T H E S I S

"Factors influencing the seasonal accumulation

of

solids in bacteria beds treating domestic sewage"

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TITLE OF THESIS

Factors affecting the seasonal accumulation of solids in bacteria beds treating domestic sewage.

SUMMARY

The literature relating to the bacteria bed method of sewage purification has been reviewed. This includes an account of its historical development and a discussion of the biological and physical factors influencing its performance.

The ecology and performance of two bacteria beds at the Langley Mill works of the Upper Tame Main Drainage Authority were studied over a period of two years. The beds treated domestic sewage at a similar overall rate of loading but at different frequencies of dosing. It was found that accumulation of film in winter was greater in the high-frequency dosed bed. There were also considerable differences in the relative proportions of the various species of macroinvertebrate grazers between the two beds. In spite of differences in the ecology, the rates of purification were mostly very similar, apart from a slightly better level of nitrification in the low-frequency dosed bed.

Laboratory experimental filters were constructed to simulate the conditions of the operational beds and their performance at different temperatures was studied both in the presence and in the absence of macrograzers, represented by the dipteran Psychoda. It was found that at 5°C the grazing activity of Psychoda was suppressed, allowing film

to accumulate, while at 20°C the flies bred actively and maintained the film at a low level. This supported the belief that film accumulation in winter is caused by the suppression of the macrograzers. However, in the absence of macroinvertebrates film levels were also greater at 5°C than at 20°C, indicating that there is a direct effect of temperature on film accumulation as well.

Studies on the carbon dioxide output of the laboratory filters were made, and by comparing these with the corresponding rates of removal of organic matter conclusions were drawn concerning the relative significance of physical and metabolic processes in sewage purification.

ACKNOWLEDGEMENTS

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CONTENTS

INTRODUCTION

The bacteria bed or percolating filter is a practical and effective method of purifying domestic and industrial effluents. The process consists basically of applying sewage, which has been strained and settled to remove solid material, to a bed containing about six feet of gravel or clinker medium. Films of microorganisms develop on the surface of the medium and the removal of dissolved and suspended organic matter from the sewage and its subsequent oxidation is primarily due to the vital activities of these organisms. The treatment is concluded with another period of settlement after the effluent has left the bed.

The process, however, suffers from the disadvantage that its performance is often seriously impaired during winter. This decline in efficiency is associated with the accumulation of solid material in the interstitial spaces of the medium which in extreme circumstances causes the complete obstruction of the bed, or "ponding". It has been demonstrated (Hawkes 1955) that in some cases this accumulation of solids can be drastically reduced by lowering the frequency of application of sewage to the surface of the bed without altering the overall rate of dosing. Hawkes (1961) later showed that if the film accumulation is controlled in this manner, the decline in efficiency at low winter temperatures is avoided. Thus it appears that the poor performance of bacteria beds in winter is due not to the direct effect of low temperature but to the accumulation of solids, an indirect effect. The

cause of the winter accumulation has usually been assumed to be the suppression of the populations of macroinvertebrates which occur in bacteria beds and whose grazing activity has been demonstrated to bring about the rapid"unloading"of accumulated solids that takes place in spring (Parkinson and Bell 1919, Reynoldson 1939a).

While there is clearly a relationship between the occurence of macroinvertebrates and the amount of solid material in the bed, it has never been shown whether the winter accumulation is purely the result of the reduced activity of macrograzers or whether there is any direct effect of temperature. The purpose of the present research was to investigate closely the causes of the increased quantities of film in bacteria beds in winter, isolating the effects of individual factors so far as possible.

The research was conducted along two lines. The first was to continue observations on the seasonal changes in film and macrofauna distribution of two bacteria beds at the Langley Mill works of the Upper Tame Main Drainage Authority, which had already been made for several years. The filters were operated at the same overall rates of loading and under conditions similar in every respect except for a wide difference between the frequencies of dosing.

The second line of research involved the construction of six small-scale percolating filters in the laboratory, which were maintained at similar rates of dosage and with a similar sewage to the Langley

Mill beds. However, certain factors which are of significance in determining the quantity of film in a filter could be controlled more closely in the laboratory than in an operational filter. These included the temperature and the presence or absence of macroinvertebrates. In this way the effect of different conditions on film accumulation were studied, and simultaneous estimates of the rate of purification and respiratory activity of the system were used to investigate the process of removal of organic matter from the sewage.

REVIEW OF THE LITERATURE

Historical Background

The application of scientific principles to the problem of sewage purification dates from the setting up of the Royal Commission on Sewage Disposal in 1898. Throughout the nineteenth century increasing urbanization and industrialization had made the collection and disposal of domestic and industrial effluents a serious problem which was emphasised by the repeated outbreak of epidemics caused by polluted water supplies and the extreme offensiveness of rivers in highly populated areas. In the latter half of the nineteenth century large construction projects were undertaken for the collection and disposal of sewage but little attention was paid to its purification.

Such methods of purification as were employed usually involved applying sewage to land, since it was understood that purification involved the removal of organic matter from the sewage and that this could enrich the soil for agricultural purposes. The increase in population, however, causing larger quantities of sewage and an increased demand for land made this process impracticable on a large scale. Early experiments on improved methods of purification usually consisted of attempts to concentrate the process of land filtration into a smaller area. The percolation of sewage through various forms of sand and soil media was employed but progress in devising a really effective means of purification was delayed by

failure to understand the nature of the processes involved. Pasteur's work had emphasised the role of microorganisms in biochemical processes of decomposition and Frankland in 1879 had recognised that sewage purification was a chemical rather than a mechanical process, but the idea of mechanical filtration died hard. For this reason early filter beds usually contained a fine medium, and inadequate ventilation to support the aerobic oxidation processes was the chief difficulty.

Research at the Lawrence Experimental Station in America demonstrated the need for adequate aeration, and the Royal Commission (1908) established the superiority of the percolating bacteria bed, in which sewage was applied continuously or intermittently to the surface of the medium, over the contact bed, a rival process in which the medium was intermittently flooded with sewage for several hours and then drained.

Considerable investigations were then undertaken into methods of improving the percolating filter. These mainly concerned the size and nature of the filter medium, the depth of medium required for optimum efficiency and the method of application of sewage to the surface of the filter. A major improvement was the development of the rotating distributor which permitted a more even application of sewage over the surface of the bed. The literature relating to the early development of the percolating filter has been reviewed by Stanbridge (1954).

Early Research

Between 1908, when the fifth report of the Royal Commission was published, and 1933 there was relatively little research into the bacteria bed and few fundamental improvements to the process were made. The introduction of the activated sludge process in 1914 for a time diverted attention from the further development of the bacteria bed. However, certain publications during this period are of particular interest.

Haigh Johnson (1914) summarised the current state of knowledge of the biology of sewage disposal. He clearly recognised the biochemical rather than mechanical nature of the process and noted that when the accumulation of film within a filter proceeded to such an extent as to choke the bed and cause "ponding", there was a rapid decline in efficiency due to the suppression of aerobic organisms. He recorded a list of organisms associated with sewage and bacteria beds and observed the changing nature of the biological film through the depth of the bed, with increasing purification of the effluent. Haigh Johnson also descibed the life histories and distribution of two of the larger invertebrates that occured in the bacteria bed, the dipteran Psychoda and the collembolan Hypogastrura (=Achorutes)

The significance of the macroinvertebrates in the functioning of the bacteria beds was investigated over a number of years by Parkinson and Bell (1919). They showed that where Hypogastrura

subviaticus was excluded from the beds, excessive accumulation of film resulting in ponding took place, while under similar conditions but with Hypogastrura present ponding was prevented.

Thompson (1925) summarised much of the information that was then available on the operation of bacteria beds, relating it in particular to his own experience at the Knostrop sewage works at Leeds. He discussed the nature of the medium, the distribution of effluent on to the bed and the question of nuisance from flies breeding in the beds.

Studies on the Ecology of Bacteria Beds.

Fundamental studies into the ecology of the bacteria bed really began with the work of Lloyd and his students at the University of Leeds starting in 1933. Much of this was done at the Knostrop Works in co-operation with Thompson. Lloyd endeavoured to discover the ecological factors influencing the activity and composition of the macroinvertebrate grazers, in view of their proven significance in the prevention of ponding. He observed that they were basically derived from a fauna characteristic of mudflats (Lloyd 1944) but the bacteria bed environment was so specialised as to exclude all but a small proportion of such species. Of sixteen species of Psychoda in the Leeds area, very similar morphologically, only three were recorded in bacteria beds(Lloyd 1945). Lloyd carried out extensive trapping of flies at the surface of bacteria beds and

noted their seasonal changes in abundance. He made laboratory studies to determine the effect of temperature on the life cycles of individual species. These studies (Lloyd 1937, Lloyd, Graham, and Reynoldson 1940) showed that temperature was a major factor influencing competition, the threshold temperature for development of Psychoda alternata , for example, being considerably higher than for the dompeting species Psychoda severini. Lloyd also concluded that interspecific competition was operative in the beds on the grounds that the seasonal abundance of species was different from what might be expected on the basis of the productivity rates at different temperatures. He suggested that this might take the form of predation but was more likely to be competition for the available food supply. The mechanical action of the sewage on the bed was also noted as a factor influencing competition and the Chironomidae were observed to be more resistant to this effect than competing species.

Reynoldson, working first with Lloyd at Leeds and then at Huddersfield, made a particular study of the ecology of the Enchytraeidae which frequently form the majority of the grazing fauna in bacteria beds. He observed that individuals of the species Lumbricillus lineatus were always dominant at Leeds and that they tended to avoid the immediate surface of the bed but accumulated just below this (Reynoldson 1939a). He discovered that in both

field and laboratory studies they responded to cold conditions by moving lower into the bed where the temperature was less extreme. He interpreted the breakdown of the film in spring as the populations of Lumbricillus moving up to the surface of the bed with warmer conditions, loosening the film and, as the detached film moved downwards, following their food supply back into the bed. Reynoldson suggested that the thigmotaxic response of Lumbricillus, a strong desire for maximum contact between its body surface and the surrounding medium, was a major cause of its tendency to avoid the immediate surface of the bed, and of its behaviour in general. This conclusion was also reached by Terry (1951) with respect to the larger Lumbricidae. Reynoldson (1943) made a comparative study of the ecology of Lumbricillus lineatus and Enchytraeus albidus, another species commonly found in bacteria beds, particularly regarding the influence of temperature. He observed that E. albidus could tolerate a wider range of temperature than L. lineatus and had a higher rate of reproduction. This led to the conclusion that other factors than temperature were involved owing to the more common occurence of L. lineatus. Reynoldson (1948) later demonstrated that the greater ability of L. lineatus to grip the medium and resist the downward flow of sewage was a major cause of its dominance. He also showed that at Huddersfield E. albidus was the dominant member of the Enchytraeidae and that this was due

to the greater susceptibility of L. lineatus to toxic chemicals (Reynoldson 1947).

Using pilot scale filters, Reynoldson (1939a) demonstrated the role of Lumbricillus lineatus in keeping bacteria beds free from excessive accumulation. In one filter he excluded all grazing fauna while in the other he introduced L. lineatus after a certain amount of film had accumulated. He observed a rapid breakdown of the film where L. lineatus had been introduced which was followed by an improvement in the nitrifying properties of this filter. This was a conclusive demonstration of the ability of this species to cause sloughing of the film and corresponded to the demonstration by Parkinson and Bell(1919) of the similar function of Hypogastrura subviaticus. Terry (1951) using similar methods showed that the lumbricid Eisenia foetida also had the ability to carry out efficient scouring of the film in the absence of any other grazing fauna. He also reported improved efficiency where lumbricid worms were present.

Lloyd (1945) summarised his own work and that of his students in a comprehensive paper. He drew attention to the role of a mixed grazing population in improving the condition of a bacteria bed and emphasised the practical applications of his research in dealing with such problems as fly nuisance.

Control of Film Accumulation

While it is accepted that excessive film accumulation causes a

deterioration in the purification capacity of a filter, there is some disagreement as to the actual film thickness required for optimum efficiency. Lloyd (1945) suggested that excessive depletion of film in summer might result in a decrease in efficiency, but more recent research suggests that only a very thin film is necessary to achieve high rates of purification (H.M.S.O. 1958, Wuhrmann 1960).

It soon became apparent that a major cause of film accumulation in bacteria beds was the strength of the applied sewage. Tomlinson (1946) concluded that the rate of growth of film depends on the strength of the sewage quite apart from the rate of application. Coinciding with Lloyd's work on the function of the grazing fauna, experiments were being made on variations in the operational procedure with a view to controlling film accumulation and increasing the capacity of bacteria beds.

One such method was "alternating double filtration" in which sewage was applied to two beds in series, with periodic changes in the order of the beds. Film accumulation tended to be much greater in the primary bed than in the secondary, but this effect was checked and reversed when the order of the beds was changed. The process was originally developed from an observation by O'Shaughnessy (1931) that the application of a clean effluent to a bed with excessive film caused a rapid improvement in its condition. The results of large scale experiments on this method are described by Wishart and

Wilkinson (1941) and Mills (1945) who showed that overall dosage rates could be increased by as much as four times. These experiments were accompanied by observations by Tomlinson (1941, 1946) on the biological changes taking place in the filters.

Another application of the ability of a weaker sewage to limit the growth of film is "recirculation", in which a proportion of the purified effluent is fed back to dilute the incoming sewage. This method has also considerably improved the overall treatment capacity of bacteria beds (Lumb and Eastwood 1958). The relationship between the film growth rate and the strength of the sewage in these processes has been explained by Hawkes (1961).

Tomlinson (1946) gave a detailed discussion of the factors involved in the accumulation of film. He stated that the three main factors are, 1) The rate of growth of the component organisms of the film and the deposition of solid material; 2) The breakdown of film by the scouring action of grazers; 3) The scouring action of the liquid flowing through the filter. He used fine gauze to exclude grazers from baskets of medium set into the surface of a filter and observed that within the baskets film accumulated faster at higher temperatures. The relative importance of grazing organisms and the mechanical action of sewage in the breakdown and removal of film has been a subject of considerable dispute between British and American workers. The British (Lloyd 1945, Reynoldson 1939a, Tomlinson 1946,

Hawkes 1957) have strongly emphasised the role of grazing organisms. Reynoldson (1941, 1942) has disputed the capacity of even high rates of flow to remove biological growths. In America most workers consider the physical scouring of the sewage to be of prime importance, the activity of the grazers being incidental or at most playing a minor role (Holtje 1943, Ingram 1949, McKinney 1962). Usinger and Kellen (1955) and Heukelekian (1945), however, have stressed the importance of the grazing fauna., The use of higher rates of application in America, combined with the generally weaker condition of the sewage, may explain the greater emphasis on hydraulic factors.

Another criterion which is of great significance in the operation of bacteria beds is the periodicity of dosing, determining whether a section of the bed receives a heavy dose occasionally or a small dose at more frequent intervals, irrespective of the overall dosage rate. Experiments on the effect of this factor are reported by Levine (1940) and Lumb and Barnes (1948). These indicated that while the frequency of dosing exerts a marked effect on the degree of purification, the optimum frequency is widely variable depending on other operational factors. Periodicity of dosing was studied in greater detail by Tomlinson and Hall (1955), accompanied by biological investigations by Hawkes (1955), who noted that at low frequencies of dosing the film accumulation at the surface of the filters was markedly reduced. This was associated with better rates of purific-

ation. He also recorded that most species of diptera were greatly reduced and replaced by Lumbricillus lineatus as the dominant grazing organism. These observations were made on beds using alternating double filtration to treat a largely industrial effluent, with a film dominated by fungus, and it cannot be assumed that the results are applicable to the treatment of domestic sewage or to beds differing in other operational factors.

Hawkes (1957) suggested that the main, and possibly only, advantage of low frequency dosing was in preventing the accumulation of film. Stanbridge(1956), considered that improved purification, especially nitrification, was due to the longer intervals between doses at low frequency, allowing more time for biochemical changes in the retained liquid. Hawkes (1961) explained the limitation of film in terms of the higher instantaneous dosage rate giving a more even vertical distribution, causing metabolism and synthesis to take place over a greater depth of the bed, rather than at the immediate surface. He showed further that a filter in which the accumulation of film was reduced by low frequency dosing maintained a high degree of efficiency throughout the year, while at high frequency film accumulated with low winter temperatures and the efficiency fell.

Physical Factors

The temperature, ventilation and the capacity of the medium to retain liquid in its interstitial spaces are all of great importance

in determining the efficiency of bacteria beds. Reynoldson (1939a) noted that the variation of temperature within a bacteria bed was considerably less than that of the surrounding atmosphere. This was explained by the incoming sewage and the vital activities of the organisms warming the bed in winter and the cooling effect of evaporation in summer. The temperature relationships of bacteria beds have been the subject of particular investigations by Bayley and Downing (1963) and Howland (1953). Ventilation of bacteria beds is to some extent dependent on the temperature differential between the bed and the atmosphere (Petru 1958) and has been studied by Mitchell and Eden (1963) using radioactive tracers.

The retention capacity of a filter is of particular significance in its ability to purify sewage as it will influence the time available for biochemical changes to take place. The retention capacity is governed by the size and shape of the bed medium and the quantity of film present and this, together with the hydraulic factors of loading and periodicity determine the retention, or contact, time. Tomlinson and Hall (1950) investigated the effect of overall loading rates on retention time, and Eden, Brendish and Harvey (1964) studied the influence of different types of medium on the retention characteristics of filters. The work of Hawkes (1961) on the relevance of periodicity to retention has already been mentioned . Contact time was considered from first principles by Sinkoff, Porges and McDermott (1959) and a

mathematical expression was derived relating retention time to the hydraulic loading rate, surface area of the medium, viscosity, gravity and the depth of the bed. A similar expression by Howland (1958) defined the retention time as being proportional to the depth and inversely proportional to the 2/3 power of the hydraulic load.

$$t \propto D/Q^{\frac{2}{3}}$$

where t = contact time, D = depth and Q = hydraulic load.

Schroepfer (1961) studied the effect of the size and shape of the medium on the porosity of the filter bed and the surface area available for colonization by biological film. Hawkes and Jenkins (1958) and Truesdale, Wilkinson and Jones (1962) investigated the purification rates that were achieved in operational beds containing different types of media. Their general conclusion was that, owing to the larger surface area exposed, the smaller media gave better efficiency in summer but in winter were more liable to ponding. The type of medium for overall optimum efficiency in a bacteria bed varied depending on the operational conditions.

Mathematical Formulations

A number of formulae have been derived to express the purification of sewage in a bacteria bed in terms of the factors already discussed. The best known is that of Velz (1948) who stated that"the rate of extraction of organic matter per interval of depth of a biological filter is proportional to the remaining concentration of organic

matter measured in terms of its removability". Expressed mathematically this becomes,

$$\frac{L_D}{L} = 10^{-KD}$$

where L = Total removable BOD in feed

L_D = Remaining removable BOD at depth D

K = Reaction constant (determined by the oxidizability of the waste)

This formula has been found to be applicable over a wide range of operational conditions.

Other formulae have been suggested by Stack (1957), Schulze (1960), Howland (1958) and Eckenfelder (1961), using mainly the factors of hydraulic load, depth, contact time and temperature to define the efficiency of a bacteria bed. A discussion of the relative merits of various formulae, and an attempt to correlate the different concepts is given by Behn (1960). He noted that the main distinction between them was the expression of BOD extraction as a function of either contact time on the one hand or depth on the other. Hawkes (1963) has stressed the significance of time rather than depth in considering the theoretical aspect of low frequency dosing.

The main criticism of these formulae is that they are expressed purely in terms of physical criteria with little regard to the biological nature of the purification process. Tomlinson (quoted by Stanbridge 1956) has suggested that the main factors that influence the

performance of a biological filter are "the amount of active film in the filter, the time of contact between the sewage and the film, the temperature at which the process of purification is taking place and the supply of oxygen". The formulae discussed employ not more than two and sometimes only one of these factors.

Criteria of purification

In assessing the strength of sewage the normal tests employed are the Biochemical Oxygen Demand and Permanganate Value and the efficiency of a system is usually expressed as the percentage reduction of these values between the feed and effluent. This is normally satisfactory as a general indication of the effectiveness of treatment. However, a reduction in the concentration of organic matter between feed and effluent does not imply that all this material has been oxidised (Levine 1940, Tomlinson and Hall 1950, Renn 1956, Bridge Cooke 1959). To assume that simultaneous oxidation was taking place would demand a rate of oxidation immensely higher than occurs in other organic systems. Renn (1956) has emphasised that there is much evidence to show that organic material is accumulated within the bed and oxidised at rates comparable to other aerobic systems. Clearly the difference between the rate of accumulation of organic matter by adsorption and its rate of removal by oxidation or other means will determine the increase or decrease of film within the bed.

While the removal of carbonaceous matter provides a useful

estimate of purification, nitrification is also important. The occurence of this process in various environments including bacteria beds has been discussed by Barritt (1933). Unlike carbonaceous oxidation, it has been demonstrated that the conversion of ammonia to nitrate can take place spontaneously as sewage passes over the filter medium (Hawkes and Jenkins 1951), but the actual circumstances in which this occurs have been the subject of much discussion. It appears that autotrophic nitrifying organisms cannot compete successfully with the more vigourously growing heterotrophic bacteria in the presence of organic nutrient (Hawkes 1963). For this reason nitrifying bacteria are believed to occur mainly in the lower half of a bacteria bed where film accumulation is usually less than at the surface. The theory that nitrifying bacteria are inhibited by lack of oxygen in a thick film is supported by the observations of Reynoldson (1939a) that the introduction of a grazing population into experimental filters caused improved nitification. Somewhat contradictory evidence is given by Heukelekian (1945c) who measured the "nitrifying capacity" of the film taken from different levels of a filter by its ability to produce nitrate under aerobic conditions in the laboratory. He found that the highest nitrifying capacity occurred in the top two feet of the bed and concluded that nitrifying organisms were not adversely affected by carbonaceous oxidation. However, the nitrifying capacity, as measured by this method, did not necessarily correspond with the actual

production of oxidised nitrogen under operational conditions. Heukelekian reported very low nitrification with high rate filters and Lumb and Eastwood (1958) observed lower nitrification with recirculation in spite of the control of film growth that is achieved with this method. It appears that the factor of contact time is important and this was emphasised by Stanbridge (1956) when considering the improved nitrification that is often achieved with low frequency dosed beds.

Microstructure of the Film

Besides considering the role of grazers and the effect of operational conditions on the efficiency of bacteria beds, much attention has been paid to the nature of the film with regard to both its component organisms and the dynamics of the metabolic processes involved in purification. Wattie (1943) isolated and cultured some of the bacteria which compose the zoogleal film. Barker (1946,1949) investigated the nature and role of the protozoa in the bacteria-bed. He demonstrated that the protozoa populations are affected by temperature, the strength of the sewage and the predatory activity of grazers. The relative importance of protozoa in purification compared with bacteria has been the subject of much controversy. Their ability to flocculate suspended matter and clarify sewage has been demonstrated in the laboratory and this may be their role in the operational bed. Pillai and Subrahmanyan (1944) have emphasised the role of protozoa to the extent of saying that "aerobic purification of sewage is

essentially due to protozoan activity; bacteria play only a secondary part", but this is regarded by most workers as an overstatement. Flocculation has been investigated more recently by Curds(1963), who identified a carbohydrate substance that was secreted by certain protozoa during the process.

A number of studies have been made on the incidence and role of fungi in bacteria beds (Tomlinson 1942, Painter 1954, Bridge Cooke 1959). This is of particular importance as ponding is often associated with a heavy surface growth of fungi. Hawkes (1965b) observed that fungi were in competition with heterotrophic bacteria and the outcome of this competition was affected by temperature, the nature and concentration of the sewage and the presence of toxic substances. Tomlinson (1942) recorded that fungi were subjected to bacterial attack and that this could be an important factor in the breakdown of the film.

In spite of the possible significance of other organisms, the oxidation of organic matter in bacteria beds is assumed to be primarily the result of bacterial metabolism, and much work has been done on the dynamics of bacterial growth developing from the classical studies of Monod (1949). The mechanism of biological oxidation has been described by McKinney (1960), Reid and Assenzo (1960) and Wilson (1963). It was noted that organic matter can be metabolised by respiration or by synthesis into living tissue and that the relative proportions of material utilized in these two processes will influence the rate of

growth of the film. Heukelekian (1945a) has mentioned the significance of endogeneous respiration in limiting the accumulation of film.

As the thickness of a bacterial film increases, the supply of oxygen to the lower layers is progressively reduced and they can therefore no longer undergo active metabolism (Sanders 1965, Tomlinson and Snaddon 1966, Bridge Cooke and Hirsch 1958). Sanders (1966) stated that the maximum rate of nutrient removal occurs when the film thickness equals the limiting thickness for the diffusion of oxygen. He suggested further that as the lower layers of a film become anaerobic their decomposition products feed the aerobic surface layers and therefore limit the removal of organic matter from the substrate. Many workers have observed that the development of an anaerobic layer could be an important cause of sloughing, though in an operational bacteria bed it is probably outweighed by other factors such as the activity of macro-grazers(Tomlinson 1941). Tomlinson and Snaddon (1966) made the significant discovery that the coefficient of oxygen diffusion showed wide differences depending on the nature of the film. It was very much greater with a fungal film, suggesting that much thicker fungal growths could develop in a bacteria bed before an anaerobic layer induced sloughing.

Experimental Techniques

Since the bacteria bed was first developed a variety of experimental procedures have been used to study its performance. In order to

investigate the course of purification and the vertical distribution of the film through the depth of the bed it was necessary to make the medium accessible at different levels. Haigh Johnson (1914) inserted panels into the side of a filter bed through which trays of medium could be removed for examination. For some studies a better method was developed by Tomlinson (1941) who had shafts set vertically into the medium of the bed, into which a series of baskets containing a similar medium were placed which could be removed to study the distribution of film and macrofauna.

In order to study the effect of operational factors such as hydraulic load, periodicity, and the nature of the bed medium, many sewage works have installed pilot plants. The degree to which environmental conditions can be controlled in such plants is limited, however, and the process has been reproduced on a laboratory scale, using cylindrical columns containing a similar medium. The control of temperature and the exclusion of macroinvertebrates is, of course, made much easier but there is a danger that the dosing procedure will no longer represent bacteria bed conditions. Gameson, Truesdale and Van Overdijk (1961) compared the variation in performance of twelve such column-filters and concluded that these filters should at least be used in triplicate to overcome the effect of individual variability.

To study the process of bacterial synthesis and oxidation, the scale of the experimental method has again been reduced and bacterial

films have been developed on alternative surfaces to the normal filter bed medium. Gloyna, Comstock and Renn (1952) ceveloped a system in which open-ended cylindrical tubes are slowly rotated at a slight incline. A sewage feed or synthetic substitute is drip-fed into the upper end and a bacterial film becomes evenly distributed on the inner surface of the tube.

Schulze (1957) has described the use of vertical wire mesh screens as an alternative medium for the development of biological films. This technique was used by Green, Cooper and Jenkins (1965) to study the purification and growth rates of films at different temperatures.

FIELD WORK - LANGLEY MILL

Description of Plant

Two circular bacteria bed filters, described as Bed A and Bed D, were operated at a similar overall dosage rate of about 80 gallons per cubic yard per day and with a similar, largely domestic, sewage. They had an identical medium in the bed, smooth gravel of nominal size 1½–2 inches. The only difference in operation was in the rate of rotation of the four-arm distributors. With Bed D the rate of rotation was controlled by a mechanised drive and was approximately 15 minutes for one complete revolution, while the distributor arms of Bed A were driven automatically by the hydrostatic pressure of the sewage siphoning from the dosing chamber and the angle of the jets on the distributor arms. With Bed A, the rate of rotation was about 1¼ minutes for a complete revolution, much faster than Bed D. Furthermore, with Bed D, sewage was only discharged from a quarter of the length of each arm and these quarters were staggered such that the bed was covered in four concentric areas, one arm covering each. In Bed A, sewage was discharged from along the whole of the length of each arm. This meant that in Bed D any part of the surface was dosed only once in every complete revolution of the distributor arms (i.e. once in about 15 minutes) while in Bed A a similar point was dosed four times for every revolution (i.e. once in about 17 seconds). (See

Plates 1 and 2).

In both beds there were intervals between successive periods of dosing while the dosing chamber recharged. The length of these intervals depended on the rate of flow of sewage which was variable throughout the day.

Plan of Observations

Regular weekly analyses of the strength and nature of the sewage feed to and effluents from, Beds A and D were made by the Upper Tame Main Drainage Authority and acknowledgement is made to the Authority for the results quoted. The tests employed in these analyses were, Biochemical Oxygen Demand (B.O.D.), Permanganate Value (P.V.), Ammonia and Oxidised Nitrogen.

In addition to the chemical analyses of the feed and effluent, the accumulation of biological film and the populations of grazing macrofauna within the medium of each bed were assessed from October 1964 to February 1967. This was done using a system of perforated cylindrical vessels containing a similar medium to the rest of the bed that were set into the bed both at the surface and in shafts running through the depth, but could be withdrawn to make the appropriate estimations. Each cylindrical container was six inches deep and six inches in diameter giving a volume of 0.1 cubic foot, open at the top and perforated in the sides and base with holes three quarters of an inch in diameter. (Plate 3).

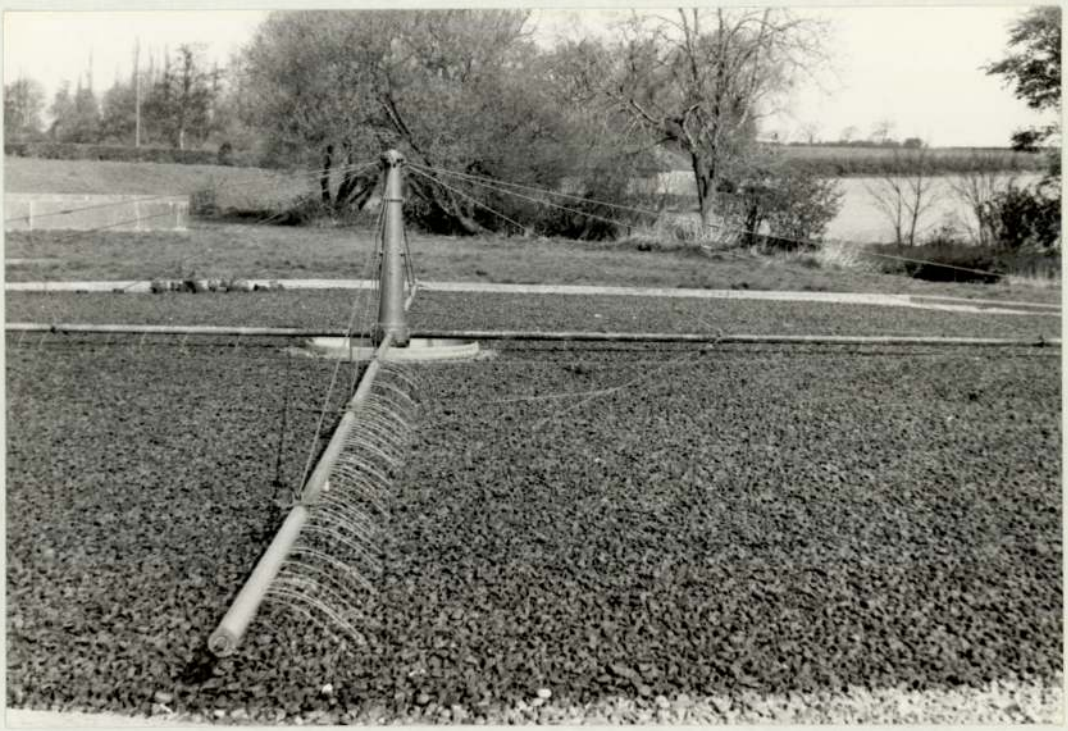


Plate 1. Bed A Langley Mill, showing sewage dosing from the whole length of each distributor arm.

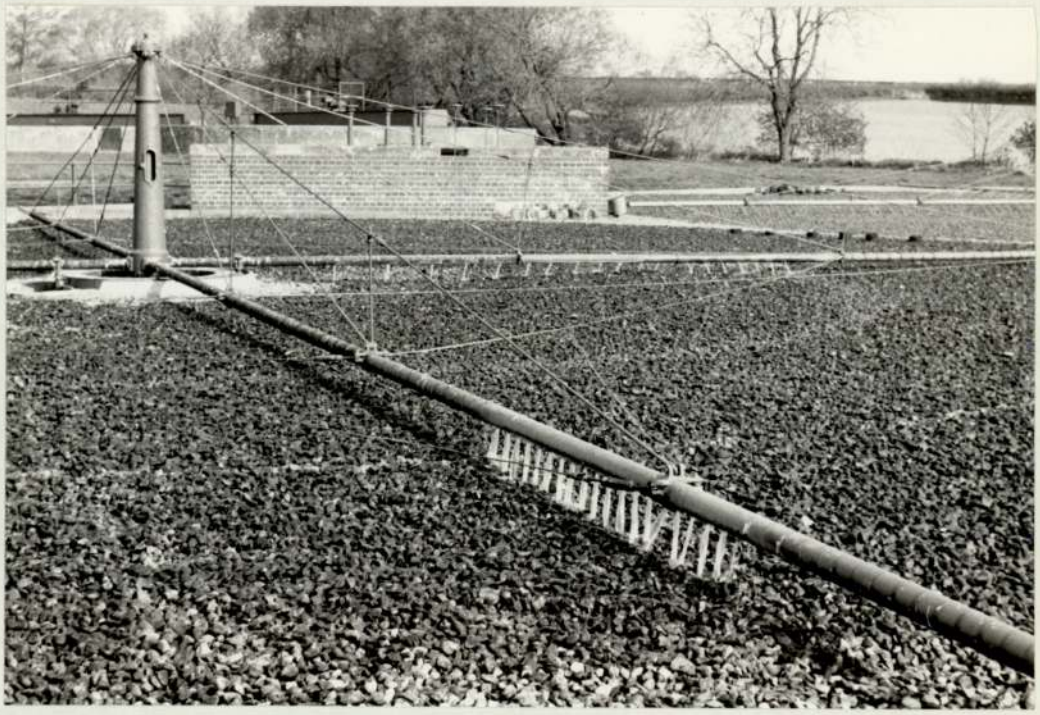
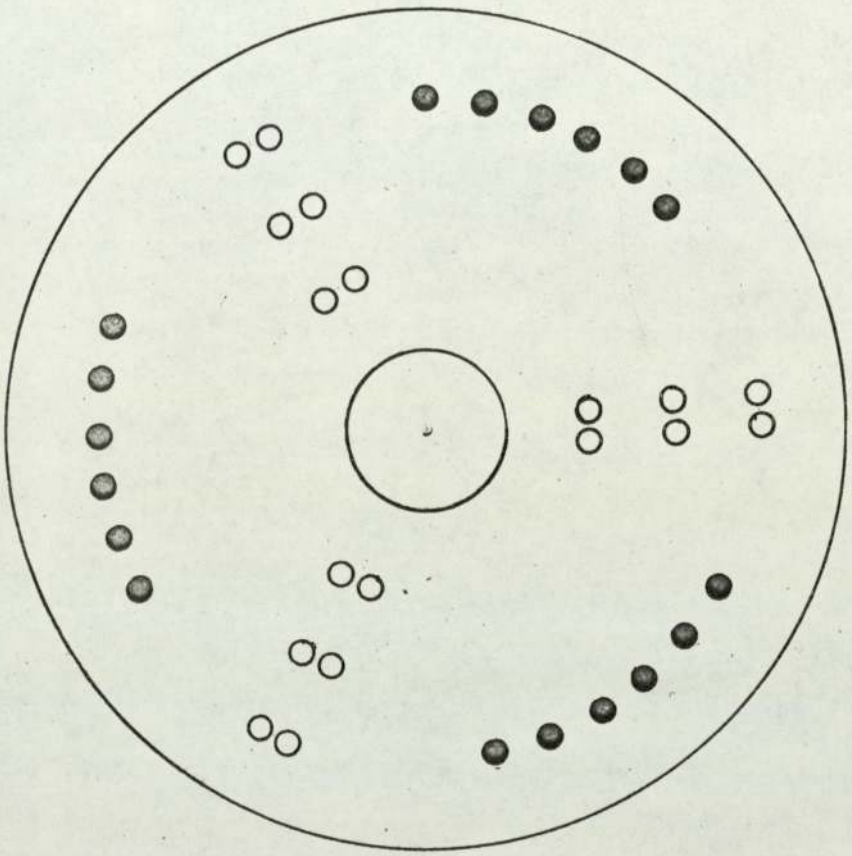


Plate 2. Bed D Langley Mill, showing sewage dosing from staggered lengths of each distributor arm.

These containers were arranged in pairs so that for any observation two samples were available, one for estimating macrofauna populations and the other for estimating film accumulation.

The pattern of arrangement of these pairs was as follows. (See Fig. 1.) For the purpose of measuring film accumulation and macrofauna populations at the surface only, pairs of containers were set into each bed to a depth of 1 foot (i.e. the length of two containers) in nine different positions. These positions were on three different radii and at three different distances from the centre. During each month the containers at three of these positions were removed and examined. The positions chosen were each on a different radius and each at a different distance from the centre, to give a random sample from the whole surface. After removal of the sample for examination, the containers were refilled with more of the medium taken from the surface and replaced. Each set of containers would thus come up for examination every third successive month, giving a three-month period to equilibrate with the conditions of the bed. In addition to the surface containers, there were nine pairs of shafts arranged concentrically towards the outer edge of each bed. (Fig. 1). These shafts, some of which are shown in position in Plate 4, each held nine containers in vertical succession. This allowed the condition of the bed to be examined throughout its full depth. The



○ Positions of surface containers

● Positions of shafts

Figure 1. Arrangement of containers in Langley Mill bacteria beds.

Plate 3. Container of the type used to sample film and macrofauna in Langley Mill beds.

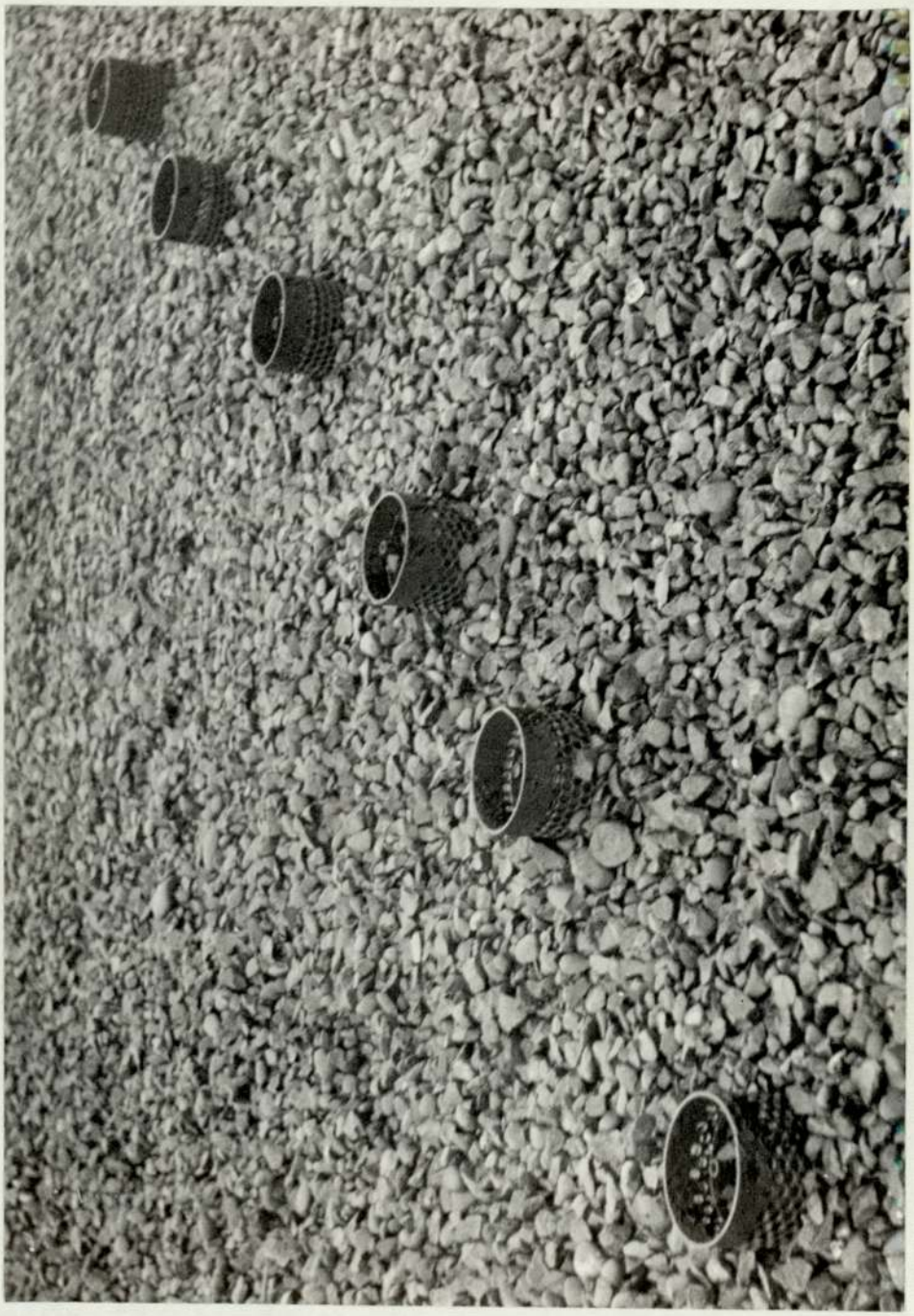


Plate 4. Six shafts of containers in position

containers from one pair of shafts in each bed were removed for examination each month. (See Plate 5). After replacement, a full year was allowed for equilibration before the same shafts were again examined. Unfortunately, this meant that conditions in the depths of the bed could only be examined during nine months of the year. The months chosen were from October of one year to June of the following, allowing the normal pattern of winter accumulation and unloading to be studied.

Methods

The analytical tests of B.O.D., P.V., Ammonia and Oxidised Nitrogen, that were made on the feed and effluent were performed according to the recommended procedure. (H.M.S.O. 1956). The results given are monthly averages of weekly samples.

The film accumulation was measured by thoroughly washing the stones removed from each container and performing the recommended test for volatile solids on a measured proportion of the washings. A correction factor was applied to the results to allow for that part of the volatile matter that was accounted for by the macro-invertebrate grazers. This factor was 0.2 gm. per 1000 individuals for Enchytraeidae, Psychodidae and Chironomidae and 0.2 gm. per 100 individuals for Anisopus. The corrected results are expressed as gm. of volatile matter per 0.1 cu. ft. of bed medium.

Both the measurement of the volatile matter and the chemical

Plate 5. Shaft removed for examination showing vertical arrangement of containers.

analyses were performed by the Upper Tame Main Drainage Authority.

The estimations of macroinvertebrate fauna were made by thoroughly washing the medium from each container, as with the film estimation, and filtering the sample through a buchner flask using a filter paper in which sectors had been cut representing one fifth of the total area of the paper. The sectors were removed and their residue washed into a petri dish. The dish was placed on a platform, illuminated from underneath, and again sectored so as to display one fifth of the area of the dish. The total volume of the sample to be counted was thus reduced by a factor of twenty-five. The results of the macrofauna estimations are expressed similarly as number of individuals per 0.1 cu. ft. of bed medium.

Results.

The temperature of the bacteria beds at Langley Mill as recorded from a depth of one foot is expressed graphically in Fig. 2 (a).

The chemical compositions of the feed throughout the period of observation are recorded in Figs. 2, 3 and 4, together with the results of corresponding tests on the respective effluents from Beds A and D. The B.O.D. of the feed was for the most part in the range of 150-250 p.p.m. There was no particular seasonal pattern of variation. Similarly the P.V. figures for the feed

FIGURE 2.

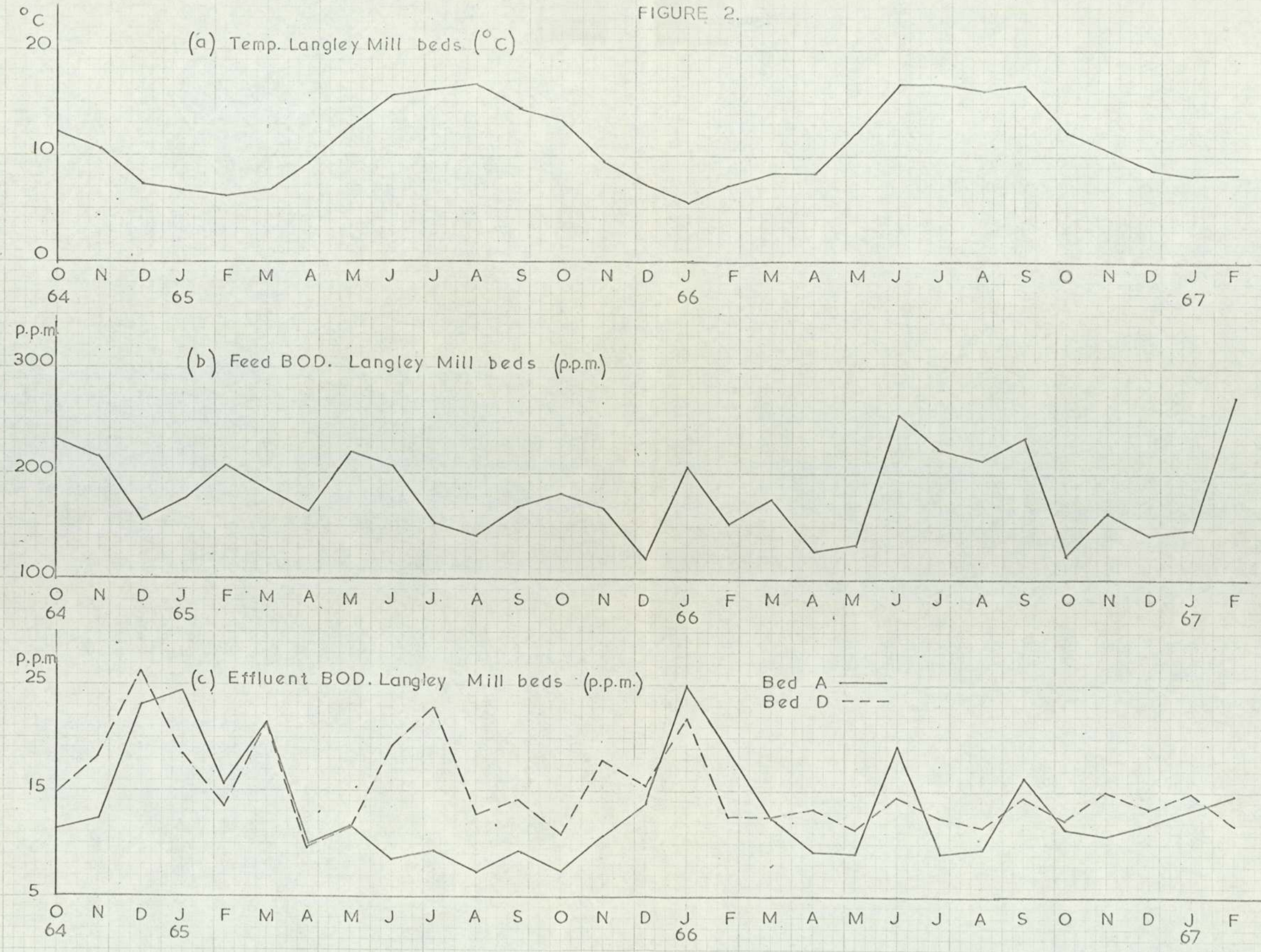

°C
20
10
0
(a) Temp. Langley Mill beds (°C)
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67
p.p.m.
300
200
100
(b) Feed BOD. Langley Mill beds (p.p.m.)
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67
p.p.m.
25
15
5
(c) Effluent BOD. Langley Mill beds (p.p.m.)
Bed A
Bed D
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67

Figure 2.

FIGURE 3.

