THE ECOLOGY OF PROTOZOAN POPULATIONS OF SLOW SAND FILTERS, WITH PARTICULAR REFERENCE TO THE CILIATES

by

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A thesis submitted to the University of London for the degree of Ph.D., 1980

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GODDARD, MARIAN R. THE ECOLOGY OF PROTOZOAN POPULATIONS OF SLOW SAND FILTERS, WITH PARTICULAR REFERENCE TO THE CILIATES.

ABSTRACT

The protozoan populations of slow sand filter beds, used by the Metropolitan Water Division of the Thames Water Authority for the purification of stored water, were studied over the period of a year. Most of the work was conducted on beds where the water approached the sand at a flow rate of 0.4 mh^{-1}, while a bed run at 0.2 mh^{-1} was used for comparative studies. Physical and chemical parameters of the water were monitored by the Thames Water Authority.

A method for the qualitative and quantitative examination of live protozoa was developed using a sedimentation technique. The time required for sedimentation limited the number of samples which could be identified, measured and counted. These were concentrated on defining the depth, rather than horizontal, distribution of protozoa as the bed functions vertically, and samples were taken as frequently as possible in order to adequately estimate the population growth rates of the protozoa. Depth distributions of particulate organic carbon content of the sand were determined, as an indication of food availability for the microorganisms, in parallel with the protozoan samples. The depth distribution of bacteria associated with the sand grains was also studied, but only over the initial period of a filter bed run. Scanning electron microscopy was employed to examine the localised distributions of bacteria on the sand grain surfaces. The availability of a pilot-scale sand column enabled interstitial water to be sampled and analysed for bacteria.

Maximum population densities of ciliates and flagellates, and variations in mean body size of the ciliates were found to be related to temperature. Maximum ciliate densities were also associated with surface accumulation of carbon, rates of head loss change and, on two occasions, with the onset of a dissolved oxygen deficit in the filtrate water. The pattern of density-depth distributions of ciliates and flagellates varied with time during the filtration runs, and deeper penetration into the sand occurred in the filter bed with the faster rate of approach water flow. Rates of growth of the ciliate and flagellate populations increased with temperature; the ciliate population Q_{10} equalled 1.6 between 4° and 20°C. Rates of growth also increased with flow rate and accummulation of particulate organic carbon in the filter beds. After maximum densities had been achieved and the ciliate population was in numerical decline, the total ciliate biovolume continued to increase, but at a more gradual rate; this was due to a succession to larger species during this part of the run.

A high initial growth rate of bacteria, doubling time equal to 10 hours, was recorded over the first few days of a filter bed run. Bacterial enumeration in the interstitial water of the pilot-scale sand column was less informative, as very little change in density distribution occurred during the initial part of a run. Oxidation-reduction profiles of the interstitial water were also found to change little with time, maintaining a characteristic increase in potential with depth.

ACKNOWLEDGEMENTS

Financial assistance was provided by the Natural Environment Research Council and Thames Water Authority. Permission to place sample cores in the slow sand filter beds at Hampton Water Treatment Works was given by the Thames Water Authority. Laboratory facilities were provided by the Zoology Department of Royal Holloway College and scanning electron microscope facilities were provided by Bedford College. I should like to thank Mr. E. Walters, Mr. P. Curzon and Mr. G. Hughes for their assistance with the field work. I am also grateful to Mr. S. Horrell and Mr. G. Rentmore for their photographic and workshop assistance.

I should like to thank Mr. J.A.P. Steel and Mr. I.P. Toms for providing me with physico-chemical data from the filter beds and for useful discussions about the study, and I should particularly like to thank Dr. D. Lodge for making available the sampling apparatus and pilot filter for my use and for discussing various aspects of the work.

Most of all, I am very grateful to my supervisor, Dr. A. Duncan, for her continual interest and useful suggestions throughout the study, and also for her helpful criticisms made during the writing of this thesis.

