1939 1 113-120

Bayley R.W. Downing A.L. 1963

J. Inst. Pub. Health Engrs. 1963 62 303-332

Bell H.D. 1926

"Research on the composition of grey slimy growth on the surface of sewage filters"

Proc. Assn. Mngrs. Sewage Disp. Wks. 1926 111

Bliss C.L. 1937

"The calculation of the time mortality curve"

Ann. Appl. Biol. 1937 24 815-852

Bridges W.R. Kallman B.J. Andrews A.K. 1963

"Persistence of DDT and its metabolites in a farm pond"

Trans. Am. Fish Soc. 1963 92 421

Bridge-Cooke W. 1959

"Trickling filter ecology"

Ecology 1959 40 2 273-291

Bridge-Cooke W. Hirsch A. 1958

"Continous sampling of trickling filter populations"

Sewage and Industrial Wastes 1958 30 138-155

Broadbent L. 1948

"Aphis migration and the efficiency of the trapping method"

Ann. Appl. Biol. 1948 35 379

Brothers W.C. 1946

"Experiments with DDT in filter fly control"

Sewage Works J. 1946 18 181

Bruce W.N. Decker G.C. 1950

"House fly tolerance for insecticides"

Soap and San. Chem. 1950 26 122-123 145-147

Bruce A.M. Merkens J.C. 1970

"Recent studies of high rate biological filtration"

Wat. Poll. Cont. 1970 69 113-139

Bruce A.M. Merkens J.C. Haynes B.A.O. 1975

"Pilot scale studies on the treatment of domestic sewage by two stage biological filtration - with special reference to nitrification"

Wat. Poll. Cont. 1975 74 80-100

Bryce D. 1960

"Studies on the larvae of the British Chironomidae (Diptera) with keys to the Chironominae and Tanypodinae"

Trans. Soc. Brit. Ent. 1960 14 19-62

Bryce D. Hobart A. 1972

"The biology and identification of the larvae of the Chironomidae (Diptera)"

Entomologists gazette 1972 23 175-217

Bull C.J. McInerney J.E. 1974

"Behaviour of juvenile Coho Salmon (Onchorhynchus kisutch) exposed to sumithion (fenitrothion) - an organophosphate insecticide"

Journal Fisheries Research Board of Canada 1974 31 12 1867-1872

Bull D.L. Whitten C.J. 1972

"Factors influencing organophosphate resistance in tobacco budworms"

J. Agricultural and food chemistry 1972 20 3 561-564

Busvine J.R. 1971

"A critical review of techniques for testing insecticides"

Commonwealth Inst. Ent. 1971

Coe R.L. 1950

"Family Chironomidae"

Handbooks for the identification of British insects Vol 1X Pt.2 Royal Ent. Soc.
London. 1950

Colyer C.N. Hammond 1968

"Flies of the British Isles"

F. Warne 1968 (2nd Ed.)

Cook E.E. Crame L. 1976

"Effects of dosing rates on trickling filter performance"

J. Wat. Poll. Cont. Fed. 1976 48 12 2723-2730

Cook E.E. Fleming M.L. 1974

"Trickling filter performance as related to media surface area"

Public Works 1974 105 1 69-72

Cook E.E. Katzberger S.M. 1977

"Effect of residence time on fixed film reactor performance"

J. Wat. Poll. Cont. Fed. 1977 49 8 1889-1895

Coppage D.L. Matthews E. 1974

"Short term effects of organophosphate insecticides on cholinesterases of
estuarine fishes and pink shrimp"

Bull. Env. Contam. Toxicol. 1974 11 5 483-488

Craft T.F. Ingols R.S. 1973

"Flow through time in trickling filters"

Water and Sewage Works 1973 120 78

Curds C.R. 1966

"An ecological study of the ciliated protozoa in Activated sludge"

Oikos 1966 15 282-289

Curds C.R. 1971

"Computer simulations of microbial population dynamics in the activated-sludge process"

Water Research 1971 5 1049-1066

Curds C.R. 1973

"The theoretical study of factors influencing the microbial population dynamics in the activated-sludge process-1"

Water Research 1973 7 1269-1284

Curds C.R. Cockburn A. 1970

"Protozoa in biological treatment processes - 11. Protozoa as indicators in the activated-sludge process"

Water Research 1970 4 237-249

Dekema C.J. Murray K.A. 1942

"Deep enclosed artificially vented filter beds v. ordinary open filter beds"

J. Inst. Sew. Purif. 1942 311-318

Del Rosario F. 1936

"The American species of Psychoda (Diptera : Psychodidae)"

Philipp J. Sci. 1936 59 85

Devonshire A.L. 1975

"Studies of the acetylcholinesterase from houseflies (Musca domestica L.)
resistant and susceptible to organophosphate insecticides"