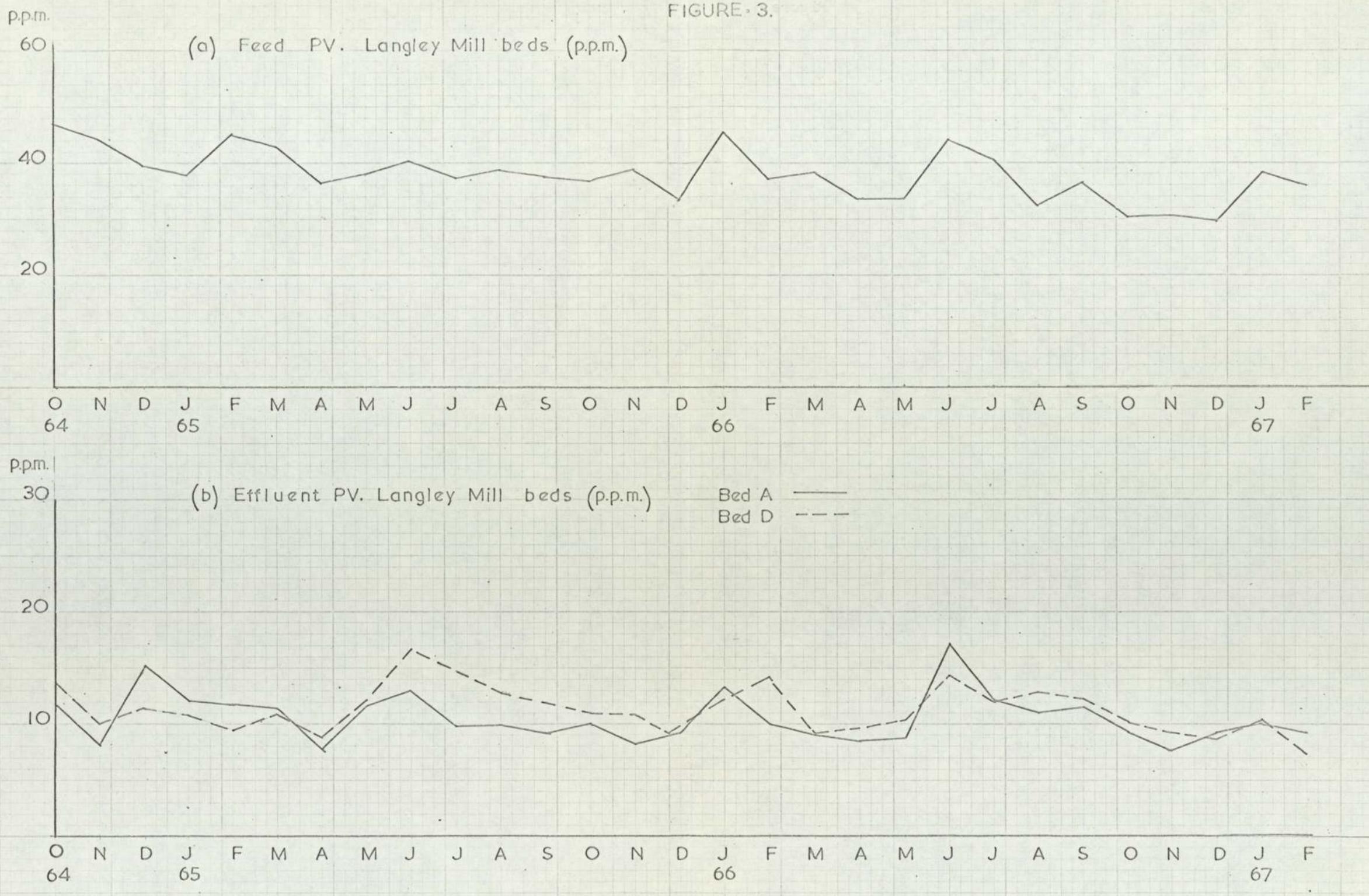
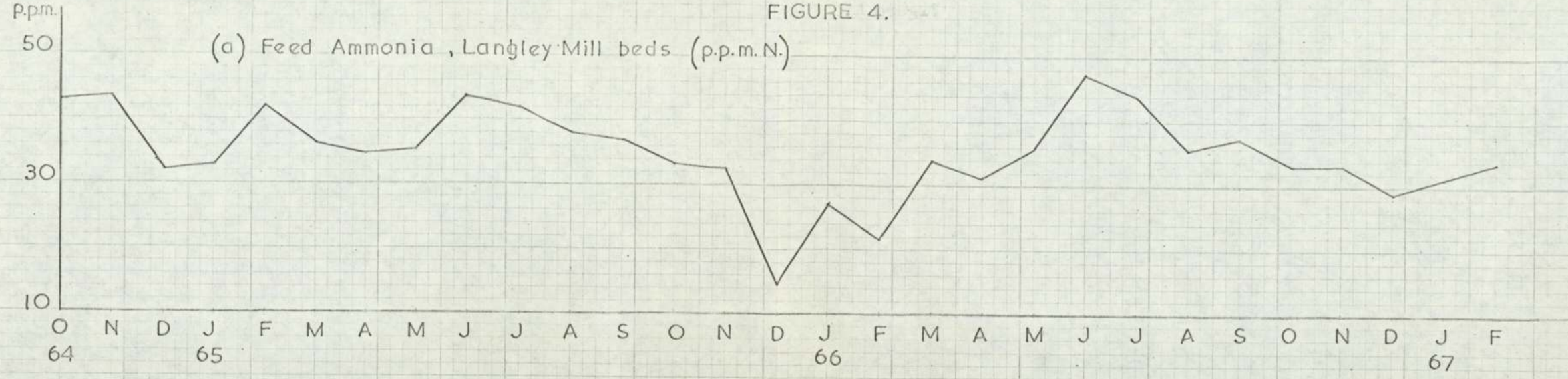
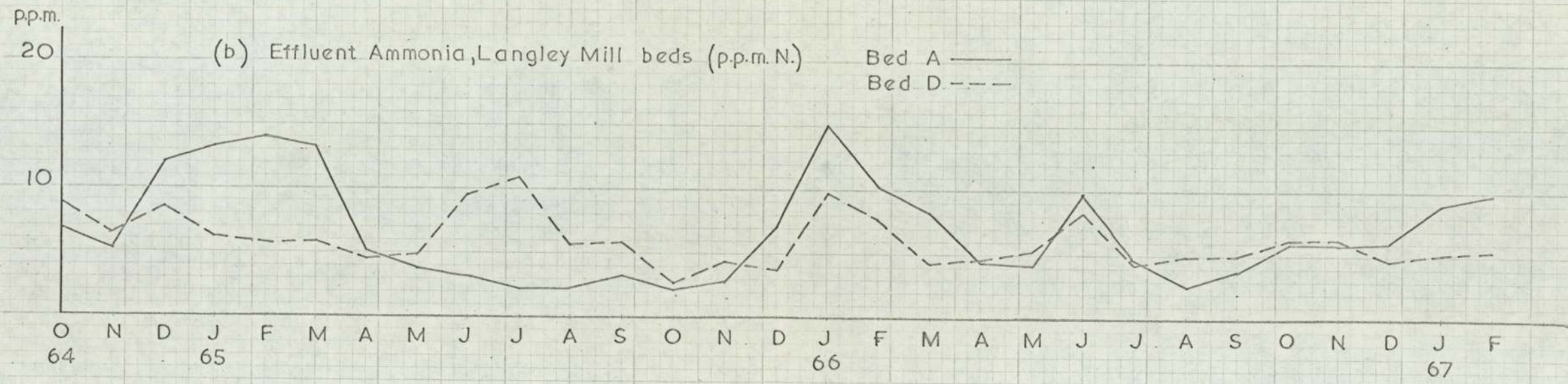

p.p.m.
60
40
20
(a) Feed PV. Langley Mill beds (p.p.m.)
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67
p.p.m.
30
20
10
(b) Effluent PV. Langley Mill beds (p.p.m.)
Bed A
Bed D
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67

Figure 3.

FIGURE 4.


p.p.m.
50
30
10
(a) Feed Ammonia , Langley Mill beds (p.p.m. N.)
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67


p.p.m.
20
10
(b) Effluent Ammonia, Langley Mill beds (p.p.m. N.)
Bed A ——
Bed D - - -
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67

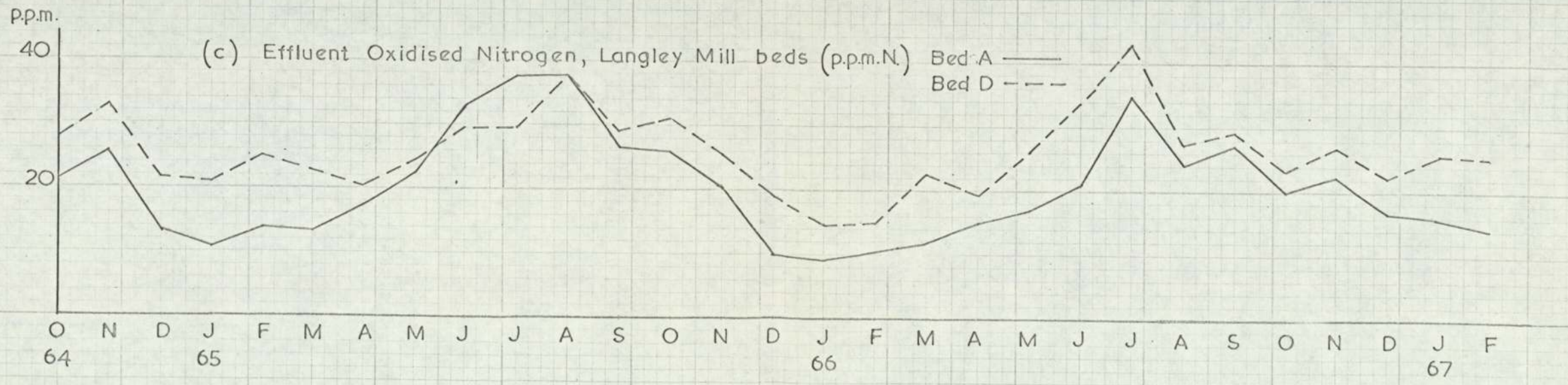

p.p.m.
40
20
(c) Effluent Oxidised Nitrogen, Langley Mill beds (p.p.m. N.)
Bed A ——
Bed D - - -
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67

Figure 4.

varied between 25 and 50 p.p.m. The concentration of ammonia in the feed was mostly steady at between 30 and 50 p.p.m. but very low figures of down to 14 p.p.m. were recorded in Dec. 1965 and Jan. and Feb. 1966. The pH of the feed varied between 7.0 and 8.0 except for Dec. 1965 when a figure of 8.5 was recorded.

The rates of purification achieved by the two beds were high throughout the period of observation. Percentage removal of B.O.D. was mostly above 90%; as would be expected, it was somewhat higher in summer than in winter. There was little difference between the rates of B.O.D. removal in the two beds with the exception of the summer and autumn of 1965 when the rate in Bed A was slightly higher than Bed D. The P.V. figures for the effluents of the two beds largely confirmed the B.O.D. figures. The concentration of the ammonia in the effluent of Bed A which was low during the summer showed fairly marked increases in winter, rising to about 15 p.p.m. These marked increases did not occur in Bed D. High levels of ammonia were, however, recorded in the effluent of Bed D during the summer of 1965. This coincided with poor rates of B.O.D. and P.V. removal showing an overall efficiency lower than in Bed A during this period. The nitrate concentrations of the effluents in both beds showed regular seasonal variations, the summer figures being approximately double those of the winter. The nitrate in the effluent of Bed D was consistently

higher than in Bed A except for three months during the summer of 1965. Nitrate was virtually absent from the feed and is not recorded.

With the macrofauna estimations, no attempt was made to determine the proportions of individual species. The main groups that occurred in the beds were the families Enchytraeidae and Lumbricidae (Oligochaeta), Psychodidae and Chironomidae (Diptera) and the genus Anisopus (Diptera). These groups may have been represented by one or more species. Occasional checks were made to determine the species of individuals from fauna samples, but it is not certain that all species present in the bed were identified. Where a family is represented by more than one species, there is no accurate information on their relative proportions.

From the family Enchytraeidae, the species Lumbricillus (=Pachydrilus) lineatus was identified and believed to be by far the dominant member of this family. The species Enchytraeus albidus from which Lumbricillus is distinguished by its curved setae and possession of haemoglobin was not discovered. Of the Psychodidae, the species Psychoda alternata was identified and believed to be the most abundant, but other members of the same genus may also have been present. The genus Anisopus was represented by the species A. fenestralis. Of the family

Lumbricidae all three species commonly associated with bacteria beds, Eisenia foetida, Dendrobaena subrubicunda and Lumbricus rubellus were discovered. The species Metriocnemus longitarsus was the only member of the Chironomidae positively identified but other representatives of this family may also have occurred.

The level of film accumulation and incidence of macroinvertebrates in the beds are recorded in two ways, as explained in the plan of operation. First, the results for samples from the surface of the beds only are expressed graphically as gm. volatile matter and numbers of individuals per 0.1 cu. ft. These figures are the average of six samples taken from three different positions in the bed, an upper and a lower sample from each position, (the upper sample being the first six inches and lower sample the second six inches from the surface of the bed). The individual figures from which these averages are derived are quoted in Appendix Tables 1 and 2. The three positions are designated 1, 2 and 3, 1 being the position nearest the circumference of the bed and 3 being that nearest to the centre. The upper and lower samples are called u. and l. Second, the results for the distribution of film and macrofauna through the depth of each bed are recorded as histograms. These are the results of one sample per month only from each bed. The samples cover nine months of the year only, from October to June, and are concluded

at June 1966.

Fig. 5 (a) shows the pattern of seasonal accumulation of film at the surface of the two beds. It can be seen that the film level in Bed A was considerably greater than in Bed D during winter. The seasonal fluctuations of macroinvertebrates are shown in Figs. 5 (b), 6 and 7. The Enchytraeidae were common in both beds and were exceptionally abundant in the spring and early summer falling to relatively low levels in the winter. Throughout the year the numbers present at the surface tended to be somewhat greater in Bed A than Bed D, but within the depths of the bed the populations in Bed D were more abundant. Psychoda were common in the surface of Bed A and reached particularly large numbers in the autumn and early winter, while in Bed D they were very scarce. Chironomidae were present in the surface of both beds, but only in insignificant quantities in Bed A. In Bed D they were usually in greater abundance than Psychoda, but both were in much smaller numbers than the Enchytraeidae. Lumbricidae were recorded at the surface of both beds, and occasionally Anisopus was noted in Bed A.

The pattern of vertical distribution of film in both beds is shown in Fig. 8. The film tended to be evenly distributed through the depth of Bed D but somewhat thicker at the surface in Bed A. The winter accumulation of film was more marked in Bed A

FIGURE 5.

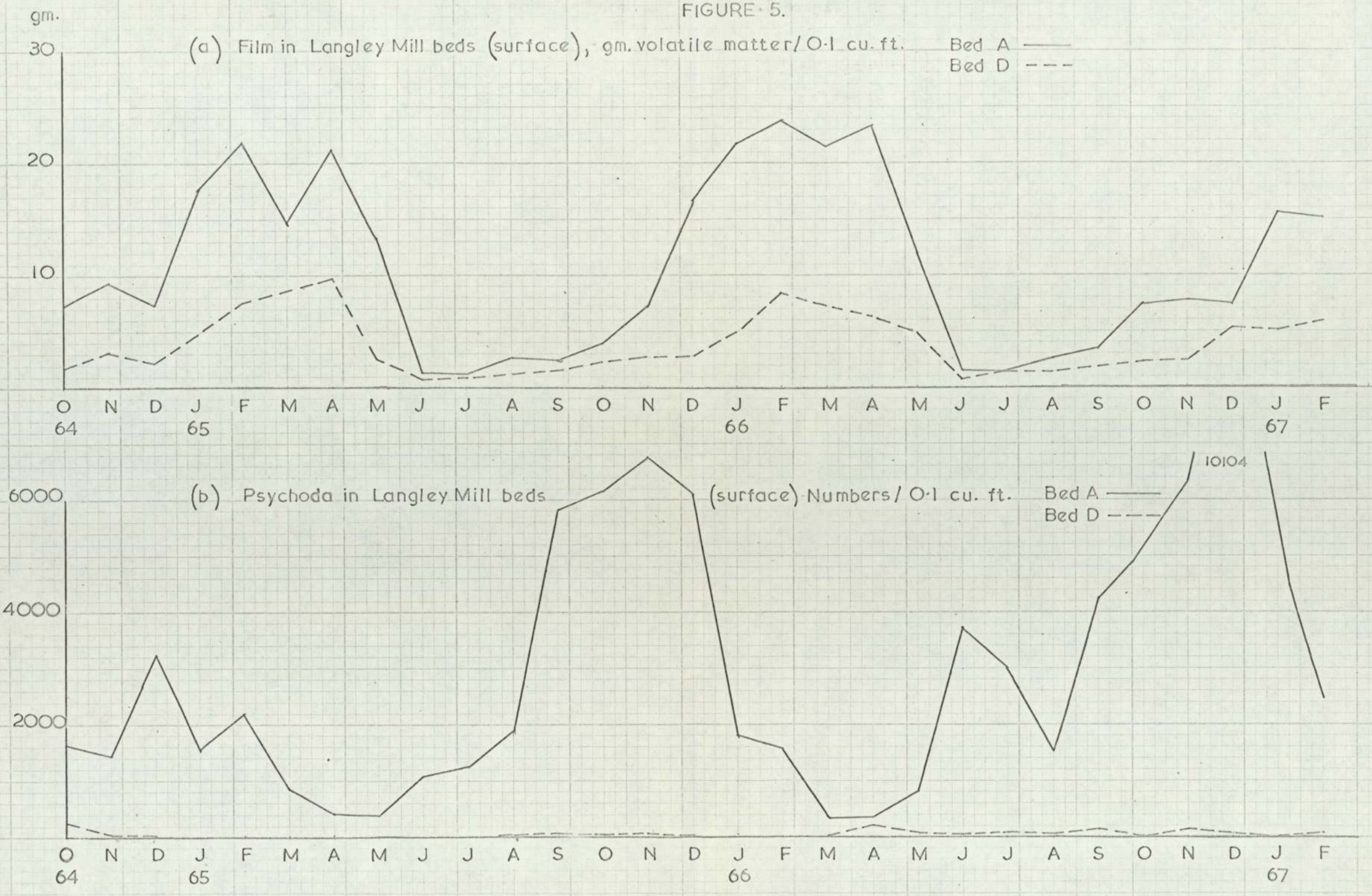
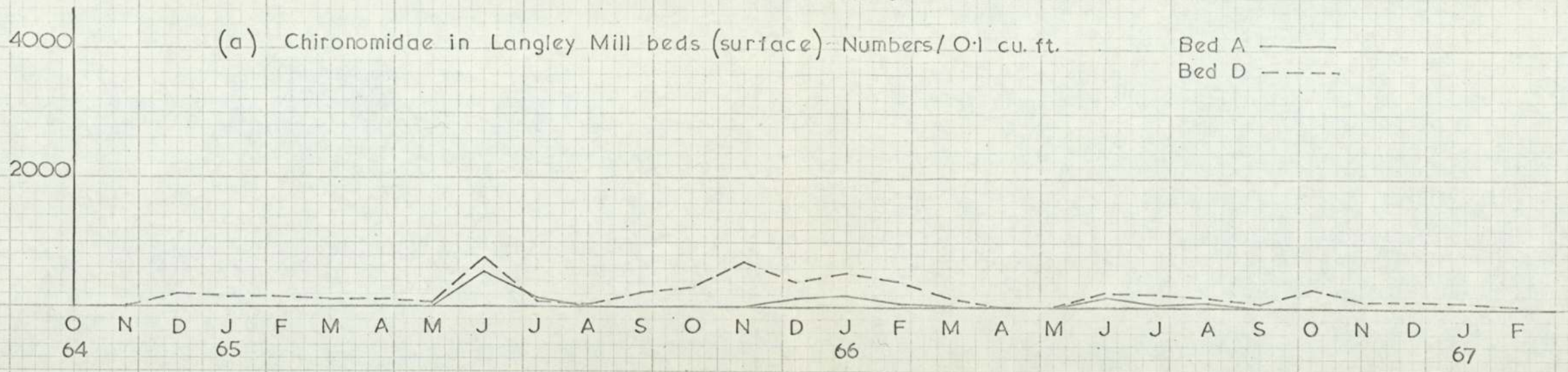

gm.
30
20
10
(a) Film in Langley Mill beds (surface), gm. volatile matter/ 0·1 cu. ft.
Bed A
Bed D
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67
6000
4000
2000
(b) Psychoda in Langley Mill beds (surface) Numbers/ 0·1 cu. ft.
10104
Bed A
Bed D
O N D J F M A M J J A S O N D J F
64
65
66
67

Figure 5.

FIGURE 6.


(a) Chironomidae in Langley Mill beds (surface) Numbers/ 0·1 cu. ft.
Bed A
Bed D
4000
2000
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67

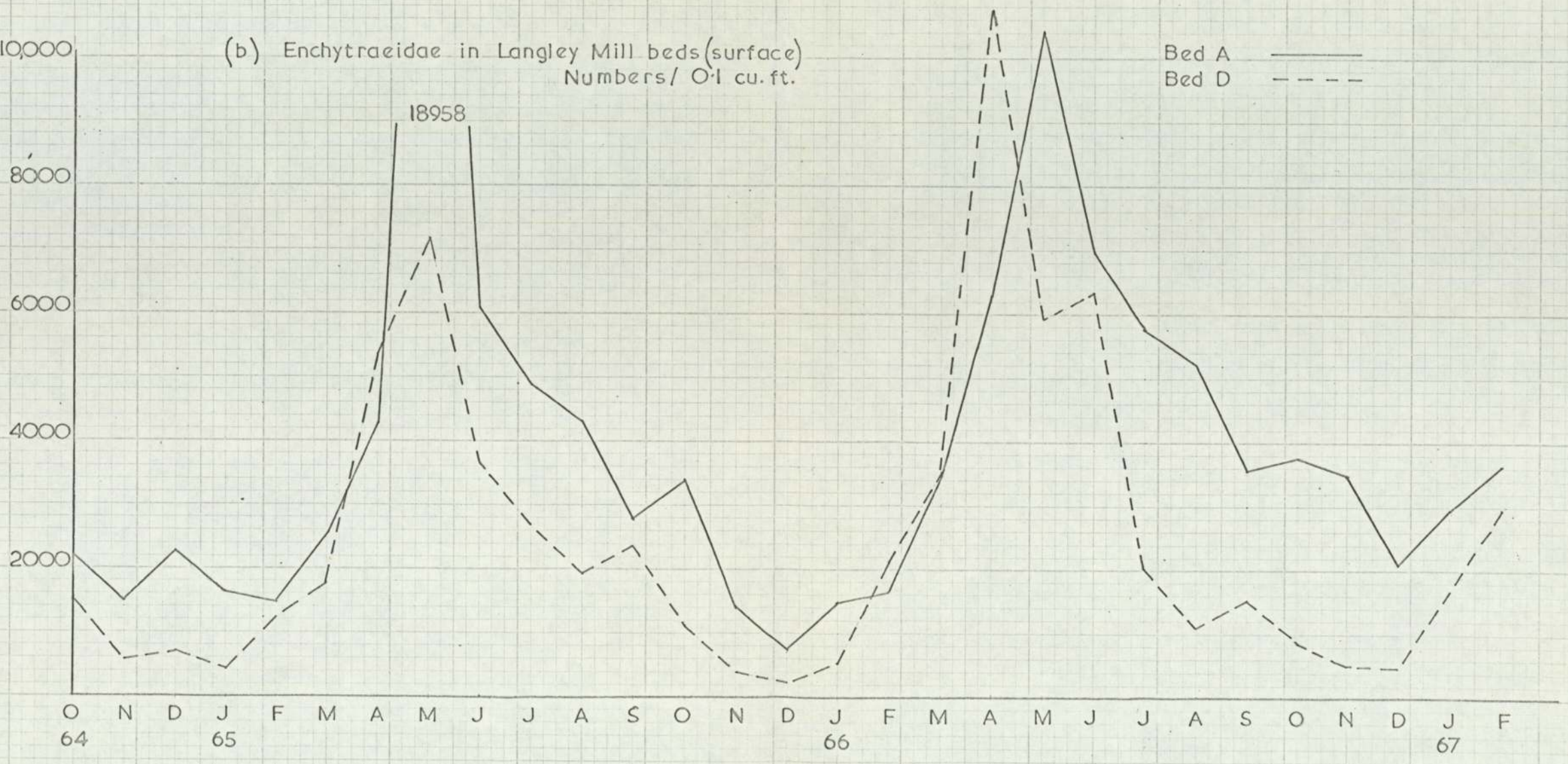
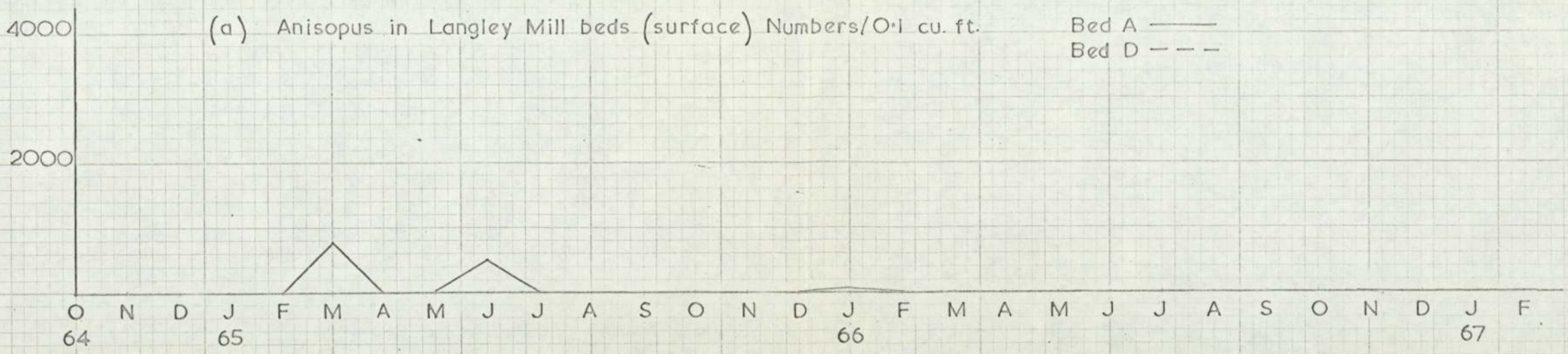
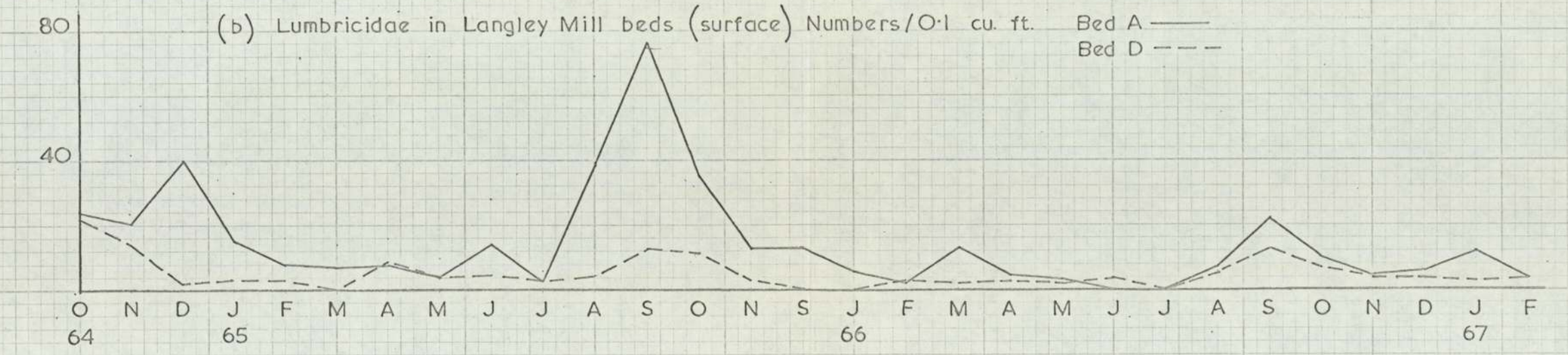

(b) Enchytraeidae in Langley Mill beds (surface)
Numbers/ 0·1 cu. ft.
Bed A
Bed D
18958
10,000
8000
6000
4000
2000
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67

Figure 6.

FIGURE 7.


4000
2000
(a) Anisopus in Langley Mill beds (surface) Numbers/0·1 cu. ft.
Bed A ——
Bed D – – –
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67


80
40
(b) Lumbricidae in Langley Mill beds (surface) Numbers/0·1 cu. ft.
Bed A ——
Bed D – – –
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67

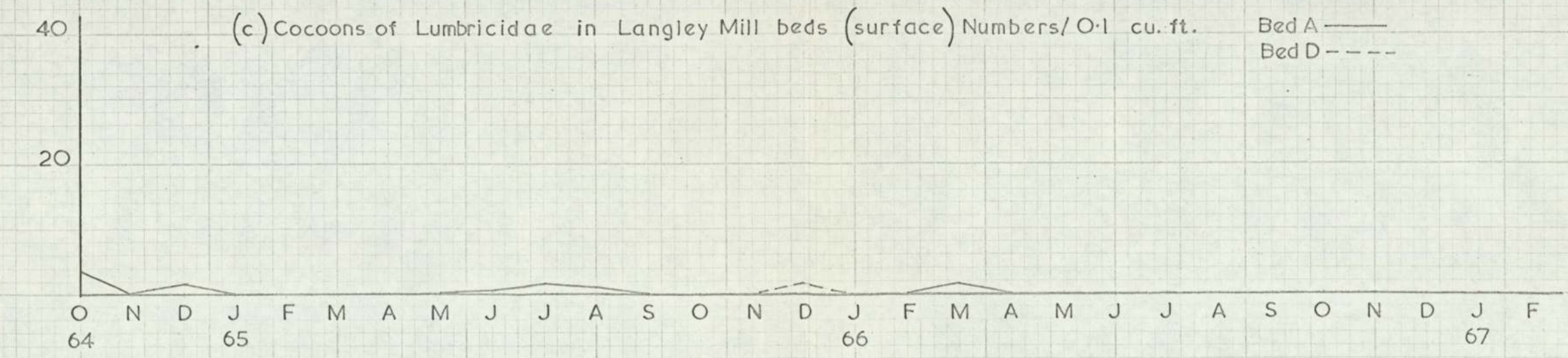
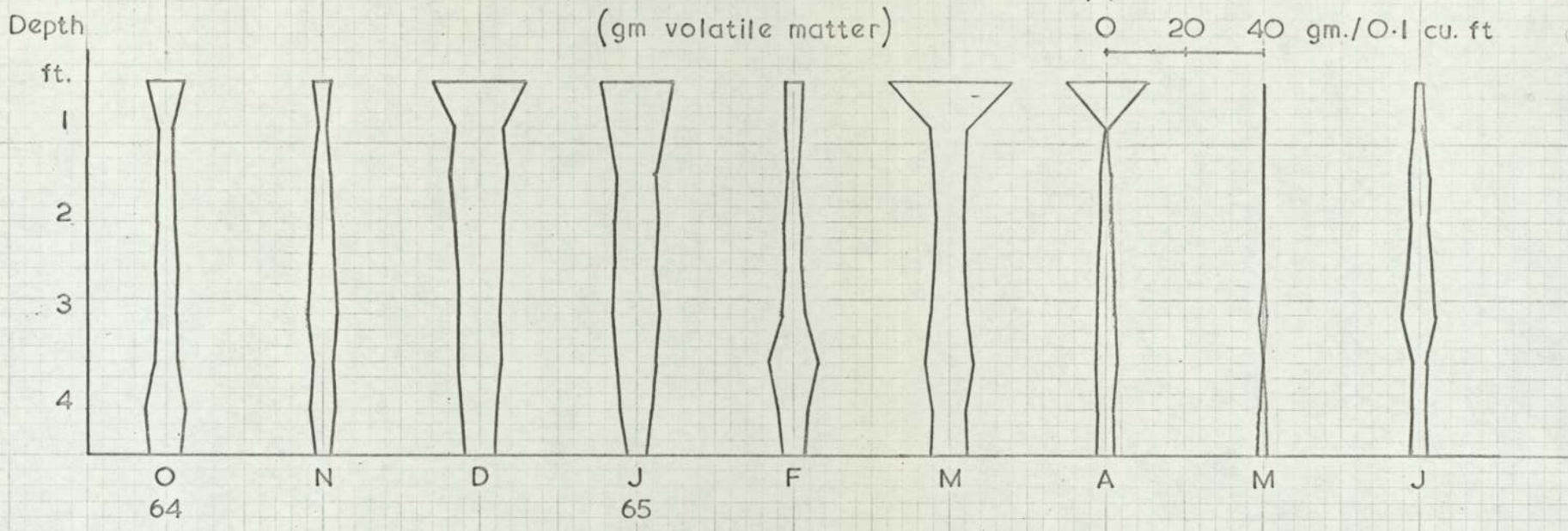

40
20
(c) Cocoons of Lumbricidae in Langley Mill beds (surface) Numbers/0·1 cu. ft.
Bed A ——
Bed D – – – –
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
64
65
66
67

Figure 7.

FIGURE 8 (pt. 1)

Vertical distribution of film in Langley Mill beds (gm volatile matter)



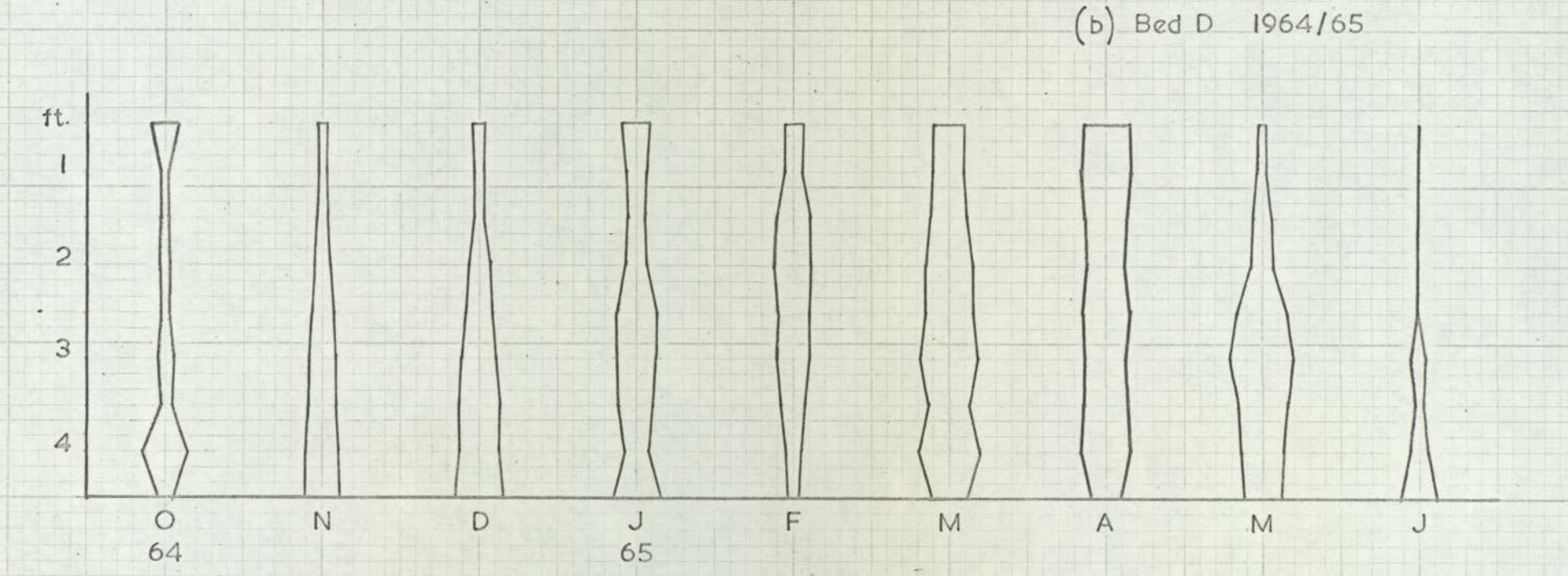
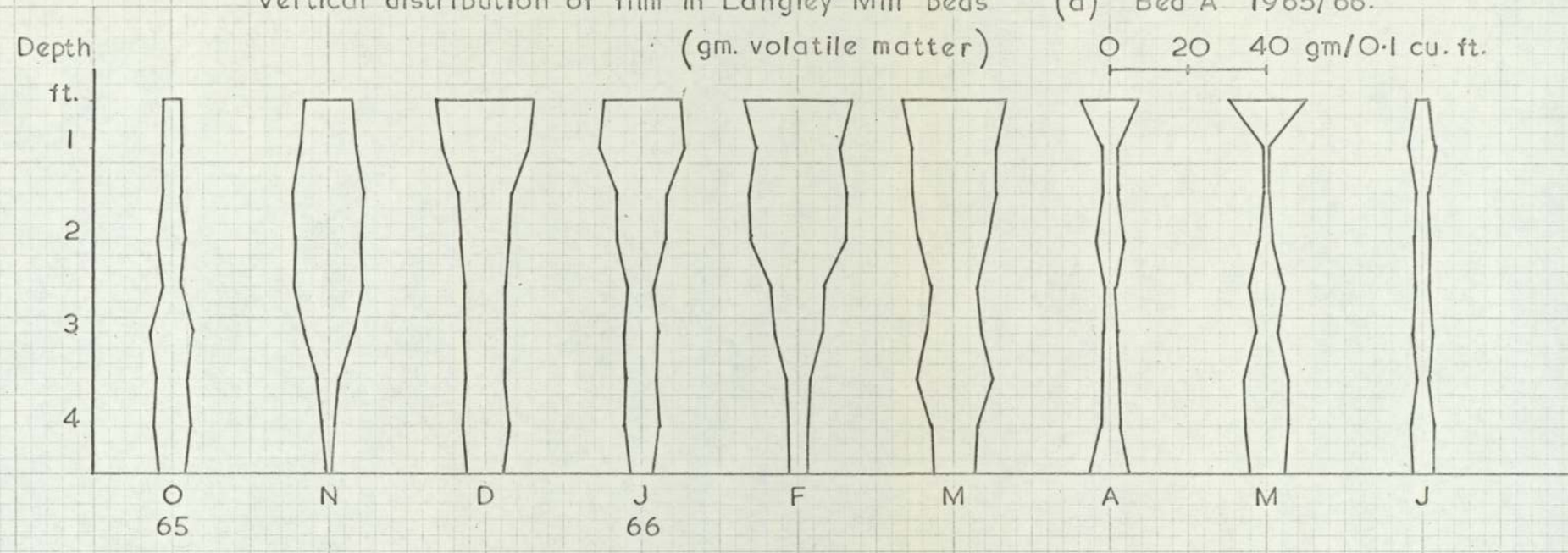


Figure 8.
(pt. 1)

FIGURE 8 (pt.2)

Vertical distribution of film in Langley Mill beds (gm. volatile matter)



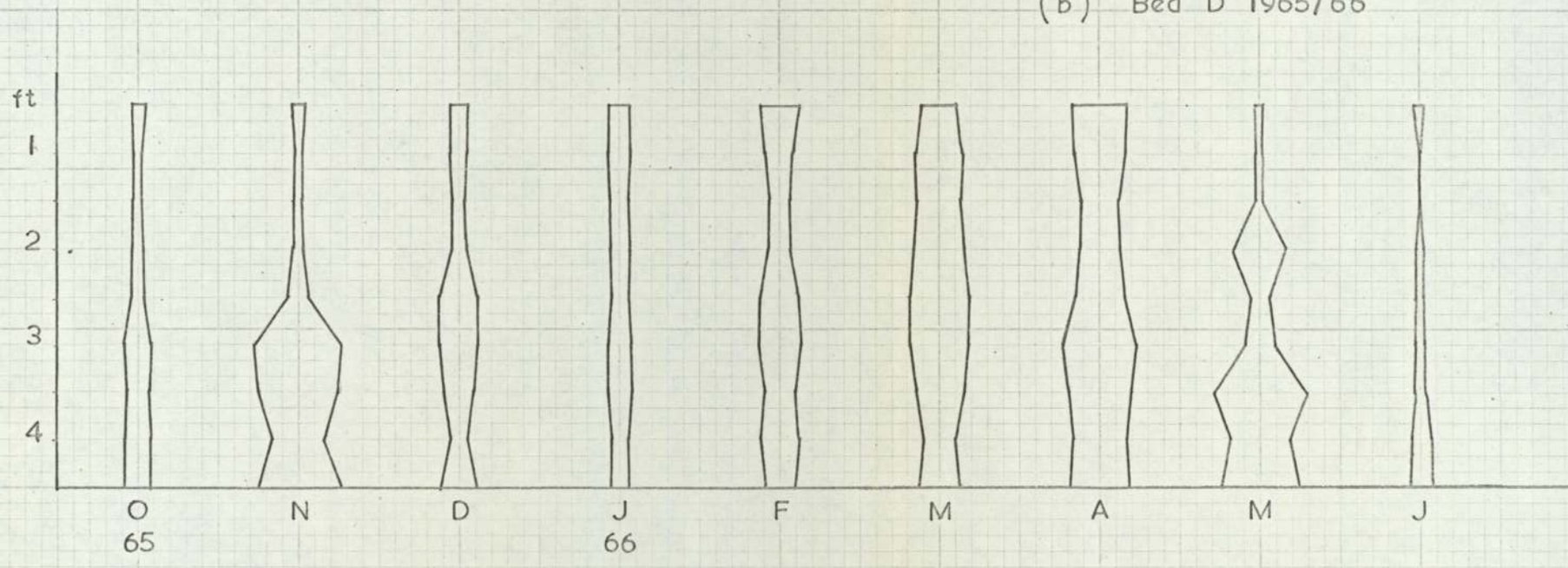


Figure 8.
(pt. 2)

and took place earlier in the winter than in Bed D, where maximum accumulations tended to occur in the early spring. Although figures for the three months July to September were not available, it is probable that the film remained at a low level throughout the summer.

The occurrence of the most important members of the grazing macrofauna through the depths of the two beds is shown in Figs. 9 and 10. Enchytraeidae are seen to increase to very large numbers in the spring and early summer in both beds. The numbers tend to be greatest in the upper half of the bed but not necessarily at the immediate surface. Numbers were generally greater in Bed D than in Bed A and this was probably associated with the relative scarcity of Psychoda in Bed D as a competing species. In Bed A Psychoda were also seen to increase in the spring and early summer in the upper half of the bed but not to such a marked extent as the Enchytraeidae. Psychoda were in particular abundance at the surface of Bed A in the autumn and early winter but these populations did not extend through the depths. Anisopus was present in Bed A during the first year of operation and was particularly abundant in May 1965, after which it was seldom recorded.

The distribution of Chironomidae in Beds A and D and Psychoda in Bed D is reproduced on a different scale in Fig. 13. Although these groups are present only in very small quantities compared

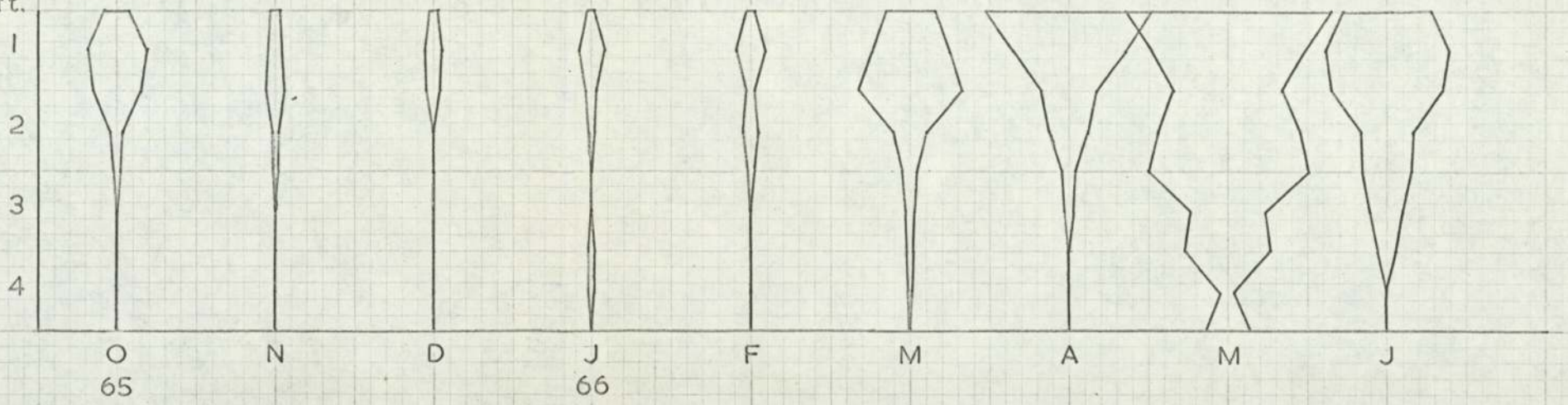
Vertical distribution of macrofauna. Bed A 1964/65 0 5 10 thousands/0·1 cu. ft.

FIGURE 9 (pt. 1)

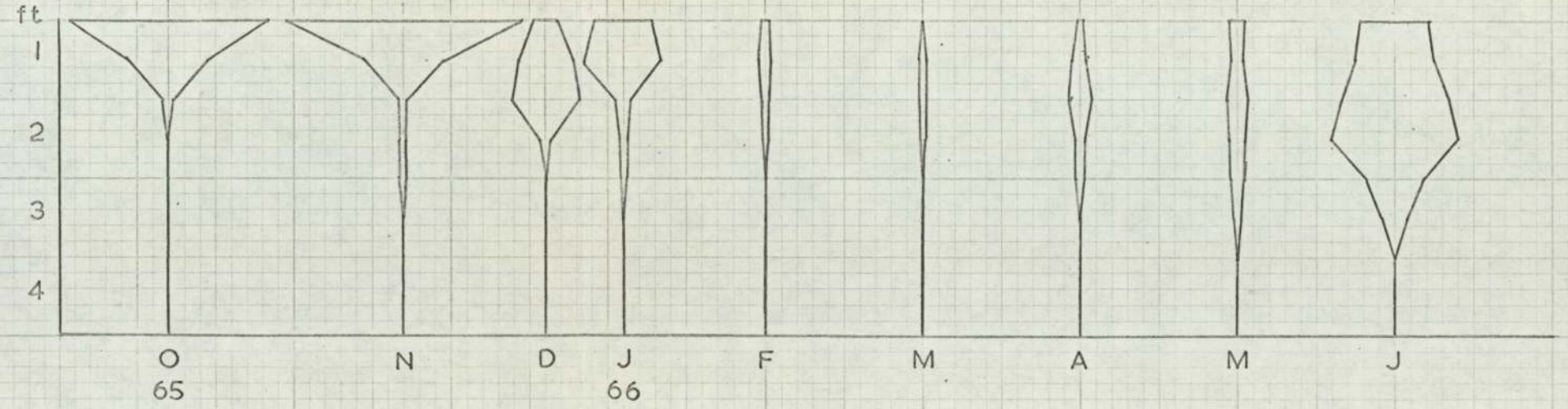
Figure 9.
(pt. 1)

Vertical distribution of macrofauna. Bed A 1965/66 0 5 10 thousands/0·1 cu. ft.