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Chapter 1 INTRODUCTION

This investigation was one of a series of research projects which have been pursued by students of Dr. A. Duncan of the Zoology Department of Royal Holloway College, in collaboration with the Metropolitan Water Division of the Thames Water Authority, formerly the Metropolitan Water Board before the reorganisation in 1974. Most of this research has involved investigations concerning the phytoplankton, zooplankton and fish populations of reservoirs belonging to the Thames Water Authority, but more recent studies have been concerned with slow sand filtration. Slow sand filters are costly to operate, mainly due to their high cleaning costs; so it was decided (Metropolitan Water Board Report No. 45, 1971 - 1973) that in order to assess the management changes likely to produce improved output and performance, it was necessary to know what was actually going on within the filter bed sand. Lloyd (1974) described the dominant microfauna and meiofauna present in the slow sand filter beds at Ashford Common and Walton Treatment Works, and investigated their depth distributions and food relations. He also examined the effect of increased flow rate on the depth distributions of the meiofauna. Lodge (1979), working at Royal Holloway College, investigated the ecology of the meiofaunal populations in the slow sand filters at Hampton Treatment Works where the present study was also carried out. She devised a sampling technique to allow collection of samples from an operational filter bed, and described the spatial and temporal variations of the meiofaunal populations within the slow sand filter. She also

designed a model filter column, used in the present study for enumeration of interstitial bacteria, which would allow the measurement of parameters which could not be obtained from an operational bed, particularly those required for energetic and respiratory studies.

The main aims of the present investigation were:

(a) To study the depth distribution of the protozoa and describe their seasonal and temporal variations.

(b) To assess the rôle of the protozoan population in the purification of water passing through the bed.

Various physico-chemical parameters were monitored by the Metropolitan Water Division, as they have been for some years, throughout the study and an attempt was made to determine whether changes occurring in the protozoan population of the filter bed sand were related to variations of these parameters measured in the top water and filtrate.

As many of the ciliates and flagellates are bacteriovorous, a study was also made of the colonisation of the filter bed sand by bacteria, particularly during the early part of a filtration run, when ciliate densities were found to be low.

Although no conclusion as to the rôle which protozoa may play in the purification of water could be extracted from the study, many interrelationships were found between temperature, dissolved oxygen, carbon content, flow rate and head loss data of the filter bed and the concurrent changes in protozoan and particularly ciliate populations within the filter bed sand.

Chapter 2 Slow sand filtration

The historical development of slow sand filtration has been described in considerable detail by Lloyd (1974) and summarised by Lodge (1979). In Britain, the first record of the use of slow sand filtration was for the treatment of bleachery waste in Paisley, Scotland in 1804; the purified water was subsequently sold to the public (Baker, 1948). James Simpson installed the first slow sand filter to treat water for public supply at the Chelsea Water Company in 1829, and since this introduction they have been used throughout the area now covered by the Metropolitan Water Division of the Thames Water Authority (MWD).

Water purification is generally achieved by a selected combination of chemical flocculation, sedimentation, microstraining, and rapid and slow sand filtration. The MWD employ a primary filtration step of either microstraining or rapid sand filtration, followed by slow sand filtration. At Hampton Treatment Works, which was used for the present study, rapid sand filtration of water supplied from local storage reservoirs in the Staines area is followed by slow sand filtration, and finally the water is chlorinated before being pumped into public supply.

Rapid sand filters operate at a higher flow rate than slow sand filters; the two rates are generally about 600 cm hr^{-1} and 20 cm hr^{-1} respectively. Larger particles suspended in the inflow water are removed by mechanical straining as the water passes through the relatively coarse sand of the rapid sand filters. The sand is washed

regularly by a reversed, upwards flow of water and air, which washes the filtered material into overflow channels. The only biological process to occur in rapid sand filtration is a certain amount of nitrification of the free ammonia present, which is performed by nitrifying bacteria associated with the surface film on the sand grains.