Biochemical Journal 1975 149 2 463-469

Devonshire A.L. Foster G.N. Sawicki R.M. 1977

"Peach-potato aphid, Myzus persicae (Sulz.) resistant to organophosphorus
and carbamate insecticides on potatoes in Scotland"

Plant Pathology 1977 26 2 60-62

Duddles G.A. Richardson S.E. Barth E.F. 1974

"Plastic medium trickling filters for biological nitrogen control"

J. Wat. Poll. Cont. Fed. 1974 46 5 937-946

Duffey J.R. O'Connell D. 1968

"DDT residues and metabolites in Canadian Atlantic coast fish"

J. Fish. Res. Board Canada 1968 25 189

Dyson J.E.B. Lloyd L. 1933

"Remarks on the flies breeding in the bacteria beds at the Knostrop
sewage works, Leeds"

Surveyor 1933 84 335-341

Dyson J.E.B. Lloyd L. 1935

"The distribution of the early stages of Metriocnemus Longitarsus Goet.
(Chironomidae) in sewage bacteria beds"

Proc. Leeds Phil. Soc. 1935 3 111 174-176

Eden G.E. Brendish K. Harvey B.R. 1964

"Measurement and significance of retention in percolating filters"

J. Inst. Sew. Purif. 1964 6 513-525

Edmonson J.H. Goodrich S.R. 1943

"The Cyclo-Nitrifying filter"

J. Inst. Sew. Purif. 1943 57-59

Edwards C.A. 1975

"Persistent pesticides in the environment"

CRC Press Inc. Cleveland Ohio. 2nd Ed. 1975 176 pp.

Edwards F.W. 1929

"British non-biting midges"

Trans. Ent. Soc. Lond. 1929 77 279-430

Eto M. 1974

"Organophosphorus pesticides - organic and biological chemistry"

CRC Press Inc. 18901 Cranwood Parkway Cleveland Ohio 44128 1974

Fair G.M. 1934

"The trickling filter fly (P.alternata) its habits and control"

Sewage Works J. 1934 6 966-981

Fleming W.E. Parker L.B. Maines W.W. Plasket E.L. McCabe P.J. 1962

"Bioassay of soil containing residues of chlorinated hydrocarbon insecticides"

USDA Tech. Bull. 1962 1266 44

Freeman P. 1950

"Handbooks for the identification of British Insects Vol 1X Pt.2
Trichoceridae, Ptychopteridae and Anisopodidae"

Trans. Roy. Ent. Soc. Lond. 1950

Frye W.W. Levine M. Becker E.R. 1931

"Effects of some insecticides on the sewage sprinkling filter fly, Psychoda alternata"

Sewage Works J. 1931 3 345

Galler W.S. Cotaas H.B. 1964

"Analysis of biological filter variables"

J. San. Eng. Div. Proc. Amer. Soc. Civ. Engr. 1964 90 SA6 59

Gameson A.L.H. Truesdale G.A. Overdijk M.J. 1961

"Variation in performance of 12 replicate small scale filters"

J. Inst. Sew. Purif. 1961 4 342-350

Gardner D.R. Bailey J.R. 1976

"Methoxychlor - its effects on environmental quality"

National research council of Canada 1976 14102 164 pp.

Gast M.K.H. 1974

"Multi stage biological treatment systems"

H₂O 1974 7 18 377-385

Georghiou G.P. Breeland S.G. Ariartnam V. 1973

"Seasonal escalation of organophosphate and carbamate resistance in
Anophles albimanus by agricultural sprays"

Environmental Entomology 1973 2 3 369-374

Georghiou G.P. Ariartnam V. Pasternak M.E. Lin C.S. 1975

"Organophosphate multiresistance in Culex pipiens quinquefasciatus in
California"

J. Economic. Ent. 1975 68 4 461-467

Gerolt P. 1976

"The mode of action of insecticides: accelerated water loss and reduced
respiration in insecticide treated Musca domestica"

Pesticide Science 1976 7 6 604-620

Gibson N.H.E. 1945

"On the mating swarms of certain Chironomidae (Diptera)"

Trans. Roy. Ent. Soc. Lond. 1945 95 263-294

Gilcreas F.F. Sanderson W.W. 1940

"The development of sewage oxidation by a trickling filter"

Sewage Works J. 1940 12 73-80

Gjullin C.M. Isaak L.W. 1957

"Present status of mosquito resistance to insecticides in the San Joaquin Valley in California"

Mosquito news 1957 17

Godsil P.J. Johnson W.C. 1968

"Pesticide monitoring of the aquatic biota at the Tule Lake National Wildlife Refuge"

Pest. Mon. J. 1968 4 21

Goldthorpe H.H. 1943

"A cubic yard of percolating bed material and a few assumptions based on experimental evidence"

J. Inst. Sew. Purif. 1943 93-102

Golightly W.H. 1940

"Factors influencing the abundance and size of Psychoda species in sewage bacteria beds"

Ann. Appl. Biol. 1940 27 406-421

Graaf J.H.J. 1976

"Can biological treatment processes be optimised in relation to temperature?"