(a) Enchytraeidae



(b) Psychoda



(c) Anisopus

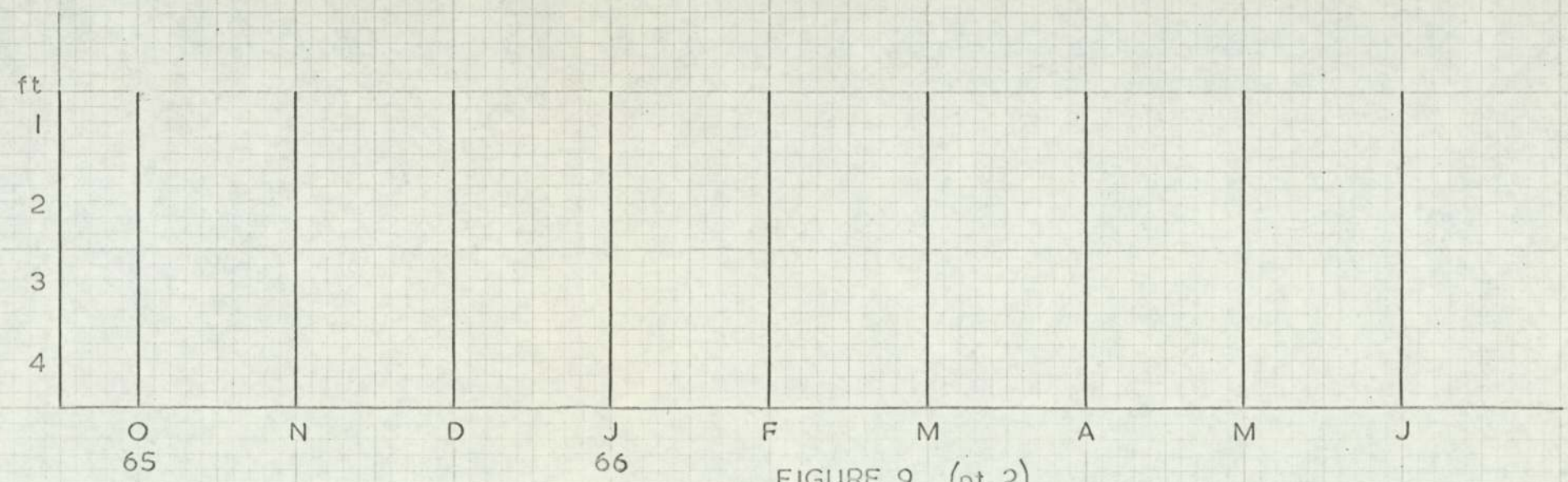


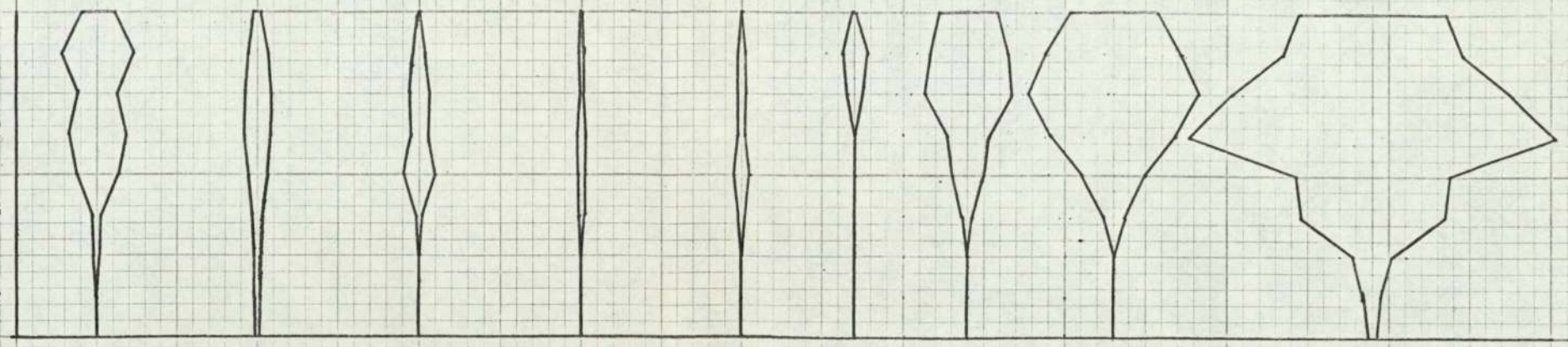
FIGURE 9 (pt. 2)

Figure 9.
(pt. 2)

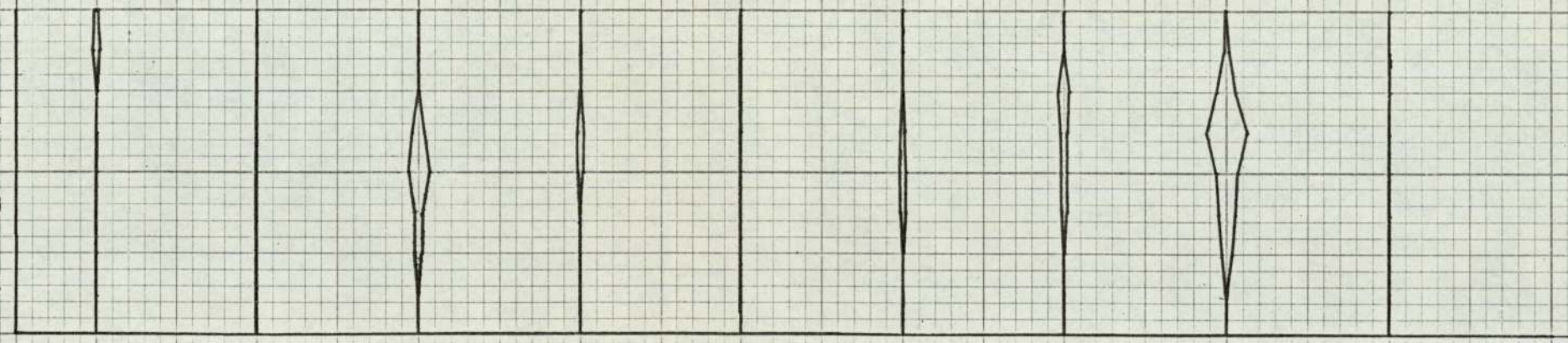
Vertical distribution of macrofauna. Bed D 1964/65 0 5 10 thousands / 0.1 cu. ft.

Depth ft

(a) Enchytraeidae



(b) Psychoda



(c) Chironomidae

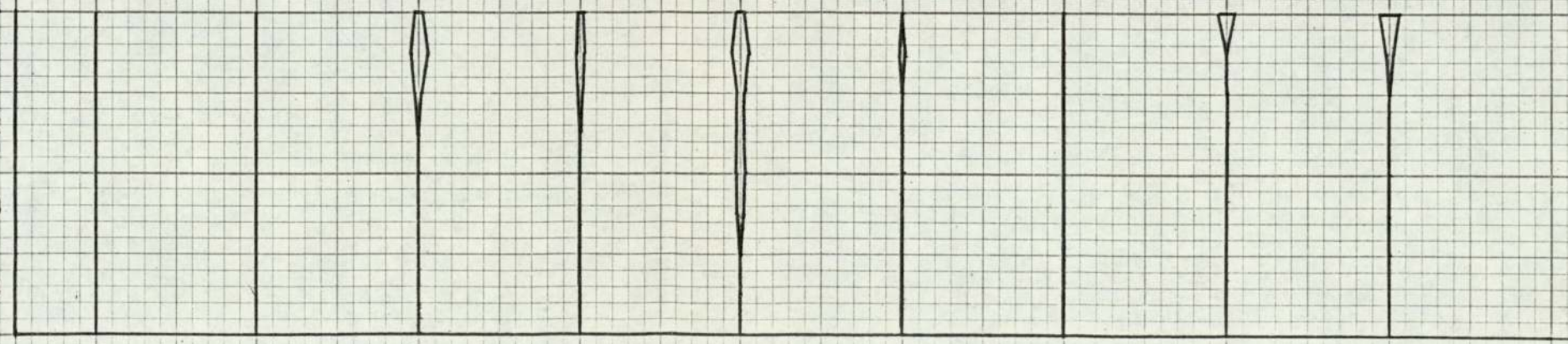


FIGURE 10 (pt. 1)

Figure 10.
(pt. 1)

Vertical distribution of macrofauna. Bed D 1965/66 0 5 10 thousands/0·1 cu. ft.

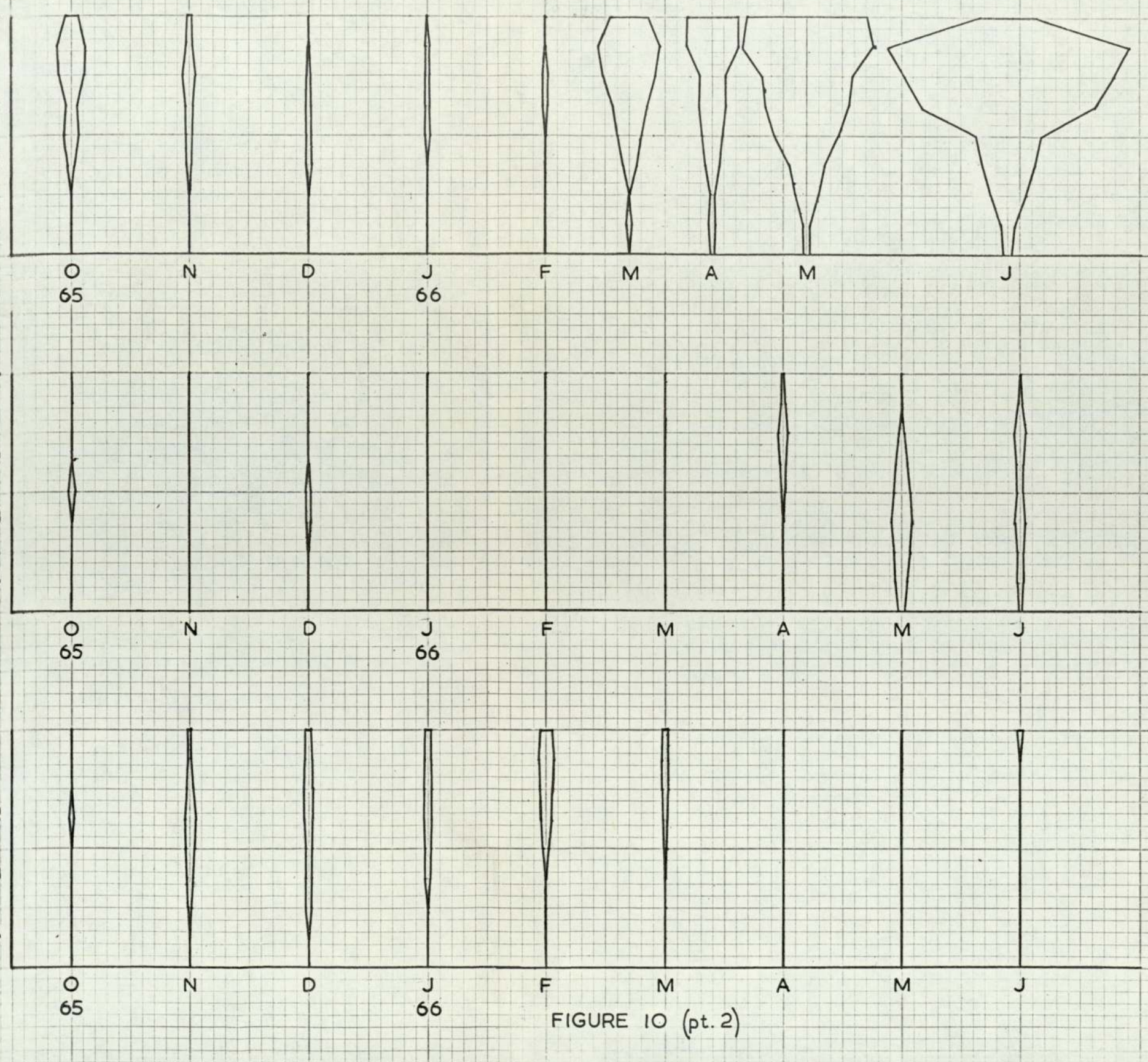
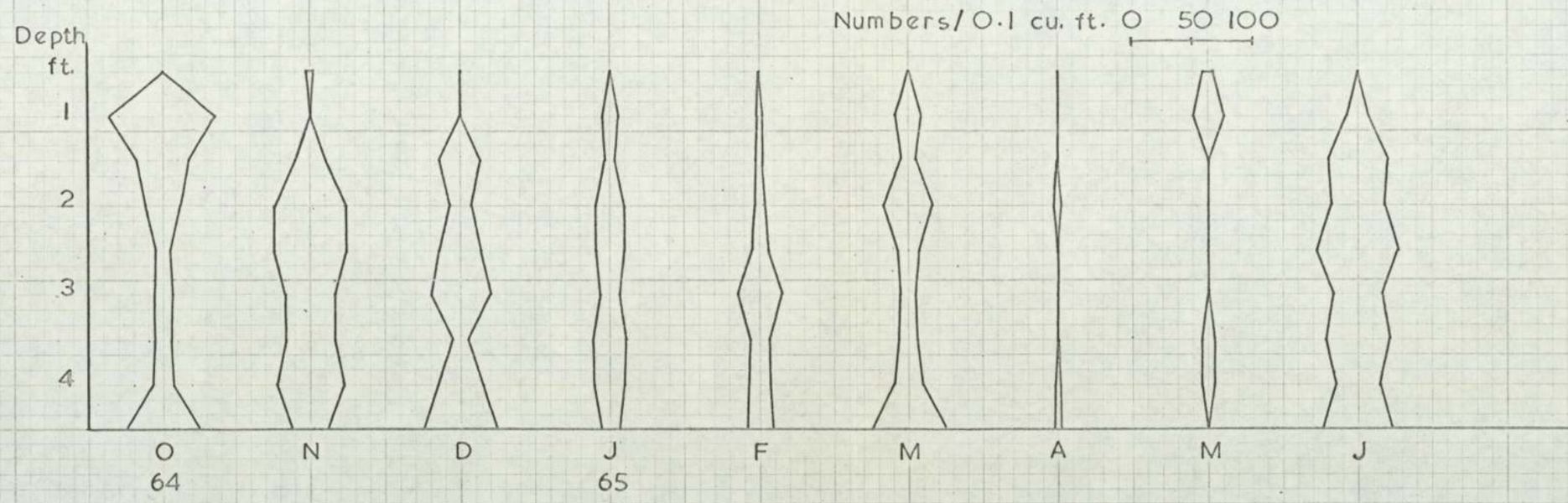


FIGURE 10 (pt. 2)

Figure 10.
(pt. 2)

FIGURE 11 (pt. 1)

Vertical distribution of Lumbricidae in Langley Mill beds (a) Bed A 1964/65



(b) Bed D 1964/65

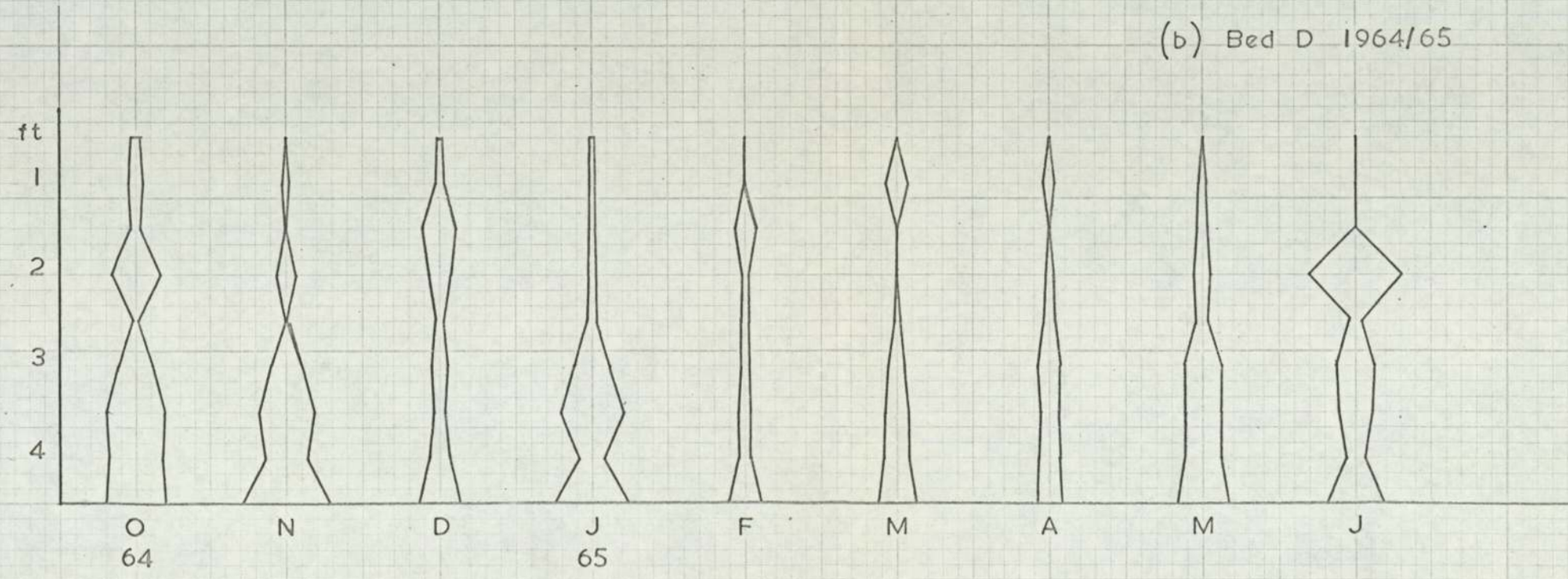
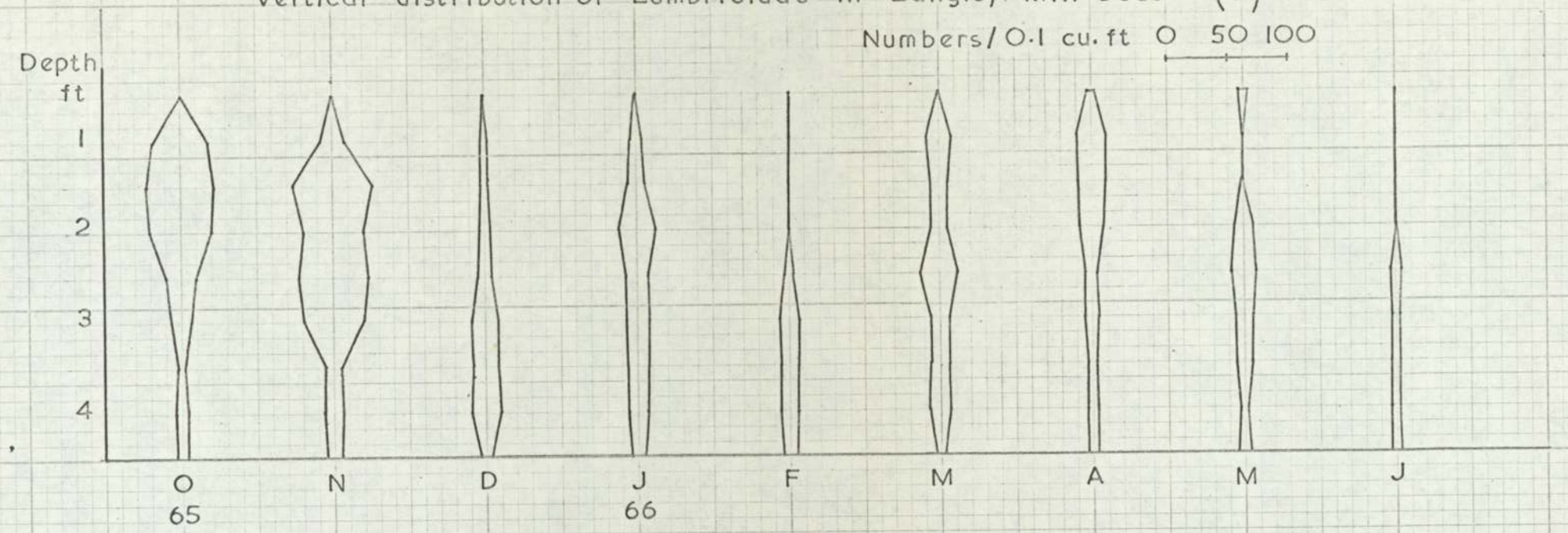


Figure 11.
(pt. 1)

FIGURE 11 (pt.2)

Vertical distribution of Lumbricidae in Langley Mill beds (a) Bed A 1965/66



(b) Bed D 1965/66

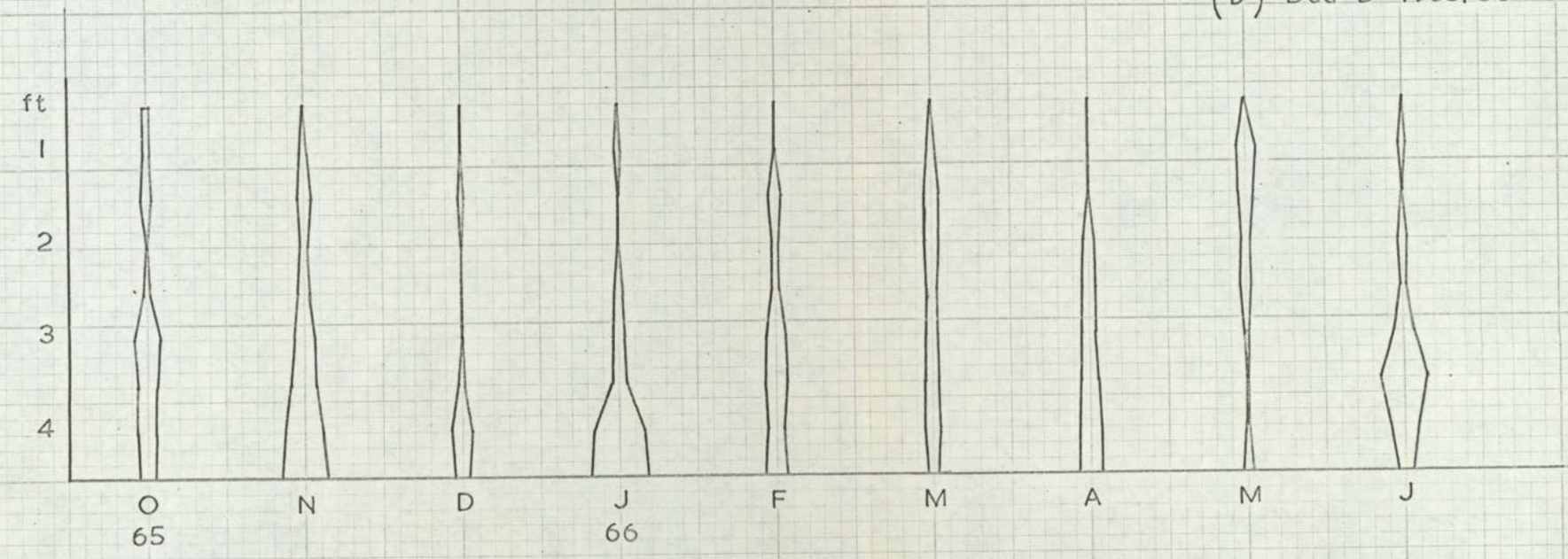


Figure 11.
(pt. 2)

FIGURE 12 (pt 1)

Vertical distribution of lumbricid cocoons in Langley Mill beds.

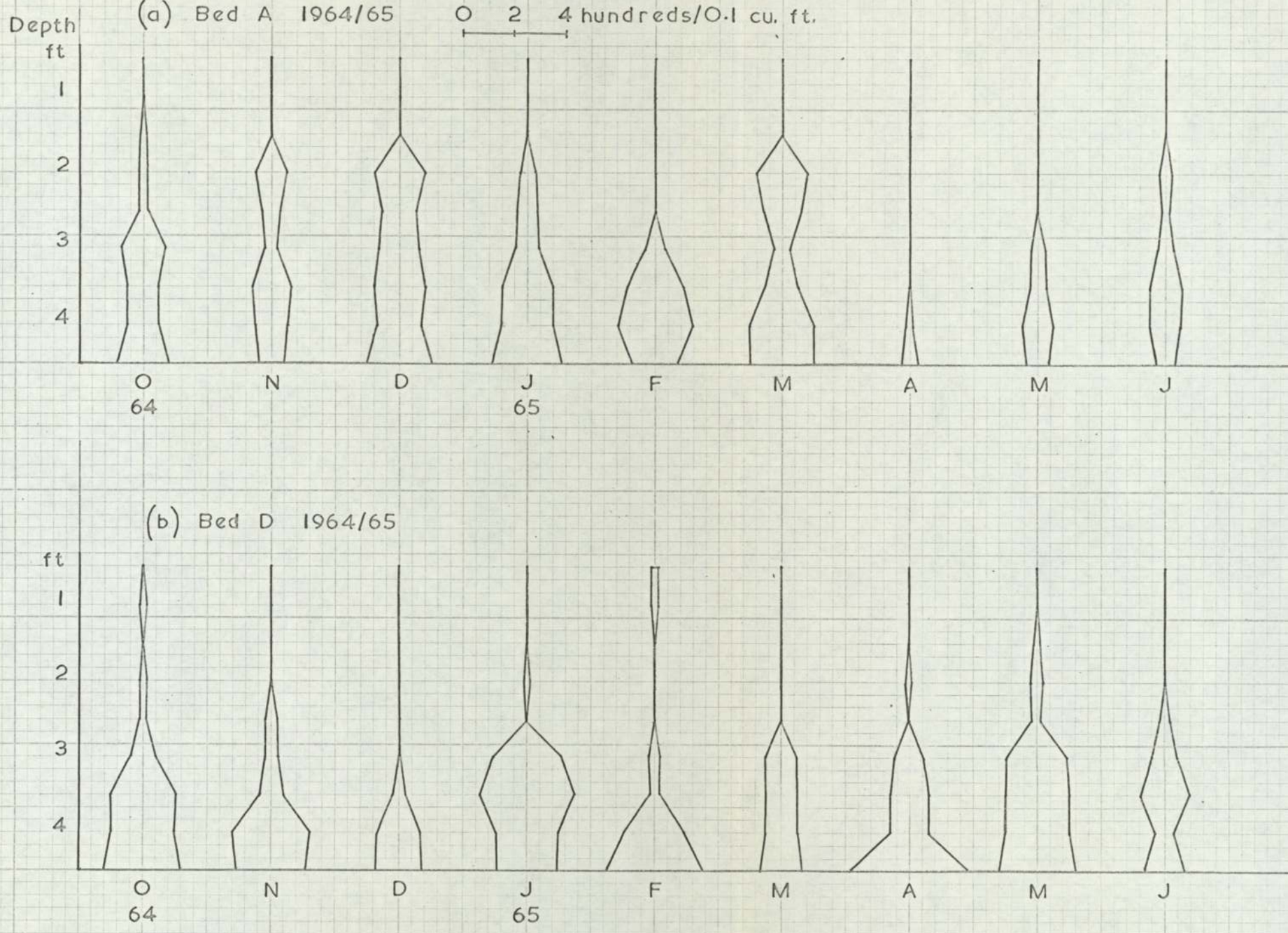


Figure 12.
(pt. 1)

FIGURE 12 (pt. 2)

Vertical distribution of lumbricid cocoons in Langley Mill beds.

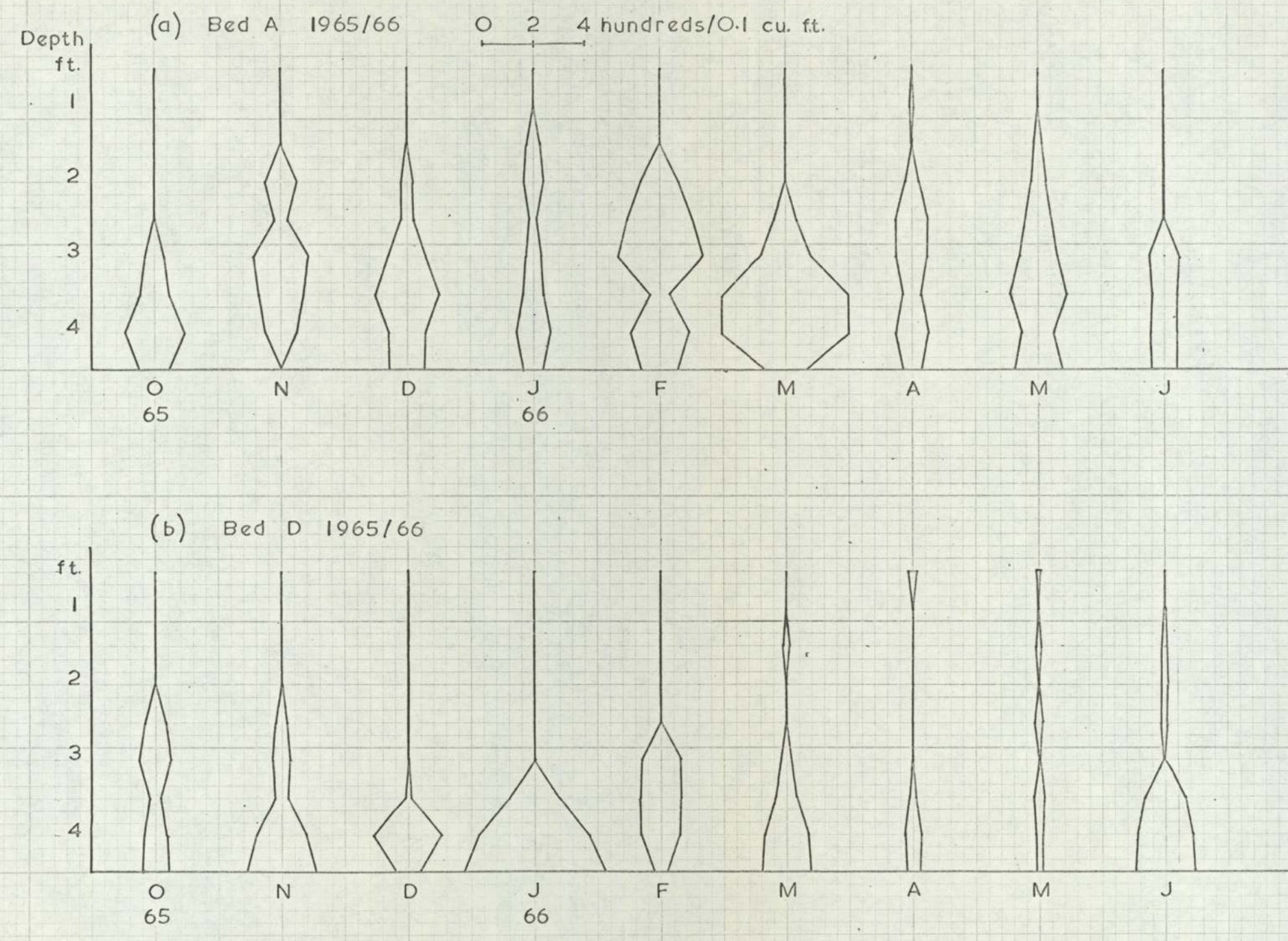
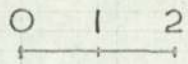
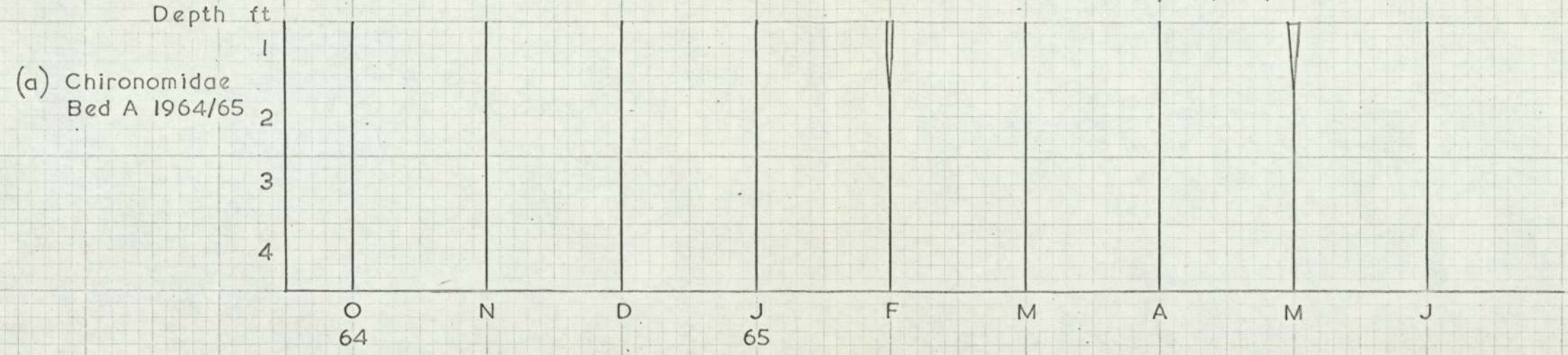
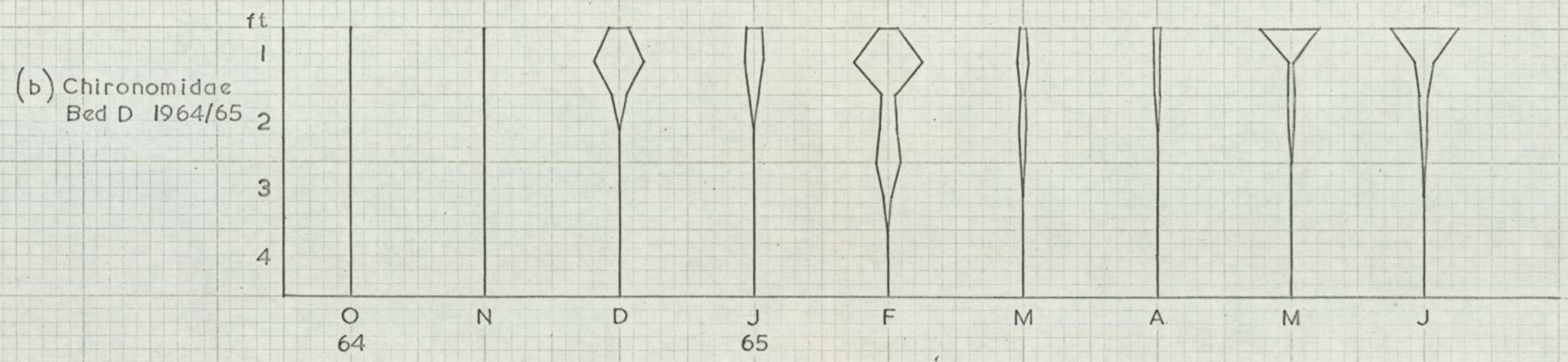


Figure 12.
(pt. 2)

Vertical distribution of macrofauna in Langley Mill beds







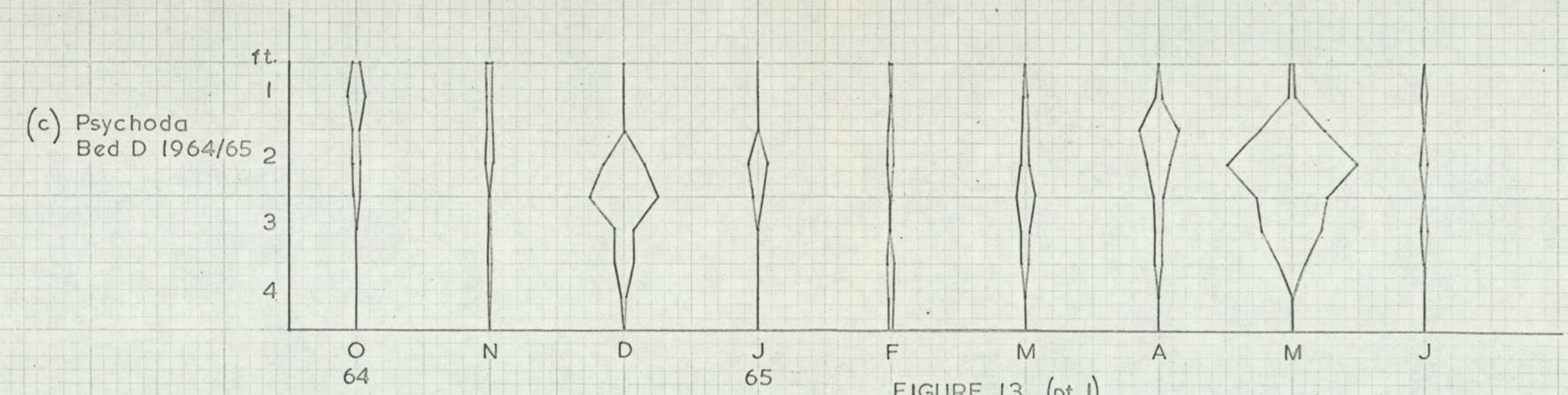


FIGURE 13 (pt. 1)

Figure 13.
(pt. 1)

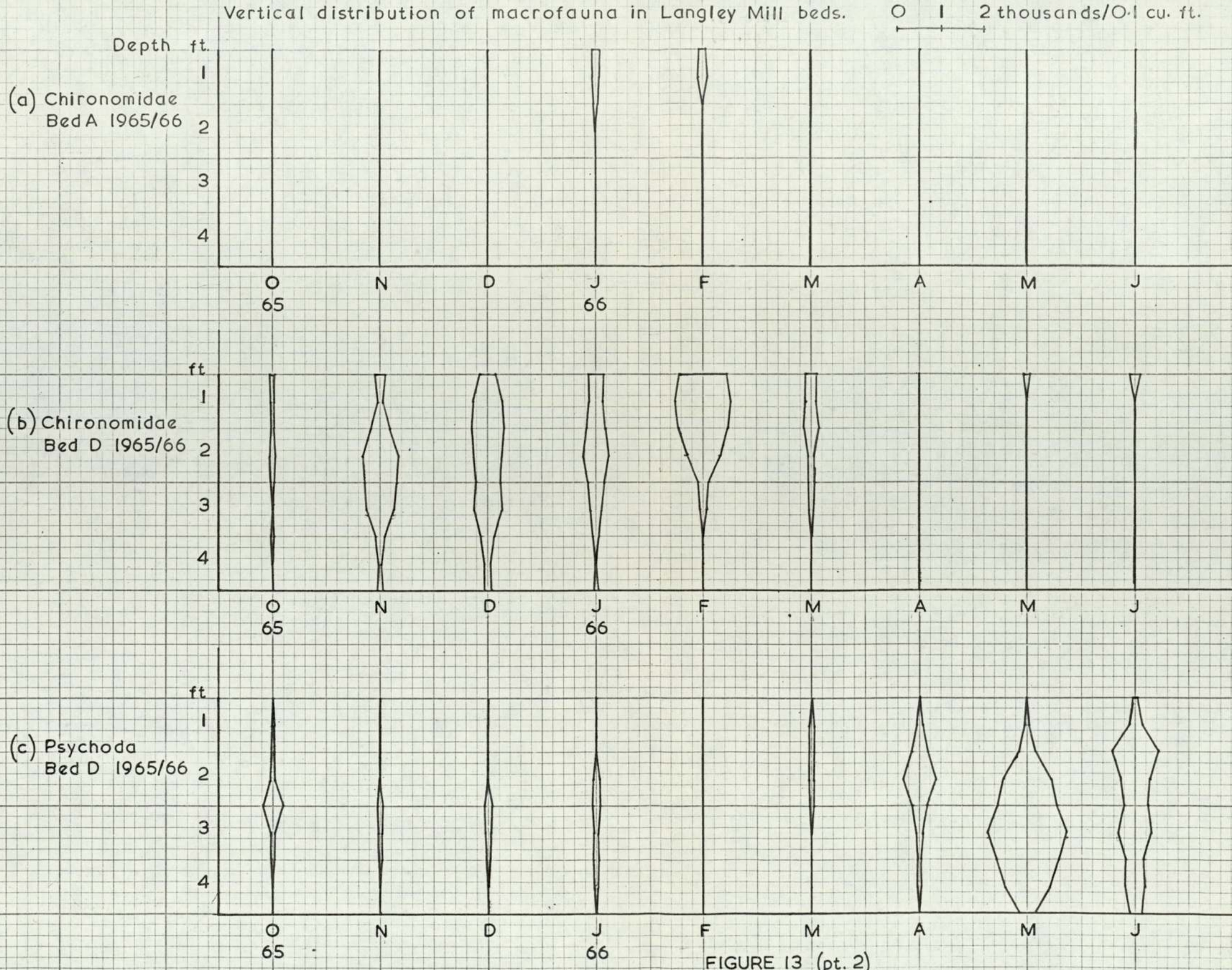


FIGURE 13 (pt. 2)

Figure 13.
(pt. 2)

with other members of the macrofauna, there appears to be a state of direct competition between then which is considered in the discussion.

The occurrence of Lumbricidae and their cocoons in the depths of the beds is shown in Figs. 11 and 12. The incidence of adults was slightly greater in Bed A than Bed D and the vertical distribution was fairly even in both beds except that they seemed to avoid the immediate surface layers. There was little distinct seasonal variation in frequency, apart from a slight increase in October and November in both beds. The cocoons tended to concentrate in the lower part of the bed, and were somewhat further down in Bed D than Bed A. As with the adults, the numbers tended to be slightly greater in Bed A than Bed D and to be a little higher in the winter.

Discussion of Results

The results of the observations at Langley Mill show that, while interesting differences in the ecology of the two beds were recorded, there was little difference in the purification rates between Bed A and Bed D. High rates of purification were achieved in Bed A, where the frequency of dosing was relatively high and there was therefore little room for improvement in Bed D, operated at a lower dosing frequency. This is in contrast to the work of Tomlinson and Hall (1955) and Hawkes (1955) at Minworth,

Birmingham, who observed a considerable improvement in the condition and efficiency of bacteria beds, when a slower rate of rotation of the distributor arms was used, up to a certain limit.

The main factor in maintaining a high rate of purification is undoubtedly the avoidance of excessive film accumulation in winter. The reason that this was avoided at Langley Mill even at a high frequency of dosing was probably concerned with the strength and nature of the sewage. At Langley Mill it was entirely domestic and of fairly low strength, while at Minworth the beds were treating a primarily industrial effluent. This appeared to give rise to a film that was largely composed of fungus whose bulky growths are frequently associated with conditions of ponding.

In Bed A the film at the immediate surface reached higher quantities in winter than in Bed D, but at lower levels the film accumulation was only slightly greater. Visual inspection showed the quantity of film at the surface of Bed A to be heavy in winter but just short of conditions of ponding. The rate of accumulation of film in Bed D appeared to be slower than Bed A, which meant that maximum levels were not reached until later in the winter, about March or April. Similarly the rate of unloading in spring was slower in Bed D, high film levels persisting longer than in Bed A. Occasionally results were recorded in both beds which did not appear to fit in with the general pattern of film accumulation. An example of this was in February 1965 where the

film levels in both Bed A and Bed D were lower than both the preceding and subsequent months. There was no apparent irregularity in the temperature or the strength of the feed for that month. It is recalled that the estimates of film and macrofauna in the shafts were made on one sample only while those at the surface were made on sample from three positions. The figures for the surface samples (Appendix Tables 1 and 2) reveal that there were occasionally considerable differences between the individual samples showing that horizontal distribution of film and macrofauna was not always uniform. It is therefore probable that the anomalous results for the shafts were not representative of the conditions in the bed as a whole.

Regular observations were made on the microscopic nature of film taken from the surface of each bed to see whether fungus was present. It was found that fungal growths were absent from the surface of Bed A during the first six months of each year but appeared in July or August and continued until December. With rare exceptions fungal growths were absent from Bed D during the whole of the year. It is thus possible that the role of periodicity in controlling film accumulation is concerned with its effect on the growth of fungus. If fungal growths in small quantities are inhibited by a lower frequency of dosing, this might well prevent the occurence of ponding where the fungus is

much more abundant.

While the rates of B.O.D. and P.V. removal are seen to be very similar between the two beds for most of the period of observation it is seen from Fig 4 (c) that Bed D produced a somewhat higher level of nitrification than Bed A. Stanbridge (1956) has suggested that the longer interval between doses is the cause of improved nitrification in beds dosed by controlled frequency distributors. Hawkes (1957), however, states that lower film levels are the most important, and possibly the only, cause of improved nitrification. Hawkes concludes further that ".... until benefits, other that the control of film have been proved for low frequency dosing there is no justification for the slowing down of distributors of filters in which there is no serious accumulation of film; the optimum period for any filter being the shortest period which suppresses the accumulation of film". This appears to apply generally to the current observations. It is seen that during the summer and autumn of 1965, the purification rate in Bed D, both in terms of B.O.D. removal and nitrification, was inferior to that of Bed A. While the effect of low frequency dosing is to control film accumulation in winter, it probably causes in addition a lower retention period and hence reduces the time available for biochemical change. Thus in summer, when the film is controlled by the action of grazers, the

disadvantages may be greater than the advantages although the inferior purification rates of Bed D during the summer of 1965 were not repeated during the following year.

The differences in populations of macroinvertebrates between the two beds, although probably having little direct effect on the efficiency are nevertheless of ecological interest. The most significant difference was the much lower populations of Psychoda larvae in Bed D especially at the surface. This was undoubtedly caused by the mechanical action of the sewage due to a higher instantaneous rate of application. Following the expected ecological pattern, the reduction of Psychoda was compensated for by a corresponding increase in other species. The populations of Enchytraeidae were somewhat greater in Bed D and chironomid larvae were recorded, particularly at the surface, though only in small numbers. Lloyd, Graham and Reynoldson (1940) observed that Chironomidae were particularly successful in withstanding the mechanical action of high sewage flow rates. While in Bed D Enchytraeidae are in the overwhelming majority there appears to be a niche which is occupied by the insect larvae. In Fig. 13, the populations of Psychoda and Chironomidae have been reproduced on a different scale to Fig. 10 to illustrate this competition. The Chironomidae in Bed A, shown in Fig. 13 (a), were virtually absent, indicating that the competitive balance in this bed was overwhelmingly in favour of Psychoda, shown in Fig 9 (b). In Bed D, the

Chironomidae were usually in superior numbers at the surface, but during summer Psychoda was dominant in the lower parts of the bed. However, in winter, the Chironomidae became dominant at all levels. This demonstrated that both the scouring action of the sewage at the surface and the lower temperatures in winter influenced the competition in favour of the Chironomidae.

Lloyd (1945) stated that Psychoda showed two peaks of abundance during the course of the year, in the spring and in the autumn. Something of this pattern was seen in Bed A, where very high populations of larvae occurred at the surface in the late autumn and early winter while in May and June large populations were present towards the middle of the bed. In Bed D the only population peak was in May or June and the numbers involved were much fewer than in Bed A. It has been pointed out (Isaac and James 1964) that the effect of temperature on grazing activity may not be so much on the number of macroinvertebrates as on their metabolism. Thus while Psychoda larvae in Bed A continued to survive in large numbers into December their grazing activity was greatly reduced and it did not prevent the build up of film during this period.

The Enchytraeidae in both beds showed a marked increase in spring, which started in March and reached a peak in May or June.

The rise in the numbers of Enchytraeidae occurred earlier in the year than with the Psychoda which was probably due, partly, to a lower threshold temperature for development and partly to the greater ability of Enchytraeidae to resist conditions of low oxygen tension brought about by the high film levels. The peak populations of Psychoda larvae normally occurred after the worst of the winter accumulation had been removed.

The occurrence of Anisopus for a limited period in Bed A only is of interest. Circumstantial evidence has tended to associate Anisopus mainly with a fungal film (Hawkes 1965 b). The presence of fungus in the film and Anisopus in the grazing fauna only in Bed A is therefore significant. However, the periods of occurrence did not coincide. Fungus was usually present in the film during the last six months of each year. Anisopus was found in significant numbers only during the first eight months of the observations up to May 1965 when it was particularly abundant. After this, the species virtually disappeared from the bed. Little can therefore be deduced from these observations concerning the relationship of Anisopus to a fungal film.

LABORATORY EXPERIMENTAL WORK

Materials and Methods

Description of Apparatus Six experimental filters were set up as two sets of three in two adjacent temperature controlled rooms. The filters were designated L1, L2, L3 in the left-hand room and R1, R2, R3 in the right hand room. Within each room the environmental conditions and operating procedure were constant so that for the purpose of statistical analysis, each set of three filters, L or R, could be considered as operating under identical conditions.

Each experimental filter consisted of a rigid white polythene cylinder of 4 in. internal diameter and 18 in. length with a solid airtight lid, pierced by two holes of 3/8 in. internal diameter, the inlet for sewage and outlet for air respectively, and at the lower end funnelled to a narrow outlet with an additional hole in the cone of the funnel for the entry of the air supply. (See Fig. 14). The cylindrical part of the vessel was separated from the funnelled part by a plastic grating, perforated by holes of 1/4 in. diameter which served to support the medium. The medium consisted of "Allplas" white polythene shperes of 3/4 in. diameter. The sewage outflow was by means of a simple water trap, consisting of two concentric glass tubes, the inner one continuous with the outlet funnel by means of a flexible joint and the outer one closed

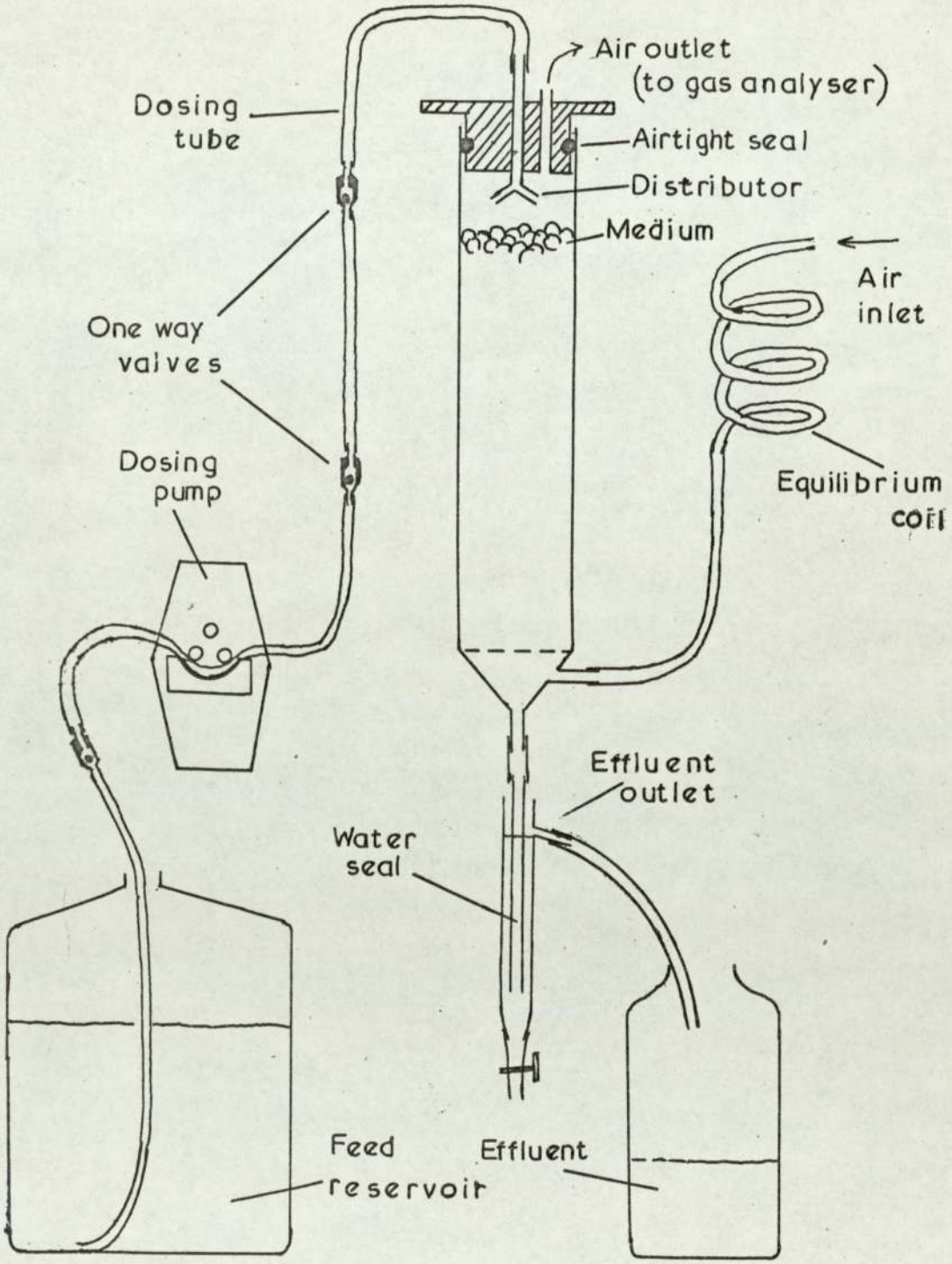


Figure 14. Diagram of single experimental filter system.

off at the lower end by means of a piece of polythene tubing and a screw clip, and with an overflow arm for the exit of the treated sewage. This arrangement formed an airtight seal which allowed for slight variations in air pressure within the filter, and also allowed for the removal of discharged humus.