2.1 Structure, maintenance and function of slow sand filters

Structure

Figure 2.1 shows a transverse section through a slow sand filter bed. The supernatant water passes through the sand layer, which varies between 0.8 and 0.5m (30 to 18 ins.) deep, at a rate of 0.2 to 0.4 m hr^{-1} (8 to 16 ins. hr^{-1}). It then percolates through the gravel, which supports the sand, through the porous base of the filter bed and into the underdrains. The flow of water is controlled by a valve which determines the rate of flow of the filtrate.

The flow of water through a filter bed depends on three factors (Fig. 2.2): the raw water head (H_1), the filtrate water head (H_2) and the rate of filtration, or velocity of flow (V_f). These factors are related, accordingly to Darcy's Law by the equation:

$$V_f = \propto (H_1 - H_2)$$

where $\propto$ depends on the hydraulic characteristics of the bed including grain size but most importantly including clogging of the interstices with filtered material and

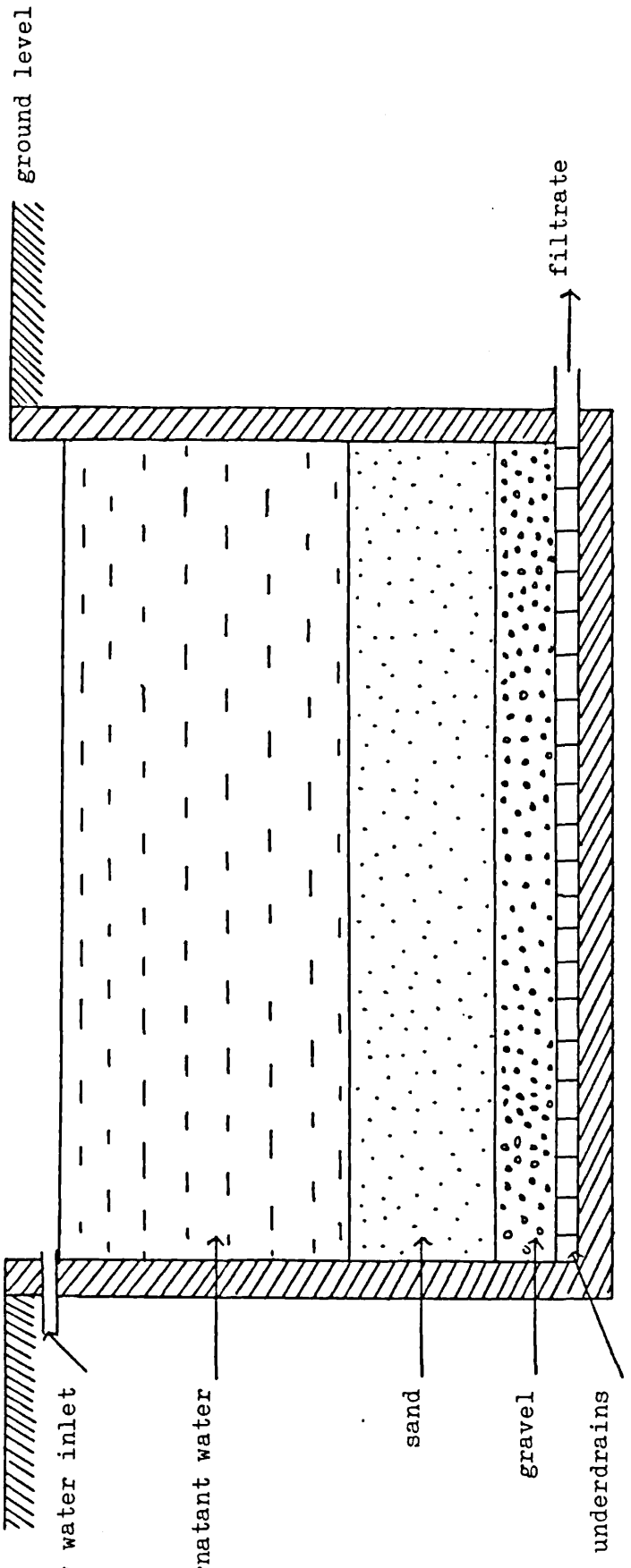
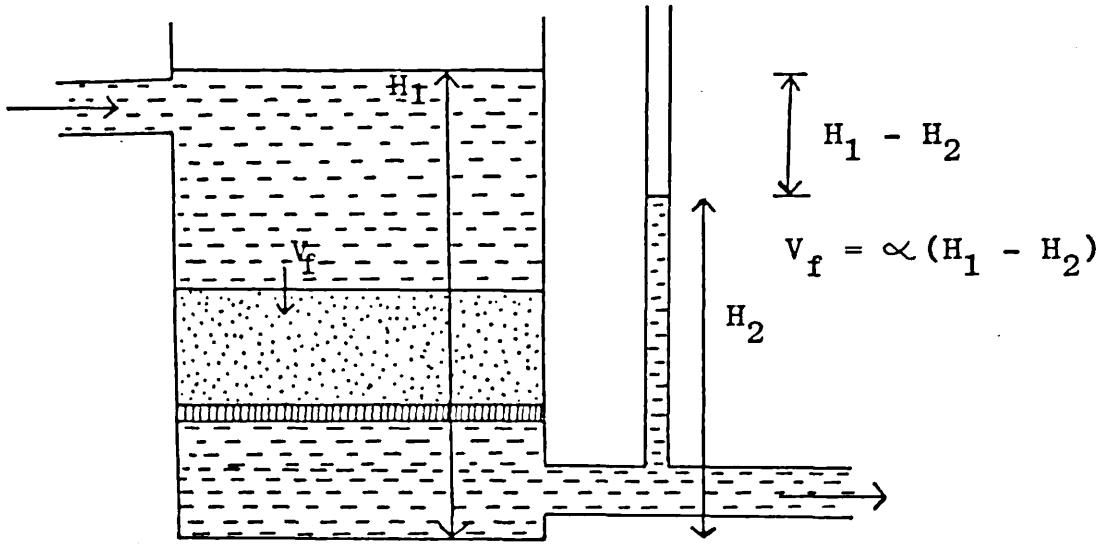