H₂O 1976 5 87-94

Green M.B. 1970

"Insect populations of sludge drying beds"

Wat. Poll. Cont. 1970 69 4 399-408

Green M.B. Willetts D.G. Bennet M. Crowther R.F. Bourton J. 1975

"Applications of toxicity testing to sewage treatment processes"

Wat. Poll. Cont. 1975 74 1 40-58

Gruhl K. 1924

"Paarungsgewohnheiten der Dipteren"

Z. Wiss. Zool. 1924 122 205-280

Gutierrez M.C. Zboray E.P. Gillies P.A. 1976

"Insecticide susceptibility of mosquitoes in California: status of organophosphate resistance in larval Aedes nigromaculis, Culex tarsalis and Culex pipiens subsp"

Calif. Vector Views 1976 23 7/8 27-33

Guzman-Varnon R. Monroe R.J. Guthrie F.E. 1974

"Evaluation of cholinesterases in strains of Helithis virescens with widely differing responses to insecticides"

J. Econ. Ent. 1974 67 2 187-189

H.M.S.O. 1972

"Analysis of raw, potable and waste waters"

D.O.E. H.M.S.O. Lond. 1972

Hannon M.R. Greichus Y.A. Applegate R.L. Fox A.C. 1970

"Ecological distribution of pesticides in Lake Poinsett, S. Dakota"

Trans. Am. Fish. Soc. 1970 99 3 496

Harvey B.R. Eden G.E. Mitchell N.T. 1963

"Neutron scattering: A technique for the direct determination of the amount of biological film in a percolating filter"

J. Inst. Sew. Purif. 1963 5 495

Hawkes H.A. 1951 a

"A study of the biology and control of Anisopus fenestralis (Scopoli 1763), a fly associated with sewage filters"

Ann. Appl. Biol. 1951 38 592-605

Hawkes H.A. 1951 b

"The ecology of Anisopus fenestralis Scop. (Diptera) in sewage bacteria beds"

Ann. Appl. Biol. 1951 39 181-192

Hawkes H.A. 1955 a

"The effect of periodicity of dosing on the amount of film and numbers of insects and worms in alternating double filters at Minworth, Birmingham"

J. Inst. Sew. Purif. 1955 1 48-50

Hawkes H.A. 1955 b

"Benefits in the filters by slow dosing"

Surveyor and Munic. County Eng. 1955 114 147-148

Hawkes H.A. 1955 c

"The effects of insecticide treatment on the macrofauna population, film accumulation and efficiency of percolating filters"

Ann. Appl. Biol. 1955 43 122-133

Hawkes H.A. 1957

"Film accumulation and grazing activity in the sewage filters at Birmingham"

J. Inst. Sew. Purif. 1957 2 88-110

Hawkes H.A. 1959

"The effect of methods of sewage application on the ecology of bacteria beds"

Ann. Appl. Biol. 1959 47 339-349

Hawkes H.A. 1961 a

"Fluctuations in the aerial density of Anisopus fenestralis Scop. (Diptera) above sewage bacteria beds"

Ann. Appl. Biol. 1961 49 66-76

Hawkes H.A. 1961 b

"An ecological approach to some bacteria bed problems"

J. Inst. Sew. Purif. 1961 2 105-132

Hawkes H.A. 1965

"Factors influencing the seasonal incidence of fungal growths in sewage bacteria beds"

Int. J. Air Wat. Pollut. 1965 9 693

Hawkes H.A. Jenkins S.H. 1951

"Biological principles in sewage purification"

J. Inst. Sew. Purif. 1951 3 300-318

Hawkes H.A. Jenkins S.H. 1955

"Comparison of four grades of sewage percolating filter media in relation to purification, film accumulation and fauna"

J. Inst. Sew. Purif. 1955 4 352-357

Hawkes H.A. Jenkins S.H. 1958

"Comparison of four grades of media in relation to purification, film accumulation and fauna of sewage percolating filters operating an alternating double filtration"

J. Inst. Sew. Purif. 1958 2 221-225

Hawkes H.A. Shephard M.R.N. 1972

"The effect of dosing frequency on the seasonal fluctuations and vertical distribution of solids and grazing fauna in sewage percolating filters"

Water Research 1972 6 721-730

Hawkes H.A. Shephard M.R.N. 1975

"Laboratory studies on the effects of temperature on the accumulation of solids in sewage percolating filters"