Sewage was applied to each filter by the intermittent operation of a peristaltic pump which forced the feed through a flexible vinyl dosing tube against a head of about three feet between the surface of the feed reservoir and the top of the cylinder. The pumps used with four of the filters were the Watson-Marlow model MHRK, and with the other two model MHRE was used which gave more accurate control of the rate of dosing. The feed entered the filter via a glass tube which passed through the central hole in the lid making an airtight seal. Within the cylinder the inner end of the glass tube formed a three-arm distributor to give an even application over three points on the surface of the medium (Plate 6). Each dosing tube was fitted with three one-way valves to maintain the head during the intervals between periods of dosing.

Air was supplied continuously to each filter at a constant rate from a compressed air system. The air entered via a tube in the funnelled outlet of the filter, having first passed through a length of copper tubing to bring the temperature into equilibrium

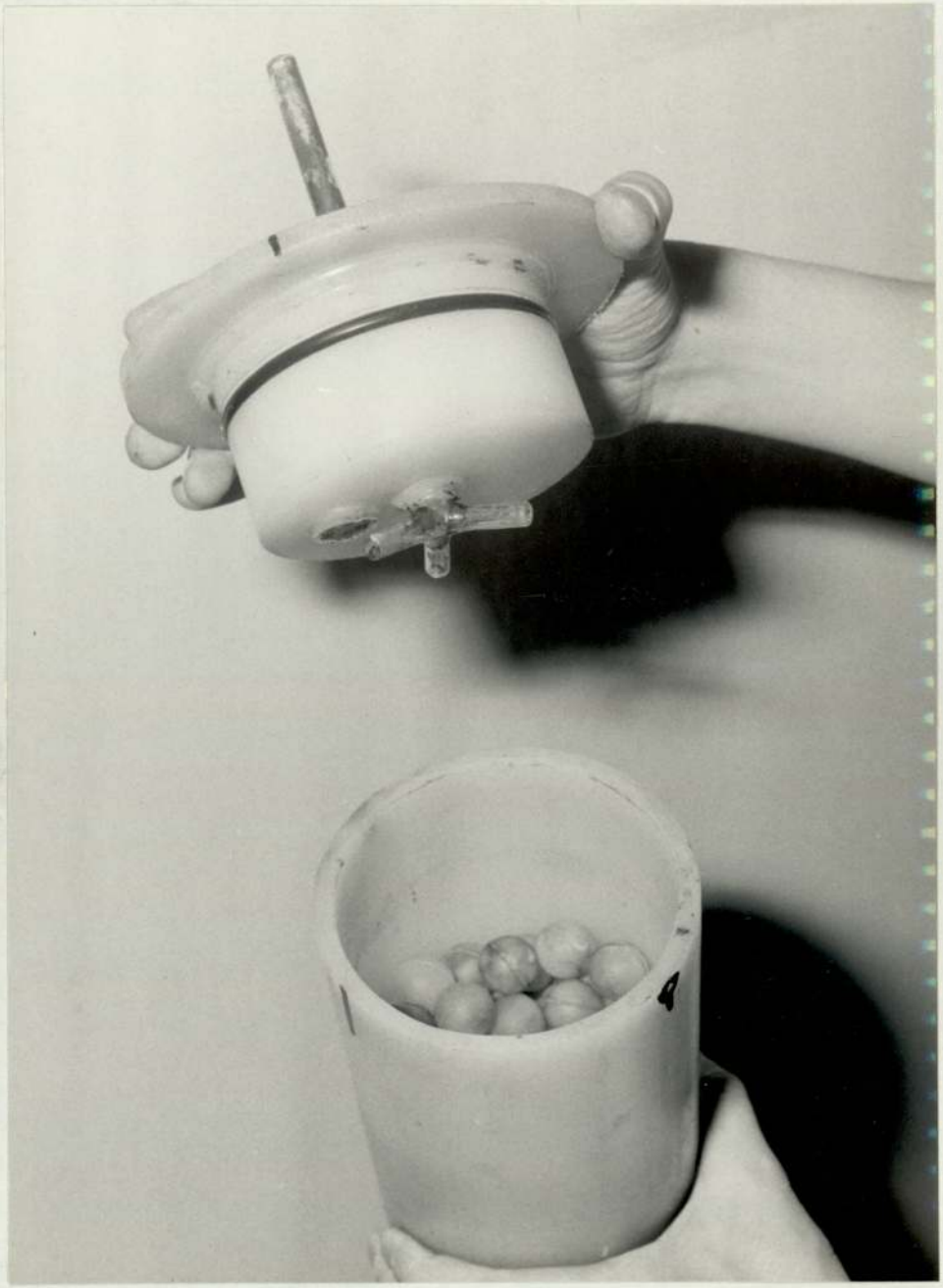


Plate 6. Experimental filter with lid removed showing glass distributor and medium of plastic spheres.

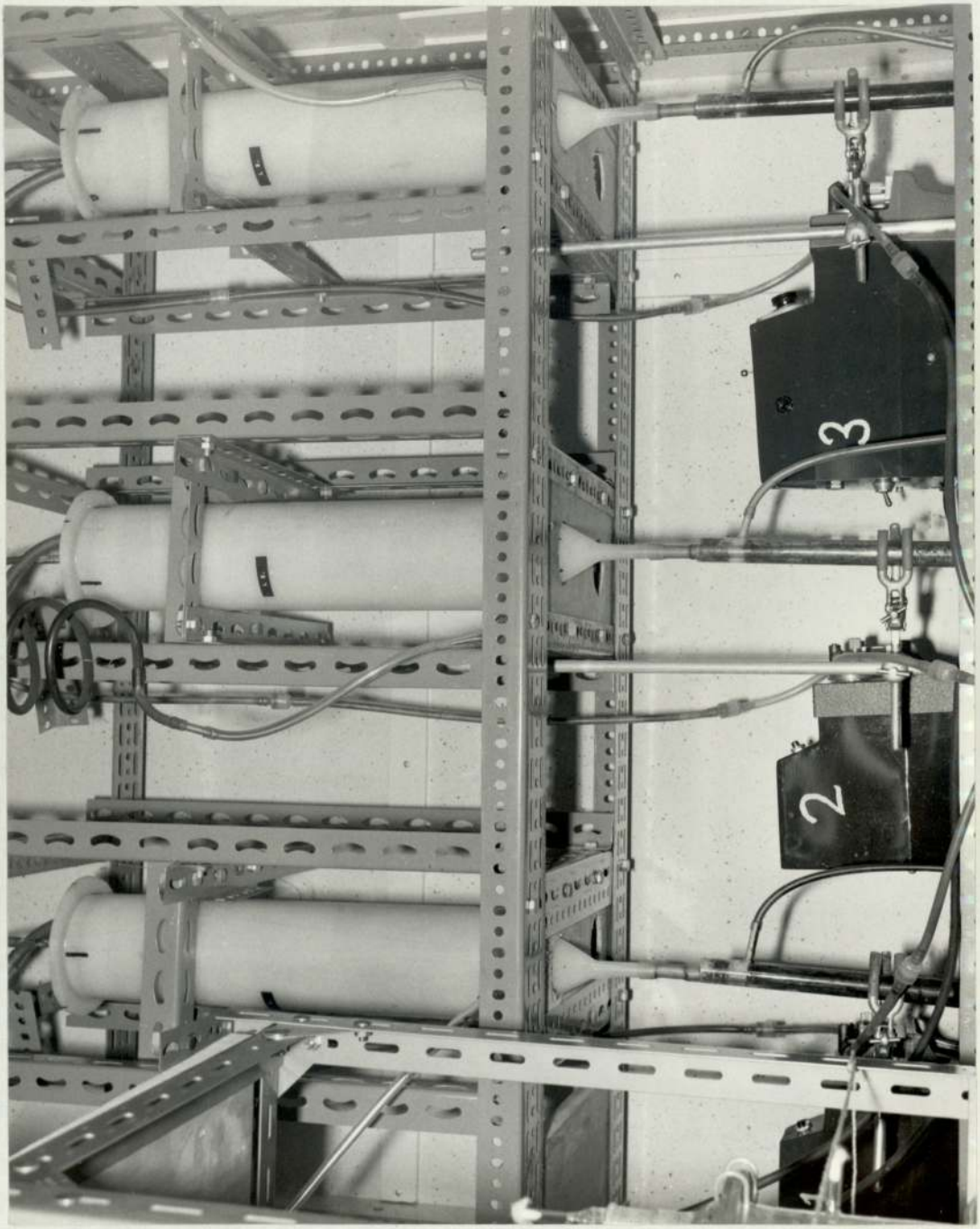


Plate 7. Three experimental filters in position.

with that of the room. The air outlet was via the second, eccentric, hole in the lid. The expelled air was conducted out of the temperature-controlled rooms by means of a considerable length of plastic tubing. Glass water traps were placed in certain positions along the length of this tubing to remove water that tended to accumulate through condensation. After leaving the temperature controlled room, the flow rate of the air was measured by means of a "Gapmeter" rotary flow meter, and could be controlled using a "Hy-flo" needle valve. The design of a single experimental unit is shown diagrammatically in Fig. 14. Three units in position are shown in Plate 7.

Operation of Experimental Filters The feed used was domestic sewage from the Langley Mill works of the Upper Tame Main Drainage Authority. Sewage deliveries were made twice a week and thus, at the time of dosing, the feed might be up to three days old. However self purification was reduced to a minimum by storing the feed at 5°C. Before dosing, the feed was filtered through a fine mesh sieve (0.0071 in. aperture) to remove any particles which might obstruct the dosing system and to prevent possible inoculation with any stages of macroinvertebrate grazers, as it was decided initially to operate the filters without the normal macrofauna population.

The three filters in each temperature controlled room were

dosed from a single vessel of about twenty litres capacity which held sufficient feed for twenty-four hours supply. The effluents were collected individually in graduated vessels and flow rates were checked by the amounts of effluent collected daily. (Plate 8). At weekends one twenty-litre vessel to contain the feed and another to collect the effluent were provided for every filter.

Each filter was operated at a rate of flow, such as to represent a section from the top quarter of a bacteria bed, being operated at 80 g.y.d. The cubic capacity of each filter was 0.1 cu. ft. but since its depth was only a quarter of a normal bed, the absolute rate of the flow was in fact 320 g.y.d. This was equivalent to 5.38 litres per day or 224 cc. per hour for each filter. The dosing pumps were operated from an electric timer on a ten-minute cycle so as to dose for a given time in each ten-minute period. It was discovered that a time of between 80 and 100 seconds in each period would give the required rate of flow. With only two of the pumps, the MHRE models, was there any facility for adjusting the rate of flow by increasing or decreasing the rate of rotation of the roller arms. With the others the rate of flow could only be controlled by altering the time of dosing within each period.

As mentioned previously, the sewage entered the filter through a glass distributor in the lid which had three arms at

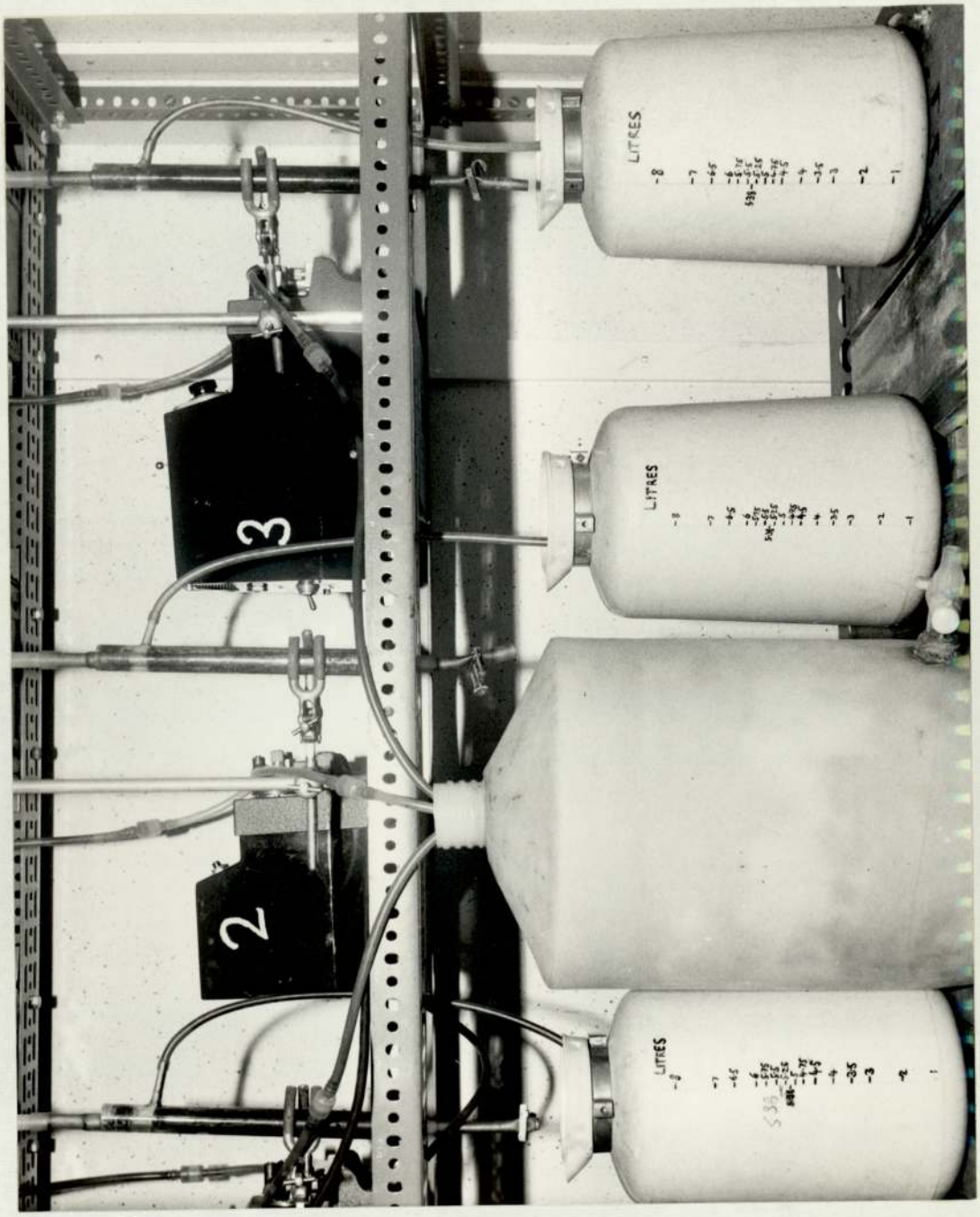


Plate 8. Large container for feed and smaller graduated containers for effluents used in dosing of experimental filters.

120° to each other designed to apply the sewage evenly over three points. Twice each week the lid of each filter, and hence the distributor, was rotated clockwise through 60° so as to dose at three intermediate pointes on the surface of the medium and to compensate for any irregularity of dosing between the three arms.

Air was supplied to each filter at a rate of approximately 1200 cc. per minute from a compressor. Apart from this controlled supply, the system was airtight and there was no other contact with the atmosphere through any joint or fitting.

Methods During the course of the experimental work, observations were made on the following criteria of each filter.

(a) The accumulation of film

(b) The Biochemical Oxygen Demand of feed and effluent

(c) The Permanganate Value of feed and effluent

(d) The carbon dioxide output

(e) The organic carbon content of the feed and effluent

(f) The discharge of humus

(g) The ammonia and oxidized nitrogen levels of feed and effluent.

(h) The microscopic nature of the film.

(a) The accumulation of film At the commencement of the experimental work, each filter, together with the medium of plastic spheres, but without its lid, was weighed by suspending it

from beneath a "Sauter-Toppan" balance which had been specially modified for this purpose. Throughout the course of the experiments, this procedure was repeated weekly and the difference between the observed weight on each occasion and the original weight was recorded as the film accumulation in grams. At the end of the experiments the medium of each filter was removed and washed free from the remaining film. It was then replaced and dried and a final reading for the weight of each system was obtained. These readings showed a small increase on the original weights which was due to the slight permeability of the medium causing condensation of liquid within some of the spheres.

(b) B.O.D. The Biochemical Oxygen Demand is a measure of the strength of a polluting substance based on the capacity of that substance to remove oxygen from the environment over a five day period at 20°C. During the course of the experimental work, the tests were performed according to the recommended procedure. (H.M.S.O. 1956).

Regular estimations were made of the B.O.D. of the feed and effluent from each filter. The sample of feed used for analysis was taken from a tap at the base of the feed reservoir. It was noticed that this often contained fairly large particles in spite of the filtering of the feed supply. After a short time the procedure was adopted of filtering the feed sample through a fine gauze mesh to remove suspended particles in order to avoid

inaccuracy that might be caused by excessive solid matter. Similarly, the effluent could be affected by the presence of humus particles especially when the film was unloading, and effluent samples were filtered through a similar gauze mesh before analysis.

(c) P.V. The Permanganate Value is an estimate of the chemically oxidisable matter present in an effluent and is measured as the amount of oxygen absorbed from acid potassium permanganate in four hours at 27°C.

Regular estimations were made of the P.V. of feed and effluent of all filters again according to the usual procedure (H.M.S.O. 1956). The samples for analysis were obtained in a similar manner to those for B.O.D.

(d) Carbon dioxide output The carbon dioxide output due to the respiratory activity in each filter was measured by means of a Grubb Parsons Infra-Red Gas Analyser (model SB2) (see Plate 9). The principle of this instrument is that infra-red radiation is projected through two similar absorption tubes on to a detector which is sensitive to any difference in absorption of radiation between the two tubes. This signal is passed to an amplifier which records it as a current reading. A continuous stream of atmospheric air was passed through one of the absorption tubes as a "background" or control, while the exhaust gas from the

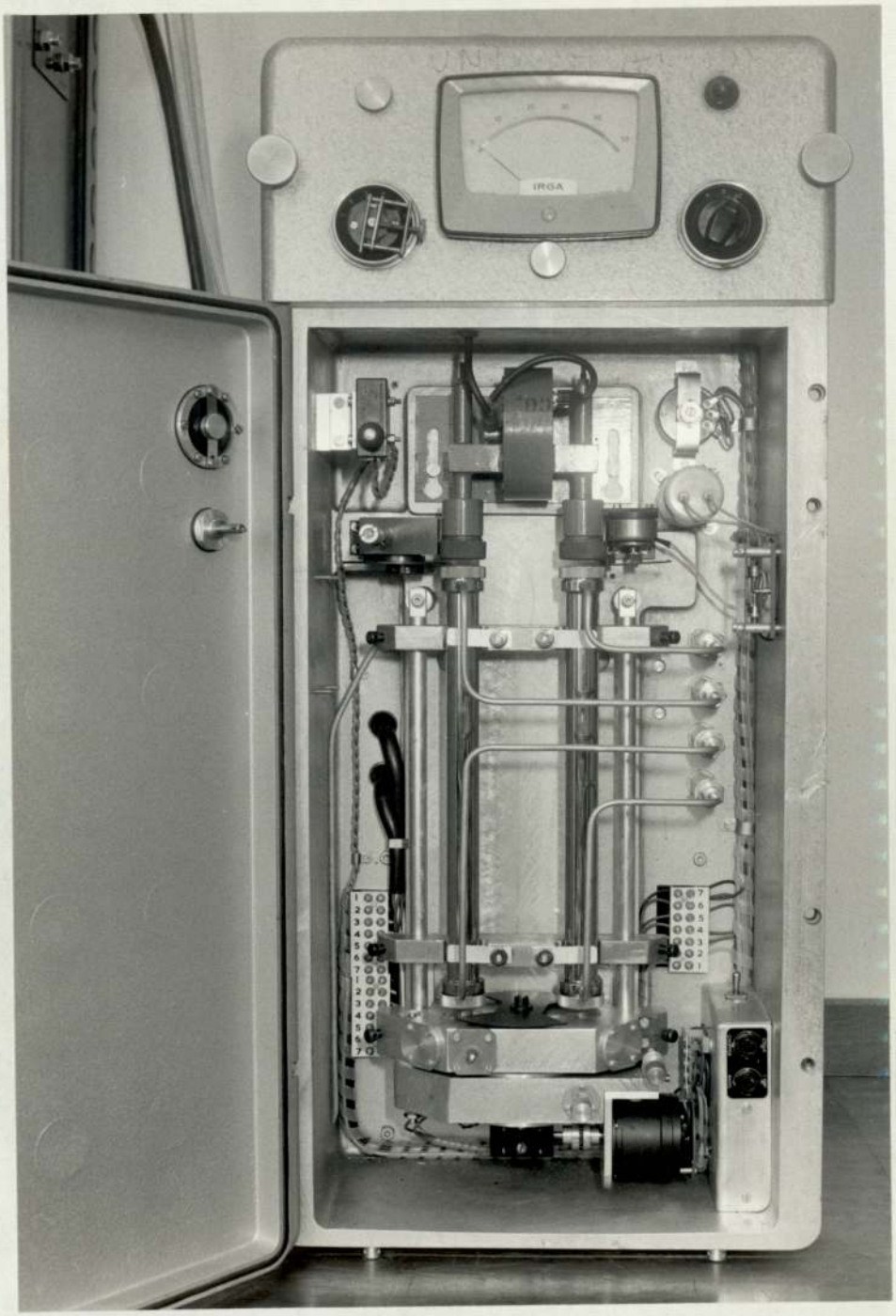


Plate 9. "Grubb Parsons" Infra-Red Gas Analyser.

appropriate filter, consisting of atmospheric air plus carbon dioxide due to respiration, was passed through the other. The instrument had a range of six sensitivities covering concentration differences between the background and sample gases of up to about 0.15% CO2. The concentration of carbon dioxide in the exhaust air naturally depended on the rate at which air was being passed through the filter and this was controlled at about 1200 cc. per minute. A similar flow rate was used for the background air.

The scale reading produced on the meter as a result of the differential absorption was recorded using a "Kent" Chart Recorder so that a continuous reading of carbon dioxide could be made over a period of time. The recorder stamped points on the chart at a frequency that could be adjusted. The frequency selected was one point every 1½ minutes approximately, so that about six points were made during each ten minute dosing period. The Gas Analyser, chart recorded and channel selector are shown in position in Plate 10.

The gas analysis system had provision for recording the output of three filters simultaneously and thus the points on the recorded chart are numbered 1, 2 and 3. In practice, however, it was found convenient to record only one filter at a time and in any particular series the different numbers refer just to the one filter that was under observation at that time. An example of a chart recording is shown in Fig.15. The concentration of carbon

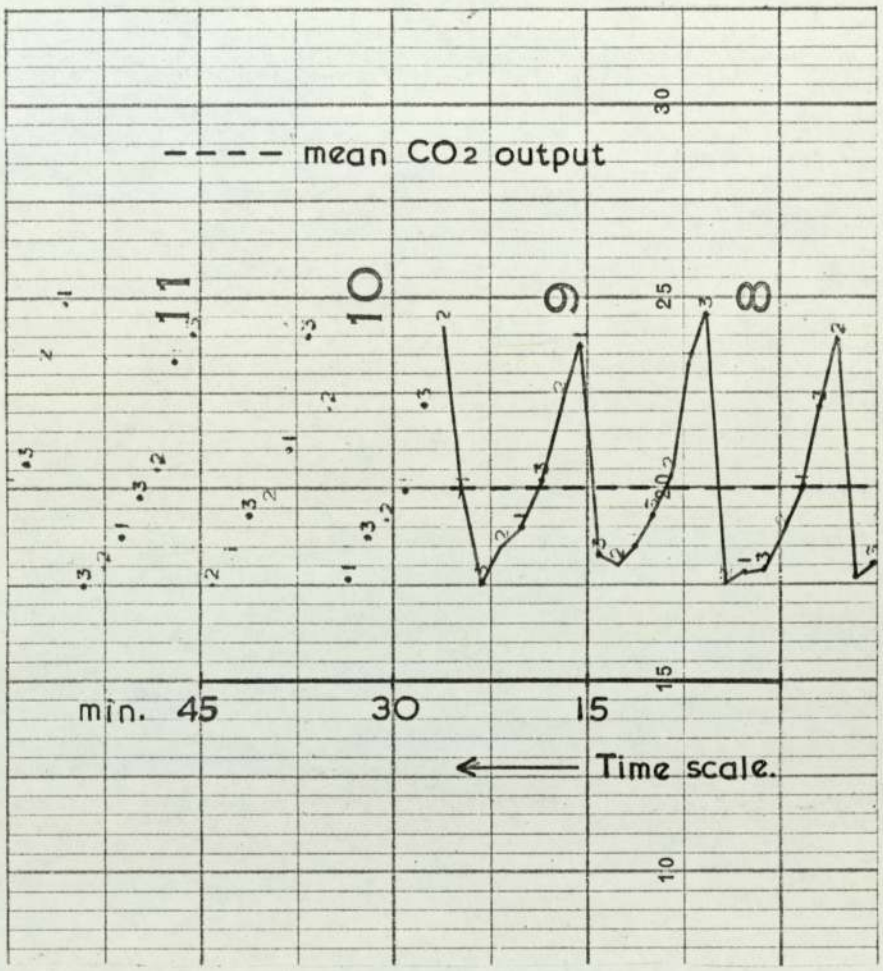


Figure 15. Pattern of carbon dioxide output from experimental filters, showing the average of the fluctuations which was taken as the measurement of respiration. (Example is taken from filter R2 at 20°C, showing average output of 54.21 mg/hr).

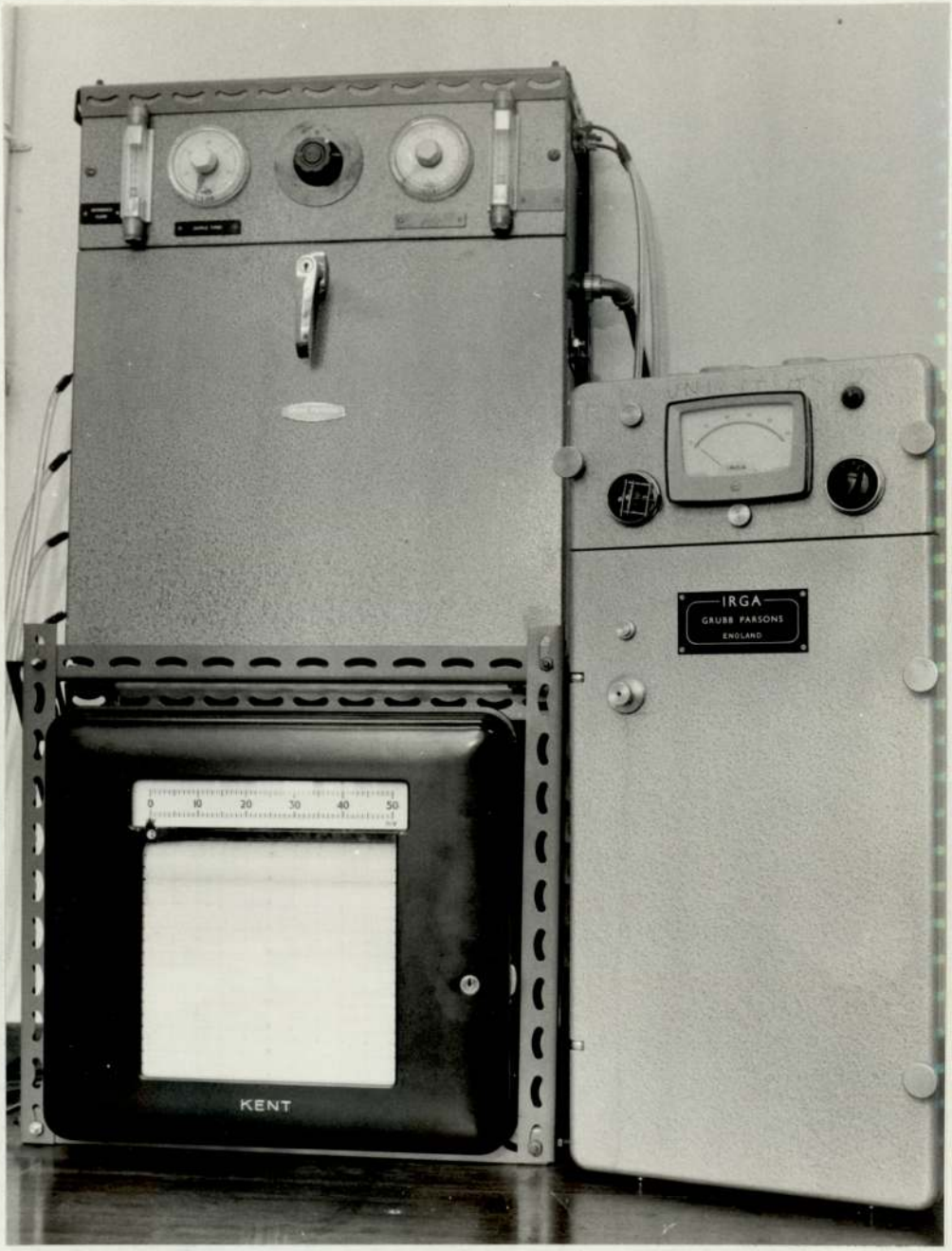


Plate 10. Infra-Red Gas Analyser (right) with channel selector (above) and "Kent" chart recorder (below).

dioxide is seen to fluctuate in regular cycles and the reading was made by taking a line representing the average of these fluctuations. The justification for using this level as the measurement of respiration is considered in the discussion.

The Gas Analyser was calibrated by passing atmospheric air through the background absorption tube and a mixture of air and 5% CO2 in nitrogen at a series of controlled dilutions through the sample tube, and obtaining a calibration curve for each sensitivity range that was used.

The average scale reading obtained during a period of analysis, was converted into a measurement of carbon dioxide concentration from the calibration curve and this in turn was converted into a reading of mg. CO2 produced per hour in which the results are expressed.

(e) Organic Carbon For a limited period during the experimental work estimates were made of the organic carbon content of the feed and effluents of the filters. The organic carbon value was determined by subtracting the inorganic carbon content, representing carbonates, bicarbonates and dissolved CO2, from the total carbon content. The method of measuring total carbon was based on the recommended test, (H.M.S.O. 1956), but with modifications developed by the Upper Tame Main Drainage Authority (Jenkins, Snaddon et al. 1965).

The apparatus consisted of a reaction flask, two absorption columns,one containing copper oxide and lead chromate and the other reduced copper, an indicator tube containing bromothymol blue and a gas absorption flask, which were all in closed circuit with a sealed pump (Charles Austen DY Mk 11A) (Plate 11). The column containing lead chromate and copper oxide had a heating coil wound round the outside adjacent to the layer of copper oxide which enabled this part of the column to be heated to about 700°C. The apparatus was first cleared of carbon dioxide by introducing a soda-lime column into the system and recirculating the air for a few minutes. The sample to be analysed was introduced into the reaction flask together with solutions of concentrated sulphuric acid and saturated chromic acid and heated to 150°C. It was maintained at this temperature for about ten minutes and then cooled to below 100°C. An excess solution of barium hydroxide was then introduced into the gas absorption flask and the gas in the system was circulated until all the carbon dioxide had been absorbed as barium carbonate. The remaining barium hydroxide was then titrated against a standard solution of hydrochloric acid, the difference between that reading and the control representing the quantity of hydroxide converted to carbonate and hence the total carbon.

The inorganic carbon content was estimated using the same apparatus, by treating a similar sample of the feed or effluent

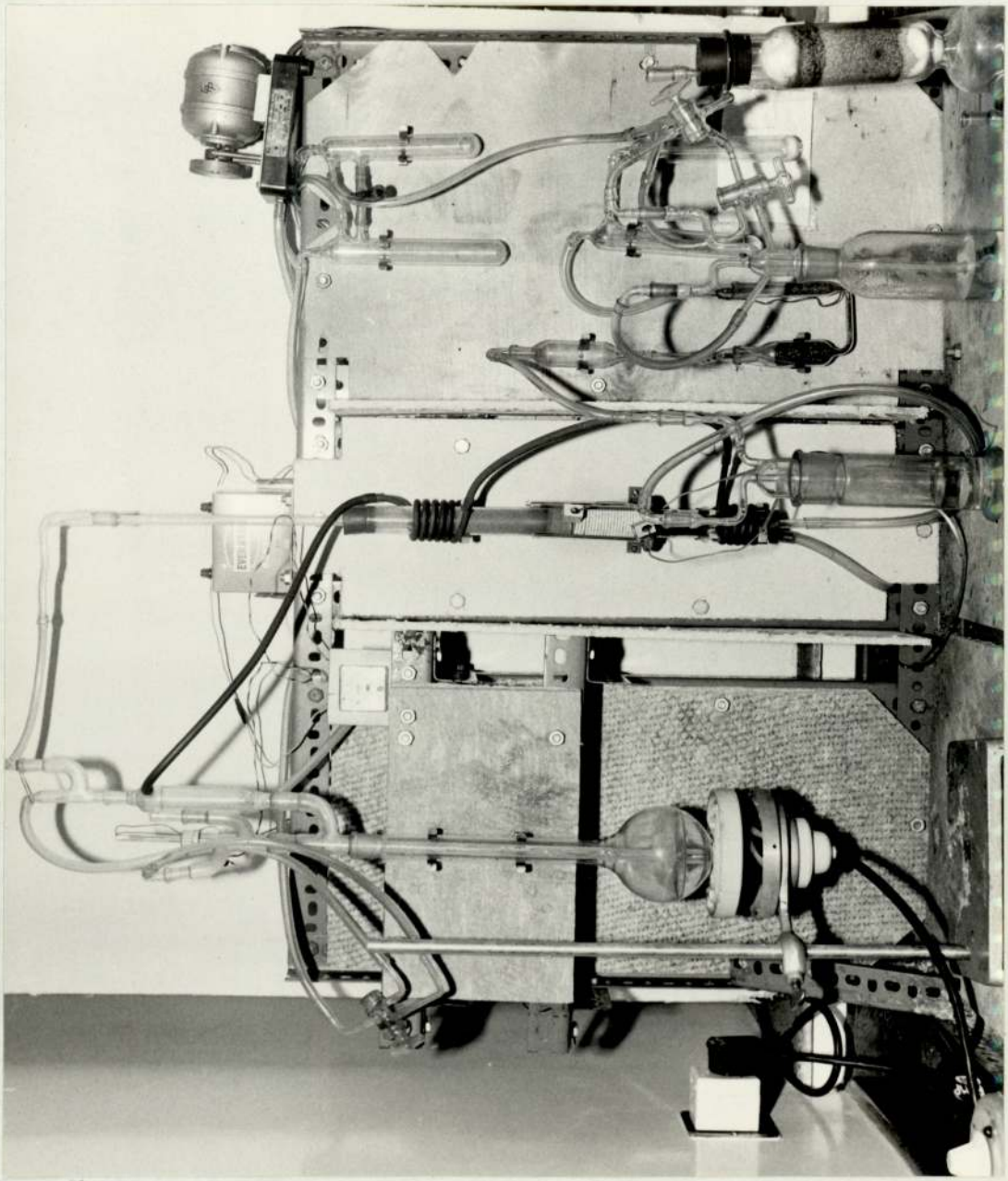


Plate 11. Apparatus for the analysis of organic carbon.

with dilute hydrochloric acid and absorbing the displaced carbon dioxide in barium hydroxide.

(f) Humus Discharged humus which accumulated in a glass chamber at the outlet of the filter was normally collected at weekly intervals in a previously weighed silica dish. The humus was dried, first on a water bath and then in an oven at just over 100°C until a steady weight was achieved. The results were recorded as grams dry weight per week.

(g) Ammonia and Oxidized Nitrogen Occasional estimations of the ammonia and oxidized nitrogen (nitrite and nitrate) present in the feed and effluents were made, but this was not a regular analytical procedure.

The method used was to distil a known volume of the sample with a small quantity of magnesium oxide powder into a solution of boric acid indicator. The boric acid indicator was then titrated against a standard solution of dilute sulphuric acid. This gave an estimate of the nitrogen present as free ammonia in the sample. A small quantity of Devarda's alloy was then added to the same sample and the volume made up with distilled water. The distillation was then repeated with a fresh quantity of boric acid indicator. The alloy acted as a reducing agent and converted oxidized nitrogen to ammonia which was estimated by titration in the same way as the free ammonia.

A blank estimation was made in each case to correct for small quantities of nitrogen in the reagents. The results are expressed as parts per million nitrogen as ammonia and as oxidized nitrogen.

(h) Microscopic nature of the film Occasional observations were made on samples of film removed from the medium of each filter at the surface. The samples were examined microscopically and rough estimates of the nature and abundance of the organisms present were recorded.

Experimental Programme

The filters were operated at the conditions described from 27th October, 1965 to 21st March, 1967. Although the operation was continuous, the results are considered as two experiments. The first, Experiment A, was from the beginning of the work to 24th August, 1966, during which macroinvertebrate grazers were excluded from all filters, and the second, Experiment B, from 24th August, 1966, when a grazing population of flies of the genus Psychoda was introduced into all the filters, until 21st March, 1967, when the experiments were concluded.

The conditions in the filters in terms of the temperature and the presence or absence of grazers is summarized in Table 1.

		Room L		Room R	
	Period	Temp.	Grazers	Temp.	Grazers
Expt. A.	14 weeks	20°C	Absent	20°C	Absent
	15 weeks	20°C	Absent	5°C	Absent
	14 weeks	20°C	Absent	20°C	Absent
Expt. B.	10 weeks	20°C	Present	20°C	Present
	14 weeks	20°C	Present	5°C	Present
	6 weeks	20°C	Present	20°C	Present

Table 1.

Experiment A. Flies absent from all filters

The purpose of this experiment was to observe the effect of temperature on the film accumulation, efficiency and respiration of the filters in the absence of macroinvertebrates. From 27th October, 1965, when the experiment was started to 1st Feb. 1966, the temperatures of the two thermostatically-controlled rooms L and R were maintained equal at 20°C. During the week ending 4th February the temperature of room R was lowered by 5°C daily during three consecutive days to 5°C, while room L remained at 20°C. These temperatures were maintained until 17th May, 1966. During the week ending 20th May, the temperature of room R was returned to 20°C in three daily increases of 5°C and the temperatures were maintained equal until the end of the experiment on 24th August.

The only instances when there were deviations from these

conditions were during occasional failures of the thermostatic control in room R. These occurred twice during Experiment A, on 11/12/65 and 1/8/66. On both occasions, room R was being maintained at 20°C but, during the failure of the control, the temperature rose to about 33°C. This occurred for about six hours on the first occasion and eight hours on the second.

The only other mishaps were two instances when the discharge of film from a filter caused blocking of the outlet and subsequent build-up of effluent within the filter. This occurred on 8/3/66 in L3 when the build up of the liquid overcame the pressure of the air supply and caused a suck-back of effluent, which cut off the ventilation to the filter. The second occurrence was on 26/6/66 when R2 became blocked. In this case there was no suck-back into the air supply system and air continued to bubble through the accumulated effluent.

During the course of the experimental work all six filters were dosed with a similar sewage and at similar conditions of loading. A sharp fall in the rate of dosing indicated that the tubing in the peristaltic pump needed replacement, or that there was some obstruction along the length of the dosing tube. The tubing normally needed to be replaced about once every six weeks.

During late June and early July 1966 chironomid flies were observed on a number of occasions in the air outlet tubes of

several of the filters. It was therefore suspected that accidental inoculation with Chironomidae had taken place and all the filters were treated with insecticide from an aerosol spray. The possibility that this might have a detrimental effect on the activity of the film was not overlooked, but there appeared to be no serious consequences. Fortunately, no populations of macro-grazers became established and the flies observed in the air outlets were either isolated cases of larvae or pupae being introduced with the feed, or more probably, adult flies entering the air outlet from the outside.

During the first few weeks of Experiment A, the B.O.D. of the feed in each room and the corresponding effluents from all six filters were estimated simultaneously twice a week. Although separate measurements of the feed B.O.D. in each room were made, the results were usually found to be very similar, since both feeds were derived from the same sewage, the only difference arising from different rates of self-purification when the two rooms were being operated at different temperatures.

Later, the procedure for B.O.D. estimation was changed so that, instead of analysing the effluents from all six filters simultaneously, the effluents were analysed in pairs on three consecutive days each week, L1 and R1 on the Wednesday, L2 and R2 on the Thursday, and L3 and R3 on the Friday. With each pair of effluents

a single analysis of the feed was made from a sample that had been previously mixed and dosed to each of the respective filters under observation.

Measurements of the P.V. of the feed and effluents of the filters were made twice each week during the early part of the experiment but this was later reduced to once.

The carbon dioxide output from the filters was measured on the same pattern as the B.O.D. estimations. The filters in the two rooms were considered as three pairs, L1 and R1, L2 and R2, L3 and R3, and their respiration rates were measured on three consecutive days respectively. Thus for each measurement of B.O.D. removal an estimate of the simultaneous respiratory rate from the corresponding filter was available.

For a limited period during Experiment A estimates were made of the organic carbon of the feed and effluents. However, this method was found to be rather laborious and time consuming, and it did not appear to contribute any more to the measurement of the purification rates than was given by B.O.D. and P.V. removal. It was therefore abandoned as a regular analytical procedure. During the period when this method was in use, analyses were made in pairs on three consecutive days, corresponding to the B.O.D. and carbon dioxide measurements.

The humus discharged from each filter was removed weekly for

estimation. Occasionally, during a period of heavy unloading, discharged humus would accumulate to such a degree as to risk the blocking of the filter outlet. In this case, humus was removed more frequently and the results added together to give a single weekly reading.

Analyses of the ammonia and oxidised nitrogen concentrations of the feed and effluents were made on about six occasions during the course of Experiment A. Previous research (Hawkes 1957) has shown that nitrifying bacteria cannot compete successfully with the more vigourously growing heterotrophic bacteria and so there is normally little nitrification in the upper section of a bacteria bed. These tests were therefore not used as a regular analytical method.

The microscopic nature of the film from the surface of each filter was examined on four occasions, once during the initial period when the temperatures of the two rooms were equal, twice while the temperature of room R was being maintained lower than that of room L, and once when the original temperatures had been restored.

Results. Experiment A.

The pattern of film accumulation in the three filters of room L is shown in Fig. 16 and those of room R in Fig. 17. The averages of the film levels in each room are compared in Fig. 18(a) These levels were measured as grams wet weight and naturally a considerable proportion of this weight was accounted for by

FIGURE 16.

Film levels in room L (gm. wet weight). Experiment A (flies absent)

Figure 16.

FIGURE 17.

Film levels in room R (gm wet weight). Experiment A (flies absent)

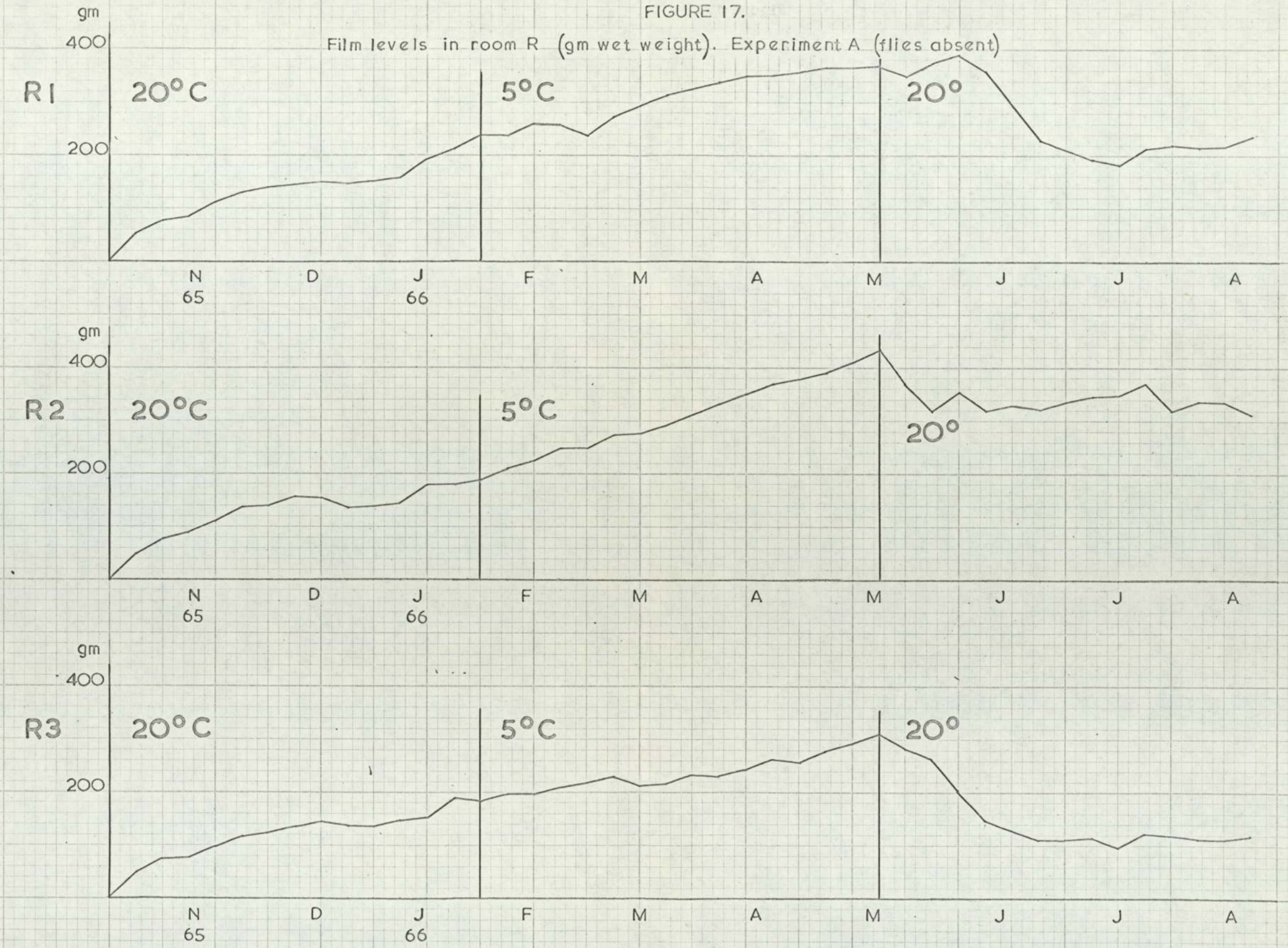


Figure 17.

liquid retained in the interstitial spaces of the medium rather than living material. The effect of this on the validity of these results as estimates of the film present is considered in the discussion.

During the initial period when the temperatures of the two rooms were equal at 20°C, the film levels showed a steady increase and there was a close similarity both between the individual levels of each set of filters and between the two averages. During this period and during the experiment as a whole, the film tended to show a faster rate of growth when the strength of the sewage was high but this effect was often masked by other factors. At the time when the temperature of room R was changed the film had reached an average of about 200 gm. per filter. With the temperature of room R at 5°C the increase in film in the filters of that room continued, but there were greater discrepancies between the individual filters, R3 tending to fall considerably behind the others. By the end of the period at 5°C, the film levels in room R had reached an average of about 400 gm. In room L during this period, the film levels tended to show a cycle of fairly rapid increase followed by a period of sloughing, when the film broke up and was discharged. These fluctuations were not necessarily in phase, and therefore at any one time, there might be a considerable difference between the quantities of film in the individual filters. The film level

in L2 tended to be consistently lower than the other filters. When the temperature of room R was raised again to 20°C, the film levels in that room tended to decrease, but only in R3 was there a complete sloughing of the film to a level of about 100 gm. R1 showed a partial decrease to about 200 gm. while R2 retained most of the film that had accumulated during the cold period and remained above 300 gm. for the whole experiment. For the final six or eight weeks of Experiment A, the film in each filter of room R remained relatively steady at widely differing levels. During the final period of the experiment the film in the filters of room L continued to fluctuate broadly as in the previous period.