Fig. 2.1

Diagram of a slow sand filter.

algal growths. This clogging of mainly the surface layers of sand increases with the length of a filtration run and depresses the value of α.

Figure 2.2 Darcy's law as applied to filtration through clean sand



In order to maintain a steady rate of filtration it is usual to keep H_1 constant and to adjust the exit head H_2 by opening the valve controlling the filtrate flow to compensate for the decrease in α. Thus H_2 is decreased during a filtration run and the head loss of the bed $(H_1 - H_2)$ simultaneously increases. Head loss is measured manometrically as the difference between the height of a column of water supported by the raw water, H_1, and the column supported by the filtered water pressure, H_2. The rate of head loss change, $\Delta (H_1 - H_2)/\Delta t$, was recorded during the present study as it was a parameter of the filter bed which varied during the different runs and seasonally between runs.

Maintenance

After a certain length of time during a filtration run, the head loss reaches the limit of 1.5 to 2.0 m, and filter cleaning becomes necessary. This is achieved by draining the filter bed and skimming the top 2 to 3 cm (1 in.) of the sand surface (Plate 2.1). The bed may only be cleaned in this way about 12 times, as the effective filter depth is reduced on each occasion, so that after 12 cleanings it has been reduced by 1 foot, and resanding becomes necessary.

Filter beds are resanded by a trenching operation. The remaining 18 ins. of mature sand in one strip of the bed is dug out, and clean sand (12 ins. deep) is placed in the trench. The mature sand in the strip next to this is placed on top of the clean sand and the trench thus made is again filled with clean sand. This process is continued until the bed has been completely resanded.