Paper presented at Inst. Wat. Poll. Cont. Meeting (West Mids. branch) 26 Mar. 1975 Univ. of Aston in Birmingham

Headlee T.J. 1919

J. Econ. Ent. 1919 12 35-41

Heukelekian H. 1945

"The relationship between accumulation, biochemical and biological characteristics of film and purification capacity of a biofilter and a standard filter"

Sewage Works J. 1945 17 23 269, 516, 743

Hockenbury M.R. Daigger G.T. Grady C.P.L. 1977

"Factors affecting nitrification"

J. Env. Eng. Div. ASCE 1977 103 EE1 9-19 Pap. No. 12713

Holden A.V. 1966

"Organochlorine insecticide residues in Salmonid fish"

J. Appl. Ecol. (Supl) 1966 3 45

Holtje R.H. 1943

"The biology of sewage sprinkling filters"

Sewage Works J. 1943 15 14-29

Horosawa I. 1950

J. Jap. Sewage 1950 148 62-67

Howard L.D. Dyer H.G. Knab F. 1912

"The mosquitoes of North and Central America and the West Indies"

Publ. Carnegie Instn. 1912 No. 159 Washington

Howell J.A. Atkinson B. 1976

"Sloughing of microbial film in trickling filters"

Water Research 1976 10 4 307-315

Hudson J.A. Phillips G.A. 1975

"Developments in sewage treatment at Leeds"

Wat. Poll. Cont. 1975 74 5 544-559

Ishaya I. Casida J.E. 1974

"Dietary TH-6040 alters compositions and enzyme activity of housefly larval cuticle"

Pest. Biochem. Physiol. 1974

Jackson R.B. 1943

Sewage Works J. 1943 15 1249

Jackson S. Brown V.M. 1970

"Effect of toxic wastes on treatment processes and watercourses"

Wat. Poll. Cont. 1970 69 3 292-313

Jenkins S.H. Baines S. Hawkes H.A. 1949

"The control of Anisopus fenestralis and factors influencing the numbers of Anisopus caught in surface traps"

J. Inst. Sew. Purif. 1949 2 178-187

Jennings P.A. Snoeyink V.L. Chian E.S.K. 1976

"Theoretical model for a submerged biological filter"

Biotechnology and Bioengineering 1976 18 9 1249-1273

Jensen S. Johnells A.G. Olsson M. Otterlund G. 1969

"DDT and PCB in marine animals from Swedish waters"

Nature 1969 224 247

Johnson J.W.H. 1914

"A contribution to the biology of sewage disposal"

J. Econ. Biol. 1914 9 3 105-127

Kallman B.J. Cope O.B. Navarre R.J. 1962

"Distribution and detoxification of toxaphene in Clayton Lake, New Mexico"

Trans. Am. Fish. Soc. 1962 91 14

Kamemoto F.I. Goodnight C.J. 1956

"The effects of various concentrations of ions on the asexual reproduction of the Oligochaete Aeolosoma hemprichi"

Trans. Amer. Micr. Soc. 1956 75 2 219

Keith J.O. 1966

"Insecticide contaminations in wetland habitats and their effects on fish eating birds"

J. Appl. Ecol. (Supl). 1966 3 71

Keith J.O. Hunt E.G. 1966

"Levels of insecticide residues in fish and wildlife in California"

Trans. 31st North Am. Wildl. Res. Conf. 1966 150

Kerrich G.J. Meikle R.D. Tebble N. 1967

"Bibliography of the key works for the identification of British flora and fauna"

The Systematics Assoc. Lond. 1967

Kessler L.H. Norgaard J.T. 1942

Sewage Works J. 1942 14 1228

Khalsa H.G. 1948

"Biology of Anisopus fenestralis Scop. A new invader of the sewage filter beds at Knostrop, Leeds"

Ph. D. Thesis Univ. of Leeds 1-61

Khan S.U. 1972

"Adsorption of pesticide by humic substances : a review"

Environmental letters 1972 3 1 1-12

Klipfontein Organic Products 1949

"Control of domestic fly breeding in digested sludge - use of BHC dust"

Publ. Hlth. Johannesburg 1949 13 153

Krige W.P. 1962

"Effects of different grades of filter medium on the purification of sewage in biological filters"

J. Inst. Sew. Purif. 1962 2 150-157

Laverack M.S. 1963

"The physiology of Earthworms"

Pergamon Press Oxford 1963

Learner M.A. 1975

"Chapter 9 Insecta"

"Ecological aspects of used water treatment Vol 1" Eds. Curds C.R. Hawkes H.A. Academic Press Lond. 1975 337 pp.