The occasional mishaps previously described, such as the failure of the temperature control and the blocking of the filter outlet, sometimes caused the film levels of the filters involved to show a sharp fall during that week. There was usually, however, an equally rapid recovery during the subsequent week and no permanent damage to the film resulted. Such a fall, and subsequent recovery, is seen in L3 in March 1966 when blocking of the outlet caused a build up of sewage within the filter.

The amounts of film present in the filters of each room during the course of the experiment were compared statistically by means of the t-test to determine whether significant differences were achieved. A separate test was made for each of the three periods of the experiment, the first when the temperatures of both rooms

were equal at 20°C, the next when the temperature of room R was at 5°C, and finally when the temperatures were again equal at 20°C. Each weekly measurement of the film in each filter was regarded as a single observation and a value of t was obtained from the formula,

$$t = \frac{\bar{L} - \bar{R}}{S\sqrt{\frac{1}{n_l} + \frac{1}{n_r}}}$$

where $\bar{L}$ = average film level of room L

$\bar{R}$ = average film level of room R

n_l, n_r = number of observations from rooms L and R respectively.

and $$S = \sqrt{\frac{\sum (L-\bar{L})^2 + \sum (R-\bar{R})^2}{n_l + n_r - 2}}$$

The value of t for each period was compared with the critical value of t indicating a probability of 0.05 for the appropriate number of degrees of freedom, obtained from the relevant t table. A value of t greater than this figure, indicating a probability of less than 0.05 was regarded as significant and a value less than this, indicating a probability of more than 0.05 was considered not significant. The values of t obtained during the three periods are shown in Table 2.

It was found that during the initial period with both rooms at 20°C there was no significant difference between the film levels in each room. During the period when room R was at 5°C the film levels in that room were significantly greater than those in room L

and these differences continued into the final period, when room R was returned to 20°C, to give a significant difference during this period also.

Period	1	2	3
Temp.	L = 20°C R = 20°C	L = 20°C R = 5°C	L = 20°C R = 20°C
$\bar{L}$	136.95	195.0	183.5
$\bar{R}$	134.28	289.6	249.0
n_l, n_r	42	45	42
t	0.263	7.642	3.736
Critical t for 0.05 P	1.99	1.99	1.99
Difference	Not sig.	Sig.	Sig.

Table 2

The figures for the BOD of the feed and effluents during Experiment A are given in Appendix Table 3. The average BOD of the feed is shown in Fig. 18(b) and the average percentage BOD removals in each room in Fig. 19(a). In the BOD analyses, where the sewage strength is calculated from the amount of oxygen decrease between two diluted samples over a five day period, there were occasions when the second sample was exhausted of oxygen due to the strength of the sample being greater than had been allowed for in the dilution factor. In these cases it was only possible to state that the strength of the sample was greater than a minimum

FIGURE 18. Experiment A.

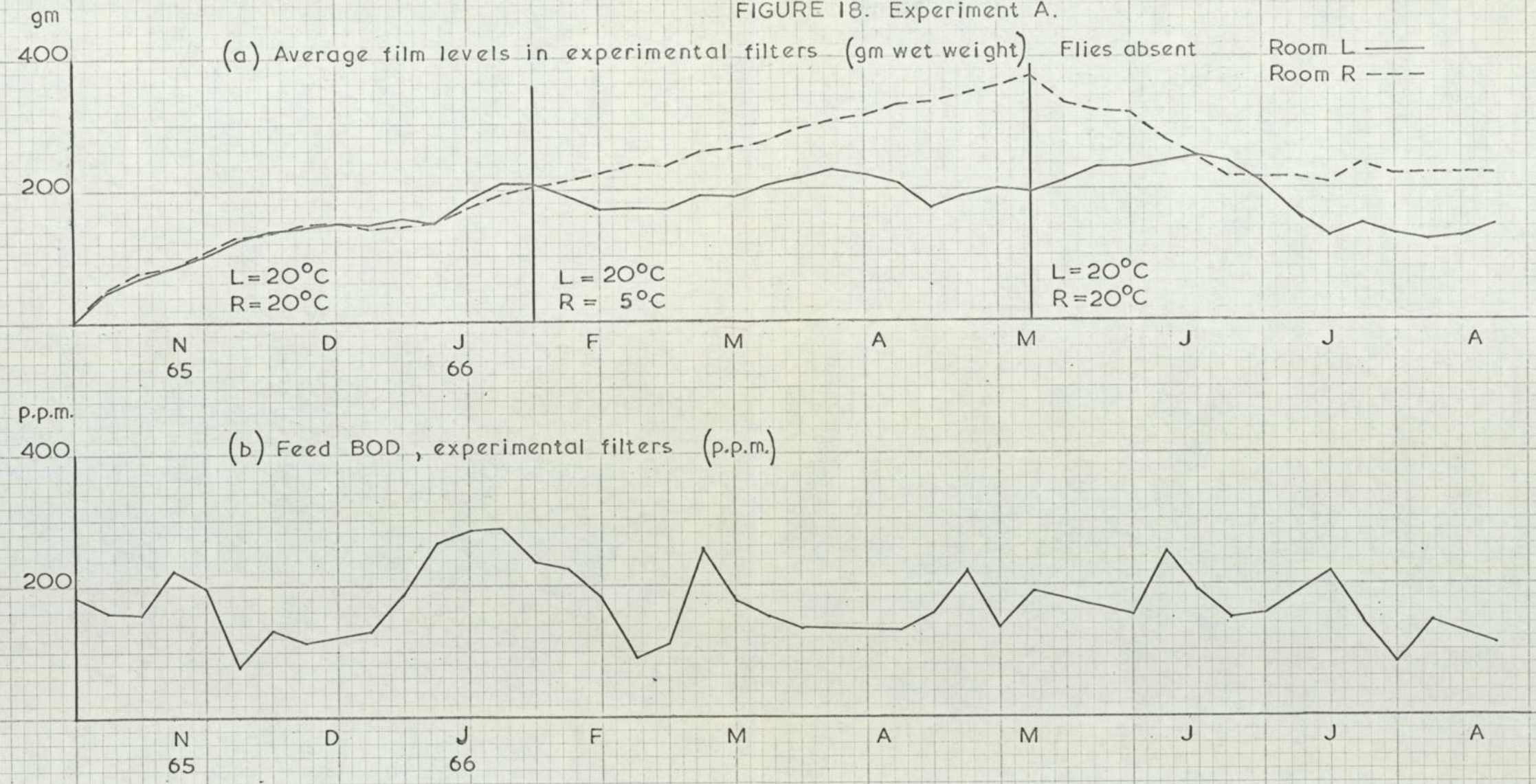


Figure 18.

FIGURE 19. Experiment A.

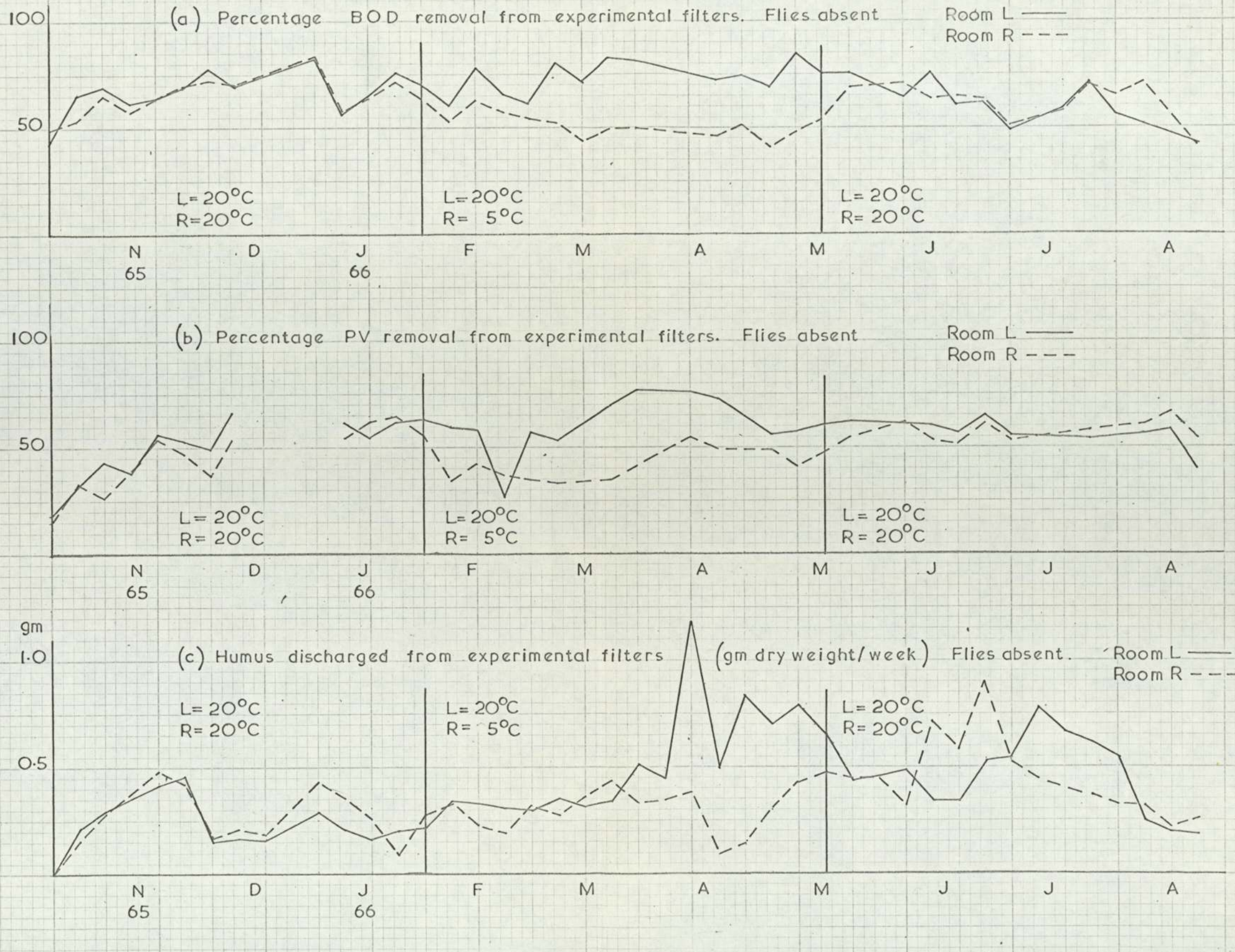


Figure 19.

value and this is indicated by the symbol $>$. However, to preserve the continuity of the graphs, this minimum value was included in the averages of BOD strength and therefore allowance must be made for slight inaccuracy. Similarly, these values have been used in the calculation of percentage removals and there will therefore be slight inaccuracies here also.

Where there was serious cause to doubt the accuracy or validity of the BOD of a feed or effluent, the figures in the table are bracketed indicating that they have not been used in the calculation of percentage removal.

Fig. 19(a) shows that during the periods of equal temperature, the average efficiencies of the filters in each room showed a close similarity and was normally in the region of 75% BOD removal. When the temperature of room R was lowered to 5°C, the efficiency of the filters dropped immediately to around 50% while in room L it was unaltered. On returning to 20°C the filters in room R rapidly recovered their original efficiency, although wide differences in the average film levels between the rooms persisted for some time. Some measurements of efficiency during this final period were rather low and these were associated with periods of sloughing, which may have caused particles of humus to be present in the effluent sample in spite of previous filtering. This would cause a high effluent BOD and consequent low efficiency. Readings tended in particular to be artificially low when a period of sloughing

coincided with an exceptionally weak feed. Apart from this effect, the efficiency of BOD removal was largely independent of the strength of the applied sewage.

The measurements of the Permanganate Value are given in Appendix Table 4 and the average percentage removal is shown in Fig. 19(b). Figures in the table marked with an asterisk show where a filter in room R was, at the time of sampling, being dosed with feed from room L and the percentage PV removal of that filter is calculated accordingly. The percentage removal of PV tended to be slightly lower than that of BOD but showed a similar pattern of variation. With the drop in temperature in room R, the percentage PV removal in that room was noticeably lower than in room L except on one occasion. As with BOD removal, artificially low figures tended to occur when an exceptionally weak feed coincided with a period of breakdown of the film.

The carbon dioxide output from the filters is given in Appendix Table 5 and the weekly averages from each room expressed as a graph in Fig. 18(c). The filters in room R showed an expected drop in CO_2 output during the period at $5^{\circ}C$. On returning to $20^{\circ}C$ the carbon dioxide output from these filters remained somewhat lower than those of room L for a few weeks but a full recovery eventually took place. A comparison of Fig. 18(c), showing CO_2 output, with Figs. 18(a) and (b), showing the average film levels and the feed BOD respectively, indicates that there was a superficial relationship

between these three criteria. Apart from temperature, the quantity of film and the strength of the applied sewage were also factors involved in determining the amount of respiratory activity. The nature of this relationship was considered in a series of regression graphs shown in Figs 20 and 21. The figures from which the points of these graphs were calculated are given in Appendix Table 6. With these figures, observations for which an exact measurement of BOD removal was not available have been omitted. For each graph the best straight line was calculated from the regression equation,

$$y = (\bar{y} - b\bar{x}) + bx$$

where $(\bar{y} - b\bar{x})$ is the intersect with the y axis and b is the regression coefficient or slope.

$$b = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sum(x - \bar{x})^2}$$

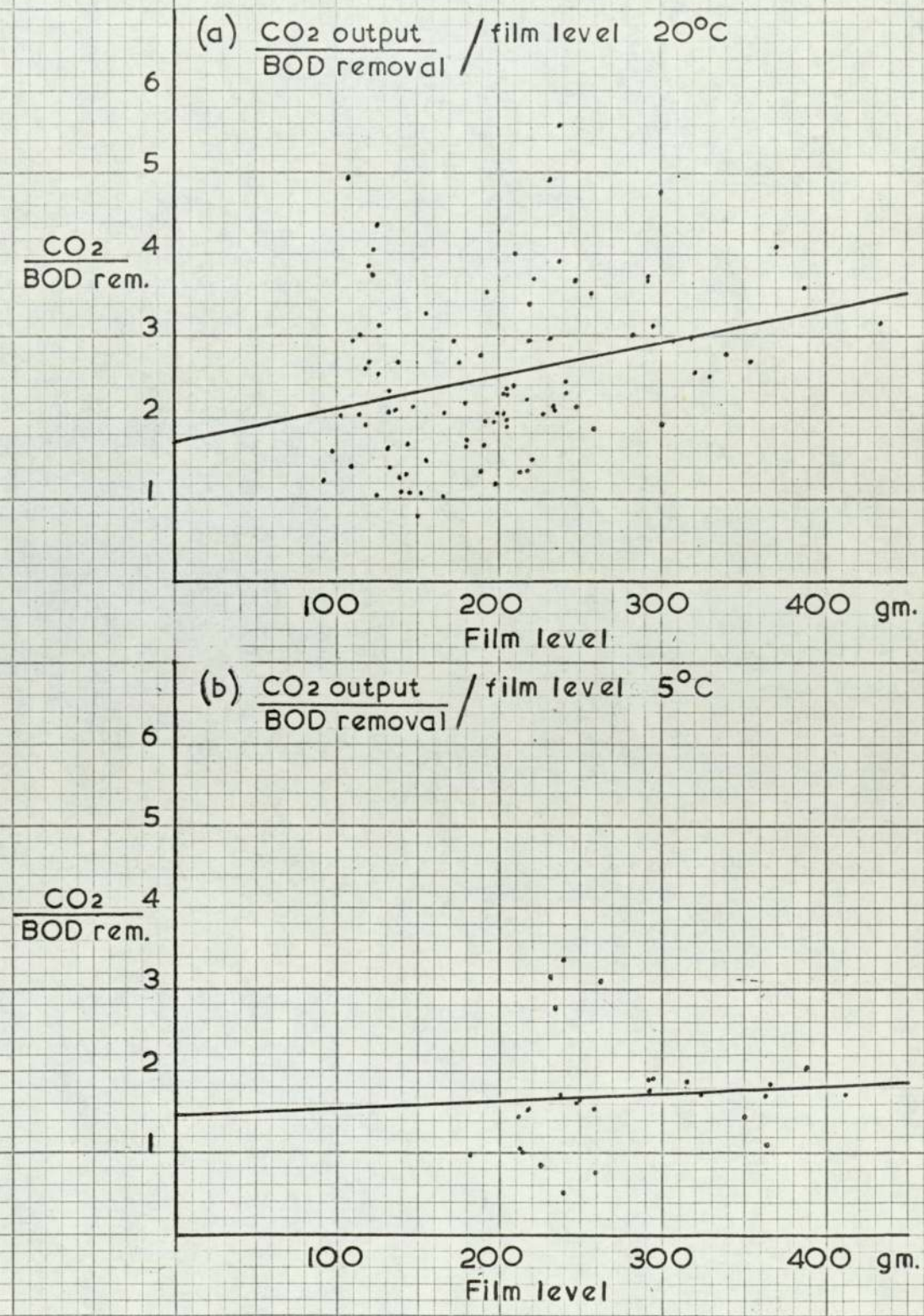
The correlation coefficient, r, indicating the linear significance of each graph was calculated from the formula,

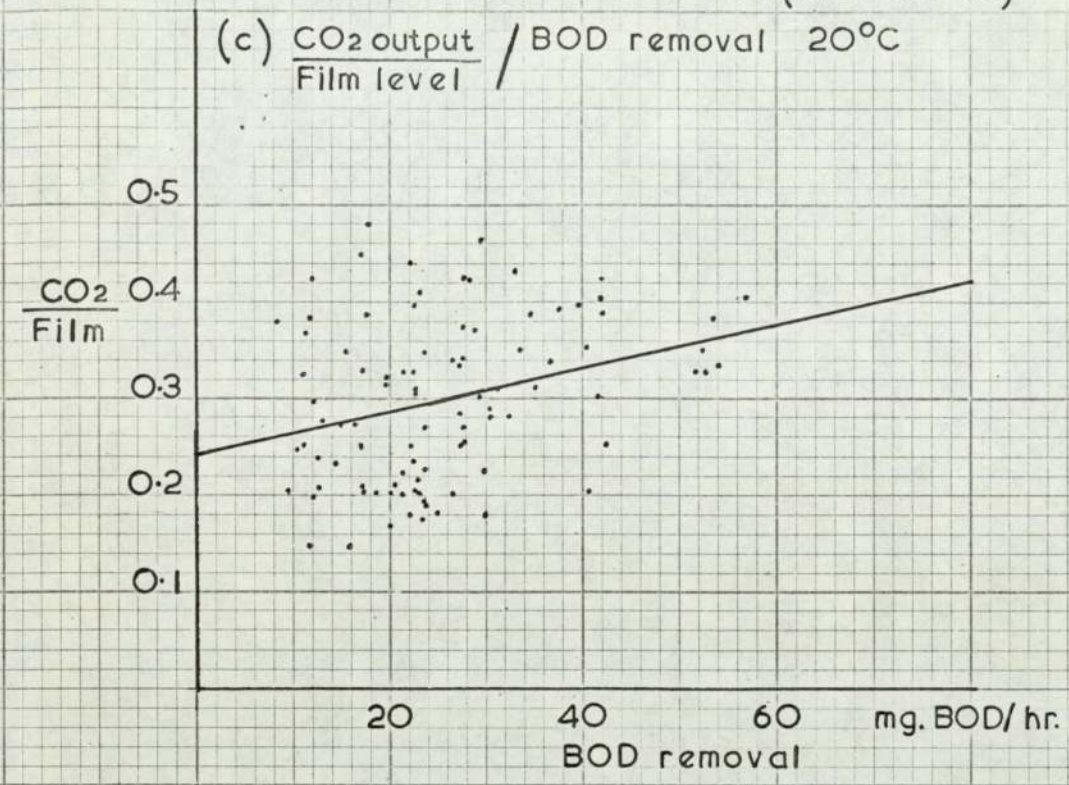
$$r = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sqrt{\sum(x - \bar{x})^2 \sum(y - \bar{y})^2}}$$

The correlation coefficient was regarded as significant or not significant, according to whether it indicated a probability of less or more than 0.05 respectively.

In Figs. 20(a) and (b)a factor given by the carbon dioxide output in mg. per hour divided by the BOD remaval measured as mg. BOD removed per hour was plotted against the film level as gm. wet

Comparison of CO_2 output (mg/hr), BOD removal (mg BOD/hr), and film levels (gm wet weight). Experiment A (flies absent)





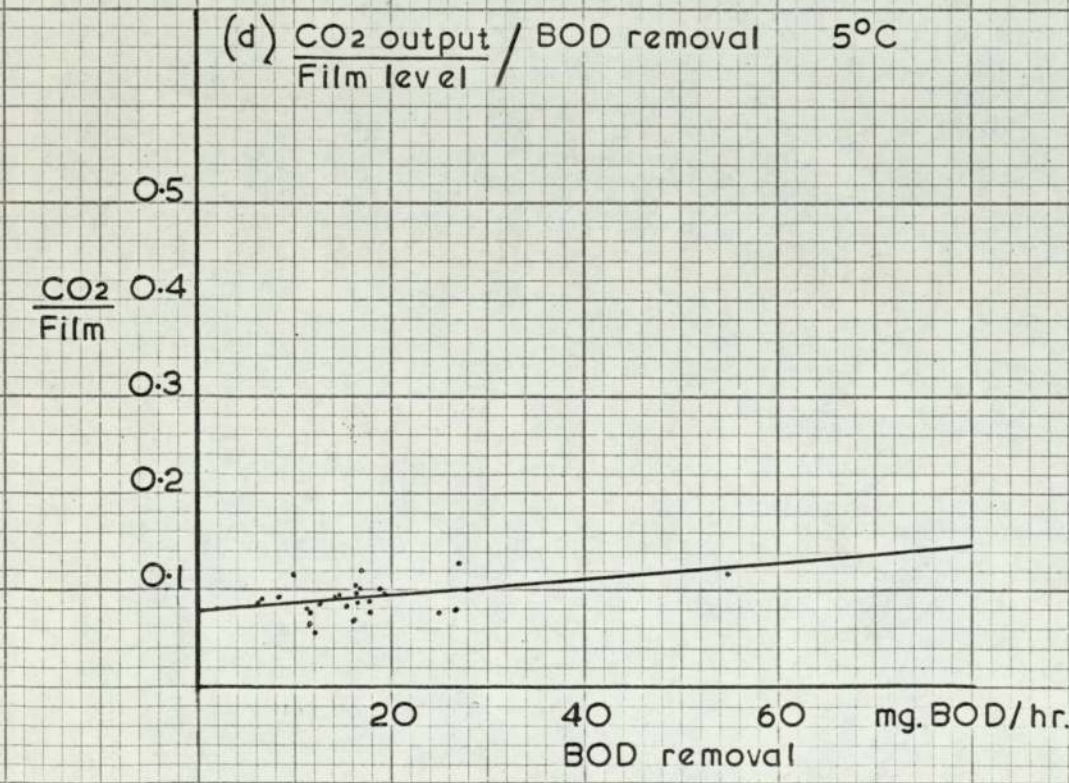


FIGURE 20.

Figure 20.

weight, at the two temperatures of 20°C and 5°C.

The regression equation of Fig. 20(a) was calculated as,

$$y = 1.6934 + 0.004053x$$

and $r = 0.3187$ ($P < 0.05$ - significant)

The critical value of r for a probability of 0.05 and 90 degrees of freedom (°F) is 0.2050.

The regression equation of Fig. 20(b) was given by,

$$y = 1.4618 + 0.000917x$$

and $r = 0.08025$ ($P > 0.05$ - not significant)

The critical value of r for 0.05P and 25°F is 0.3809

In Figs. 20(c) and (d), the respiration rate per unit weight of film in mg. CO2/gm wet weight/hour has been plotted against the rate of BOD removal as mg. BOD removed/hour at the two temperatures.

The equation representing Fig 20(c) was,

$$y = 0.2412 + 0.00219x$$

and $r = 0.2944$ ($P < 0.05$ - significant)

and that representing Fig. 20(d) was given by,

$$y = 0.0786 + 0.00072x$$

and $r = 0.3885$ ($P < 0.05$ - significant)

It was found from these graphs that there was a clear relationship between the carbon dioxide output and the factors of BOD removal and film quantity, but that this relationship was not linear over the whole range of values encountered. For example, a particularly weak feed would give rise to a low rate of BOD

removal, but the CO2 output would not fall in proportion, due to the respiration of previously synthesized film. Similarly, while carbon dioxide output was greater with high levels of film, this increase was not in proportion to the quantity of film, and the CO2 output per unit weight of film was correspondingly lower. In Fig. 21, the results are considered as the relationship between the CO2 output per $\sqrt{\text{film}}$ and $\sqrt{\text{BOD}}$ removed. The units used are the same as before.

The line of Fig. 21(a) was given by the equation,

$$y = 1.781 + 0.4545x$$

and the correlation coefficient, $r = 0.4893$ ($P < 0.05$ - significant)

The line of Fig. 21 (b) was calculated as,

$$y = 1.069 + 0.1054x$$

and $r = 0.401$ ($P < 0.05$ - significant)

A comparison between the correlation coefficients of Figs. 21(a) and (b) and those of Figs. 20(c) and (d) shows that the coefficient was considerably higher in Fig. 21(a) than in Fig. 20(c), indicating a greater linear significance using this treatment of the results obtained at 20°C. However, there was little difference between the correlation coefficients of Figs. 21(b) and 20(d) which considered the results obtained at 5°C.

The factor given by CO2 output per BOD removed which is used as one of the co-ordinates in Figs. 20(a) and (b) was considered separately by a direct statistical comparison between the values

Comparison of CO_2 output (mg/hr), $\sqrt{\text{BOD removal}}$ (mgBOD/hr), and $\sqrt{\text{film levels}}$ (gm. wet weight). Experiment A (flies absent)

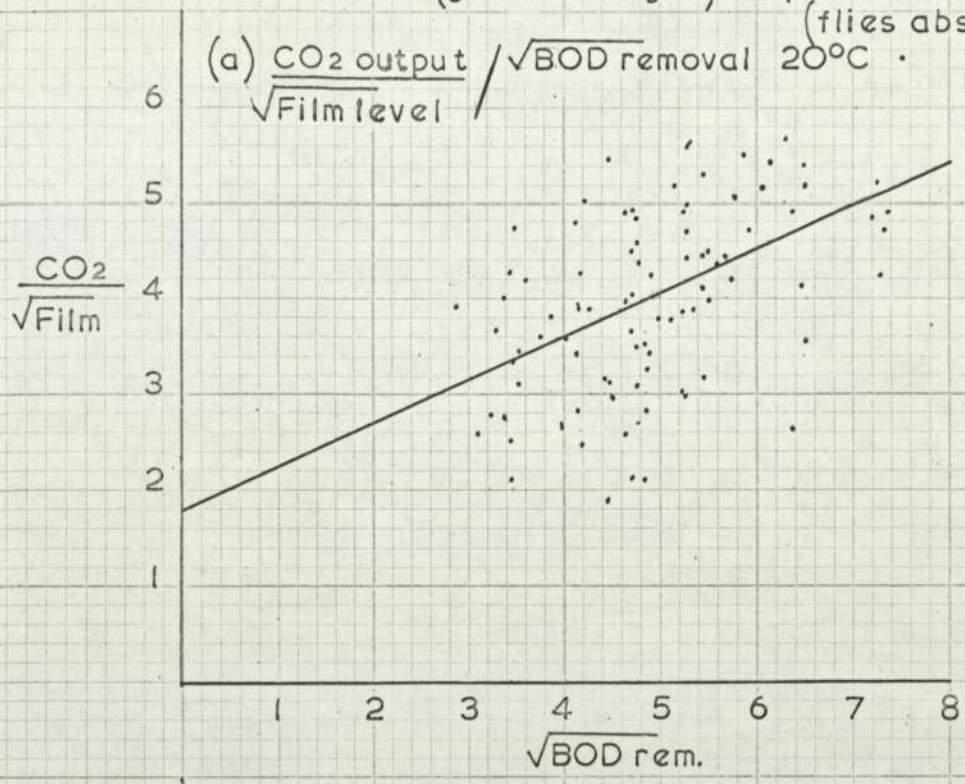


FIGURE 21.

obtained at 20°C and 5°C. The factor describes the carbon dioxide output in proportion to the degree of purification and can thus be used comparatively to indicate to what extent a given rate of BOD removal is due to direct respiration or to physical and biophysical adsorption. It was found that the average value of this factor for 92 readings at 20°C was 2.502, while at 5°C it was 1.707 for 27 observations. Comparing these two factors by means of the t-test, it was found that the value of t was 4.281. The critical value of t, indicating a probability of 0.05 at 120°F was 1.98. This shows a markedly significant difference between the factors at the two temperatures and indicates that the rate of BOD removal at 5°C was greater than would be expected from the corresponding CO2 output, as compared with similar criteria at 20°C. Thus BOD removal must be accounted for by adsorption of organic matter on to the film.

The figures for the organic carbon levels of feed and effluents for the period when these tests were being used are given in Table 3. The results show a similar pattern to BOD removal rates. An average rate of approximately 75% removal was achieved at 20°C and this was reduced to about 50% at 5°C, although the individual figures varied widely. While the results were insufficient to give a continuous estimate of the purification rate during Experiment A, they were nevertheless significant in considering the carbon balance of the filters. This is dealt with in the discussion.

The average weekly discharge of humus from the filters of each

TABLE 3.

Organic carbon content of feed and effluents (p.p.m.)
(with weekly averages)

Experiment A (flies absent)

Feed L	Effluents L1	L2	L3	Av. L. Eff.	Feed R	Effluents R1	R2	R3	Av. R. Eff.
Week ending 21/1/66 L = 20°C, R = 20°C									
220.0	38.0	-	-		220.0	-	-	-	
132.0	-	68.0	-		132.0	-	20.0	-	
230.0	-	-	20.0		230.0	-	-	80.0	
Av.194.0	38.0	68.0	20.0	42.0	194.0	-	20.0	80.0	50.0
Week ending 4/2/66 Temp of room R changed to 5°C, L = 20°C									
128.0	38.0	-	-		15°C 128.0	32.0	-	-	
66.0	-	-	-		10°C 66.0	32.0	-	-	
148.0	24.0	-	-		5°C 148.0	62.0	-	-	
Av.114.0	31.0	-	-	31.0	114.0	42.0	-	-	42.0
Weeks ending 11/2/66 to 5/5/66 L = 20°C, R = 5°C									
96.0	22.0	-	-		96.0	44.0	-	-	
58.0	-	34.0	-		58.0	-	62.0	-	
94.0	-	-	22.0		94.0	-	-	-	
Av. 82.7	22.0	34.0	22.0	26.0	82.7	44.0	62.0	-	53.0
80.0	28.0	-	-		80.0	38.0	-	-	
68.0	-	24.0	-		68.0	-	20.0	-	
52.0	-	-	-		52.0	-	-	-	
Av. 66.6	28.0	24.0	-	26.0	66.6	38.0	20.0	-	29.0
Av. 78.0	42.0	-	-	42.0	78.0	70.0	-	-	70.0

TABLE 3 (Cont.)

Feed L	Effluents L1	L2	L3	Av. L. Eff.	Feed R	Effluents R1	R2	R3	Av. R. Eff.
L = 20°C, R = 5°C									
104.0	16.0	-	-		104.0	56.0	-	-	
82.0	-	58.0	-		82.0	-	66.0	-	
78.0	-	-	-		78.0	-	-	28.0	
Av. 88.0	16.0	58.0	-	37.0	88.0	56.0	66.0	28.0	50.0
172.0	50.0	-	-		172.0	104.0	-	-	
138.0	-	54.0	-		138.0	-	110.0	-	
Av.155.0	58.0	54.0	-	56.0	155.0	104.0	110.0	-	107.0
Week ending 17/6/66 L = 20°C, R = 20°C									
114.0	40.0	-	-		114.0	46.0	-	-	
134.0	-	52.0	-		134.0	-	64.0	-	
154.0	-	-	40.0		154.0	-	-	78.0	
Av. 134.0	40.0	52.0	40.0	44.0	134.0	46.0	64.0	78.0	63.0

room is shown in Fig. 19(c). These results show that the highest levels of humus discharge were recorded where the film was unloading, as would be expected. Where the film was accumulating, such as in the filters of room R during the cold period, the lowest levels of humus were recorded. Allowance must be made for occasional error in the results, as the vessel in which the discharged humus was collected also served as a water seal to allow for slight increases in pressure within the filters. If, for any reason, the pressure became excessive, the water seal was broken and the accumulated humus was displaced into the effluent.

The results of the ammonia and oxidised nitrogen tests are shown in Table 4. The figures are given in pairs, one giving the concentration of ammonia and the other of oxidised nitrogen, as applied to the same sample of feed or effluent. For the most part nitrification was fairly low, but rates of up to 50% were recorded at 20°C. During the cold period, nitrification rates of the filters in room R at 5°C were lower than the corresponding rates in room L.

Microscopic examination of film taken from the surface of each filter was made on four occasions during Experiment A. The film itself was invariably bacterial rather than fungal. A variety of microinvertebrates occured within the film and these may well have had the effect of reducing the film level by grazing

TABLE 4.

Ammonia and Oxidised Nitrogen concentrations of feed and effluents (p.p.m. N)

Experiment A (flies absent)

	Feed L	Effluents L1	L2	L3	Feed R	Effluents R1	R2	R3
	20°C				20°C			
NH_3	69.8	63.6	65.6	64.0	68.8	62.8	61.8	63.6
Ox.N	0.8	0.2	0.8	0.6	0.4	0.4	1.0	0.2
NH_3	63.2	60.8	61.0	60.4	64.6	63.8	62.4	63.0
Ox.N	0.0	0.4	0.2	0.4	1.0	1.6	0.4	0.4
NH_3	32.2	24.6	23.8	20.6	27.0	23.0	12.8	19.8
Ox.N	3.2	5.2	3.8	4.6	0.4	3.6	3.2	3.0
NH_3	45.4	23.6	38.2	28.2	48.0	43.8	40.0	42.2
Ox.N	0.0	18.2	4.4	10.2	0.0	4.6	6.0	2.0
	20°C				5°C			
NH_3	42.8	20.0	29.2	18.4	32.0	28.2	27.8	27.4
Ox.N	0.2	19.2	12.2	19.0	0.6	8.2	6.8	4.2
NH_3	42.6	26.0	28.8	7.4?	43.2	37.6	36.2	36.6
Ox.N	0.0	11.2	6.0	9.8	0.4	2.8	3.6	3.6
	20°C				20°C			
NH_3	34.1	20.6	23.4	22.0	30.6	14.4	16.8	17.8
Ox.N	0.9	12.9	9.8	9.2	0.0	12.4	9.6	12.2

in a similar manner to macroinvertebrates though on a much reduced scale. Nematode worms were usually present in considerable numbers and protozoa of the classes Ciliata and Flagellata were common. Mites were occasionally present but only in very small numbers. Rotifers showed wide fluctuations in quantity, varying from abundant to apparently absent. They also tended to be more frequent in the filters of room L than those of room R. The lowering of the temperature in room R did not appear greatly to affect the composition of the microfauna, but the organisms present showed much less activity. It should be noted that the composition of the microfauna was not necessarily uniform at all levels. There may have been differences in the abundance of certain organisms at lower levels compared with the surface, where the samples were taken, in the same way that the composition of the macrofauna varies throughout the depth of an operational bed.

Experiment B. Flies present in all filters.

On 24th August 1966, about a hundred flies of the genus Psychoda, taken from the bacteria beds at Langley Mill, were introduced into each of the experimental filters. Although the filters were operated without a break this marked the end of Experiment A and the beginning of Experiment B. Whilst no attempt was made to identify all the flies exactly, they were mostly of the species P. alternata. The initial inoculation appeared to be unsuccessful in filters L1 and R1 and a second population was introduced about

two weeks later. However, within four weeks of the start of the experiment a breeding population of flies was established in all the filters.

The experiment began with the temperatures of both rooms at 20°C. This was continued until 1st November 1966. During that week the temperature of room R was lowered to 5°C in three daily stages as in Experiment A, and maintained at this temperature until 7th February 1967. In the week ending 10th February, the temperature was raised to 20°C in three stages and this was continued until the end of the experiment on 21st March 1967. When the temperature of room R was returned to 20°C from 5°C, the medium of each filter in that room was observed closely to see whether the fly population had survived a prolonged period below the threshold required for the continuation of the life cycle. Within about a week several flies had been seen in R2 and this population continued to develop without any reintroduction. One fly was seen in R1, but in this filter and in R3, although individuals may have survived, a full breeding population did not develop When this became apparent, larvae and adult flies were introduced into these filters in order to re-establish the population. This, however, proved difficult to achieve. Repeated introductions were necessary before the flies became established in R1, and in R3 a breeding population had still not developed when the experimental work was concluded.

As in Experiment A, there were occasional failures of the

temperature control in room R. On 16/9/66 and 21/9 /66 while room R was set at 20°C, there were abrupt rises in temperature to about 35°C for four hours on the first occasion and twelve hours on the second. Over the weekend 12-14/11/66, while room R was set at 5°C, there were repeated failures of the temperature control. First, the temperature rose to about 37°C for eight hours before returning to normal. This was followed by a failure fo the power supply for a period of about twenty hours causing the temperature of room R to rise gradually to the external room temperature of about 20°C. The failure of the power supply also affected the thermo-control of room L, but since this room was being operated at 20°C there was little change in temperature. After the power was restored there was another failure of the thermostatic control in room R when the temperature rose to 37°C for about twelve hours. Finally, on 4/1/67 the temperature rose from 5°C to about 37°for twelve hours.

On 19/11/66, the compressor supplying a continuous stream of air to all the filters broke down and was not repaired until 23/11/66. During this period the film, especially in the filters of room L which was at 20°C, showed signs of becoming anaerobic and the efficiency of purification fell considerably. It also appeared that the population of flies in these filters was severely affected. When the air supply was restored, the population in L1 recuperated, but in L2 and L3 the flies had been destroyed. A new

population was therefore reintroduced into these two filters. The effect of the loss of ventilation on the filters of room R which was then at 5°C was less marked, but there was nevertheless a deterioration in the efficiency of purification. It was not possible to determine whether the fly population had been affected as it was already reduced and inactive due to the low temperatures. However,a new population of Psychoda larvae was introduced into each filter in case lack of oxygen had destoyed the original one. The effect of this failure of the air supply system is considered in more detail in the results.

Throughout Experiment B, the estimation of the various factors described in the methods was continued essentially as in Experiment A. Weekly measurements were made of the film levels, the discharged humus and the PV of the feed and effluents. The BOD removal and carbon dioxide output of corresponding filters in room L and room R were measured in pairs on three consecutive days each week. Occasional analyses were made on the ammonia and oxidised nitrogen concentrations of the feed and effluents, and the microscopic nature of the film was examined periodically. The only method which was not used in Experiment B was the analysis of organic carbon. Although this method had given reasonably satisfactory results for a limited period during Experiment A, it was found to be too laborious to use as a regular procedure and had been abandoned before Experiment B was started.

Results. Experiment B.

The film levels in the three filters of room L during Experiment B are shown in Fig. 22 and those of room R in Fig. 23. The averages of the film levels in each room are compared in Fig. 24(a). Experiment B began with the filters in both rooms at 20°C. the introduction of flies was found to cause rapid breakdown of film to a level of about 100 gm. per filter as soon as the population became established. In room L where the temperature was kept at 20°C for the whole of Experiment B, the film remained steady at this low level for the rest of the experiment. When the temperature of room R was lowered to 5°C, the film levels began to rise at a rate similar to that during the cold period of Experiment A. This indicated that the low temperatures had caused virtually the complete sippression of the grazing activity of the flies. By the end of the cold period, the film in the filters of room R had reached an average of just over 200 gm. per filter. During the final period, when the temperature of room R was returned to 20°C, the film levels of the individual filters in that room differed considerably depending on the survival of the fly population. In R2 where the fly population survived without any reintroduction, the film showed a fairly rapid fall to a level similar to that of the filters of room L. In R1 the fly population did not survive the cold period, but with several reintroductions it eventually became established and the film level fell markedly during the

FIGURE 22.

Film levels in room L (gm wet weight). Experiment B (flies present)

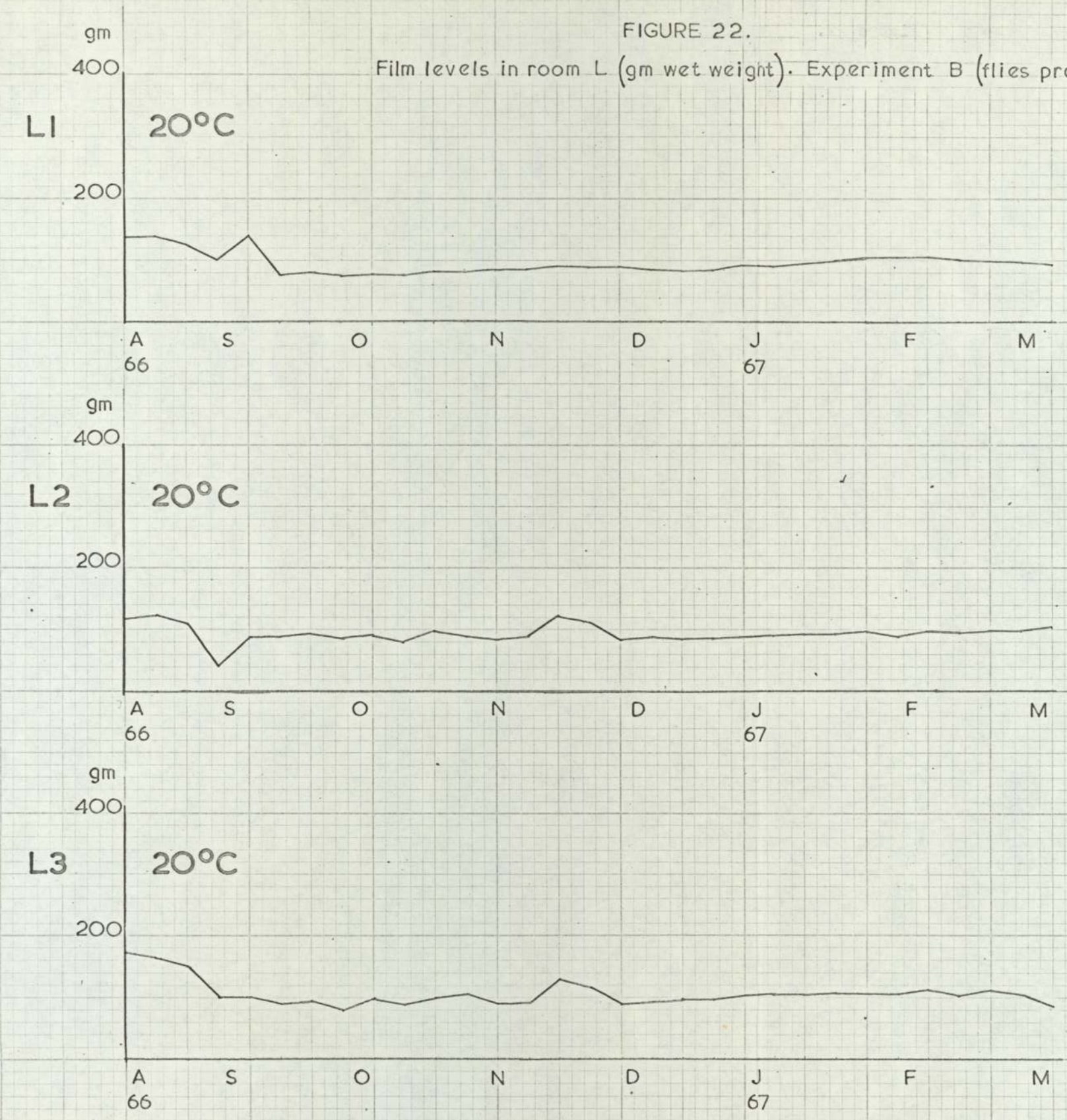


Figure 22.

FIGURE 23.

Film levels in room R (gm wet weight). Experiment B (flies present)

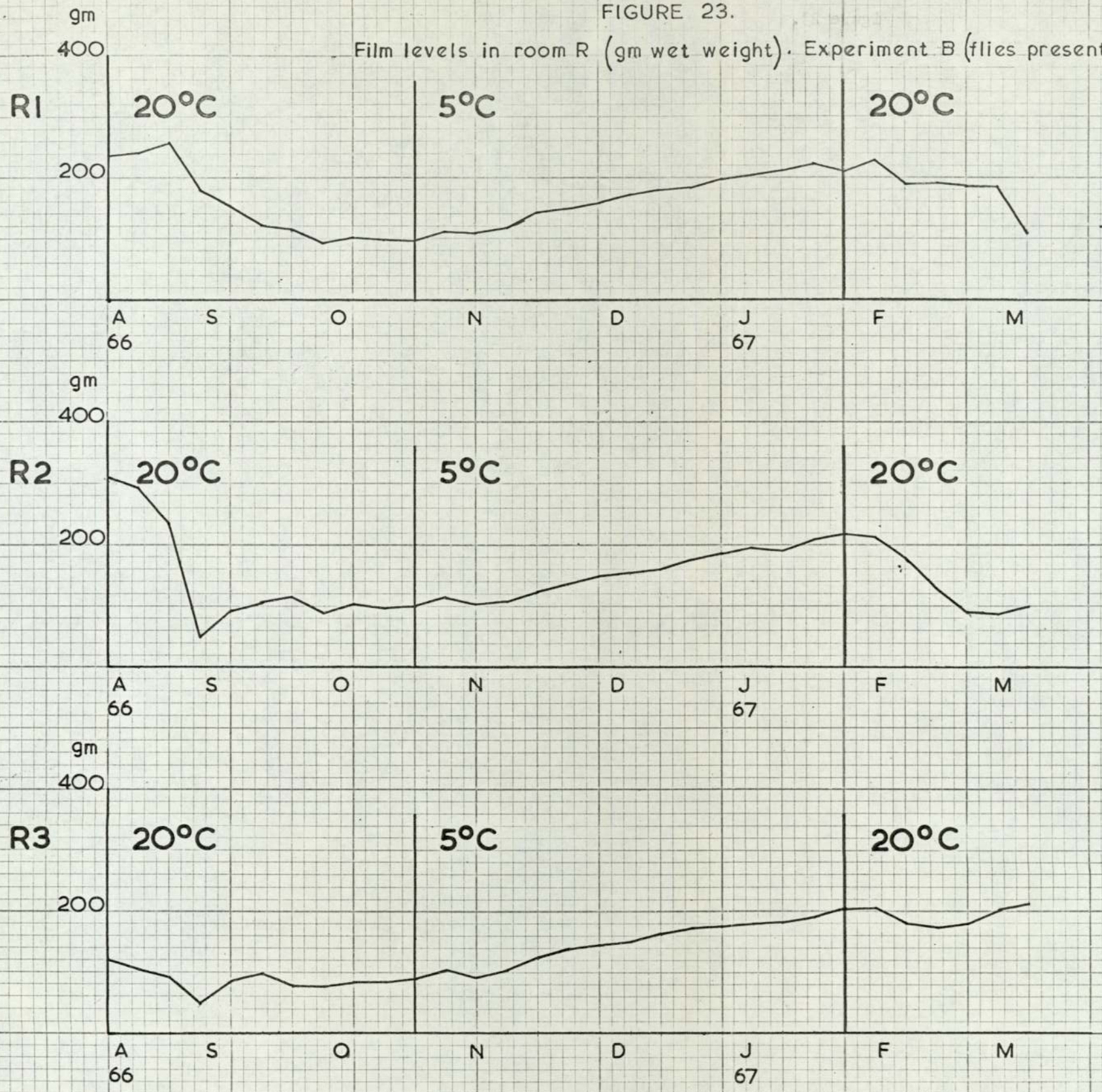


Figure 23.

final week of the experiment. In R3, the fly population was also destroyed by the cold but, despite repeated introductions of adults and larvae, failed to become reestablished. The film showed a slight fall initially when the temperature was raised to 20°C, but it later rose and at the end of the experiment was higher than at any time during the cold period.

As in Experiment A, the occasional failures in temperature control caused temporary setbacks in the pattern of film accumulation, but no permanent damage was done. This is seen in November 1966, shortly after the cold period in room R had begun, where the failure of the temperature control caused the loss of most of the film that had accumulated.

The failure of the compressed air system towards the end of November 1966 is of interest concerning its effect on the filters of room L. In L2 and L3 where the fly populations were destroyed, a sharp rise in the film levels occurred, but they returned to normal during the subsequent two weeks as a reintroduced population became established. In L1 where the fly population survived, no rise took place.

The average levels of film accumulation in the two rooms were compared statistically by means of the t-test. The results are shown in Table 5. A value of t indicating a probability of less than 0.05 was regarded as significant and that indicating a probability of more than 0.05 was regarded as not significant.