In situ sand washers which do not involve the removal of sand from the filter bed have been described by Laval (1952) and Lewin (1961). Burman and Lewin (1961) examined some of the biological effects of both types of cleaning operation on subsequent filtration runs, and found that algal regrowth occurred more rapidly on those beds where the sand was cleaned in situ.

Function

Slow sand filters have been found to be extremely effective at reducing colour, turbidity and taste in

Plate 2.1 Cleaning of a slow sand filter bed by removing the surface inch of sand

addition to bacterial and viral densities and traces of many organic chemicals in the supernatant filter water (Kemna, 1899; Pascoe, 1956; Burman, 1962; Metropolitan Water Board Report Nos. 43 1967 - 1968, 44 1969 - 1970, 45 1971 - 1973). Mechanisms put forward for the removal of these impurities from the water include physical filtration, surface force mechanisms, and chemical and biological activity. Gravity is a significant removal mechanism for microscopic particles of greater density than water (Ives and Gregory, 1967), as they collect preferentially on the top surface of the grains, while particles of a density similar to water may be brought, by hydrodynamic forces, in to contact with a grain surface. In order for the particles to be removed from the laminar flow of water within the sand, they must adhere to the sand grain once they have gained contact.

The forces between the sand grain and low density particles may arise from the overlap of electrical double layers or from interactions of van der Waals forces. At the boundary between a sand grain and the interstitial water, a dilute solution of ions, the sand surface carries a negative charge which is balanced by an appropriate number of positively charged ions in the adjacent solution, forming what is known as an electrical double layer. Particles in the solution have a similar double layer, and since the sand surfaces and the great majority of colloidal impurities in water have negative charges, the double layer interaction will usually be one of repulsion. However, this depends on the concentration of dissolved salts in the water, and in Thames Water, the range of

electrical repulsion will only be about 10^{-6} cm (Ives and Gregory, 1967). At this range, the attractive forces of the van der Waals type become significant and may overcome the repulsion. These are the universal attractive forces between atoms and molecules (Ives and Gregory, 1966) and act over a range of about 5×10^{-6} cm.

Pascoe (1956) suggested that the sand particles possessed a small positive charge resulting from the breaking of the SiO_2 lattice, and that his observation of adsorption of negatively charged bacteriophages to the sand grains, was due to the attractive electrostatic forces between the phage and grains.

The main stream of water passing through the filter bed flows in a path through the centre of the interstices, the velocity of flow decreasing, due to frictional forces, closer to the sand grains (Ives, 1980). Consequently, flattened ciliates, characteristic protozoan fauna of this environment, are able to live in close association with the grains and their surface bacterial colonisation without being constantly swept along in the filter bed water.

The importance of biological processes on the efficiency of slow sand filtration has not been examined as thoroughly as the physical effects. Kemna (1899) suggested that biological mechanisms occurring at the sand surface contributed greatly to the purification of water and Burman (1962) described the formation of a zoogloeal layer on the surface of the filter, which was thought to be mainly responsible for bacterial removal, as one of the most important factors in the efficient performance of a slow sand filter.

A complex biological community has been found to develop in the filter beds, including bacteria, algae, protozoa, rotifers, oligochaetes and other macrofauna (Bellinger, 1968; Lloyd, 1974; Lodge, 1979). The contribution which many of the flora and fauna make to the purification of the filter bed supernatant is not well understood as research has been mainly concerned with only the bacterial and algal populations.

2.2 Biology of slow sand filtration

Immediately following the cleaning and recharging of a filter bed, the filtration is limited to its mechanical action of straining, but after a few days the filter bed becomes chemically and biologically active at the water/sand interface with the development of algae and bacteria. Kemna (1899) likened the bacterial slimes to a spider's web: the 'microbes becoming caught in the slimy layer on the surface of each grain of sand'. The river water abstracted from the Thames to supply the storage reservoirs has a particulate organic carbon (P.O.C.) content of between 1,000 and 12,000$\mu g l^{-1}$. This is reduced by 75% during storage so that the feed water to the primary filters has a P.O.C. content of 250 to 3,000μg C l^{-1}. This is further reduced by the rapid sand filtration to 25 to 300 μg C l^{-1} in the feed water to the slow sand filters. With such low concentrations of organic material as are present in the primary filtered water the only possible means for rapid biological action and removal of impurities is in the presence of solid surfaces enabling the surface concentration of nutrients and bacteria. Consequently the development of the

bacterial slime on the sand surfaces provides a perfect site for biological oxidation to take place (Heukelekian and Heller, 1940).