Litchfield J.R. Wilcoxon F.W. 1949

"A simplified method of evaluating dose-effect experiments"

J. Pharmac. Exp. Ther. 1949 96 99-113

Lloyd L.I. 1935

"The bacteria beds of sewage works as an environment for insects"

Proc. R. Ent. Soc. 1935 10 34

Lloyd LI. 1937

"Observations on sewage flies, their seasonal incidence and abundance"

J. Inst. Sew. Purif. 1937 1 150-165

Lloyd LI. 1941

"The seasonal rhythm of a fly (Spaniotoma minima) and some theoretical considerations"

Trans. Roy. Soc. Trop. Med. Hyg. 1941 35 93-104

Lloyd LI. 1943 a

"Materials for a study in animal competition - The fauna of sewage bacteria beds. Part II"

Ann. Appl. Biol. 1943 30 47-60

Lloyd LI. 1943 b

"Materials for a study in animal competition Part III. The seasonal rhythm of a fly Psychoda alternata (Say) and an effect of intraspecific competition"

Ann. Appl. Biol. 1943 30 358-364

Lloyd LI. 1943 c

"Town planning and the small sewage purification plant"

Nature 1943 151 475

Lloyd L.I. 1944 a

"Biological investigations in relation to incipient ponding"

J. Inst. Sew. Purif. 2 71-76

Lloyd L.I. 1944 b

"Sewage bacteria bed fauna in its natural setting"

Nature 1944 154 397

Lloyd L.I. 1945

"Animal life in sewage purification processes"

J. Inst. Sew. Purif. 1945 1 119-139

Lloyd L.I. Graham J.F. Reynoldson T.B. 1940

"Materials for a study in animal competition, the fauna of sewage bacteria beds"

Ann. Appl. Biol. 1940 27 122-150

Lloyd R. 1960

"The toxicity of Zinc sulphate to Rainbow Trout"

Ann. Appl. Biol. 1960 48 84-94

Lumb C. Barnes J.P. 1948

"The periodicity of dosing percolating filters"

J. Inst. Sew. Purif. 1948 1 83-98

Lundie M. 1940

"The control of flies in percolating filters"

J. Inst. Sew. Purif. 1940 212-218

Marshall J.F. 1938

"The British Mosquitoes"

London 1938

McKinney R.E. Gram A. 1956

Sew. Ind. Wastes 1956 28 1219-1237

McNicholas J. Kirkwood J.W. 1953

"Some definitions, facts and figures relating to sewage treatment"

J. Inst. Sew. Purif. 1953 1 42-49

Meltzer D. 1962

"Investigation of the time factor in biological filtration"

J. Inst. Sew. Purif. 1962 2 169-175

Micks D.W. Rougeau D. 1977

"Organophosphorus tolerance in Culex pipiens quinquefasciatus in Texas"

Mosquito News 1977 37 2 233-239

Missiroli A. 1947

"Riduzione o eradicazione degli anofeli"

Riv. di. Parassitol. 1947 8 125-126

Motoyama N. Dauterman W.C.

"The role of nonoxidative metabolism in organophosphate resistance"

Dep. Ent. N. Carolina State Univ. Raleigh N.C. 27607 U.S.A 350-356

Mulla M.S.

"Resistance in culicine mosquitoes in California - countermeasures"

Dep. Ent. Calif. Univ. Riverside Calif. U.S.A.

Murray K.A. 1939

"The control of filter flies on percolating filters"

J. Inst. Sew. Purif. 1939 2 350-354

Niani D. 1955

"Spray plate controls filter flies"

Wastes Engng. 1955 26 77

Novelli G. 1975

"The use of biological filters"

Ingegneria Ambientale 1975 4 6 576-583

Olroyd H. 1964

"The natural history of flies"

Wiederfeld and Nicholson London 1964

Oppenoorth F.J. Smitsaert H.R. Welling W. Pas L.J.T. Van der, Hitman K.T.
1977

"Insensitive acetylcholinesterase, high glutathione - S - transeferase and
the hydrolytic activity as resistance factors in a tetrachlorovinphos
resistant strain of house fly"

Pest. Biochem. Physiol. 1977 7 1 34-47

Ordman D. 1946

"Bronchial asthma caused by trickling sewage filter fly (Psychoda) :
inhalent insect allergy"

Nature Lond. 1946 157 441

O'Shaughnessy F.R. 1931

"Some considerations in the oxidation of sewage"

Proc. Ass. Sew. Disp. Wks. 1931 1 74

Otter den C.J. 1959

"Investigations into an ecological method for control of Psychoda spp. at
waste water treatment plants in the Netherlands"

Report to Rijksinstituut voor Zuivering van Afval water Voorburg, The
Netherlands. 1959.