Period	1	2	3
Temp.	L = 20°C R = 20°C	L = 20°C R = 5°C	L = 20°C R = 20°C
$\bar{L}$	97.85	94.99	100.10
$\bar{R}$	118.83	159.81	168.80
n_l, n_r	30	42	18
t	1.747	10.81	6.343
Critical t for 0.05P	2.00	1.99	2.035
Difference	Not sig.	Sig.	Sig.

Table 5.

Itwas found that during the initial period at 20°C, the difference between the film levels of the filters in each room was not significant. During the second period, while room R was at 5°C, the difference was significant,and the value of t was greater than for the corresponding period of Experiment A, owing to the greater uniformity between the individual film levels in each room. During the final period, the differences between the film levels in each room persisted sufficiently to give a significant difference between the means during this period also.

The BOD values of the feed and effluents during Experiment B are given in Appendix Table 7. The average weekly BOD of the feed is shown in Fig. 24(b) and the average percentage BOD removal of

the two rooms is compared in Fig. 25 (a). The strength of the feed varied between less than 100 p.p.m. at one extreme and 300 p.p.m. at the other. The efficiency of purification was rather erratic in both rooms at the beginning of the experiment and this was associated with a heavy unloading of the film following the introduction of flies. After this, room L maintained a steady level of efficiency, not falling below 65% and usually around 80% BOD removal. This was an improvement on the performance of the filters in Experiment A which was probably because the film was kept at a uniformly low level and did not undergo the cycles of synthesis and breakdown, which sometimes gave rise to anomalous results in Experiment A. During the cold period in room R the efficiency of the filters was considerably lower than those of room L, but nevertheless somewhat higher than the corresponding period of Experiment A. During one week, exceptionally high efficiency of nearly 80% was recorded. On returning to equal temperatures, the filters in room R took a few weeks to recover to the efficiency of those in room L.

The results of the PV analyses are given in Appendix Table 8 and the weekly averages of percentage PV removal in Fig. 25 (b). The validity of some of the levels of efficiency as estimated by this method must give rise to serious doubt. There are often wide discrepancies between the efficiency as measured by the rates

FIGURE 24. Experiment B

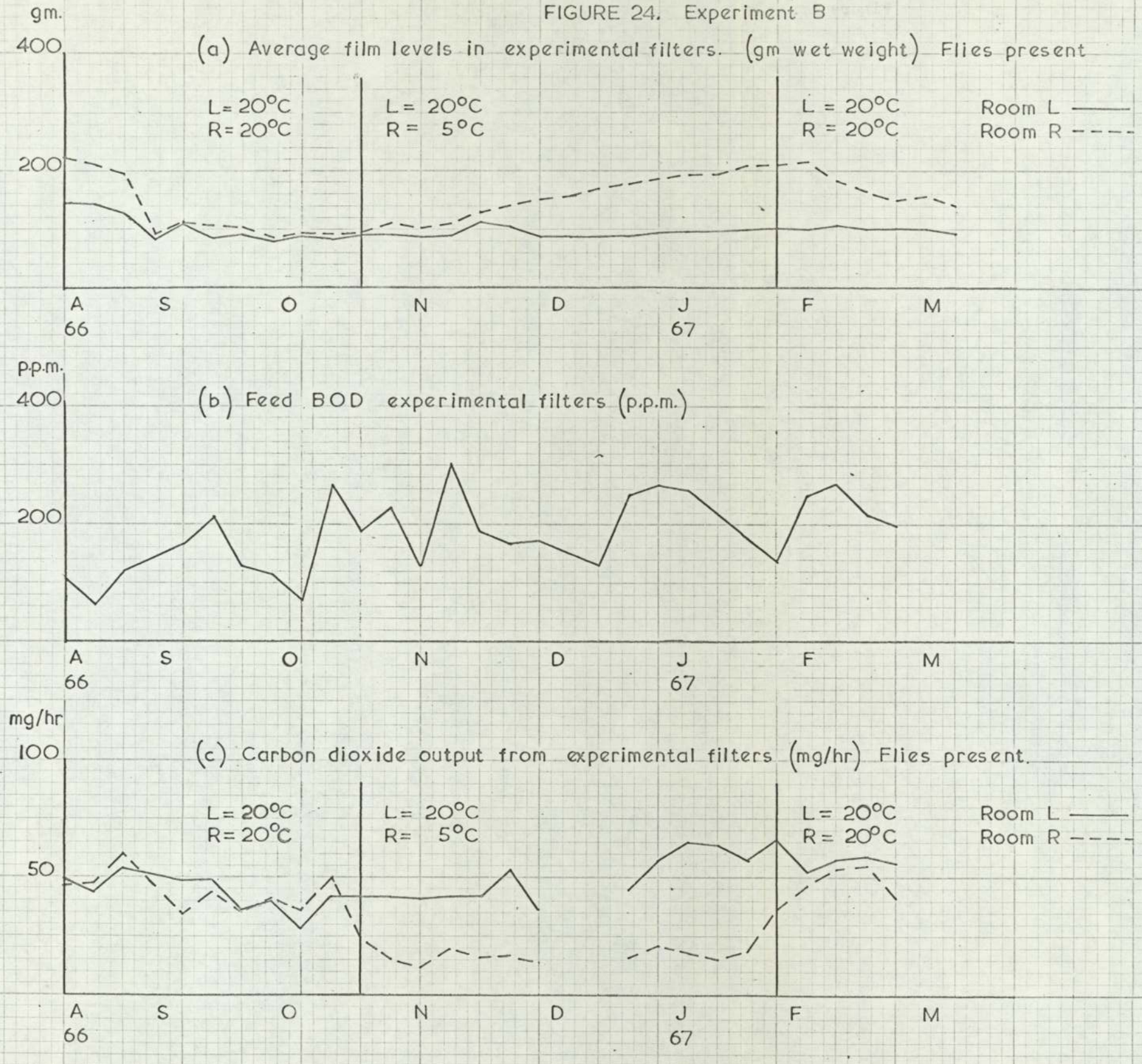


Figure 24.

FIGURE 25. Experiment B

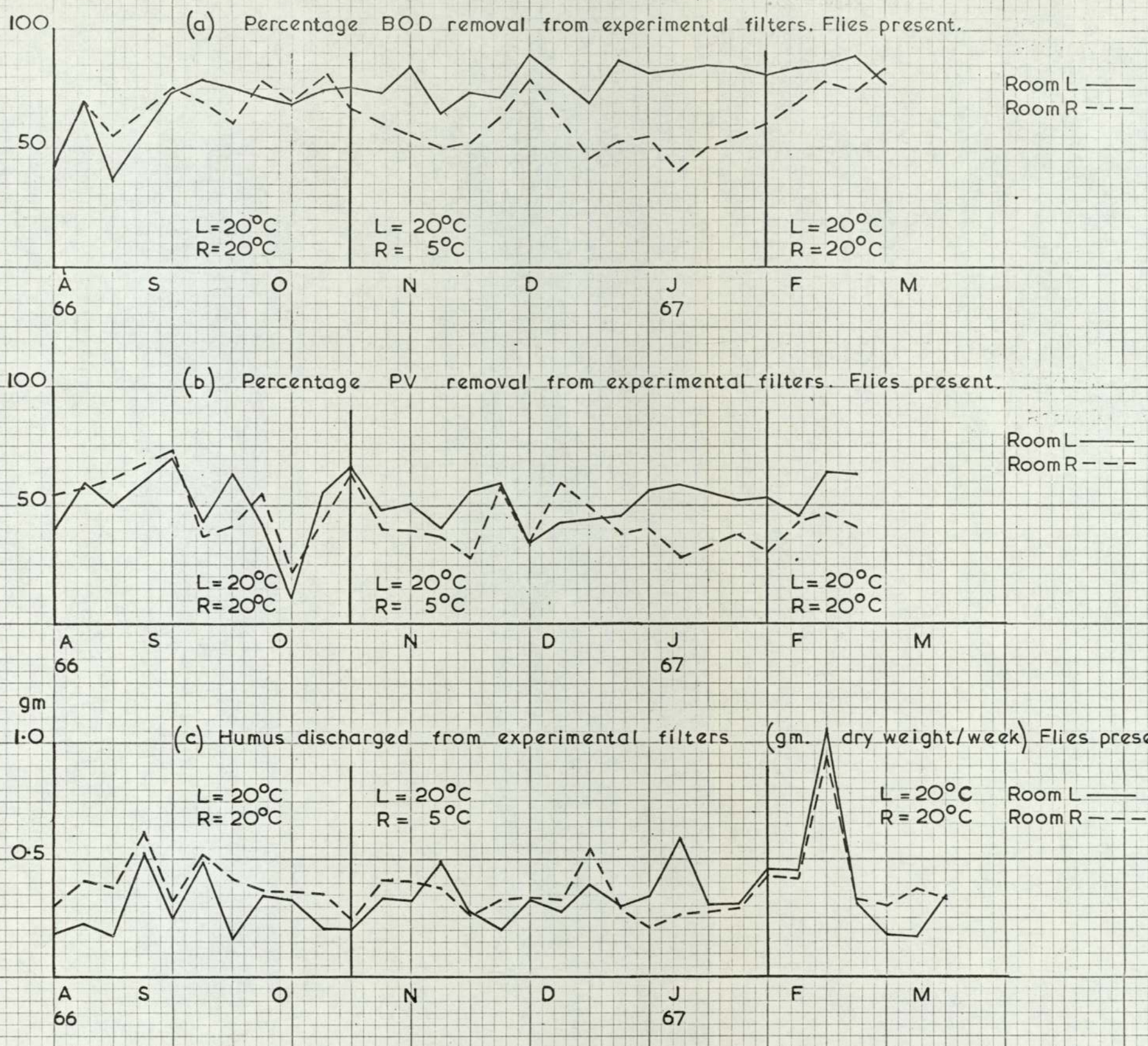


Figure 25.

of BOD removal and PV removal respectively, and the percentage BOD removal is probably the more accurate figure. The percentage PV removal tended to be abnormally low when the feed was particularly weak. During the period when room R was at 5°C, percentage removal in these filters was mostly below those of room L, but on a few occasions there was little difference between the rooms and during one week it was apparently higher in room R than in room L. These abnormal results were contradicted by the corresponding BOD removal figures.

The rates of carbon dioxide output are given in Appendix Table 9 and the weekly averages are shown in Fig. 24 (c). As would be expected, the outputs from the filters of the two rooms were similar during periods of equal temperature and that of room R fell considerably during the period at 5°C. As in Experiment A, the relationship between the film levels, the absolute rate of BOD removal and the CO2 output has been considered in a series of regression graphs in Figs. 26 and 27. The figures from which the points of these graphs have been calculated are given in Appendix Table 10. For each graph the equation representing the best straight line has been determined and the correlation coefficient, describing its linear significance, has been calculated.

In Figs. 26 (a) and (b), the CO2 output divided by the rate of BOD removal has been plotted against the film level at 20°C and 5°C.

The units are the same as used in Experiment A.

The regression equation for Fig. 26 (a) was calculated as

$$y = 1.362 + 0.0032x$$

and the correlation coefficient, $r = 0.0578$. This indicated a probability of more than 0.05 and was therefore considered not significant. The critical value of r for 0.05P at 60 degrees of freedom (^{o}F) is 0.2500. Since the film in the filters at 20°C during Experiment B was kept low by the grazing activity of the flies, this graph did not give a very wide dispersion of values along the axis representing the film level and hence the significance of this line was very small.

The regression equation for Fig. 26 (b) was

$$y = 0.3480 + 0.00275x$$

and $r = 0.4803$ ($P < 0.05$ - significant)

The critical value of r, indicating a probability of 0.05 at 18°F was 0.4438.

In Figs. 26 (c) and (d), the respiration rate per unit weight of film was plotted against the rate of BOD removal at the two temperatures of 20°C and 5°C, using the same units as before.

The equation representing Fig. 26 (c) was

$$y = 0.3655 + 0.00408x$$

and $r = 0.4797$ ($P < 0.05$ - significant),

and the equation for Fig. 26 (d) was

$$y = 0.0707 + 0.001849x$$

and $r = 0.387$ ($P > 0.05$ - not significant)

As in Experiment A, the relationship between the CO2 output per $\sqrt{\text{film}}$ and the $\sqrt{\text{BOD}}$ removed was considered and the graphs obtained at 20°C and 5°C are shown in Fig. 27 (a) and (b) respectively.

The equation of Fig. 27 (a) was calculated as

$$y = 2.064 + 0.5005x$$

and $r = 0.5136$ ($P < 0.05$ - significant)

and that of Fig. 27 (b) as

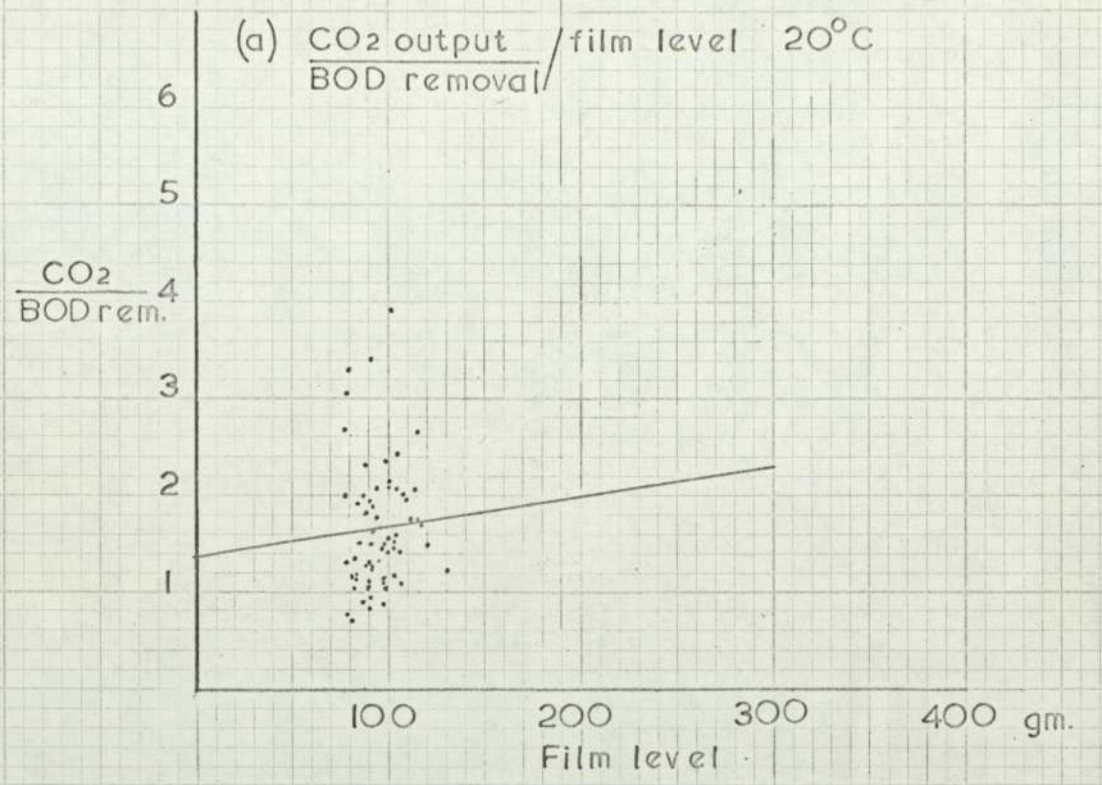
$$y = 0.3100 + 0.2179x$$

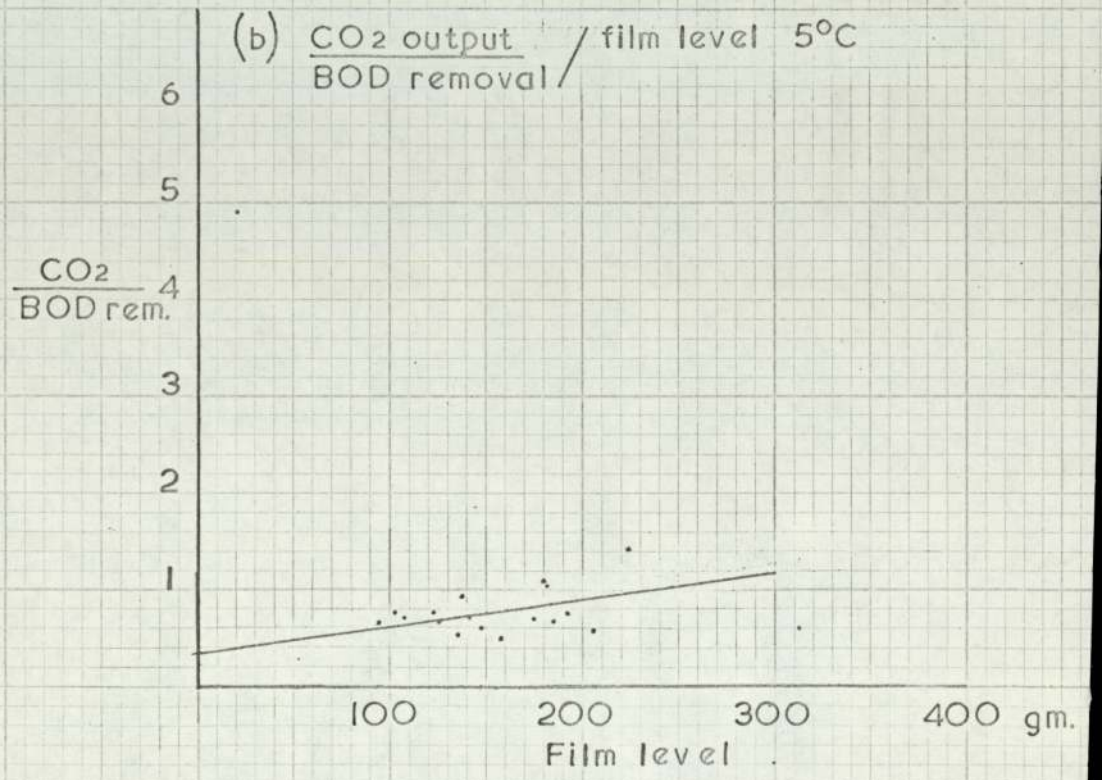
and $r = 0.5877$ ($P < 0.05$ - significant)

The correlation coefficients of these equations are higher at both 20°C and 5°C than the corresponding coefficients of Figs. 26 (c) and (d) indicating that a greater linear significance was given by this treatment of the results.

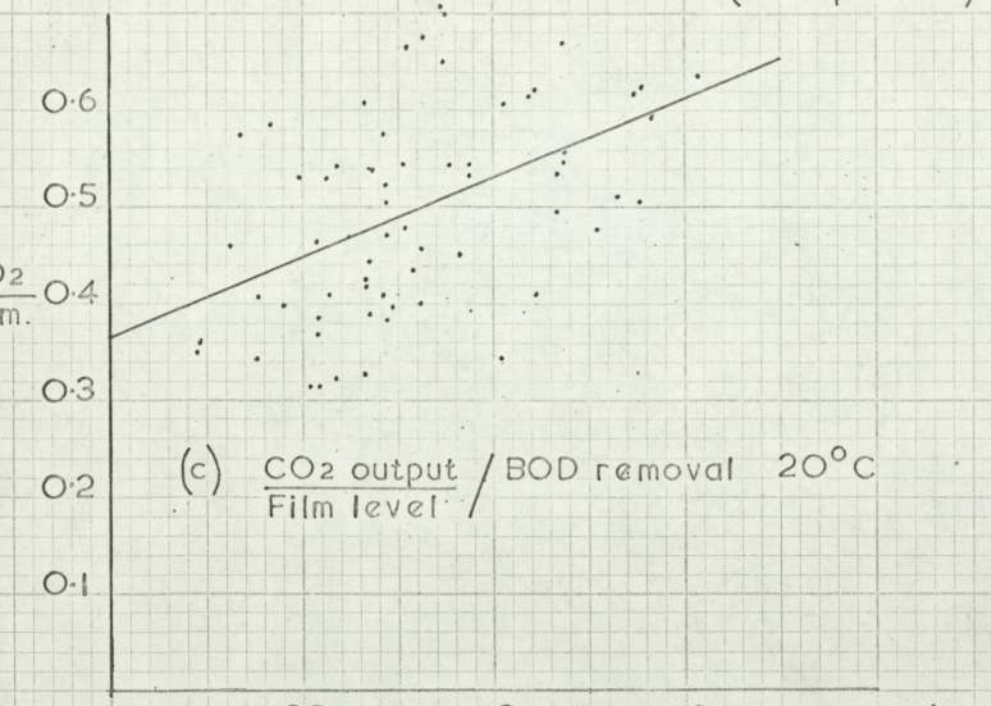
As in the previous experiment, each reading of CO2 output was divided by the corresponding result for BOD removal to give a factor relating purification rate to respiratory activity of the film. The values of this factor obtained at 20°C were compared statistically with those at 5°C by means of the t-test. The average value for 63 readings at 20°C was 1.668 and for 18 readings at 5°C it was 0.761. The value of t was found to be 8.142 compared with a critical value of t, for a probability of 0.05, of 1.99

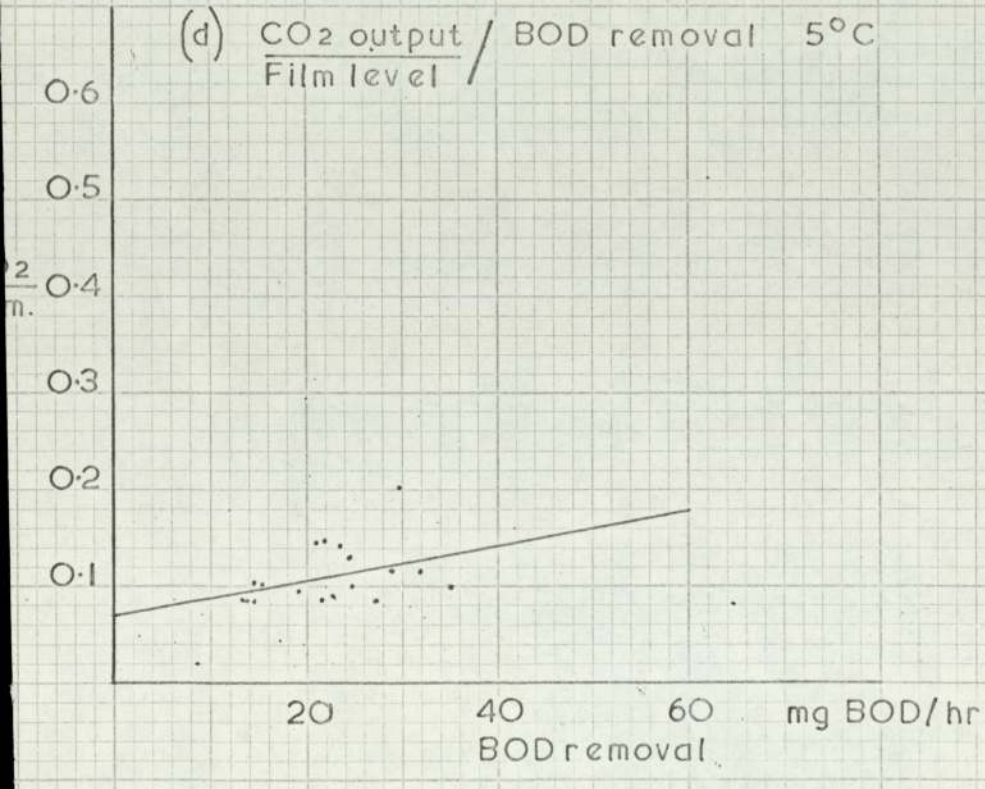
Comparison of CO_2 output (mg/hr) BOD removal.





/hr) and film levels (gm wet weight). Experiment B.
(flies present)

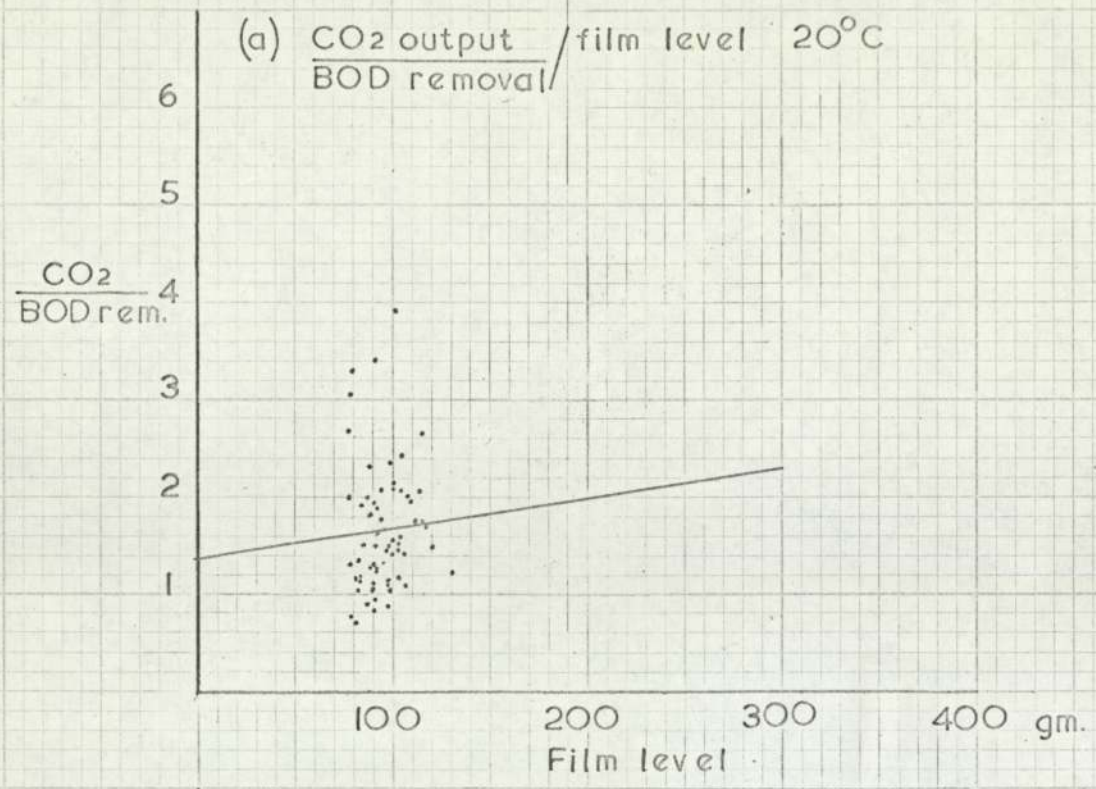


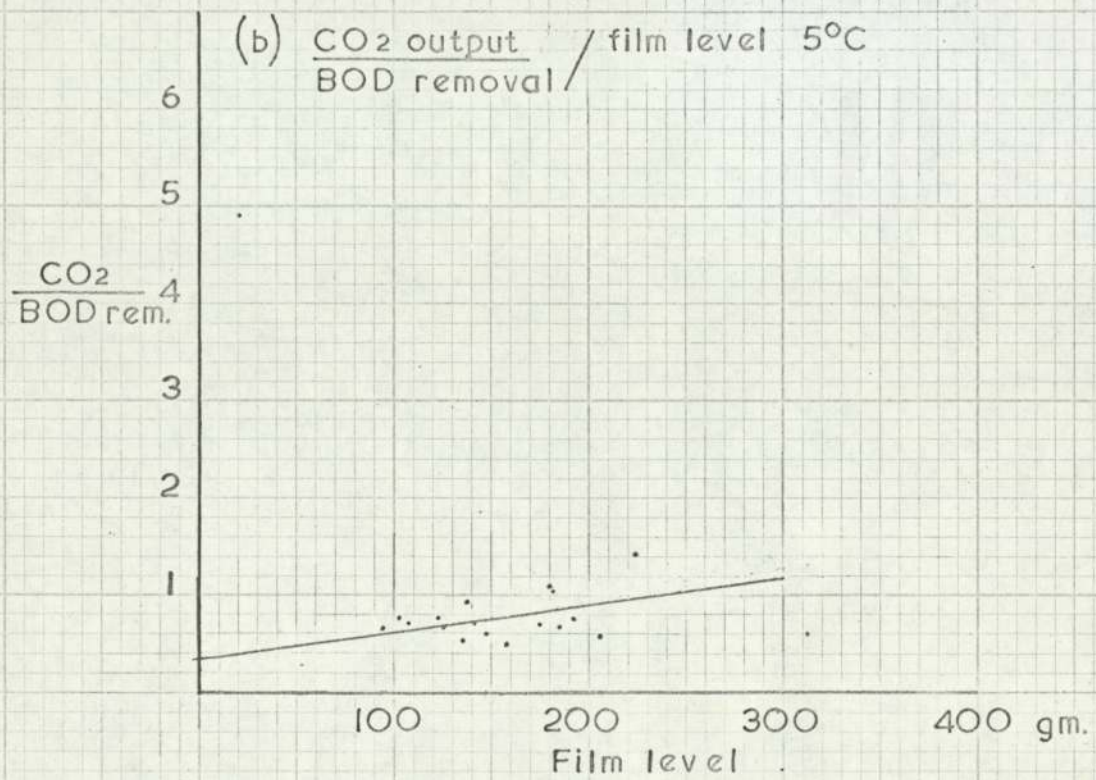


RE 26.

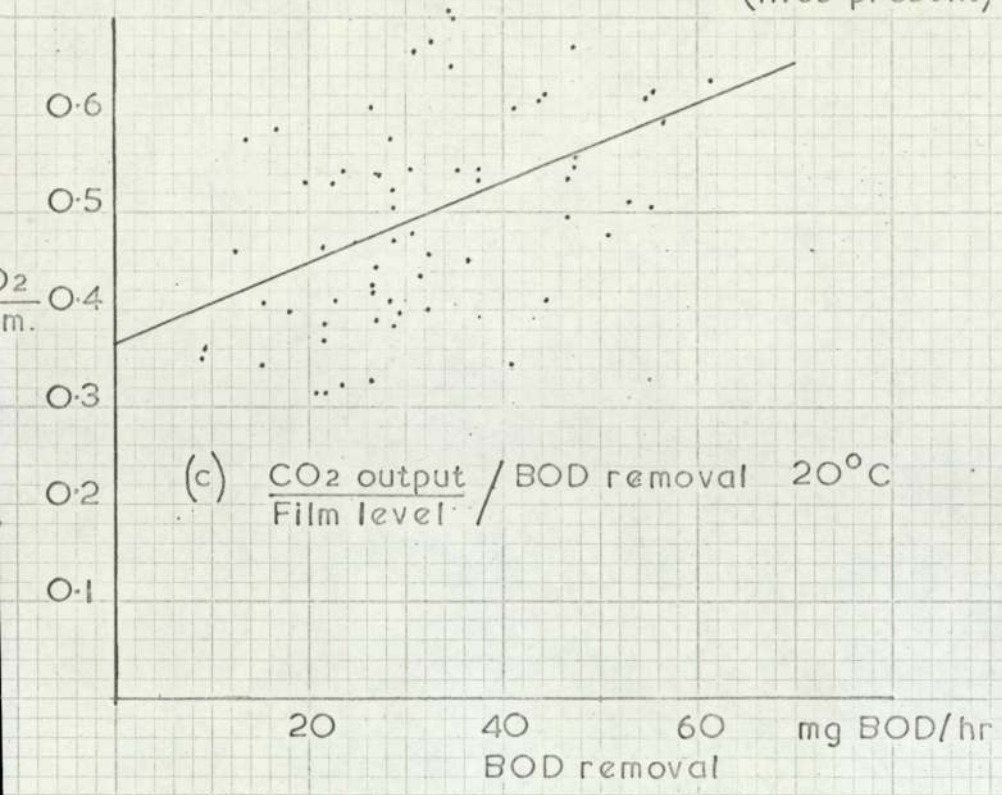
Figure 26.

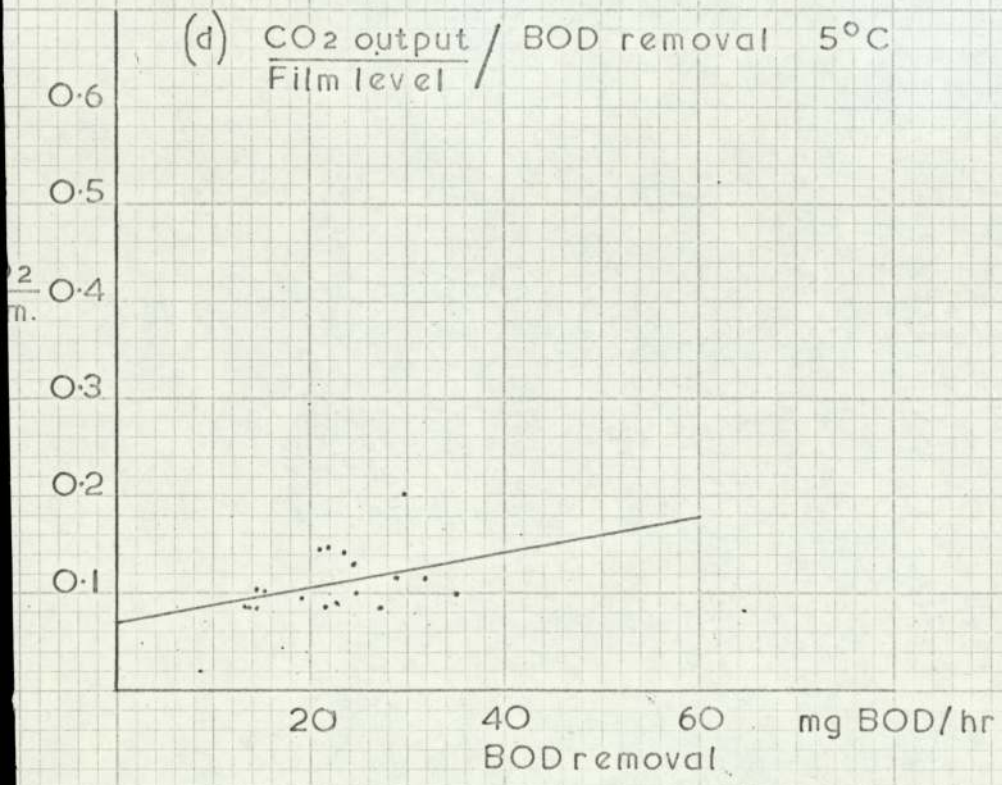
Comparison of CO_2 output (mg/hr) BOD removal.





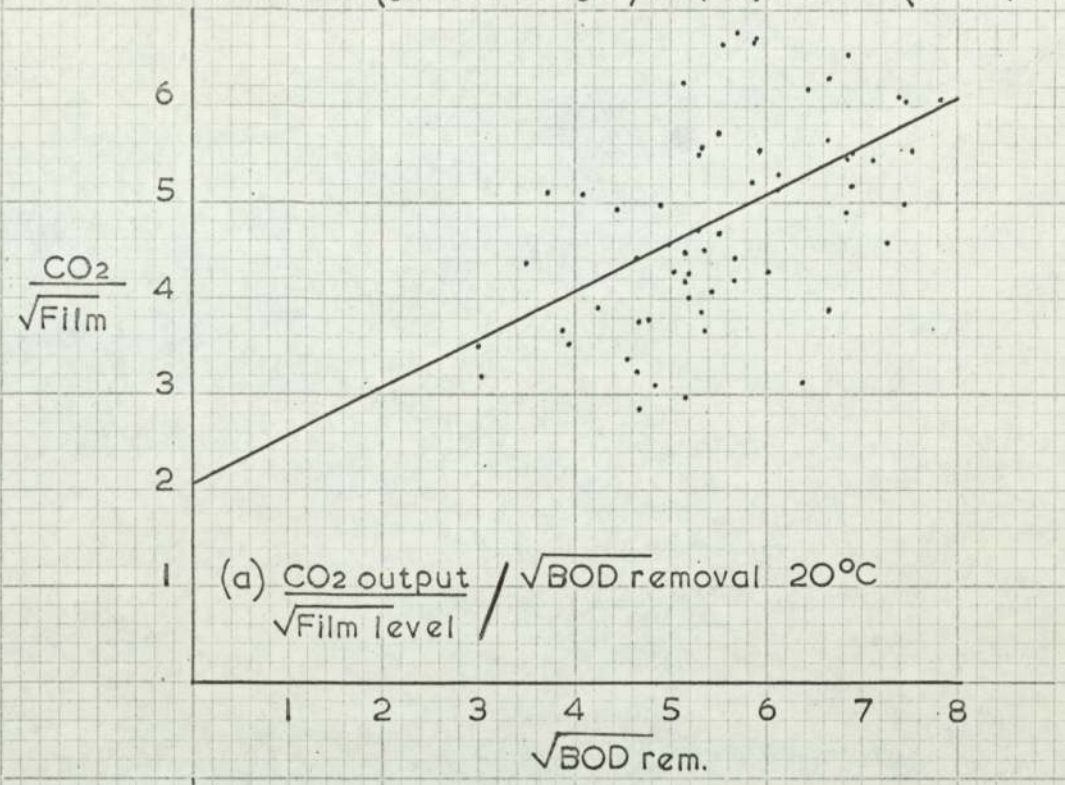
O/hr) and film levels (gm wet weight). Experiment B.
(flies present)





RE 26.

Comparison of CO_2 output (mg/hr.), $\sqrt{\text{BOD removal}}$ (mg BOD/hr) and $\sqrt{\text{film levels}}$ (gm wet weight). Experiment B (flies present)



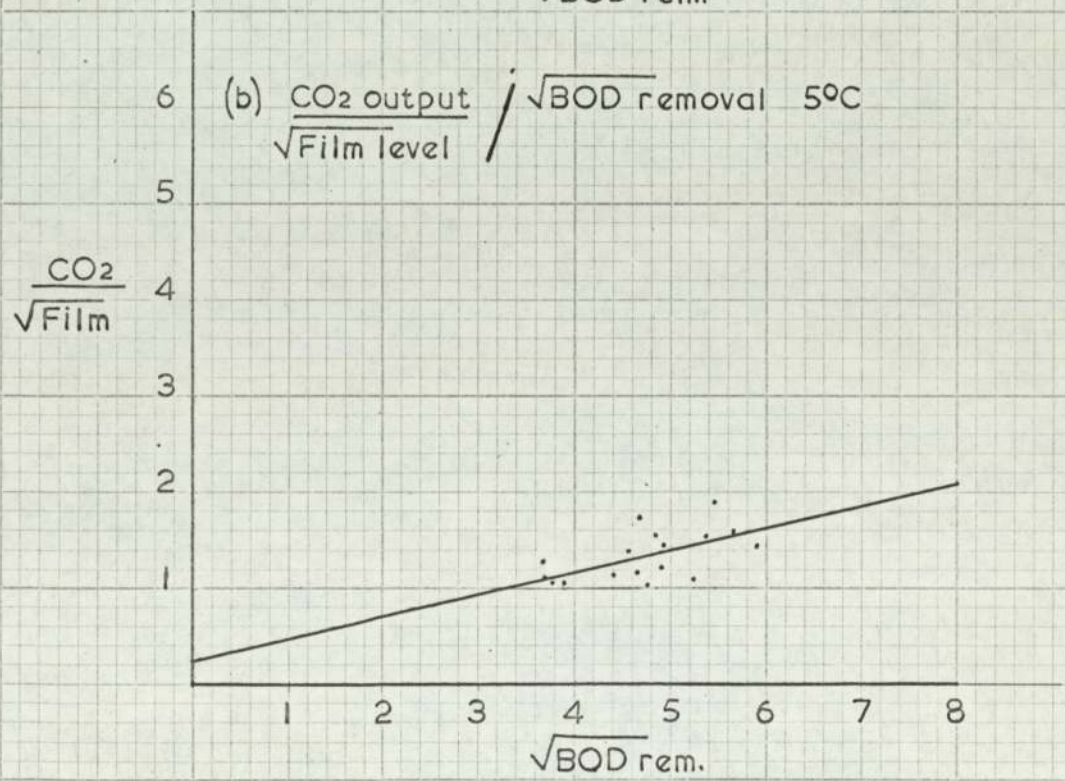


FIGURE 27.

for 80 degrees of freedom. As in Experiment A, this shows that some of the purification capacity at 5°C is due to factors other than spontaneous respiration, presumably biophysical absorption of organic matter on to the film.

The average discharge of humus from the filters of each room is shown in Fig. 25 (c). The results, however, must be treated with caution owing to the loss of humus from the collecting vessel into the effluent. Higher levels of humus discharge were recorded during periods of unloading of the film, such as shortly after the initial introduction of flies, but there was little difference between the rates of humus discharge from the filters of the two rooms while at different temperatures in contrast to what had been observed in Experiment A.

The ammonia and oxidised nitrogen levels of the feed and effluents were analysed on two occasions during Experiment B, the first during the initial period with both rooms at 20°C and the second while room R was at 5°C. The results are shown in Table 6.

	F1	L1	L2	L3	FR	R1	R2	R3
	20°C				20°C			
NH_3	28.8	21.4	15.6	19.4	30.2	17.6	20.5	19.9
OxN	0.3	6.6	14.4	11.4	0.0	14.1	11.7	10.6
	20°C				5°C			
NH_3	53.4	44.2	46.0	42.8	43.8	41.1	38.9	40.6
OxN	1.4	9.5	4.9	7.8	0.8	1.4	1.0	0.8

Table 6.

It was found that at 20°C nitrification rates of up to 50% were achieved, but were usually lower. The nitrification at 5°C was negligible.

Microscopic examination of film taken from the surface of each filter was made on two occasions during this experiment. While the film itself was bacterial rather than fungal as before, considerable differences were found in the nature and abundance of the micrograzers. Nematodes and rotifers which had usually been frequent in Experiment A were largely absent. This suggested that the macroinvertebrate grazers, i.e. the flies, were in direct competition with the micrograzers at the same trophic level. This was supported by the observation that during the cold period in which the macrograzers were apparently suppressed, nematodes and rotifers reappeared in one of the filters of room R. Protozoa, mostly flagellates, were usually present during Experiment B, but not in such great abundance as before. Mites were virtually absent. As mentioned previously, it should not be assumed that the composition of the film at the surface, where the sample was taken, was the same as in all parts of the filter. It was observed that rotifers and nematodes occurred in considerable numbers in the discharged humus and were therefore flourishing in some parts of the system.

Discussion of Results

Validity of Experimental Criteria. The film levels in the filters during the course of the experimental work were measured as the difference between the weight of the filter at the time of observation and its original dry weight. There were naturally certain disadvantages to this means of estimation. A considerable proportion of the weight was accounted for by liquid retained in the interstitial spaces of the filter and also by condensation within the plastic spheres of the medium. At the end of the experiment the increase in weight of the filters due to this condensation was measured and found to be between 17 and 30 gm. per filter. In addition, the proportion of the estimate of film weight represented by retained liquid was found, as might be expected, not to be constant at different levels of film. At the end of the experiments, measurements of the volatile matter were made on the film remaining in the filters and it was found that while a wet weight of 100 gm. produced a reading for volatile matter of approximately 1.2 gm., a wet weight of 200 gm. gave about 4.5 gm. volatile matter. Hence, at low film levels a much higher proportion of the wet weight was accounted for by retained liquid. Thus, while the weights were not an absolute measurement of film present, it was not practicable to apply any correction factor; the figures were still of significance in comparing the levels of

film between different filters.

The most acceptable means of measuring the polluting strength of an effluent has for long been a subject of discussion. The BOD test is widely employed and was used as the standard analytical test during the experimental work. The results were mostly satisfactory, the main problem being the preparation of the sample for analysis, rather than the test itself. The sample for the feed was taken from a tap at the base of the feed vessel and sometimes contained particles of suspended matter which had settled to the bottom. The sample was therefore filtered through find gauze mesh to remove material that might tend to give an exaggerated result. Similarly, a period of breakdown of the film occasionally caused suspended matter to appear in the effluent and produce an exceptionally high result, giving an unrealistic figure for the efficiency of the filter. This was especially the case when the feed was particularly weak. For this reason, effluent sample also were filtered through a similar mesh.

The Permanganate Value is also widely used as a standard test for pollution, and was employed during the experimental work as a supporting test to the B.O.D. analyses but on several occasions the results were not satisfactory. An abnormally high result for feed or effluent might occur for no apparent reason, giving an unrealistic measurement of the efficiency. The standard methods

book (H.M.S.O. 1956) admits that this test "... may at times give misleading results unless further information is available".

Another criterion used in the assessment of pollution was the concentration of organic carbon but the method for this was found to be too laborious for regular analyses to be made. The results however are an absolute measurement of the carbon present rather than an empirical value based on oxygen absorption as in the previous two tests, and it is possible that as the procedure for this test is improved it may become a much more acceptable method.

The concentration of carbon dioxide in the exhaust air from the filters was not uniform but showed regular fluctuations, or "surges" coinciding with the period of dosing. The exact cause of these fluctuations is discussed later on, but their effect on the validity of the carbon dioxide output as a measurement of respiration must be considered here. One possible explanation was that dissolved carbon dioxide was displaced from the sewage feed as it came into contact with the air stream. In this case a certain proportion of the recorded CO_2 output would be due to inorganic sources rather than the respiration of the film. This would be reflected by a difference in the levels of inorganic carbon between the feed and the effluent. However, during the period when the level of inorganic carbon in the feed and effluent was being measured, little difference was found and thus it appeared that any

dissolved carbon dioxide that was released from the feed during dosing was replaced during its passage through the filter. This confirms that the line representing the average of the carbon dioxide fluctuations was a valid estimate of the respiratory rate.

The temperatures of 20°C and 5°C, at which the relative efficiency and behaviour of the experimental filters were compared, were chosen because Hawkes (1961) had reported that a steady rate of purification was maintained throughout the year in an operational bacteria bed provided that film accumulation was avoided. These temperatures may have been slightly outside the maximum and minimum seasonal temperatures of an operational bed but it was desired to observe the performance of the filters at a temperature difference sufficient to affect the purification rates.

The number of filters in each room was chosen as three on the basis of the work of Gameson, Truesdale and Van Overdijk (1961) who suggested this as the minimum number required for valid statistical comparisons to be made between groups of filters because of the effect of variability within each group.

The Performance of the Experimental Filters The first point that is of significance in the film measurements, and an important conclusion of the experimental work, is that both in the presence and in the absence of Psychoda, the levels of film accumulation achieved at 5°C were significantly higher than those at 20°C. The

"winter accumulation" of film in operational beds has long been recognised as detrimental to their efficiency, particularly if this accumulation proceeds to such an extent as to cause "ponding". It has usually been assumed that the cause of winter accumulation is the suppression of the grazing activity of the macrofauna at lower temperatures. It is usually assumed further that in the absence of a grazing population the rate of growth of film would be faster at high summer temperatures than in winter. Tomlinson (1946) excluded macrograzers from baskets of medium set into the surface of a bacteria bed and found that higher rates of growth occurred with increased temperature over short periods. However, the current experiments show that with a prolonged absence of macrograzers other factors are involved in determining the levels of film achieved at different temperatures.

The higher film levels during the cold period should be considered in the light of several factors. The first is the behaviour of zoogleal slimes in continuous culture. It has been shown that zoogleal slimes growing on a surface follow the classical pattern of bacterial growth described by Monod (1949), where, after an initial latent period, there is an acceleration growth phase, followed by a period of exponential growth. However, as the thickness of the film increases, the lower layers are deprived of oxygen and anaerobic bacteria develop which cause the attachment

of the film to the medium to be weakened and eventually the film breaks up (Sanders 1966, Bridge Cooke and Hirsch 1958). This accounts for the cycles of accumulation and sloughing experienced at 20°C in the absence of flies. At lower temperatures the activity of the anaerobic bacteria was suppressed allowing larger quantities of film to accumulate.

Another factor is suggested by the frequent observation (Renn 1956, Bridge Cooke 1959, Eckenfelder 1961) that purification of sewage consists of two processes, namely the adsorption of dissolved and suspended matter on to the surface of the film by physical and biophysical processes, which takes place very rapidly with the passage of the sewage over the medium and the subsequent biochemical oxidation of the organic material which is spread over a longer period of time. The effect of a sharp fall in temperature would be to reduce the metabolic activity of the film and hence delay the rate of biochemical oxidation. However, the process of biophysical adsorption would probably not be so severely affected and therefore organic material would be removed from the sewage at a greater rate than it was oxidised, causing accumulation of solid matter. This is discussed later when corresponding respiration and B.O.D. removal rates are compared. The importance of self-oxidation as a factor controlling film accumulation has seldom been investigated but Heukelekian (1945a) mentions it as being of

significance.

In accounting for the greater film accumulation at lower temperatures, a third factor which must be considered is that, in the absence of macroinvertebrates such as fly larvae and enchytraeid worms, their grazing activity may to a limited extent be replaced by that of microinvertebrates such as nematodes, rotifers and protozoa. These organisms may reduce the quantity of film at higher temperatures, both by direct consumption and by mechanically loosening the accumulated material. Hence, the suppression of micrograzers at low temperatures in the experimental filters probably contributed to the greater film accumulation, though the effect of their grazing activity was very much less than that of macrograzers in an operational bed, whose suppression in winter is normally assumed to be the main cause of film accumulation.