Bacterial accumulation has been observed at the sand surface of slow sand filters (Burman and Lewin, 1961) and a rapid reduction in densities from 10^7 g^{-1} sand at the surface to 10^4 g^{-1} sand was found to occur in the top 10 to 15 cm with little further reduction occurring down to 100 cm (Schmidt, 1963). The interstitial bacterial density, however, decreased exponentially with depth throughout the top 100 cm of sand. Lodge (1979) who studied the depth distributions of meiofauna, particulate organic carbon, dissolved oxygen and redox conditions in slow sand filter beds also found that biological activity, measured as oxygen consumption per g carbon, was far greater per unit weight of carbon in the upper 2 cm of sand than it was at depths between 2 and 5 cm and at depths below 5 cm, with mean daily consumption rates of 0.6, 0.08 ard 0.01 g O_2 $g^{-1}C$ respectively.

Studies on the algal flora of slow sand filter beds have shown diatoms to be the dominant colonisers of the filter bed sand surface (Kemna, 1899; Brook, 1953; Bellinger, 1968), with a few of the smaller species, less than 35μ maximum dimension, able to penetrate through the top 2" of sand, e.g. Stephanodiscus astraea and Navicula sp. (Bellinger, 1968). Kemna described a theory, attributed to Strohmeyer, suggesting that a moderate evolution of gas by the filamentous algae keeps them just above the sand surface, so that they do not clog the interstices of the filter bed, and at the same time perform a preliminary straining of the water.

Brook (1954) observed a succession of sand surface algae occurring during each filter bed run, and after examination of the gut contents of chironomid larvae, he related this to increased predation by such insect larvae. Ridley (1971) however, described the succession of algae during a summer run as occurring in three stages:

1. Proliferate growth in the supernatant water during the first seven days.
2. Surface seeding with diatoms and other algae from the inflow or from those which survived the cleaning process in the sand, after five days.
3. Growth of larger filamentous species after ten days.

Bellinger (1968) also found that diatoms were able to survive the cleaning process of the filter bed below the sand surface (i.e. heterotrophically), and to seed the bed as it was re-filled.

Studies on the predation of algae by protozoa (Brook, 1952) revealed that two ciliate species, Chilodon sp. and Oxytricha sp. commonly found in the filter beds, were strictly diatom feeders and that if present in sufficient densities they could have a marked effect on the composition of the algal flora of the bed surface. Protozoa have also been reported to be very effective in the removal of viruses from filter bed water (Metropolitan Water Board Report, No. 44, 1969 - 1970). By examining the feeding rates of Vorticella spp., Lloyd (1974) concluded that the predation

by these ciliates on the interstitial bacteria was a significant secondary role to the removal of bacteria by physical adsorption. He observed that predation was 2 to 5 times more efficient at the slower flow rate of 20 cm hr^{-1} than at 40 cm hr^{-1}, with 55% and 87% of seeded bacteria removed from an experimental sand cell by 50 and 200 individuals, respectively, of V. campanula at 20 cm hr^{-1}. The corresponding predation figures at 40 cm hr^{-1} were 11% and 39%. Studies by Ritterbusch (1976) similarly concluded that the metazoan populations of slow sand filters were not of primary importance in the purification of water passing through the beds on the basis of chemical analyses conducted on the water.