Otter den C.J. 1966

"A physical method for the permanent control of Psychoda pests at wastewater treatment plants"

J. Wat. Poll. Cont. Fed. 1966 38 156-164

Painter H.A. 1970

"A review of the literature on inorganic nitrogen metabolism in micro-organisms"

Wat. Res. 1970 4 393

Parkinson W.H. Bell H.B. 1919

"Insect life in sewage filters"

Sanitary Pub. Co. London 1919

Peach J.P. 1957

"Some percolating filter experiments at Cheltenham"

J. Inst. Sew. Purif. 1957 2 111-115

Peterle T.J. 1967

"Translocation and bioaccumulation of Cl^{36} DDT in a freshwater marsh"

VII Congress des Biologistes du Gibier. Beograd Ljubljana 1965 1967

Plapp F.W. 1976

"Biochemical genetics of insecticide resistance"

Ann. Rev. Ent. 1976 21 179-197

Quarterman K.D. Schoof H.F. 1958

"The status of insecticide resistance in Athropods of public health importance in 1956"

Amer. Jour. Trop. Med. Hyg. 1958 7 74

Rao T.R. Russel P.F. 1938

"Some field observations on the swarming and pairing of mosquitoes particularly A. annularis in South India"

J. Malaria Inst. India 1938 1 395-403

Rawn A.M. 1949

"Eradication of sludge flies"

Wat. Sewage Wks. 1949 96 260

Reay R.C. 1969

"Insects and Insecticides"

Contemporary Science Paperbacks, Oliver and Boyd Edinborough 1969

Reinke J. Uthe J.F. Jamieson D. 1972

"Organochlorine pesticide residues in commercially caught fish in Canada - 1970"

Pest. Mon. J. 1972 6 1 43

Remaudiere G. Iperti G. Leclant F. Lyon J.P. Michel M.F. 1973

"Biology and ecology of aphids and their natural enemies, application of integrated control in orchards"

Entomorphaga 1973 Mem. Hors. Ser. 6 35pp.

Reynoldson T.B. 1941

"The biology of the macrofauna of a high rate double filtration plant"

J. Inst. Sew. Purif. 1941 109-128

Reynoldson T.B. 1942

"Further studies on the biology of a double filtration plant at Huddersfield"

J. Inst. Sew. Purif. 1942 116-139

Reynoldson T.B. 1948

"An ecological study of the enchytraeid worm populations of sewage bacteria beds : Synthesis of field and laboratory data"

J. Anim. Ecol. 1948 17 27

Richards A.G. Cutkomp L.K. 1946

"Correlation between the possession of a chitinous cuticle and sensitivity to DDT"

Bio. Bull. 1946 90 97-108

Richards O.W. 1930

"Key to the Sphaeroceridae"

Proc. Zoo. Soc. Lond. 1930 261

Riedmuller S. 1957

"Die Bedeutung der "Tropfkörperfliege" Psychoda für die
Reinigungswirkung des Tropfkörpers"

Paper presented at meeting of Bayer. Biol. Versucharstalt, Munich, Germany
1957.

Roslatseva S.A. Golovfin G.V. Ivanova G.B. Aldoshina T.I. 1975

"A study of the epicula protective characteristics in Musca domestica L.
races resistant to organophosphate insecticides"

All Union Research Inst. Chem. Plant Protection Moscow USSR 1975 133-135

Sacca G. 1947

"Sull esistenza di Mosche domestiche resistenti al DDT"

Riv. di Parassitol 1947 8 127-128

Satchell G.H. 1947

"The ecology of the British species of Psychoda (Diptera : Psychodidae)"

Ann. Appl. Biol. 1947 34 611

Satchell G.H. 1947

"The larvae of the British species of *Psychoda* (Diptera : Psychodidae)"

Parasitology 1947 38 51-69

Sawicki R.M. 1973

"Resynthesis of multiple resistance to organophosphate insecticides from strains with factors of resistance isolated from the S.K.A. strain of houseflies"

Pesticide Science 1973 4 2 171-180

Schaeffer C.H. Dupras E.F. 1976

"Factors affecting the stability of Dimilin in water and persistence of Dimilin in field waters"

J. Agric. Food. Chem. 1976 24 4 733-738

Scheltinga H.M.J. 1977

"Application of public nuisance act to sewage works and pumping stations"

H₂O 1977 10 9 198-203

Scouller W.D. Goldthorpe H.H. 1932

Publ. Health Congr. Lond. Paper no. 11

Shephard M.R.N. 1967

"Factors influencing the seasonal accumulation of solids in bacteria beds treating domestic sewage"

M.Sc. Thesis 1967 Univ. of Aston in Birmingham

Shriver L.E. 1975

"Operational practices to upgrade trickling filter plant performance"