The presence of flies in Experiment B outweighed all other factors in the determination of the film levels. At 20°C the flies were able to breed actively and the film was maintained at a consistently low level. The other factors, previously discussed, which were involved in the control of film in the absence of flies did not arise here. However, at 5°C, the grazing activity of the flies appeared to be suppressed altogether and the film accumulation was probably governed by the same factors as in Experiment A. It would have been of interest to have used intermediate temperatures between 20°C and 5°C to determine at what level the rate of

accumulation of the film and its consumption by the flies were in equilibrium. Although the flies introduced into the filters were not identified individually, they appeared to be mostly Psychoda alternata, since this species has a relatively high threshold temperature for development, while P. severini can continue to breed at temperatures down to 3°C (Lloyd 1937).

The difficulty in getting flies re-established in the filters of room R after the end of the cold period of Experiment B is hard to explain. While flies survived in R2 several reintroductions were necessary before a breeding population developed in R1 and they failed completely to become established in R3. Reynoldson (1939a) reported difficulty in getting enchytraeid worms to develop in an already overloaded bed, but it seems unlikely that excessive film prevented the development of the flies since at the end of Experiment A there was more film present in some filters than was present in R3 at the end of the cold period and little difficulty was encountered in getting the flies established at the beginning of Experiment B.

The efficiency of purification as determined by percentage B.O.D. removal, tended to be somewhat higher during Experiment B than Experiment A both at 20°C and 5°C. This raises the question of whether the presence of macroinvertebrates and consequent lower level of film improves the rate of purification. Lloyd (1945)

expressed the opinion that while excessive film accumulation is detrimental to efficiency, the film might be so severely reduced by grazing that it was unable to produce a satisfactory effluent. This however has not been supported by subsequent research and the present work confirmed other reports that only very thin films of bacteria are necessary to produce the highest levels of efficiency (H.M.S.O. 1958, Wuhrmann 1963). The slightly lower efficiency in Experiment A was partly due to the intermittent build-up and sloughing of the film causing suspended matter to appear in the effluent, giving a high B.O.D. value and consequent low efficiency. Apart from this, reasonably steady levels of efficiency were achieved over a wide range of film thickness. Sanders (1966), however, has suggested that in theory the efficiency of a zoogleal film is impaired with increased thickness, since only the surface layer of the film is actively respiring, and lower layers consist of accumulated organic matter which replaces the organic matter in the sewage as the metabolic substrate. Hawkes (1961) has mentioned that the quality rather than the quantity of film is important in the efficiency of filters.

Hawkes also reported an experiment in which the efficiency of an operational filter was shown to decrease in winter where film accumulation took place, but in a similar filter where the accumulation of film was controlled by reducing the frequency of

dosing, no drop in efficiency occurred. This might appear to suggest that film accumulation is the only factor involved in efficiency and that temperature is unimportant. However, the temperatures encountered in Hawkes' work were less extreme than those used in the present experimental work, where low temperatures were seen to reduce the efficiency directly. Also, it is recalled that the purification of effluents obeys Velz' Law (Velz 1948) which states that organic matter removed per unit depth of a bed is proportional to the remaining concentration. The experimental filters were only one quarter of the depth of a normal bed and therefore the different rates of purification that took place in these filters at different temperatures would become less marked over the full depth of a bed.

As has been stated in the results, the carbon dioxide output from the filters at a given temperature was related to both the quantity of film present and to the absolute rate of B.O.D. removal which in turn depended on the strength of the applied sewage. In a series of graphs a factor given by the carbon dioxide output per unit weight of film was plotted against the B.O.D. removal. (Figs. 20c and d, 26c and d). Since the film levels were much lower in Experiment B than Experiment A, while the carbon dioxide output was only slightly lower, the value of this factor was considerably greater in Experiment B, especially at

20°C. It should also be noted that at low levels of film a greater proportion of the weight was accounted for by liquid retained within the interstitial spaces of the medium thus tending to emphasise this difference still further. When these measurements were plotted in the same way but using the square roots of the film levels and B.O.D. removal rates (Figs. 21 and 27), the regression lines showed a greater degree of linear significance and there was a much greater similarity between the lines obtained in Experiment A and in Experiment B.

The factor given by the carbon dioxide output per B.O.D. removed, used as one of the co-ordinates in Figs. 20a and b and 26a and b, was found to be considerably higher in Experiment A than in Experiment B. This was caused by the higher output of carbon dioxide in Experiment A associated with higher levels of film and a slightly lower rate of B.O.D. removal. The statistical comparison of this factor at 20°C and 5°C revealed that in both experiments a significantly higher value was obtained at 20°C. This indicates that at cold temperatures, the rate of B.O.D. removal is greater than would be expected from the rate of respiration, implying removal of the organic matter by biophysical adsorption on to the film. This is an important conclusion of the experimental work.

Carbon Balance. While the organic carbon estimations in

Experiment A were not adequate to provide a continuous estimate of efficiency, they were nevertheless sufficient to make a brief investigation of the carbon balance of the system at the two temperatures of 20°C and 5°C. In Table 7, corresponding readings for the organic and inorganic carbon levels of the feed and effluent and the simultaneous carbon dioxide output are recorded for a number of occasions at 20°C. The left hand side shows the "applied" carbon in mg/hr as organic and inorganic carbon in the feed. The inorganic carbon represents bicarbonates and dissolved carbon dioxide in the feed and was measured by displacement with dilute hydrochloric acid and absorption in barium hydroxide. The organic carbon is the difference between the total carbon and the inorganic carbon. The procedure for measuring total carbon has been described in the Methods. On the right hand side of the table the "recovered" carbon is shown as organic and inorganic carbon in the effluent and carbon dioxide in the exhaust air. The carbon dioxide is recorded as mg carbon evolved per hour and hence the results are proportional to, but not equal to, the figures in Appendix Table 5, where the respiration of the filters during Experiment A was recorded as mg carbon dioxide per hour.

In Table 8, the carbon balance at 5°C is recorded in a similar manner to Table 7. In both tables, the difference between the applied and recovered carbon in each experiment is given in the

TABLE 7.

Carbon balance. Experiment A 20°C 19/1/66 to 17/6/66.

	Applied Carbon (mg/hr)			"Recovered" Carbon (mg/hr)				Diff. (A-R)
	Feed			Effluent				
	Org C	Inorg C	Total	Org C	Inorg C	C as CO_2	Total	
L1	49.28	6.27	55.55	8.51	5.38	16.11	30.00	+25.55
L2	29.57	16.58	46.14	15.23	5.82	18.53	39.59	+ 6.55
R2	29.57	16.58	46.14	4.48	11.20	19.40	35.08	+11.09
L3	51.52	9.86	61.38	4.48	9.41	14.80	28.69	+32.69
R3	51.52	9.86	61.38	17.92	11.20	15.84	44.96	+16.42
L1	21.50	12.99	34.50	4.93	6.72	15.16	26.81	+ 7.69
L2	12.99	10.30	23.30	7.62	11.65	8.09	27.35	- 4.05
L3	21.06	9.86	30.91	4.93	10.30	11.43	26.66	+ 4.25
L1	17.92	4.48	22.40	6.27	4.48	12.91	23.66	- 1.26
L2	15.23	7.62	22.85	5.38	6.72	8.09	20.19	+ 2.66
L2	18.37	12.54	30.91	12.99	7.62	14.44	35.05	- 4.14
L1	38.53	8,96	47.49	11.20	10.30	18.93	40.43	+ 7.06
L2	30.91	12.54	43.46	12.10	10.30	15.89	38.29	+ 5.17
L1	25.54	14.34	39.87	8.96	9.86	19.40	38.22	+ 1.65
R1	25.54	14.34	39.87	10.30	14.34	17.55	42.19	- 2.32
L2	30.02	11.65	41.66	11.65	7.62	21.58	40.84	+ 0.82
R2	30.02	11.65	41.66	14.34	10.75	15.52	40.61	+ 1.05
L3	34.50	13.44	47.93	8.96	8.96	28.62	46.54	+ 1.39
R3	34.50	13.44	47.93	17.47	14.34	13.71	45.52	+ 2.41

TABLE 8.

Carbon balance. Experiment A 5°C 9/2/66 to 5/5/66.

	Applied Carbon (mg/hr)			"Recovered" Carbon (mg/hr)				Diff. (A-R)
	Feed			Effluent				
	Org C	InorgC	Total	Org C	Inorg C	C as CO2	Total	
R1	21.51	12.99	34.50	9.86	11.20	5.19	26.25	+ 8.25
R2	12.99	10.30	23.30	13.89	8.96	5.51	28.36	- 5.06
R1	17.92	4.48	22.40	8.51	5.38	4.83	18.72	+ 3.68
R2	15.23	7.62	22.85	4.48	6.72	5.15	16.35	+ 6.50
R1	17.47	13.89	31.36	15.68	11.65	8.52	35.84	- 4.48
R1	23.30	9.41	32.70	12.54	11.65	8.89	33.08	- 0.38
R2	18.37	12.54	30.91	14.78	10.75	8.45	33.99	- 3.08
R3	17.47	11.65	29.12	6.27	15.68	7.52	29.47	- 0.35
R1	38.53	8.96	47.49	23.30	9.86	7.43	40.58	+ 6.91
R2	30.91	12.54	43.46	24.64	10.30	9.17	44.11	- 0.65

column on the extreme right. A positive figure indicated that more carbon had been applied than recovered during the period of observation. This difference might be accounted for by the rate of removal of organic carbon from the feed by absorption and synthesis exceeding the overall rate of respiration of the system, or by the loss of carbon as humus. An attempt was made to determine the quantity of carbon that was represented by the humus discharged. It was found that 50 mg dry weight of humus contained about 15 mg of carbon. The dry weight of humus discharged from the filters normally averaged less than 0.5 gm. per week, equivalent to 150 mg carbon. Although, as has been mentioned, the measurements of humus may at times have been inaccurate owing to the loss of humus in the effluent, it appears that, except during periods of heavy unloading, only a relatively small proportion of the applied carbon was accounted for by this factor.

A negative difference between the applied and recovered carbon indicated that carbon was being lost at a higher rate that it was being applied, presumably by the respiration of previously synthesized material or by the loss of such material in the effluent during the sloughing of the film.

With one or two exceptions the differences between the applied and recovered carbon at 20°C were remarkably small. The nineteen readings showed an average positive difference of 6.04 mg/hr. At

5°C, the difference was even smaller; in ten measurements, the applied carbon exceeding the removed by an average of 1.34 mg/hr. The larger amount of unrecovered carbon at 20°C than at 5°C was contrary to what would have been expected from the greater accumulation of film that took place at low temperatures. There appeared to be no correlation between the unrecovered carbon and the rate of film accumulation either in the individual readings or in the overall results at the two different temperatures.

It is of interest to compare these results with those of Wilson and McLachlan (1940) for a similar experiment. They found that the "recovered" carbon occurred as inorganic and organic carbon in the effluent and carbon dioxide in the exhaust air in roughly similar proportions to those of the current experimental work. However, a variable proportion of applied carbon averaging about 30% was unaccounted for and therefore presumably retained as film or discharged as humus. However, the report does not define the conditions of the filters or indicate the circumstances of the experiment in sufficient detail to draw any conclusions from the comparison.

The Pattern of Carbon Dioxide Output. It has been mentioned that carbon dioxide was not evolved from the filters at a uniform rate, but that the concentration of the gas in the exhaust air showed regular fluctuations, each consisting of a rapid surge

followed by a more gradual decline. At any given period their amplitude and shape were reasonably constant but with different temperatures and different strengths of the feed, there were considerable variations.

The cause of these fluctuations was investigated in a series of experiments. First, it was noticed that their period coincided with the period of dosing, which was kept constant at ten minutes during Experiments A and B. Fig. 28 shows the effect of changing the dosing period to twenty minutes, without altering the overall rate of dosing. As expected, the period of the fluctuations changed correspondingly and their amplitude was greater, but the average level of carbon dioxide output was unchanged.

It appeared from this and further observations that the sudden surge in carbon dioxide output coincided with the actual dosing of the feed onto the medium and the more gradual decline with the interval between successive periods of dosing. There were two possible explanations for this pattern. The first was that organic matter in the sewage was being spontaneously oxidised as soon as it came in contact with the film and the second was that carbon dioxide dissolved in the sewage was released by the air stream as the feed entered the filter.

A number of simple observations and experiments were made to determine which of these two processes was the cause of the

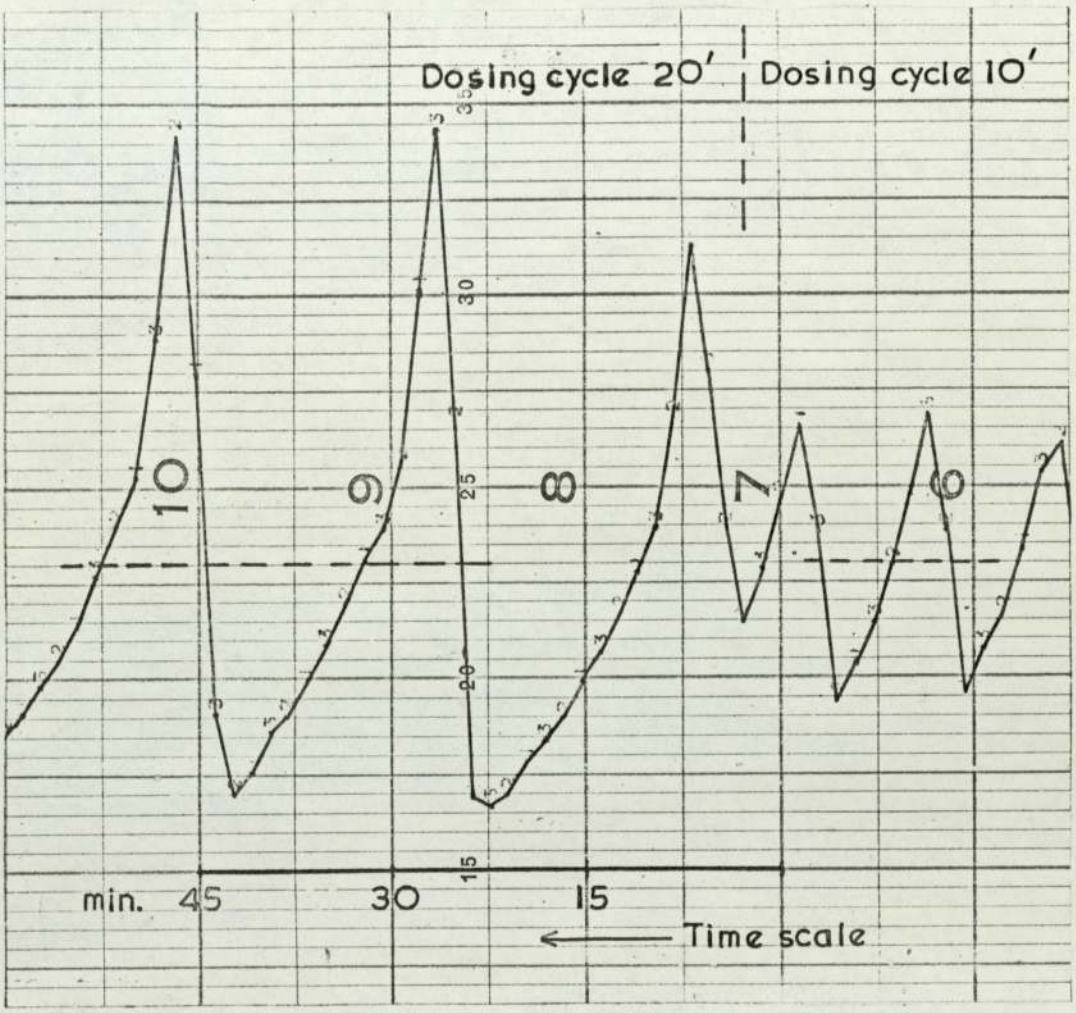


Figure 28. Effect on carbon dioxide output of changing dosing period from 10 to 20 minutes; overall sewage flow rate constant. (Filter at 20°C)

"surging" effect. It was noticed from measurements made during the experimental work that the height of these surges differed considerably when the same sewage was applied to filters at different temperatures. Figure 29 shows that the filter at 5°C, besides giving a lower overall rate of respiration, showed fluctuations of much smaller amplitude than the filter at 20°C in spite of being dosed with a similar sewage at the same rate. This may be partly accounted for by the lower solubility of carbon dioxide with increased temperature, thus allowing a greater quantity of the gas to be displaced in the airstream at this temperature than in the cold filter. However, it seems unlikely that this effect would account for the whole difference and it is probable that the surge was partly caused by spontaneous oxidation of organic matter, the level of which was reduced at lower temperatures.

In another experiment (Fig. 30), a filter was operated at 20°C at the usual rate of dosing. During the course of the run, the lid of the filter, and thus the three-arm sewage distributor, was rotated through 60°, causing the feed to be applied at three different points of the surface of the medium. The immediate effect was a sharp rise in the height of the surges, but they returned to normal during the course of the next few periods of dosing. The sudden increase was clearly caused by the direct respiration of the new part of the film on the application of the

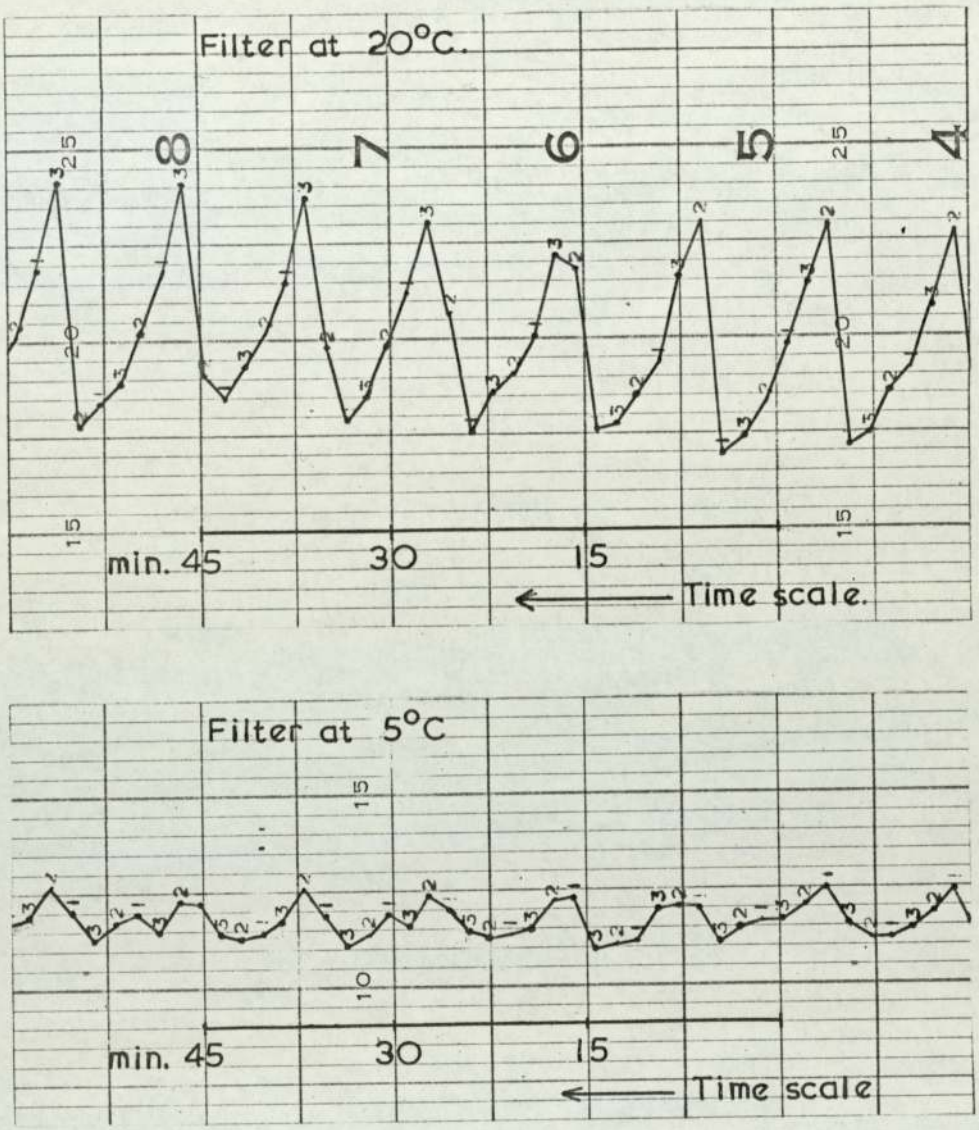


Figure 29. Effect on carbon dioxide output of dosing the same sewage onto two filters at different temperatures. (Examples are filter L2 at 20°C and filter R2 at 5°C. B.O.D. of sewage = 150.7 p.p.m.)

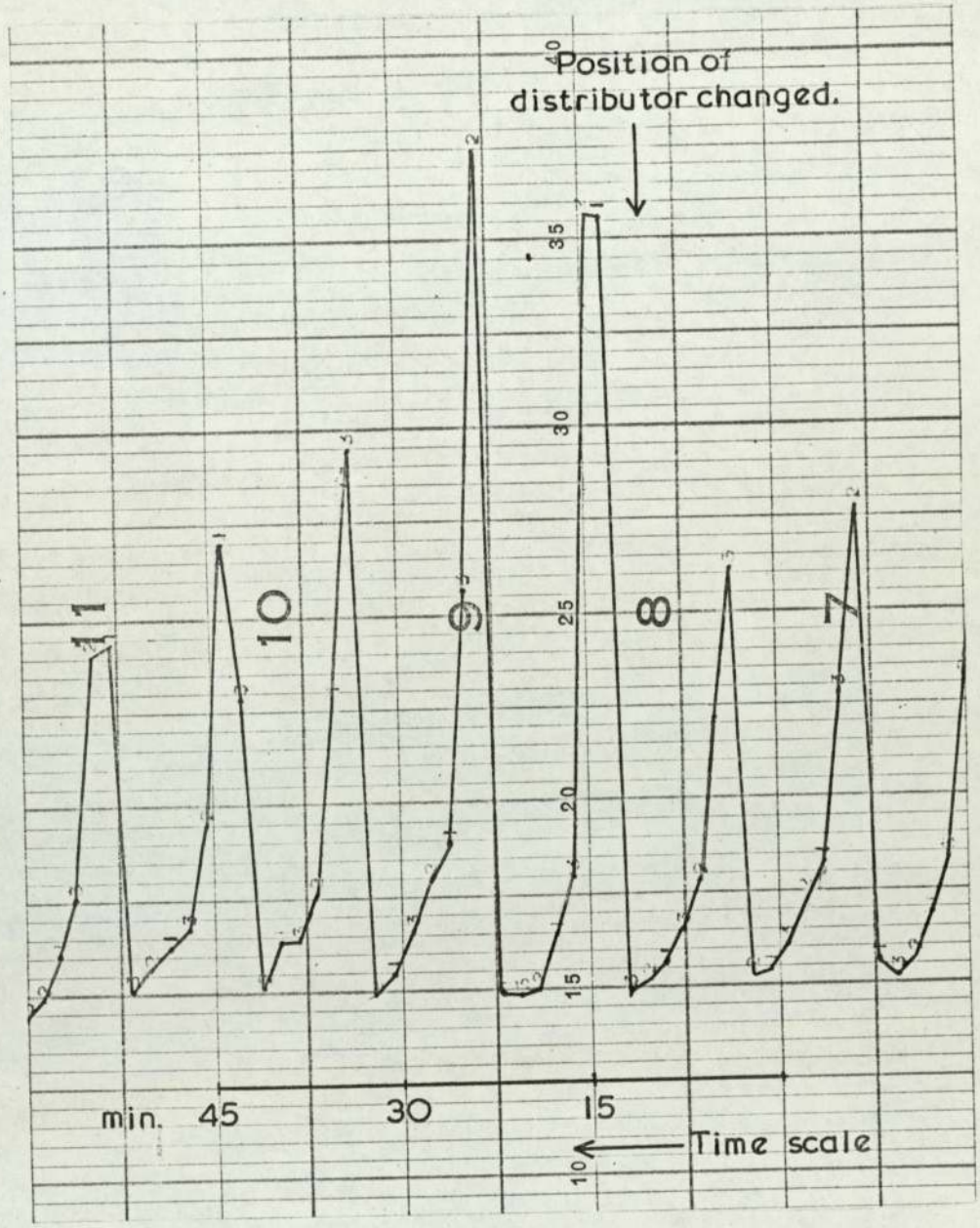


Figure 30. Effect on carbon dioxide output of changing position of distributor, thus dosing new part of the film. (Filter at 20°C).

feed.

In another experiment a solution of synthetic sewage was prepared from the following formula supplied by the Water Pollution Research Laboratory, Stevenage.

Nutrient Broth (Oxoid)	120 mg/litre
Dextrin	150 "
Glucose	100 "
Soluble Starch	100 "
Ammonium Chloride	130 "
Potassium Sulphate	8.3 "
Detergent Dobane JNX	60 "

The solution contained no possible source of inorganic carbon and was sterilized by boiling to prevent the development of any organisms which might form carbon dioxide by respiration. The dosing tubes and feed container were also sterilized and the absence of inorganic carbon confirmed by analysis. The carbon dioxide output from a filter dosed with this solution at 20°C is shown in Fig. 31. While the height of the surge is considerably smaller than usually occurred with normal sewage at this temperature, its presence confirms previous observations that this phenomenon is partly due to the spontaneous oxidation of organic matter. This does not alter the hypothesis, discussed earlier, that the rapid removal of organic matter from sewage during the relatively short

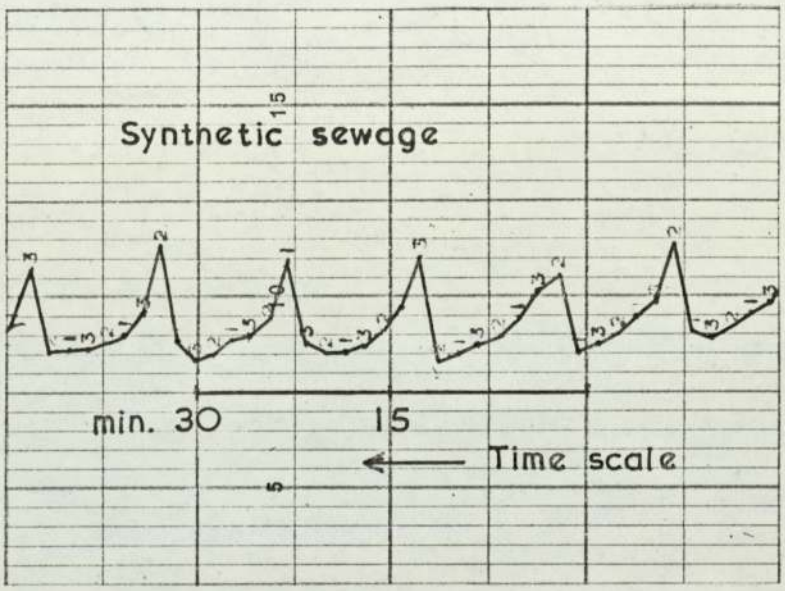


Figure 31. Pattern of carbon dioxide output at 20°C using sterile synthetic sewage, free from dissolved CO2 and inorganic carbon.

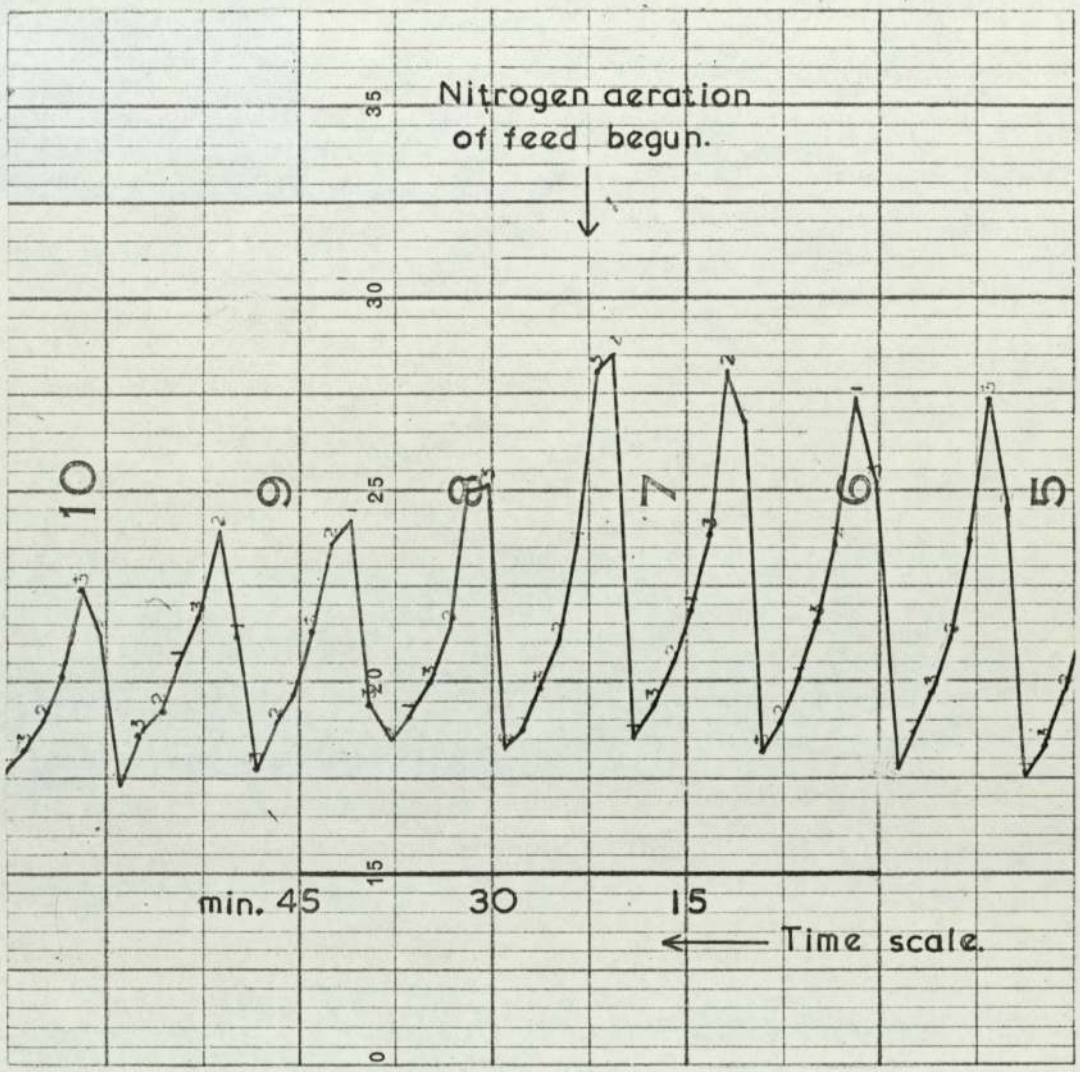


Figure 32. Effect of aerating feed with nitrogen gas on carbon dioxide output of filter at 20°C.

period of contact with the film is principally due to the biophysical adsorption of dissolved and suspended matter on to the film and that immediate oxidation only accounts for a small part.

Against these observations must be set certain experimental evidence that the surges are partly due to the release of dissolved carbon dioxide from solution. The results of one experiment are shown in Fig 32. A filter at 20°C was dosed with normal sewage at the usual rate. After a certain period, nitrogen gas was bubbled through the feed to displace the dissolved carbon dioxide without influencing the organic nature of the sewage. The amplitude of the surges was seen to decrease steadily during the period of aeration. This showed clearly that they were partly caused by the release of dissolved carbon dioxide, but the size of this proportion was not determined since it could not be assumed that the nitrogen aeration had completely removed all the dissolved CO2.

The overall conclusion of these experiments was that both dissolved and metabolic carbon dioxide showed increases coinciding with the dosing of the filter. The relative proportion of each was not determined and probably varied under different operational conditions.

SYNTHESIS OF RESULTS FROM FIELD AND LABORATORY WORK.

Although the operational conditions of the laboratory filters were as far as possible based on the Langley Mill bacteria beds, the scope for direct comparison of the results from these two parts of the experimental work is somewhat limited. While the rates of dosage in terms of quantity of sewage applied per cubic volume of bed medium were the same in both systems, differences in other factors such as the dosing frequency, ventilation and nature of the medium inevitably affected the criteria being studied.

The results at Langley Mill concerned the purification rates and the distribution of film and macroinvertebrates in two beds being operated at different dosing frequencies. The dosing periods were about fifteen minutes and less than one minute respectively. The dosing period in the laboratory experiments was kept constant at ten minutes but the mechanical effect of the applied sewage was inevitably much less than with the gravity-fed distributors of the operational beds.

The change in temperature from 20°C to 5°C over a period of three days in the experimental filters was not, of course, comparable to the seasonal fall in temperature in the Langley Mill beds, but it was deliberately decided to make this sharp change so that the effect of a lower temperature on the performance of the filters would rapidly become apparent and not be masked by other factors.

While the Langley Mill beds contained a mixed population of macro-invertebrates which showed considerable variations in the relative proportions of different species both with seasonal temperature changes and at the different dosing frequencies, it was decided to use Psychoda only in the laboratory filters in investigating the effect of a grazing population. It was not possible to estimate the numbers of macro-invertebrates present in the laboratory filters and therefore the outcome of interspecific competition could not have been studied if a mixed population had been introduced . The Psychoda populations bred satisfactorily at 20°C and kept the film at a low level, while at 5°C their development was suppressed and film accumulation took place. It is possible that if other species with lower temperature thresholds had been present, grazing activity could have continued at this low temperature and film accumulation would have been less marked.

The film levels in the Langley Mill beds were measured as grams of volatile matter, whilst those in the experimental filters were measured as grams wet weight. At the end of the laboratory work the film remaining in each filter was washed from the medium, and an estimate was made of the volatile matter, so that some comparison could be made between the film levels in the two systems. It was found that a film level of 200 gm. wet weight gave a reading of about 4.5 gm. volatile matter. The experimental filters corresponded to the top quarter of an operational bed, and the film readings can therefore be compared with those

from the surface samples of the Langley Mill beds. It is seen from Fig. 5(a) that in winter the film levels in Bed A reached about 20 gm. volatile matter per 0.1 cu. ft., while the corresponding levels in Bed D did not exceed 10 gm. Fig. 18(a) shows that in the absence of flies, the maximum film levels in the filters of room R during the cold period averaged less than 400 gm. wet weight, which corresponded to about 10 gm. volatile matter per 0.1 cu. ft. according to the above estimate. In the presence of flies, the maximum average film level during the cold period was only slightly over 200 gm. wet weight. At 20°C, the film in the experimental filters with flies present maintained a steady level of around 100 gm. which was comparable to the figure of about 2 gm. volatile matter, that was experienced in both Langley Mill beds during summer.

Thus it appears that, in spite of more gradual changes in temperature, the winter accumulation of film in the Langley Mill beds was more rapid and proceeded to a greater extent than in the experimental filters. Differences in the operational procedure which might account for this have already been mentioned. The different methods of application of sewage to the medium of each system was one possible cause; another was that air was fed through the experimental filters at a rate probably many times greater than the ventilation due to mormal air movements in the operational beds. The nature of the medium probably influenced the degree of film accumulation as well. However, the

plastic spheres of the experimental filters were smaller than the stones of the operational beds and therefore exposed a larger surface area for colonization by bacteria. This might have been expected to favour larger growths of film in the laboratory filters, which was the reverse of what actually occured.

While the results from the experimental work did not account for the absolute levels of film encountered in the Langley Mill beds, they can be used to explain the actual causes of the seasonal incidence of film accumulation. The laboratory work showed that in the absence of macrograzers, the filters being operated at 5°C achieved a higher degree of film than those at 20°C. Possible explanations for this have been considered but the main cause was probably that the metabolic activity of the film was more severely affected by low temperatures than the corresponding rate of adsorption of organic matter on to the film, which has been shown to be the initial process in purification. It was also shown by the laboratory work that, whilst at 20°C the presence of macrograzers kept the film down to a very low level, their activity was suppressed at 5°C allowing the film to accumulate. This supports the general assumption that the ponding of bacteria beds in winter is due in some degree to the suppression of macroinvertebrates.

At Langley Mill the winter temperatures in the beds fell to very nearly 5°C . Although some species with a lower development threshold than this may have been present, there was undoubtedly a very considerable suppression of grazing activity, as in the experimental filters.

This is confirmed by the lower populations of macrograzers of all species during the coldest months of the year. Even those individuals that survived would show a much lower rate of metabolism.

It is concluded therefore that there is both a direct effect of temperature on the accumulation of film in winter and an indirect effect due to the suppression of the macrograzers. The sloughing of the film in the spring in an operational bed is undoubtedly due to the renewed grazing activity of the macroinvertebrates, as their populations recover. In the absence of grazers, there was some tendency for film levels to decrease when the temperature was returned to 20°C from 5°C, but this effect was rather indecisive.

It was not practicable to make any comparison between the purification rates of the two systems. Velz' law states that a constant proportion of the remaining oxidizable organic matter in the sewage is removed per interval of depth of a bed, and therefore any direct comparison between beds of different depths is unsatisfactory. Furthermore, it has been shown that different biochemical processes of purification take place at different levels. Nitrification, for example, appears to take place in the lower layers of a bacteria bed. A comparison between the rates of nitrification in systems of different depths would therefore be invalid.

CONCLUSIONS

The main conclusions of the research may be summarised as follows:-

1. Over a two year period of observation, the accumulation of film in winter in the surface layers of a bacteria bed treating domestic sewage at a high frequency of dosing was considerably greater than that of a similar bed operating at a lower frequency of dosing, but at a similar overall rate of loading. In the lower layers of the medium the difference in winter accumulation was less marked, but that of the high-frequency dosed bed was slightly greater for most of the winter period except at the very end, where the spring unloading occured earlier than in the bed dosed at low frequency.

This confirmed that lowering the frequency of dosing is of great significance in the control of excessive film accumulation.

2. Maximum populations of macroinvertebrates occured in spring, coinciding with the rapid unloading of the accumulated film. Macro-grazers were present only in very small numbers during the coldest months of the winter. This was circumstantial evidence that film accumulation in winter is caused by the suppression of grazing activity and that spring unloading is due to the increased populations of macro-grazers with higher temperatures.

There were considerable differences in the composition of the macrofauna in the two beds, the most significant feature being the much smaller numbers of Psychoda in the low-frequency dosed bed, particularly in the surface layer. This was compensated for by an increase in the numbers of other species, notably oligochaete worms of the family

Enchytraeidae. In the low-frequency dosed beds there were also small numbers of Chironomidae mostly near the surface, apparently favoured by their ability to withstand the greater scouring effect of the sewage.

3. Over the period of observation there was little difference in the level of B.O.D. removal between the two beds, except for about four months during the first summer period when the efficiency of the low-frequency dosed bed was somewhat inferior. It was therefore concluded that, provided conditions of ponding are avoided, larger quantities of film in a filter do not adversely affect the level of B.O.D. removal.

The level of nitrification was, with occasional exceptions, somewhat greater in the low- frequency dosed bed. This difference tended to be more marked in winter. It appeared therefore that nitrification was favoured by smaller quantities of film in the bed.

4. Experiments using laboratory filters showed that both in the presence and in the absence of macrograzers, greater film accumulation took place at 5°C than at 20°C over a prolonged period. This demonstrated that, while the suppression of grazers in winter is an important factor in causing the accumulation of film, there is also a direct influence of temperature which promotes the same effect independently of the macrograzers. The differential effect of temperature on the removal of organic matter from sewage and its oxidation is one possible explanation. Another is that an anaerobic region is formed at the base of a layer of film causing its attachment to the medium to be weakened. This effect would be reduced at low temperatures, allowing greater quantities of film to accumulate.

On returning the temperature of the laboratory filters to 20°C after a period at 5°C, sloughing of the film was found to be much more rapid and complete in the presence of macrograzers than in their absence. Grazing activity of macroinvertebrates is therefore the probable cause of the spring unloading in operational beds treating domestic sewage.

5. The rate of carbon dioxide output from the laboratory filters was found to be influenced not only by temperature, but also by the quantity of film present and the strength of the applied sewage. The rate of respiration was found not to be proportional to the degree of B.O.D. removal at different temperatures. This indicated that some of the purification was not due to spontaneous biochemical oxidation of the sewage, but to the biophysical adsorption of organic matter on to the film followed by its oxidation which was not necessarily proceeding at the same rate. When the temperature of the filters was lowered from 20°C to 5°C, the carbon dioxide output fell by a greater proportion than the corresponding B.O.D. removal rate, showing that the biophysical adsorption was less affected by temperature than the respiration.

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APPENDIX

TABLE 1.

Bed A. Incidence of film and macrofauna in surface layers (gms. and numbers per 0.1 cu. ft.)

		Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Oct.64	1.u.	2950	0	0	1625	13.44
	1.	750	0	0	1450	7.73
	2.u.	2350	0	0	1675	9.00
	1.	-	0	0	2000	3.75
	3.u.	800	0	0	3850	5.31
	1.	1400	0	0	2700	4.68
	Av.	1650	0	0	2216	7.32
Nov.64	1.u.	1050	0	0	1000	14.81
	1.	1675	15	0	1025	7.83
	2.u.	2575	0	0	2125	11.76
	1.	1050	0	0	1400	4.93
	3.u.	850	0	0	2250	12.78
	1.	1625	0	0	1150	3.63
	Av.	1471	2	0	1491	9.29
Dec.64	1.u.	2425	0	0	2000	16.79
	1.	3025	0	0	3475	6.66
	2.u.	2775	0	0	2900	8.80
	1.	2625	0	0	1675	3.32
	3.u.	3400	0	0	1925	5.01
	1.	5125	0	0	1700	3.63
	Av.	3229	0	0	2279	7.37

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jan.65 1.u.	1200	0	0	1400	17.65
1.	2275	0	0	3125	15.77
2.u.	1525	0	0	700	22.55
1.	950	0	0	1075	15.10
3.u.	1250	0	0	1550	18.21
1.	2125	0	0	1875	16.48
Av.	1554	0	0	1621	17.63
Feb.65 1.u.	2675	75	0	1525	27.15
1.	4750	25	0	1175	14.46
2.u.	1375	0	0	375	23.51
1.	2600	0	0	2175	13.46
3.u.	625	0	50	1250	26.47
1.	1150	0	0	2425	26.64
Av.	2196	17	8	1487	21.95
Mar.65 1.u.	300	1225	0	1475	10.67
1.	1075	3475	0	2325	3.64
2.u.	300	55	0	2200	23.95
1.	1725	0	0	5550	16.99
3.u.	850	0	0	1925	18.13
1.	975	0	0	1725	14.66
Av.	871	792	0	2533	14.68

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Apr.65 1.u.	20	0	0	4875	28.63
1.	725	0	0	4650	8.23
2.u.	75	0	0	2800	26.04
1.	925	0	0	5175	19.15
3.u.	550	0	25	4675	24.09
1.	300	0	0	3500	21.21
Av.	429	0	4	4279	21.22
May 65 1.u.	25	0	50	17150	12.14
1.	400	0	0	5075	4.73
2.u.	300	0	0	30375	21.21
1.	1050	5	0	9775	3.84
3.u.	25	0	0	32875	15.85
1.	575	0	0	18500	21.59
Av.	396	1	8	18958	13.23
Jun.65 1.u.	25	850	275	3100	1.27
1.	-	2025	0	4800	-
2.u.	550	150	750	8225	1.84
1.	4650	0	100	5950	2.49
3.u.	300	0	1975	5450	2.58
1.	925	0	175	9000	0.05
Av.	1075	504	546	6087	1.37

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
July 65 1.u.	200	0	0	2350	1.07
1.	1125	0	0	6100	1.05
2.u.	425	0	150	7650	2.47
1.	250	0	50	6200	1.26
3.u.	3750	0	550	3250	0.39
1.	1750	40	100	3850	0.62
Av.	1250	7	142	4900	1.14
Aug.65 1.u.	2375	0	0	2850	2.61
1.	3850	0	0	7050	3.58
2.u.	1650	0	0	4825	3.15
1.	1375	0	0	5500	1.74
3.u.	1275	0	0	4600	2.16
1.	700	0	0	1115	2.54
Av.	1871	0	0	4323	2.63
Sept 65 1.u.	11850	0	0	900	3.46
1.	2825	0	0	1750	3.03
2.u.	3175	0	0	2550	4.60
1.	4050	0	50	2650	0.00
3.u.	8750	0	0	3325	1.66
1.	4200	0	75	5575	1.76
Av.	5808	0	21	2791	2.42

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Oct.65 1.u.	10120	0	0	2675	6.32
1.	2800	0	0	3075	4.02
2.u.	8675	0	0	2650	4.40
1.	5125	0	0	-	2.86
3.u.	5750	0	0	3750	4.32
1.	4500	0	0	4725	2.00
Av.	6161	0	0	3375	3.99
Nov.65 1.u.	9725	0	0	1175	9.06
1.	7975	0	0	1750	7.30
2.u.	10475	0	0	1675	8.82
1.	6450	0	0	1475	3.21
3.u.	1950	0	0	1400	9.59
1.	3925	0	0	1050	5.00
Av.	6750	0	0	1421	7.16
Dec.65 1.u.	11,875	0	150	500	22.18
1.	5475	0	25	400	18.49
2.u.	6025	0	25	550	19.05
1.	3500	0	75	750	12.97
3.u.	2600	0	375	1050	17.71
1.	7025	0	300	1225	9.86
Av.	6083	0	158	746	16.71

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jan.66 1.u.	800	100	250	-	24.05
1.	2900	50	0	-	12.82
2.u.	800	0	400	575	28.06
1.	2200	0	25	2700	22.11
3.u.	1700	25	375	800	21.40
1.	2425	0	100	1800	22.50
Av.	1806	29	191	1469	21.82
Feb.66 1.u.	1425	0	0	175	27.36
1.	3575	0	100	4025	11.52
2.u.	1275	0	250	650	29.18
1.	1325	0	0	1625	22.24
3.u.	425	0	25	875	26.63
1.	1350	0	25	1675	25.47
Av.	1563	0	67	1621	23.73
Mar.66 1.u.	325	0	0	2225	14.01
1.	725	0	0	1975	11.36
2.u.	275	0	0	1900	32.24
1.	225	0	0	8725	18.26
3.u.	225	0	0	1325	33.11
1.	400	0	0	4850	19.95
Av.	363	0	0	3483	21.49

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Apr.66 l.u.	100	0	0	2300	28.19
l.	525	0	0	5925	16.97
2.u.	150	0	0	8350	29.51
l.	250	0	0	8775	17.13
3.u.	225	0	0	5400	25.11
l.	950	0	0	7000	22.33
Av.	366	0	0	6291	23.21
May 66 l.u.	150	0	0	12075	18.97
l.	1100	0	0	7400	3.20
2.u.	325	0	25	13775	14.36
l.	2950	0	0	9975	0.59
3.u.	150	0	0	9025	23.47
l.	150	0	0	10300	11.13
Av.	804	0	4	10425	11.95
Jun.66 l.u.	725	0	375	11250	1.04
l.	5325	0	100	14500	0.02
2.u.	2725	0	300	4950	1.32
l.	2225	0	75	5000	2.67
3.u.	4800	0	125	2150	2.56
l.	6525	0	75	3950	2.11
Av.	3721	0	175	6966	1.62

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jul.66 1.u.	3200	0	100	4175	1.37
1.	6325	0	75	9050	1.56
2.u.	1475	0	100	4800	1.57
1.	3425	0	0	8200	1.60
3.u.	900	0	0	3325	1.22
1.	2675	0	0	4950	1.31
Av.	3000	0	46	5750	1.44
Aug.66 1.u.	1125	0	100	2175	1.95
1.	2175	0	0	7625	2.73
2.u.	300	0	200	5075	3.08
1.	1100	0	25	4425	2.59
3.u.	2000	0	50	4925	3.74
1.	2600	0	0	6975	1.60
Av.	1550	0	63	5200	2.62
Sept,66 1.u.	12775	0	0	3150	5.79
1.	1600	0	0	2975	2.32
2.u.	6275	0	0	3125	2.72
1.	2725	0	0	4875	2.91
3.u.	1025	0	0	3425	3.89
1.	1150	0	0	3800	3.31
Av.	4258	0	0	3558	3.32

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Oct.66 1.u.	10050	0	0	3375	13.12
1.	4175	0	0	5100	5.16
2.u.	7400	0	0	2350	9.89
1.	3000	0	0	3875	3.39
3.u.	2675	0	0	2650	8.07
1.	1850	0	0	5125	5.23
Av.	4858	0	0	3746	7.48
Nov.66 1.u.	11050	0	0	2375	13.47
1.	3125	0	0	3325	7.45
2.u.	10075	0	0	3175	4.85
1.	4200	0	0	1825	4.19
3.u.	4500	0	0	4925	6.32
1.	5150	0	0	5200	10.35
Av.	6350	0	0	3470	7.77
Dec.66 1.u.	7725	0	0	1450	14.96
1.	7625	0	0	2600	6.89
2.u.	13575	0	0	2025	3.47
1.	17025	0	0	1725	2.78
3.u.	3825	0	0	1875	10.66
1.	10850	0	0	2975	5.85
Av.	10104	0	0	2108	7.44

TABLE 1 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jan.67 1.u.	1950	0	0	650	23.14
1.	3450	0	0	1500	13.87
2.u.	4150	0	0	2650	22.36
1.	10100	0	0	5175	9.50
3.u.	1450	0	0	2250	17.24
1.	5800	0	0	5475	7.20
Av.	4483	0	0	2950	15.55
Feb.67 1.u.	950	0	0	950	2o.94
1.	1025	0	0	4650	10.17
2.u.	4075	0	0	1125	15.32
1.	6625	0	0	2200	6.00
3.u.	500	0	0	2075	19.74
1.	1700	0	0	10725	18.65
Av.	2479	0	0	3621	15.14

TABLE 2.