Meiofaunal populations of a slow sand filter bed run at 20 cm hr^{-1} were found to develop very slowly over the first two weeks of a run (Lodge, 1979), achieving densities of 10 - 100, of predominantly oligochaetes, per 40 cm^3 of sand. Numbers continued to increase slowly throughout a run, reaching highest densities of 1,000 per 40 cm^3 after 8 weeks of a summer run. Nematodes were not very common, and chironomids were abundant only at the sand surface during the summer.

There were very few patterns of meiofaunal depth distribution although the enchytraeid worms occasionally exhibited surface avoidance and the nematodes and chironomids were most abundant at the surface. Little is known of the feeding habits of these meiofaunal populations although they appear to graze on detritus, thus consuming bacteria and probably protozoa associated with this organic debris.

Chapter 3 Interstitial Ciliate Ecology

The ciliates are very cosmopolitan in their distribution, with many common genera represented in the most diverse of habitats. Three common species Cyclidium glaucoma, Cinetochilum margaritaceum and Chilodonella cucullulus have been found in a number of environments including fresh water, marine, an activated sludge aeration tank and an Imhoff tank (Lackey, 1938). Ciliates have been found to dominate the protozoan fauna of both percolating filters and activated sludge plants with 53 different species isolated from the former and 67 from the latter (Curds and Cockburn, 1970).

Ciliates are also very common in a number of benthic environments including brackish water marshes (Webb, 1956) and freshwater lakes and Scottish lochs (Webb, 1961; Bryant and Laybourn, 1972/1973; Finlay et al, 1979). Although less common in sand and gravel, ciliates have been isolated from a number of marine sands including those of islands off the German and Danish coasts (Hartwig, 1973) and in the Baltic Sea (Fenchel, 1966). They have also been recorded in coastal sands of Germany (Hartwig, 1974), North Yorkshire (Hartwig and Parker, 1977), Florida (Borror, 1963), Bermuda (Hartwig, 1977), the Waltair and Orissa coast of India (Rao and Ganapati, 1968; Rao, 1969), the Caspian Sea (Agamaliev, 1972) and the White Sea (Burkovsky, 1969). The only reported study of ciliate populations in fresh water sands and gravels is that of Lüpkes (1976) who identified 19 species from the Fulda-River benthos.

Ciliates are well adapted to the interstitial life of sand environments (Fauré - Fremiet, 1950). In sand of 0.1 to 0.3 mm particle diameter, the microporal ciliates have certain general characteristics: they are thigmotactic, the adoral membranelle acting as an adhesive organ and the ciliation allowing them to glide along surfaces; they also have a flattened and often elongated body with a prolonged caudal region, the total body length reaching up to 3 mm, and some have the ability to contract the frontal region very rapidly, e.g. Lacrymaria olor. The mesoporal ciliates, which are found in medium and coarse sands (particle diameter greater than 0.4 mm) are generally small to medium size and flattened, swimming alternately very fast and then suddenly stopping when in contact with a solid surface (Fauré-Fremiet, 1951) e.g. Glaucoma sp. Some typical interstitial species are euryporal, being widespread in both fine and coarse sands, while the algal detritus immediately above the sand surface fosters ciliates generally wider and more circular in cross section being uniformly ciliated and non-thigmotactic (Borror, 1968) e.g. Tetrahymena sp. The ciliates identified during the present study concurred with the above classification and fell mainly into the microporal and mesoporal groups.

Fenchel (1978) found that ciliates completely outnumbered other individuals of all other species in marine sand with a median grain size between 0.1 mm and 0.2 mm, but in sands consisting of larger grains, although they were more common, they were also accompanied by a rich metazoan fauna.

3.1 Factors effecting the distribution of interstitial ciliates

Ciliates are extremely tolerant to wide ranges of physical and chemical environmental factors including temperature, pH, salinity, dissolved oxygen, free carbon dioxide, ammonia and sulphide (Noland, 1925; Bick, 1957, 1968; Dillon et al 1968; Bick and Kunze, 1971; Stössel, 1979). However, Dragesco (1960) concluded that temperature and salinity, together with grain size, organic matter content and mechanical agitation of the sand were important in the control of ciliate distributions.