J. Wat. Poll Cont. Fed. 1975 47 11 2640-2651

Sidwick J.M. Preston J.R. 1976

"The cost of producing effluents to varying standards by biological treatment techniques"

Eff. Wat. Treat J. 1976 16 5 238-241 244-247

Simpson W.J. 1933 Sewage Works J. 1933 5 103

Smallman B.N. Mansingh A. 1969

"The cholinergic system in insect development"

Ann. Rev. Ent. 1969 14 387

Solbé J.F. de L.G. Ripley P.G. Tomlinson T.G. 1974

"The effects of temperature on the performance of experimental percolating filters with and without mixed macroinvertebrate populations"

Wat. Res. 1974 8 557-573

Solbé J.F. de L.G. Tozer J.S. 1971

"Aspects of the biology of Psychoda alternata Say. and P.severeni parthenogenetica Tonn. (Diptera) in a percolating filter"

J. Appl. Ecol. 1971 8 835-844

Solbé J.F. de L.G. Williams N.V. Roberts H. 1967

"The colonisation of percolating filters by invertebrates and their effect on the settlement of humus solids"

Wat. Poll. Cont. 1967 66 5 423-448

Stanbridge H.H. 1956

"Thoughts on biological filtration"

J. Inst. Sew. Purif. 1956 3 270

Stanbridge H.H. 1958

"Experiment at Ewell sewage works to determine the effect of low frequency dosing on filter performance"

J. Inst. Sew. Purif. 1958 2 226-227

Steinhaus E.A. Brinley F.J. 1957

"Some relationships between bacteria and certain sewage inhabiting insects"

Mosquito News 1957 17 229-302

Stover E.L. Esfandi A. Little H. Kincannon D.F. 1976

"Inhibiting nitrification in waste water treatment plants"

Wat. Sew Wks. 1976 123 8 56-59

Styles P.D. 1975

"The effect of dosing frequency on the vertical distribution of solids and grazing fauna in sewage percolating filters"

Undergrad 3rd year project report 1975 Univ. of Aston in Birmingham

Sudderuddin K.I. Tan K.H. 1973

"Some hydrolases and their involvement in insecticide resistance"

PANS 1973 19 1 24-35

Symposium 1960

"Maturing of percolating filters"

J. Inst. Sew. Purif. 1960 4 366-375

Tariq M.N. 1975

"Retention time in trickling filters"

Prog. Wat. Tech. 1975 7 2 225-234

Taylor L.R. 1951

"An improved suction trap for insects"

Ann. Appl. Biol. 1951 38 582

Taylor T.H.C. 1955

"Biological control of insect pests"

Ann. Appl. Biol. 1955 42 190-196

Tedeschi F.B. Lucas R.W. 1956

"Enclosed natural draught filter beds"

J. Inst. Sew. Purif. 1956 2 161-166

Terry R.J. 1952

Proc. Leeds Phil. Soc. 1952 6 104-111

Terry R.J. 1956

"The relations between bed medium of sewage filters and the flies breeding in them"

J. Anim. Ecol. 1956 25 1 6-14

Thirugnanam M. Forgash A.J. 1977

"Environmental impact of mosquito pesticides : toxicity and anticholinesterase activity of chlorpyrifos to fish in a salt marsh habitat"

Arch. Env. Cont. Toxicol. 1977 5 4 415-425

Thompson J.T. 1942

"High rate dosing of gravel percolating beds"

J. Inst. Sew Purif. 1942 140-151

Thompson J.T. Watson H. 1944

"Notes on means of increasing the work done by percolating beds"

J. Inst. Sew. Purif. 1944 65-76

Thomson W.T. 1973

"Agricultural Chemicals Book 1 - Insecticides"

Thomson Publications Indianapolis Indiana 1973

Tomlinson T.G. 1941

"The treatment of settled sewage in percolating filters in series with periodic changes in the order of the filters"

J. Inst. Sew. Purif. 1941 39-76

Tomlinson T.G. 1943

"Biological control of a fly population in sewage filters"

Nature Lond. 1943 152 52

Tomlinson T.G. 1946

"Animal life in percolating filters"

Wat. Poll. Res. Tech. Pap. no. 9 H.M.S.O. London

Tomlinson T.G. Grindley J. Collet R. Muirden M.J. 1949

"Control of flies breeding in percolating sewage filters (Part 2)"

J. Inst. Sew. Purif. 1949 127-139

Tomlinson T.G. Hall H. 1950

"Some factors in the treatment of sewage in percolating filters"

J. Inst. Sew. Purif. 1950 4 338-350

Tomlinson T.G. Hall H. 1951

"Treatment of settled sewage in percolating filters, experiments at Minworth, Birmingham 1947-1950"