Bed D. Incidence of film and macrofauna in surface layers.
(gms and numbers per 0.1 cu. ft.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Oct.64 1.u.	300	0	0	2450	1.81
1.	550	0	0	1600	1.86
2.u.	575	0	0	1725	2.04
1.	125	0	0	1900	2.09
3.u.	0	0	0	950	2.19
1.	125	0	0	450	1.51
Av.	279	0	0	1512	1.92
Nov.64 1.u.	50	0	0	850	3.33
1.	100	0	0	600	3.12
2.u.	0	0	0	350	3.41
1.	50	0	0	950	2.52
3.u.	50	0	0	200	3.45
1.	50	0	0	500	3.03
Av.	50	0	0	575	3.14
Dec.64 1.u.	60	0	675	250	2.12
1.	40	0	225	1400	2.29
2.u.	20	0	145	425	3.29
1.	10	0	70	950	3.32
3.u.	30	0	100	600	1.66
1.	15	0	30	550	1.15
Av.	29	0	207	697	2.31

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jan.65 1.u.	20	0	240	105	2.90
1.	10	0	250	270	4.11
2.u.	10	0	200	275	5.14
1.	45	0	115	575	5.52
3.u.	15	0	125	430	7.55
1.	0	0	40	1050	4.21
Av.	16	0	161	451	4.90
Feb.65 1.u.	50	0	275	1275	9.03
1.	100	0	125	1250	9.11
2.u.	10	0	210	1425	6.27
1.	50	0	125	700	8.70
3.u.	0	0	285	575	8.05
1.	0	0	75	2150	4.00
Av.	35	0	182	1229	7.53
Mar.65 1.u.	25	0	20	265	7.71
1.	0	0	125	1650	10.62
2.u.	0	5	50	1050	5.67
1.	0	0	400	5500	9.30
3.u.	0	5	45	60	8.00
1.	0	0	95	65	12.43
Av.	4	2	122	1765	8.62

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Apr.65 1.u.	0	0	150	5400	11.61
1.	100	0	75	7900	11.70
2.u.	0	0	50	6150	8.42
1.	25	0	375	6375	9.81
3.u.	0	0	50	2025	9.78
1.	50	0	75	4100	7.21
Av.	29	0	128	5325	9.75
May 65 1.u.	25	0	200	6275	1.48
1.	75	0	150	13650	0.0
2.u.	-	-	-	-	-
1.	0	10	40	7125	1.33
3.u.	0	0	15	4325	3.28
1.	0	0	15	4525	6.98
Av.	20	2	84	7180	2.61
Jun.65 1.u.	0	0	1775	1350	0.46
1.	0	0	350	4100	0.39
2.u.	0	0	925	4325	0.93
1.	0	40	650	5750	0.43
3.u.	15	5	525	3100	1.71
1.	175	0	350	3400	1.13
Av.	31	7	762	3671	0.84

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jul.65 1.u.	0	0	150	3125	0.52
1.	0	0	75	4600	0.26
2.u.	0	0	75	700	1.74
1.	0	0	100	3950	0.30
3.u.	0	0	0	1750	1.79
1.	0	0	50	1950	1.17
Av.	0	0	75	2679	0.96
Aug.65 1.u.	90	0	20	1155	2.20
1.	40	0	15	2475	0.83
2.u.	20	0	100	2650	1.93
1.	50	0	15	1925	0.80
3.u.	0	0	5	1450	1.73
1.	35	0	25	1900	0.43
Av.	39	0	30	1926	1.32
Sept 65 1.u.	175	0	175	4100	1.60
1.	125	0	250	3000	0.76
2.u.	25	0	400	3275	1.80
1.	35	0	175	1975	2.86
3.u.	40	0	120	1175	1.85
1.	20	0	235	675	0.63
Av.	70	0	226	2366	1.58

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Oct.65 1.u.	50	0	250	2525	3.07
1.	150	0	550	2175	1.12
2.u.	25	0	275	1050	2.49
1.	30	0	190	450	1.83
3.u.	50	0	425	450	3.46
1.	10	0	250	95	1.24
Av.	52	0	323	1124	2.20
Nov.65 1.u.	125	0	475	375	3.31
1.	75	0	525	600	1.70
2.u.	75	0	1250	150	3.75
1.	100	0	550	450	1.55
3.u.	100	0	700	200	4.03
1.	25	0	775	650	2.39
Av.	83	0	712	406	2.79
Dec.65 1.u.	35	0	280	20	3.79
1.	10	0	500	250	2.61
2.u.	10	0	475	225	4.78
1.	20	0	450	475	2.77
3.u.	0	0	375	100	2.12
1.	5	0	350	250	1.05
Av.	13	0	405	220	2.85

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jan.66 1.u.	75	50	400	425	6.62
1.	75	0	750	350	5.58
2.u.	50	0	675	225	5.25
1.	75	25	1200	250	5.90
3.u.	0	0	250	1200	3.61
1.	50	0	25	925	2.72
Av.	54	12	550	563	4.95
Feb.66 1.u.	75	0	200	425	8.69
1.	25	0	975	875	8.09
2.u.	0	0	400	1900	7.87
1.	0	0	675	1675	13.28
3.u.	0	0	125	2675	6.07
1.	0	0	250	5150	6.77
Av.	16	0	437	2117	8.46
Mar.66 1.u.	0	0	75	3950	8.77
1.	0	0	200	3450	6.98
2.u.	0	0	225	3800	10.74
1.	0	0	50	2800	7.21
3.u.	0	0	200	1000	8.08
1.	0	0	150	6250	1.73
Av.	0	0	150	3542	7.25

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Apr.66 l.u.	25	0	100	14200	10.79
l.	550	0	0	15450	7.99
2.u.	25	0	0	9600	2.60
l.	300	0	50	7600	4.11
3.u.	125	0	0	6575	8.61
l.	225	0	0	11025	3.88
Av.	208	0	25	10741	6.32
May 66 l.u.	0	0	10	2625	2.47
l.	0	0	5	4575	0.96
2.u.	5	0	5	4950	3.19
l.	125	0	0	11250	13.72
3.u.	50	0	0	3050	3.14
l.	250	0	0	9125	6.17
Av.	79	0	3	5929	4.94
Jun.66 l.u.	5	0	195	6025	0.95
l.	70	0	85	14850	0.00
2.u.	0	0	875	4275	0.19
l.	50	0	260	6550	1.08
3.u.	10	0	50	2350	1.44
l.	45	0	45	4025	1.36
Av.	30	0	251	6346	0.84

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jul.66 1.u.	100	0	675	1050	1.69
1.	125	0	105	2575	0.88
2.u.	20	0	260	1750	1.84
1.	165	0	135	2550	1.20
3.u.	75	0	80	1100	2.11
1.	65	0	50	3125	0.97
Av.	91	0	217	2025	1.45
Aug.66 1.u.	75	0	450	650	1.58
1.	225	0	100	1525	0.87
2.u.	15	0	150	500	2.26
1.	0	0	250	775	1.55
3.u.	0	0	75	1300	1.57
1.	30	0	50	1925	0.79
Av.	58	0	179	1113	1.44
Sept.66 1.u.	55	0	0	1300	3.73
1.	375	0	0	2475	0.83
2.u.	100	0	350	1600	2.08
1.	275	0	75	2100	1.16
3.u.	25	0	0	1125	1.30
1.	175	0	0	550	2.13
Av.	168	0	71	1525	1.87

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Oct.66 1.u.	50	0	425	300	2.62
1.	50	0	275	1775	0.94
2.u.	0	0	275	1100	2.86
1.	0	0	100	900	1.74
3.u.	0	0	175	750	3.06
1.	0	0	700	325	2.31
Av.	17	0	325	858	2.26
Nov.66 1.u.	150	0	100	650	4.35
1.	200	0	25	375	2.97
2.u.	75	0	250	825	2.44
1.	175	0	125	875	1.79
3.u.	75	0	0	200	1.67
1.	50	0	0	200	1.51
Av.	121	0	83	521	2.45
Dec.66 1.u.	125	0	100	275	8.42
1.	100	0	150	925	3.26
2.u.	0	0	225	775	4.26
1.	0	0	25	400	3.56
3.u.	150	0	200	400	6.69
1.	25	0	75	75	5.73
Av.	67	0	129	475	5.32

TABLE 2 (Cont.)

	Psychodidae	Anisopus	Chironomidae	Enchytraeidae	Film
Jan.67 1.u.	50	0	300	1075	5.71
1.	25	0	75	1550	7.11
2.u.	0	5	200	1150	4.95
1.	0	0	50	1550	4.73
3.u.	0	0	50	1875	4.43
1.	25	0	50	2750	3.25
Av.	17	1	121	1658	5.03
Feb.67 1.u.	0	0	75	1200	7.07
1.	75	0	50	3675	6.23
2.u.	25	0	25	3925	5.61
1.	50	0	25	5075	6.81
3.u.	75	0	0	825	5.44
1.	200	0	0	3175	4.47
Av.	71	0	29	2979	5.94

TABLE 3

B.O.D. values of feed and effluents (with weekly averages)

Experiment A (flies absent)

Weeks ending 29/10/65 to 28/1/66 L = 20°C, R = 20°C									
Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
150.0	-	-	-		160.0				
210.0	125.0	125.0	111.6		214.5	107.1	111.6	107.1	
Av.180.0	125.0	125.0	116.0	120.5	187.2	107.1	111.6	107.1	108.6
165.0	49.0	68.7	74.2		106.5	64.2	52.2	76.7	
189.2	57.5	57.5	62.5		179.5	62.5	44.7	65.7	
Av.177.1	53.2	63.1	68.3	61.5	143.0	63.3	48.4	71.2	61.0
124.0	42.1	37.0	39.5		124.0	59.8	37.0	57.2	
181.2	44.3	63.2	56.9		196.0	54.4	53.1	60.6	
Av.152.6	43.2	50.1	48.2	47.2	160.1	57.1	45.0	58.9	53.7
243.0	84.8	>88.5	>88.5		293.0	>87.3	>89.8	>88.5	
199.2	78.7	84.9	79.9		164.3	>89.9	89.9	>92.4	
Av.221.1	81.7	>86.7	>84.5	>84.2	228.6	>88.6	> 89.9	>90.4	>89.6
191.5	88.6	104.6	76.8		191.5	-	92.2	104.6	
209.0	52.2	62.2	52.2		189.0	58.2	32.4	66.2	
Av.200.2	70.4	83.4	64.5	72.8	190.2	58.2	62.3	85.4	68.6
78.5	21.6	21.6	32.1		-	-	-	-	
-	-	-	-		73.5	24.0	25.3	20.0	
Av. 78.5	21.6	21.6	32.1	25.3	73.5	24.0	25.3	20.0	23.1

TABLE 3 (Cont.)

L = 20°C, R = 20°C

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
153.0	28.1	-	-		153.0	32.3	-	-	
106.3	-	24.0	-		106.3	-	27.0	-	
142.6	-	-	38.7		142.6	-	-	53.2	
Av.134.0	28.1	24.0	38.7	30.3	134.0	32.3	27.0	53.2	37.4
90.5	28.8	25.8	24.5		90.5	27.1	19.3	25.8	
143.7	44.7	48.7	42.1		127.9	43.4	48.7	34.2	
Av.117.1	36.6	37.7	33.3	35.7	109.2	35.2	34.0	30.0	33.1
-	-	-	-		(64.2	38.9	16.8	43.0)	
(195.7	66.8	69.5	49.5)		-	-	-	-	
Av.195.7	66.8	69.5	49.5	61.9	64.2	38.9	16.8	43.0	32.9
216.9	36.2	-	-		216.9	36.2			
149.8	-	28.4	-		149.8	-	25.8	-	
Av.183.3	36.2	28.4	-	32.3	183.3	36.2	25.8	-	31.0
294.4	>81.4	-	-		294.4	>82.7	-	-	
(>306.1	-	>71.4)			(>306.1	-	>73.9)		
196.9	-	-	>117.8		196.9	-	-	>113.6	
Av >265.8	>81.4	>71.4	>117.8	>90.2	>265.8	>82.7	>73.9	>113.6	>90.1
255.9	116.7	-	-		255.9	87.5	-	-	
264.1	-	83.3	-		264.1	-	>124.1	-	
323.9	-	-	89.1		323.9	-	-	85.2	
Av.281.3	116.7	83.3	89.1	96.4	281.3	87.5	>124.1	85.2	>98.9

TABLE 3 (Cont.)

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
L = 20°C, R = 20°C									
317.1	85.8	-	-		317.1	76.1	-	-	
275.3	-	41.6	-		275.3	-	88.3	-	
268.9	-	-	80.4		268.9	-	-	82.8	
Av.287.1	85.8	41.6	80.4	69.3	287.1	76.1	88.3	82.8	82.5
Week ending 4/2/67 Temp of room R changed to 5°C, L = 20°C									
161.3	45.5	-	-		15°c 161.3	50.3	-	-	
172.8	67.8	-	-		10°c 172.8	73.8	-	-	
374.5	85.1	-	-		5°c 374.5	125.2	-	-	
Av.236.2	66.1	-	-	66.1	236.2	83.1	-	-	83.1
Weeks ending 11/2/66 to 13/5/66 L = 20°C, R = 5°C									
115.4	52.3	-	-		115.4	65.7	-	-	
139.3	-	61.4	-		139.3	-	53.0	-	
125.0	-	-	34.0		125.0	-	-	56.8	
Av.126.6	52.3	61.4	34.0	49.2	126.6	65.7	53.0	56.8	58.8
187.2	31.7	-	-		187.2	68.9	-	-	
182.9	-	54.6	-		182.9	-	58.2	-	
166.2	-	-	31.4		166.2	-	-	72.6	
Av.178.7	31.7	54.6	31.4	39.2	178.7	68.9	58.2	72.6	66.6
97.4	21.1	-	-		97.4	46.5	-	-	
87.7	-	36.9	-		87.7	-	35.6	-	
93.1	-	-	37.3		93.1	-	-	37.3	
Av. 92.7	21.1	36.9	37.3	31.7	92.7	46.5	35.6	37.3	39.8

TABLE 3 (Cont.)

L = 20°C, R = 5°C									
Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
78.5	12.1	-	-		78.5	51.6	-	-	
103.2	-	66.8	-		103.2	-	40.1	-	
151.2	-	-	52.8		151.2	-	-	50.4	
Av.111.0	12.1	66.8	52.8	43.9	111.0	51.6	40.1	50.4	47.4
>388.6	-	37.3	-		>388.6	-	68.8	-	
126.9	-	-	37.2		126.9	-	-	>97.8	
Av>257.7	-	37.3	37.2	37.3	>257.7	-	68.8	>97.8	>83.3
150.2	26.9	-	-		150.2	77.7	-	-	
150.7	-	68.3	-		150.7	-	>103.5	-	
228.2	-	-	51.3		228.2	-	-	107.0	
Av.176.4	26.9	68.3	51.3	48.8	176.4	77.7	>103.5	107.0	>96.1
159.3	-	27.5	-		159.3	-	84.7	-	
144.4	-	-	25.9		144.4	-	-	69.9	
Av.151.8		27.5	25.9	26.7	151.8	-	84.7	69.9	77.3
139.7	11.2	-	-		139.7	55.7	-	-	
134.9	-	34.2	-		134.9	-	62.0	-	
126.2	-	-	30.0		126.2	-	-	82.0	
Av.133.6	11.2	34.2	30.0	25.1	133.6	55.7	62.0	82.0	66.6
148.8	72.1	-	-		148.8	77.5	-	-	
133.9	-	27.6	-		133.9	-	54.7	-	
114.1	-	-	15.3		114.1	-	-	78.3	
Av.132.3	72.1	27.6	15.3	38.3	132.3	77.5	54.7	78.3	70.2

TABLE 3 (Cont.)

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
$L = 20^{\circ}C$, $R = 5^{\circ}C$									
138.8	44.0	-	-		138.8	89.7	-	-	
171.9	-	52.6	-		171.9	-	73.9	-	
163.6	-	-	23.8		163.6	-	-	63.7	
Av.158.1	44.0	52.6	23.8	40.1	158.1	89.7	73.9	63.7	75.8
267.4	79.5	-	-		267.4	156.6	-	-	
181.5	-	57.0	-		181.5	-	108.4	-	
Av.224.4	79.5	57.0	-	68.2	224.4	156.6	108.4	-	132.5
123.3	18.2	-	-		162.4	108.8	-	-	
137.5	-	14.1	-		137.5	-	57.3	-	
127.7	-	-	26.3		127.7	-	-	63.2	
Av.129.5	18.2	14.1	26.3	19.5	142.5	108.8	57.3	63.2	76.4
Week ending 20/5/66 Temp.of room R changed to $20^{\circ}C$, $L = 20^{\circ}C$									
181.5	27.5	-	-		10°C 181.5	110.2	-	-	
183.9	-	58.6	-		15°C 183.9	-	73.0	-	
203.2	-	-	53.8		20°C 203.3	-	-	79.0	
Av.189.5	27.5	58.6	53.8	46.6	189.5	110.2	73.0	79.0	87.4
Weeks ending 27/5/66 to 12/8/66 $L = 20^{\circ}C$, $R = 20^{\circ}C$									
Av.178.5	-	-	43.2	43.2	178.5	-	-	56.5	56.5
121.2	63.5	-	-		121.2	24.9	-	-	
154.9	-	59.6	-		154.9	-	37.9	-	
183.9	-	-	40.1		183.9	-	-	78.7	
Av.153.3	63.5	59.6	40.1	54.4	153.3	24.9	37.9	78.7	47.2

TABLE 3 (Cont.)

L = 20°C, R = 20°C

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R Eff.
231.2	74.8	-	-		231.2	>100.1	-	-	
212.4	-	48.8	-		212.4	-	80.1	-	
309.2	-	-	56.1		309.2	-	-	>92.2	
Av.250.9	74.8	48.8	56.1	59.9	250.9	>100.1	80.1	>92.2	>90.8
231.7	96.7	-	-		231.7	131.4	-	-	
197.9	-	74.1	-		197.9	-	65.6		
145.0	-	-	56.1		145.0	-	-	23.6	
Av.191.5	96.7	74.1	56.1	75.6	191.5	131.4	65.6	23.6	73.5
144.5	101.2	-	-		144.5	68.7	-	-	
145.0	-	44.0	-		145.0	-	39.1	-	
159.4	-	-	24.4		159.4	-	-	57.0	
Av.149.6	101.2	44.0	24.4	56.5	149.6	68.7	39.1	57.0	54.9
Av.149.8	76.6	-	-	76.6	159.4	79.0	-	-	79.0
217.9	-	>91.9	-		203.1	-	>90.7	-	
217.9	-	-	88.5		242.7	-	-	>94.7	
Av.217.9		>91.9	88.5	>90.2	222.9	-	>90.7	>94.7	>92.7
183.3	48.6	-	-		183.3	56.0	-	-	
114.7	-	35.8	-		114.7	-	33.4	-	
(128.9	-	-	>152.8)		(148.8	-	-	52.0)	
Av.142.3	48.6	35.8	(152.8)	42.2	142.3	56.0	33.4	52.0	45.5

TABLE 3 (Cont.)

$L = 20^{\circ}C$, $R = 20^{\circ}C$

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
89.4	41.3	-	-		89.4	33.9	-	-	
(84.5	-	>95.6)	-		(84.5	-	14.1)		
74.6	-	-	31.3		74.6	-	-	23.8	
Av. 82.8	41.3	(95.6)	31.3	36.3	82.8	33.9	14.1	23.1	23.9
163.5	64.7	-	-		163.5	32.6	-	-	
143.8	-	>96.8	-		143.8	-	38.8	-	
124.0	-	-	47.3		124.0	-	-	48.5	
Av.143.8	64.7	96.8	47.3		143.8	32.6	38.8	48.5	

Brackets indicate where, due to a suspected inaccuracy, figures have not been used in the calculation of percentage B.O.D. removal rates.

TABLE 4

P.V. of feed and effluents (with weekly averages)

Experiment A(flies absent)

Weeks ending 29/10/65 to 28/1/66 L = 20°C, R = 20°C

*Effluents from filters in room R marked thus were dosed with feed taken from room L and percentage removals were calculated accordingly.

Feed L	Effluents L1	L2	L3	Av L. Eff.	Feed R	Effluents R1	R2	R3	Av. R Eff.
65.5	-	-	-		54.8	-	-	-	
64.1	50.5	54.9	52.5		59.9	50.2	57.5	44.9	
Av.64.8	50.5	54.9	52.5	52.6	57.3	50.2	57.5	44.9	50.9
46.3	31.2	30.4	31.0		54.2	31.5	33.3	37.1	
44.9	30.7	30.7	32.3		45.2	34.3	29.1	32.9	
Av.45.6	30.9	30.5	31.6	31.0	49.7	32.9	31.2	35.0	33.0
Av.35.7	18.7	18.5	23.5	20.2	35.0	28.0	21.5	27.5	25.7
Av.58.5	36.5	36.7	35.5	36.2	35.5	36.7	35.2	35.2	35.8
60.7	31.5	30.0	24.7		65.2	41.7	24.7	32.7	
60.5	24.7	25.7	24.2		63.5	21.5	18.7	36.5	
Av.60.6	28.1	27.8	24.4	26.8	64.3	31.6	21.7	34.6	29.3
Av.29.6	11.5	19.5	10.7	13.8	28.3	15.5	13.4	16.3	15.1
51.0	19.5	-	-		51.0	23.8	-	-	
39.0	-	21.6	-		39.0	-	27.5	-	
30.8	-	-	17.9		30.8	-	-	22.7	
Av.40.3	19.5	21.6	17.9	19.7	40.3	23.8	27.5	22.7	24.7
One set of readings per week only, was taken after this time									
57.7	19.7	17.9	20.9	19.5	39.5	15.1	21.9	17.2	18.1

TABLE 4 (Cont.)

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
L = 20°C, R = 20°C									
83.0	33.8	32.8	27.6	31.4	65.3	32.1*	27.1	36.3	31.8
59.7	24.8	21.9	33.4	26.7	70.8	23.5	24.0	32.6	26.7
76.5	30.7	27.1	29.2	27.0	73.9	26.3*	25.1	27.6	26.3
50.6	18.9	18.6	18.3	18.6	48.1	24.3	17.8	21.7	21.3
Temp. of Room R changed to 5°C, L = 20°C									
Weeks ending 4/2/66 to 13/5/66									
40.1	17.3	15.0	16.5	16.3	32.0	20.4*	18.3	28.4	22.4
47.6	19.0	21.7	18.7	19.8	40.2	27.4	20.0*	25.4	24.3
24.9	17.2	20.2	17.0	18.1	27.1	21.5	12.1	17.8	17.1
36.0	14.9	14.1	17.1	15.4	28.1	23.9*	17.3	19.0	20.1
46.0	24.0	18.6	21.5	21.4	55.8	36.7	33.3*	35.2	35.1
51.9	12.2	15.9	19.8	16.0	44.8	25.9	28.4	35.7	30.0
49.2	11.6	-	11.2	11.4	31.9	16.6	18.3	21.4	18.8
43.9	11.3	12.6	7.7	10.5	37.7	17.0	13.6	20.7	17.1
55.2	17.0	18.5	10.1	15.2	41.2	26.1*	19.0	24.4	23.2
61.0	20.9	28.1	31.5	26.8	49.2	31.4	22.4	22.1	25.3
40.9	11.1	26.4	14.8	17.4	34.1	23.7	16.7	23.9*	21.4
Temp of room R changed to 20°C, L = 20°C									
Weeks ending 20/5/66 to 19/8/66									
63.2	17.9	29.7	26.7	23.8	58.7	31.9	38.6	23.9*	31.5

TABLE 4 (Cont.)

L = 20°C, R = 20°C

Feed L	Effluents L1	12	13	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av. R. Eff.
58.7	22.7	19.7	20.5	21.0	63.1	31.8*	28.6	21.9	27.4
-	18.1	-	17.9	18.0	47.6	16.4	17.5*	20.5	18.1
62.1	24.4	26.8	22.8	24.7	61.1	22.6	30.3*	31.8	28.2
59.7	25.3	29.0	22.8	25.7	72.7	34.3*	30.3	34.3	33.0
56.2	20.5	20.8	17.0	19.4	-	21.8*	20.9	29.9	24.2
43.4	21.2	18.5	17.5	19.1	57.2	27.1*	20.4	24.4	24.0
50.8	21.7	24.6	24.0	23.4	54.5	22.4*	21.1	24.0	22.5
40.8	16.3	18.3	19.0	17.9	62.8	16.1	16.0	24.9	19.0
52.9	21.7	24.1	21.5	22.4	62.5	19.6	20.5	22.9	21.0
36.0	20.8	23.9	20.7	21.8	44.4	13.9	22.5	23.9	20.1

TABLE 5

Carbon dioxide output (mg/hr). Weekly readings

Experiment A (flies absent)

Weeks ending 26/11/65 to 28/1/66 L = 20°C, R = 20°C							
L1	L2	L3	Av.L.	R1	R2	R3	Av.R.
-	40.53	-	40.53	-	-	31.31	31.31
-	-	-	-	-	-	23.10	23.10
24.90	-	25.32	25.11	-	-	21.05	21.05
-	29.90	33.54	31.72	-	-	-	-
-	36.13	39.06	37.59	32.27	35.34	29.83	32.48
48.63	51.55	35.34	45.17	50.23	65.10	31.19	48.84
61.11	67.89	54.21	61.07	74.88	71.08	58.04	68.00
71.08	77.32	50.22	66.21	71.67	72.68	56.68	67.01
Week ending 4/2/66 Temp. of room R changed to 5°C, L = 20°C							
-	-	-	-	15°c -	-	-	
-	-	-	-	10°c 38.05	-	-	
-	-	-	-	5°c 28.54	-	-	
Av.				33.29			33.29
Weeks ending 11/2/66 to 13/5/66 L = 20°C, R = 5°C							
55.54	29.62	41.86	42.34	19.02	20.19	15.05	18.09
-	40.53	56.87	48.70	20.36	22.98	-	21.67
47.30	29.62	50.22	42.38	17.68	18.86	17.90	18.15
52.88	40.53	52.88	48.76	20.36	22.98	-	21.67

TABLE 5 (Cont.)

L = 20°C, R = 5°C							
L1	L2	L3	Av.L.	R1	R2	R3	Av.R.
-	-	48.76	48.76	-	-	20.61	20.61
-	52.88	80.78	66.83	31.22	30.95	27.53	29.90
66.43	61.11	79.06	68.87	-	29.62	26.18	27.90
-	52.88	-	52.88	32.56	30.95	27.53	30.35
37.73	51.55	69.48	52.92	23.17	32.28	24.81	26.75
69.35	58.19	62.44	63.33	27.20	33.61	32.95	31.25
54.21	56.87	47.30	52.79	20.36	30.95	27.53	26.28
Week ending 20/5/66 Temp. of room R changed to 20°C, L 20°C							
79.05	-	-		10°C -	48.76	-	
-	74.27	-		15°C -	66.43	-	
-	-	72.68		20°C -	79.05	-	
Av.		75.33	75.33		64.75		64.75
Weeks ending 27/5/66 to 19/8/66				L = 20°C, R=20°C			
91.01	84.09	69.48	81.53	54.66	69.48	82.45	68.86
63.77	72.67	69.48	68.64	77.96	71.08	46.78	65.27
71.08	79.05	104.83	84.99	64.29	56.87	50.22	57.13
61.11	82.37	94.86	79.45	60.13	74.27	44.07	59.49
-	75.73	-	75.73	-	61.11	46.78	53.95
-	-	59.65	59.65	-	-	50.64	50.64
-	52.88	47.30	50.09	-	74.88	44.07	59.47
40.53	37.73	32.28	36.85	46.21	47.30	44.07	45.86
45.84	50.22	45.84	47.30	65.10	65.10	51.25	60.48

TABLE 6

Corresponding results for film level (gm. wet weight), carbon dioxide output (mg/hr) and BOD removal (mg BOD/hr) used for statistical comparison.

Experiment A (flies absent) 20°C

Film	CO2	BOD rem.	Film	CO2	BOD rem.
139.0	24.90	22.19	220.0	77.32	52.39
196.0	61.11	31.20	144.5	29.62	17.46
216.5	71.08	51.85	109.0	40.53	28.76
237.5	55.54	14.15	116.5	29.62	11.39
189.0	47.30	17.10	107.0	40.53	8.16
192.5	52.88	14.88	124.5	52.88	12.06
180.5	37.73	17.19	132.0	61.11	29.55
179.0	69.35	42.12	133.5	52.88	22.57
202.5	54.21	23.56	147.5	51.55	23.83
205.0	79.05	34.52	136.5	58.19	27.91
231.5	63.77	12.93	166.5	56.87	27.66
227.0	71.08	35.06	175.0	74.27	28.09
124.5	40.53	10.78	219.5	72.67	21.36
104.5	45.84	22.15	234.0	79.05	36.68
126.5	37.73	11.97	219.5	82.37	27.75
93.5	40.53	32.91	246.0	75.73	22.64
133.5	29.90	21.30	110.5	52.88	17.69
143.5	36.13	27.21	123.0	47.30	11.68

TABLE 6 (Cont.)

Experiment A 20°C

Film	CO2	BOD rem.	Film	CO2	BOD rem.
191.0	67.89	40.53	145.0	25.32	23.29
155.5	33.54	22.77	387.5	77.96	21.59
166.0	54.21	52.63	292.0	60.13	22.48
198.5	50.22	42.25	221.0	46.21	12.44
199.0	41.86	20.40	216.0	65.10	29.34
204.5	56.87	30.22	257.0	79.05	22.26
210.0	50.22	12.51	139.0	35.34	27.80
209.0	52.88	22.06	180.5	72.68	41.92
241.0	48.76	20.11	434.5	79.05	24.86
203.5	80.78	39.65	354.5	71.08	26.23
232.0	79.06	26.56	319.5	56.87	29.66
295.5	69.48	22.15	329.0	74.27	29.66
234.0	47.30	22.73	320.5	61.11	23.74
206.0	72.68	33.49	370.5	74.88	18.23
241.0	69.48	30.33	319.0	47.30	15.78
248.0	69.48	32.23	338.5	65.10	23.54
259.0	104.83	56.74	292.0	43.19	11.68
300.5	94.86	19.93	236.0	58.19	10.40
156.0	32.28	9.70	97.5	31.31	19.48
138.5	45.84	17.19,	117.0	23.10	11.99
173.0	47.30	16.06	124.5	21.05	20.04

TABLE 6. (Cont.)

Experiment A 20°C

Film	CO2	BOD rem.	Film	CO2	BOD rem.
150.0	32.27	40.51	152.0	58.04	53.51
191.0	74.88	37.75	188.5	56.68	41.72
213.0	71.67	54.00	307.0	82.45	27.84
281.5	82.45	27.35	125.5	44.07	17.24
205.0	46.78	23.57	120.0	44.07	11.39
131.0	44.07	27.21	114.0	51.25	16.93
114.0	46.78	22.95	120.0	46.75	17.48

Experiment A 5°C

239.0	28.54	54.76	292.0	29.62	16.72
238.0	19.02	11.14	314.5	30.95	16.34
259.5	20.36	26.52	366.5	32.28	17.75
258.5	17.68	11.41	388.0	33.61	16.39
239.0	20.36	6.03	411.0	30.95	17.98
294.5	31.22	16.25	182.0	15.05	15.29
323.5	32.56	18.83	211.0	17.90	12.51
350.5	23.17	15.98	231.0	20.61	6.53
364.5	27.20	24.84	214.5	27.53	27.17
363.0	20.36	12.02	217.5	26.18	16.70
212.5	20.19	19.35	234.5	27.53	9.91
226.5	22.98	27.95	263.0	24.81	8.03
248.5	18.86	11.68	291.0	27.53	14.46
249.5	22.98	14.15			

TABLE 7

B.O.D. values of feed and effluents (with weekly averages).

Experiment B (flies present)

Weeks ending 26/8/66 to 28/10/66 L = 20°C, R = 20°C									
Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
85.3	-	58.2	-		85.3	-	64.4	-	
134.4	-	-	62.6		134.4	-	-	56.4	
Av. 109.9	-	58.2	62.6	60.4	109.9	-	64.4	56.4	60.4
(47.6	47.7)	-	-	-	(47.6	16.7)	-	-	
75.2	-	23.1	-		75.2	-	23.1	-	
Av. 61.4	(47.7)	23.1	-	23.1	61.4	16.7	23.1	-	19.9
119.6	66.2	-	-		119.6	20.3	-	-	
118.6	-	85.3	-		118.6	-	72.2	-	
125.0	-	-	78.9		125.0	-	-	70.2	
Av. 121.1	66.2	85.3	78.9	76.8	121.1	20.3	72.2	70.2	54.2
156.0	-	34.8	-		156.0	-	51.8	-	
171.0	-	-	52.9		171.0	-	-	27.4	
Av. 163.5	-	34.8	52.9	43.9	163.5	-	51.8	27.4	39.6
264.0	31.1	-	-		264.0	55.9	-	-	
199.0	-	45.4	-		199.0	-	67.8	-	
179.0	-	-	53.1		179.0	-	-	66.8	
Av. 214.0	31.1	45.4	53.1	43.2	214.0	55.9	67.8	66.8	63.5

TABLE 7 (Cont.)

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R Eff.
L = 20°C, R = 20°C									
149.0	51.4	-	-		149.0	82.3	-	-	
139.0	-	28.0	-		139.0	-	46.5	-	
99.5	-	-	19.3		99.5	-	-	30.5	
Av. 129.2	51.4	28.0	19.3	32.9	129.2	82.3	46.5	30.5	53.1
134.0	37.8	-	-		134.0	36.5	-	-	
120.5	-	32.1	-		120.5	-	24.8	-	
90.5	-	-	28.6		90.5	-	-	16.3	
Av. 115.0	37.8	32.1	28.6	32.8	115.0	36.5	28.4	16.3	25.9
55.0	13.5	-			55.0	14.8	-	-	
89.5	-	-	35.2		89.5	-	-	31.5	
Av. 72.2	13.5	-	35.2	24.4	72.2	14.8	-	31.5	23.1
>258.5	>67.5	-	-		>258.5	>67.5	-	-	
298.0	-	62.4	-		298.0	-	53.8	-	
242.5	-	-	75.1		242.5	-	-	46.9	
Av.>266.3	>67.5	62.4	75.1	>68.3	>266.3	>67.5	53.8	46.9	>56.1
Week ending 4/11/66 Temp. of room R changed to 5°C, L = 20°C									
168.5	50.1	-	-		15°c 168.5	45.3	-	-	
179.5	-	42.9	-		18°c 179.5	-	63.9	-	
209.0	-	-	41.3		5°c 209.0	-	-	75.9	
Av. 185.7	50.1	42.9	41.3	44.8	185.7	45.3	63.9	75.9	61.7

TABLE 7 (Cont.)

Weeks ending 11/11/66 to 3/2/67 L = 20°C, R = 5°C									
	Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
	249.0	66.6	-	-		249.0	>71.6	-	-	
	228.5	-	67.1	-		228.5	-	>78.2	-	
	208.5	-	-	51.0		208.5	-	-	>80.6	
Av.	228.7	66.6	67.1	51.0	61.5	228.7	>71.6	>78.2	>80.6	>76.8
	128.5	26.5	-	-		128.5	61.0	-	-	
	123.5	-	17.0	-		123.5	-	60.1	-	
	143.0	-	-	15.4		143.0	-	-	49.9	
Av.	131.7	26.5	17.0	15.4	19.6	131.7	61.0	60.1	49.9	57.0
	329.0	103.4	-	-		329.0	>151.0	-	-	
	319.0	-	114.4	-		319.0	-	>150.0	-	
	259.5	-	-	98.4		259.5	-	-	>146.2	
Av.	302.5	103.4	114.4	98.4	105.4	302.5	>151.0	>150.0	>146.2	>149.1
	174.5	46.6	-	-		174.5	88.0	-	-	
	194.0	-	50.3	-		194.0	-	89.8	-	
	198.0	-	-	51.8		198.0	-	-	89.4	
Av.	188.8	46.6	50.3	51.8	49.6	188.8	88.0	89.8	89.4	89.1
	172.5	62.3	-	-		172.5	51.1	-	-	
	167.0	-	38.8	-		167.0	-	69.4	-	
	162.0	-	-	42.0		162.0	-	-	60.4	
Av.	167.2	62.3	38.8	42.0	47.7	167.2	51.1	69.4	60.4	60.3

TABLE 7 (Cont.)

$L = 20^{\circ}C$, $R = 5^{\circ}C$

Feed L	Effluents L1	L2	L3	Av.L Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
143.0	16.4	-	-		143.0	21.3	-	-	
137.5	-	17.3	-		137.5	-	28.3	-	
229.0	-	-	20.3		229.0	-	-	62.8	
Av. 169.8	16.4	17.3	20.3	18.0	169.8	21.3	28.3	62.8	37.4
Av. 119.0	41.4	42.6	27.7	37.2	139.0	84.6	71.0	72.3	76.0
Av. 248.0	-	-	32.0	32.0	248.0	-	-	117.6	117.6
267.5	47.5	-	-		267.5	>98.1	-	-	
287.5	-	35.5	-		287.5	-	145.0	-	
242.5	-	-	58.8		242.5	-	-	113.2	
Av. 265.8	47.5	35.5	58.8	47.3	265.8	>98.1	145.0	113.2	>118.8
322.5	48.1	-	-		322.5	166.8	-	-	
193.0	-	-	37.4		193.0	-	-	131.5	
Av. 257.8	48.1	-	37.4	42.8	257.8	166.8	-	131.5	149.2
211.0	37.4	-	-		211.0	102.6	-	-	
187.0	-	28.0	-		187.0	-	91.2	-	
133.5	-	-	15.5		133.5	-	-	69.4	
Av. 177.2	37.4	28.0	15.5	27.0	177.2	102.6	91.2	69.4	87.7
124.5	24.6	-	-		124.5	64.4	-	-	
154.0	-	-	18.9		154.0	-	-	57.8	
Av. 139.3	24.6	-	18.9	21.8	139.3	64.4	-	57.8	61.1

TABLE 7 (Cont.)

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
Week ending 10/2/67 Temp. of room R changed to 20°C, L = 20°C									
238.0	40.3	-	-		10°C 238.0	87.5	-	-	
258.0	-	47.8	-		15°C 258.0	-	96.0	-	
199.0	-	-	44.4		20°C 199.0	-	-	90.3	
Av. 231.7	40.3	47.8	44.4	44.2	231.7	87.5	96.0	90.3	91.3
Weeks ending 17/2/67 to 10/3/67 L = 20°C, R = 20°C									
244.0	34.6	-	-		244.0	81.2	-	-	
259.5	-	47.6	-		259.5	-	>76.1	-	
Av. 251.8	34.6	47.6	-	41.1	251.8	81.2	>76.1	-	>78.6
288.0	39.2	-	-		288.0	51.6	-	-	
248.5	-	40.4	-		248.5	-	65.1	-	
Av. 268.2	39.2	40.4	-	39.8	268.2	51.6	65.1	-	58.3
168.5	23.4	-	-		168.5	59.4	-	-	
269.5	-	22.1	-		269.5	-	43.6	-	
Av. 219.0	23.4	22.1	-	22.8	219.0	59.4	43.6	-	51.5
202.0	64.4	-	-		202.0	37.9	-	-	
231.0	-	18.6	-		231.0	-	34.3	-	
163.0	-	-	44.6		163.0	-	-	29.0	
Av. 198.7	64.4	18.6	44.6	42.5	198.7	37.9	34.3	29.0	33.7

Brackets indicate where, due to a suspected inaccuracy, figures have not been used in the calculation of percentage BOD removal rates.

TABLE 8

P.V. of feed and effluents (Weekly readings)

Experiment B (flies present)

Weeks ending 26/8/66 to 28/10/66 L= 20°C, R= 20°C

*Effluents from filters in room R marked thus were dosed with feed taken from room L and percentage removals were calculated accordingly.

Feed L	Effluents L1	L2	L3	Av. L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
38.1	16.9	15.6	13.8	15.4	35.1	11.8	15.9	18.6*	15.4
46.8	21.5	24.0	25.0	23.5	56.0	16.9*	19.4	25.0	20.4
62.3	17.7	20.2	18.2	18.7	66.3	17.3	21.6	14.4	17.8
42.7	22.6	26.2	24.4	24.4	44.9	23.6	27.9	32.8	28.1
49.2	16.9	18.9	18.2	18.0	41.3	22.7	-	26.5	24.6
31.5	18.1	16.5	20.9	18.5	42.0	20.6	18.4	17.1	18.7
23.5	20.8	22.5	19.0	20.8	31.8	22.8	23.9	27.5	24.7
50.6	23.8	21.4	21.1	22.1	-	31.5	26.1	26.3	28.0
61.0	20.9	20.5	20.5	20.6	59.2	20.8	20.9	24.0	21.9
Weeks ending 11/11/66 to 3/2/67 L= 20°C, R= 5°C									
51.7	32.0	27.1	22.1	27.1	51.2	29.1	31.4	31.8	30.8
38.4	17.8	20.8	18.3	19.0	37.7	24.5	23.5	21.0	23.0
60.8	34.5	34.2	40.2	36.3	64.2	42.7*	38.5	39.2	40.1
44.7	21.3	17.9	-	19.6	44.4	31.5	32.7	32.3	32.2
55.3	26.1	20.6	20.3	22.3	49.8	22.3*	20.5	22.5	21.8

TABLE 8 (Cont.)

Feed L	Effluents L1	L2	L3	Av.L. Eff.	Feed R	Effluents R1	R2	R3	Av.R. Eff.
L = 20°C, R = 5°C									
33.7	21.4	26.2	18.8	22.1	32.2	21.3	20.9	20.9	21.0
44.5	27.1	26.1	23.5	25.6	69.5	27.5	27.2	27.5	27.4
43.3	25.4	22.3	23.6	23.8	44.0	27.4	26.4	27.9	27.2
64.3	32.4	25.3	25.9	27.9	57.8	32.3	33.8	37.6	34.6
52.0	25.3	19.8	19.6	21.6	43.5	33.5	31.3	35.0*	33.3
39.8	19.8	19.3	18.3	19.1	40.0	27.8	23.3	23.8	25.0
56.0	28.5	25.8	23.8	26.0	51.5	37.2	34.5	-	35.9
Weeks ending 10/2/67 to 3/67 L = 20°C, R = 20°C									
59.3	26.1	32.1	25.6	27.9	67.3	37.3	39.6	39.3	38.7
64.2	25.8	19.8	22.9	22.8	52.2	26.3*	34.4	28.1	29.6
52.3	22.3	17.4	18.0	19.2	39.5	22.0*	23.0	25.5	23.5

TABLE 9

Carbon dioxide output (mg/hr). Weekly readings

Experiment B (flies present)

Weeks ending 26/8/66 to 28/10/66 L = 20°C, R = 20°C							
L1	L2	L3	Av.L.	R1	R2	R3	Av.R.
-	48.76	47.30	48.03	-	44.51	46.75	45.63
36.54	47.30	44.51	42.78	68.45	43.19	30.24	47.29
37.73	66.43	55.54	53.23	79.05	58.19	41.84	59.69
-	54.21	41.86	48.03	-	29.62	38.51	34.07
30.95	62.44	52.88	48.76	46.21	41.84	41.84	43.30
25.64	44.51	37.73	35.96	39.39	36.40	30.95	35.58
28.30	45.84	45.84	39.99	35.37	41.84	44.51	40.57
28.30	29.62	-	28.96	35.37	37.73	-	36.55
36.40	40.52	48.76	41.89	38.05	60.15	51.25	49.82
Week ending 4/11/66 Temp. of room R changed to 5°C, L = 20°C							
26.97	-	-		15°C 29.88	-	-	
-	45.83	-		10°C -	26.97	-	
-	-	52.88		5°C -	-	17.90	
Av.			41.89				24.92
Weeks ending 11/11/66 to 3/2/67				L = 20°C, R = 5°C			
28.30	40.52	56.87	41.89	13.66	16.21	16.21	15.36
35.07	45.83	43.17	41.36	10.85	10.76	13.69	11.77
25.64	52.88	48.76	42.43	20.36	18.86	20.61	19.94

TABLE 9 (Cont.)

L = 20°C, R = 5°C							
L1	L2	L3	Av.L.	R1	R2	R3	Av.R.
35.07	48.42	-	41.74	13.66	17.53	16.21	15.80
-	59.65	45.84	52.74	-	20.19	12.09	16.14
36.40	36.40	-	36.40	13.66	14.75	-	14.20
-	44.50	-	44.50	-	16.21	-	16.21
-	51.55	62.44	56.99	-	21.65	20.61	21.13
58.19	-	72.68	65.43	20.36	-	15.05	17.71
-	-	63.69	63.69	-	-	15.05	15.05
52.88	59.65	59.65	57.39	19.02	20.19	16.55	18.59
Week ending 10/2/67 Temp. of room R changed to 20°C, L = 20°C							
63.77	-	-		10°C 29.88	-	-	
-	63.77	-		15°C -	39.06	-	
-	-	69.48		28C -	-	41.84	
Av.			65.67				36.93
Weeks ending 17/2/67 to 10/3/67 L = 20°C, R = 20°C							
55.54	48.76	-	52.15	44.88	47.69	-	46.28
62.44	48.76	61.11	57.44	56.68	54.21	47.69	53.53
67.89	48.76	-	58.32	47.69	62.44	-	55.06
66.43	54.21	47.30	55.98	50.22	36.40	37.73	41.45

TABLE 10

Corresponding results for film level (gm. wet weight), carbon dioxide output (mg/hr) and B.O.D. removal (mg. B.O.D./hr) used for statistical comparison.

Experiment B (flies present) 20°C

Film	CO2	BOD rem.	Film	CO2	BOD rem
81.0	25.64	21.88	79.0	40.52	52.81
76.0	28.30	21.57	96.0	45.83	30.62
78.5	28.30	9.30	90.0	40.52	36.18
82.5	26.97	26.54	84.5	45.83	23.88
82.5	28.30	40.86	120.5	48.42	32.21
85.5	35.07	22.87	114.0	59.65	28.74
91.0	35.07	28.67	82.5	36.40	26.94
89.5	36.40	28.38	86.5	51.55	56.49
91.0	58.19	61.51	95.0	63.77	47.12
99.5	52.88	22.40	89.0	48.76	47.50
102.5	63.77	44.32	98.5	48.76	46.65
103.5	55.54	46.94	96.0	48.76	55.46
106.5	62.44	55.77	97.0	54.21	47.61
100.5	67.89	32.53	100.0	41.86	26.48
100.5	66.43	30.85	92.0	52.88	28.22
86.0	54.21	27.17	94.5	37.73	17.98
88.0	62.44	34.43	79.5	45.84	13.88
94.5	44.51	24.88	89.5	48.76	37.53

TABLE 10 (Cont.)

Experiment B 20°C

Film	CO2	BOD rem.	Film	CO2	BOD rem.
86.0	45.84	19.82	99.0	52.88	37.59
104.5	56.87	35.31	92.0	29.62	23.36
91.5	43.17	28.60	105.5	41.84	29.41
117.5	45.84	26.90	115.5	36.40	20.73
102.5	62.44	41.18	90.0	41.84	21.45
104.0	72.68	34.88	97.0	60.15	54.74
104.5	63.69	26.45	130.5	62.44	50.64
109.0	59.65	30.28	89.0	36.40	44.09
107.0	69.48	34.65	91.0	41.84	12.28
111.0	47.30	26.54	84.0	38.51	32.19
115.5	39.39	14.95	96.0	41.84	25.15
92.5	35.37	21.85	76.0	30.95	15.46
101.0	35.37	9.01	76.0	44.51	16.63
83.0	51.25	43.85			

Experiment B 5°C

107.5	10.85	15.13	185.5	21.65	31.94
141.5	13.66	19.39	89.0	17.90	29.84
158.5	13.66	27.28	94.0	13.69	20.87
205.5	20.36	34.90	125.0	16.21	24.35

TABLE 10 (Cont.)

Experiment B 5°C

Film	CO2	BOD rem.	Film	CO2	BOD rem.
224.0	19.02	13.47	135.0	12.09	22.77
103.0	10.76	14.21	174.5	20.61	28.98
123.5	17.53	23.36	180.0	15,05	13.79
137.0	20.19	21.86	181.0	15.05	14.37
147.0	14.75	24.48	192.0	16.55	21.57