J. Inst. Sew. Purif. 1951 1 110-116

Tomlinson T.G. Hall H. 1955

"The effect of periodicity of dosing on the efficiency of percolating filters"

J. Inst. Sew. Purif. 1955 1 40-54

Tomlinson T.G. Jenkins S.H. 1947

"Control of flies breeding in percolating sewage filters"

J. Inst. Sew. Purif. 1947 2 94-114

Tomlinson T.G. Muirden M.J. 1948

"Control of flies breeding in sewage percolating filters"

J. Inst. Sew. Purif. 1948 1 168-179

Tomlinson T.G. Stride G.O. 1945

"Investigations into the fly populations of percolating filters"

J. Inst. Sew. Purif. 1945 2 140-148

Tonnoir A. 1940

"A Synopsis of British Psychodidae (Diptera) with descriptions of new species"

Trans. Soc. Brit. Ent. 1940 7 2

Toobey T.E. Hursey P.A. Alabaster J.S. 1975

"The acute toxicity of 102 pesticides and miscellaneous substances to fish"

Chemistry and Industry 1975 12 523-526

Trapido H. 1952

"Modified response of Anophles albimanus to DDT residual house spraying
in Panama"

Am. J. Trop. Med. Hyg. 1952 1 853-861

Trapido H. 1954

"Recent experiments on the possible resistance to DDT by Anophles
albimanus in Panama"

Bull. Wld. Hlth. Org. 1954 11 885-889

Truesdale G.A. Wilkinson R. 1962

"A comparison of the behaviour of various media in percolating filters"

J. Inst. Sew. Purif. 1962 4 325-340

Truesdale G.A. Wilkinson R. Jones K. 1961

Inst. Publ. Hlth. Engrs. J. 1961 60 273

Tsukamoto M. 1963

"The log dosage probit mortality curve in genetic researches of insect
resistance to insecticides"

Botyu - Kagaku 1963 28 91-99

U.S. Env. Res. Agency. 1974

"Pesticide degradation in water"

Chem. Eng. News 1974 15 26

Usinger R.L. Kellen W.R. 1955

"The role of insects in sewage disposal beds"

Hilgardia 1955 23 263-321

Uvarov B.P. 1931

"Insects and Climate"

Trans. Roy. Ent. Soc. Lond. 1931 79 19 1-246

W.H.O. 1975

"Instructions for determining the susceptibility or resistance of adult mosquitoes to organophosphate and carbamate insecticides"

W.H.O. Geneva. Switz. 1975 7 2pp.

W.H.O. 1976

"Resistance of the vectors and reservoirs of disease to pesticides, 22nd report of the W.H.O expert committee on insecticides"

W.H.O. Technical Report Series 1976 585 88pp.

Wallace R.R. Hynes H.B.N. Merritt W.F. 1976

"Laboratory and field experiments with Methoxychlor as a larvicide for Simuliidae (Diptera)"

Environmental Poll. 1976 10 4 251-269

Water Pollution Research 1956

H.M.S.O. Lond. 1957 42

Watson W. Fishburn F. 1964

"Insecticide resistance of filter flies"

J. Inst. Sew. Purif. 1964 5 464-467

Wheatley A.D. Williams I.L. 1976

"Pilot scale investigations in to the use of random pack plastic filter media in the complete treatment of sewage"

Wat. Poll. Cont. 1976 75 4 468-486

Whitten B.K. Goodnight C.J. 1966

"Toxicity of some common insecticides to Tubificids"

J. Wat. Poll. Cont. Fed. 1966 38 227-235

Wichmand H. 1953

"Control of multi resistant house flies"

Nature 1953 172 758-759

Williams C.B. 1940

"An analysis of 4 years captures of insects in a light trap Part II: the effect of weather conditions on insect activity; and the estimation and forecasting of changes in the insect population"

Trans. Roy. Ent. Soc. Lond. 1940 90 227-306

Williams N.V. Taylor H.M. 1968

"The effect of Psychoda alternata Say. (Diptera) and Lumbricillus rivalis Levinson. (Enchytraeidae) on the efficiency of sewage treatment in percolating filters"

Wat. Res. 1968 2 139-150

Wolfe N.L. Zepp R.G. Gordon J.A. Baughman G.L. Cline D.M. 1977

"Kinetics of chemical degradation of malathion in water"

Env. Science and Technol. 1977 11 1 88-93

Woods D.R. Williams J.M. Croyden J. 1976

"Fly nuisance at Tamworth Reclamation Works"

Paper presented at Inst. Wat. Poll. Cont. Meeting (East Mids. Branch) 26th Oct. 1976 Kegworth

Woods D.R. Williams J.M. Croydon J. 1978

"Fly nuisance control in treatment systems"

Wat. Poll. Cont. 1978 77 2 259-270