The availability of sufficient food of the required type seems to be the most important deciding factor for successful ciliate colonisation, this being linked in particular to the state of oxygenation of the sediment. Fenchel (1969) found that algal feeding ciliates dominated shallow water marine sands in the summer, the period of algal growth, while bacterivorous species dominated the deeper sites all year round, and also the shallower areas between autumn and spring.

The characteristic vertical zonation of ciliates in beach sand, with highest densities occurring at the surface and these decreasing rapidly with depth in the top few centimetres, was found to correlate with a redox potential gradient (Fenchel, 1966) and oxygen and sulphide concentrations (Webb, 1961; Burkovsky, 1971). Sands oxidised throughout, for example those mixed by surf did not show these typical ciliate zones which were explained by a combination of oxygen requirements, tolerance to

various reduced chemicals, and specialisation to feed on microorganisms requiring particular redox conditions. Indeed, in a study of Scandinavian marine sand (Fenchel, 1967), the largest populations of ciliates were found in strongly reducing sediments with a rich growth of sulphur bacteria. Goulder (1974) also found higher populations at deeper sites in a freshwater lake. However, the sediment was oxygenated and its highly flocculant nature provided abundant interstitial space giving both shelter and a large surface area for bacterial colonisation, and hence a plentiful food supply for the ciliates.

3.2 Rôle of ciliates in the decomposition of organic matter

Ciliates have been reported to enhance the decomposition of organic matter through a number of direct and indirect actions. They may stimulate bacterial decomposition of organic matter in marine and fresh water sediments by their predation on the bacterial population, keeping it at a level such that each bacterial cell performs at its maximum chemical efficiency (Mare, 1942; Johannes, 1965; Barsdate and Prentki, 1974). Johannes (1965) found that protozoa directly enhanced the regeneration of phosphorus in a marine environment, by excreting that obtained in bacterial food in a form available for incorporation into primary productivity. In the absence of such bacterial grazers, nutrients and in particular phosphates, would become tied up in bacterial biomass. Curds et al (1968) observed that a reduction in bacterial density and turbidity of the effluent from

a model activated sludge plant corresponded with an increase in ciliate density. These improvements were thought to be due to the direct predation on bacteria by the ciliates in addition to flocculation of the suspended matter and bacteria caused by ciliate secretions. A similar effect was recorded by Reid (1969) with Vorticella spp. in an activated sludge plant. The feeding currents of the vorticellids are thought to bring the sludge bacteria into contact with those coated with mucous secretions produced by the ciliates, thus producing a more settleable floc and improving the quality of the effluent.

3.3 Depth distributions of benthic ciliates

Very little data has been published concerning the variation of ciliate densities with depth in benthic habitats. Most work has either been largely descriptive giving information as to the presence or absence of species at a number of depths, e.g. Lüpkes (1976), or it has been concerned with the position of the ciliates in a whole ecosystem relative to other organisms and chemical and biological factors, e.g. an activated sludge aeration tank (Curds et al, 1968; Curds, 1971a) or a pond (Bamforth, 1958). Indeed Fenchel (1966) was the first to describe the vertical distribution of different ciliate species in the benthic environment of a brackish water beach.

Where densities of ciliates have been examined with depth, they have generally been expressed in terms of the number found in the sampling cores at a certain depth.

Consequently, as the diameter of the corers vary, the resultant densities are not directly comparable.

Highest densities are generally found in the surface cm of all sediments. Fenchel found 98 ciliates per 5.3 cm^3 of sand in the top 1 cm of the brackish-water sand sediment, while densities as high as 82,895 cm^{-3} have been recorded in the surface cm of sediment in a small eutrophic loch (Finlay, 1980). Goulder (1971) counted 5,500 individuals cm^{-3} of one species, Loxodes striates, in a freshwater lake sediment, however Bryant and Laybourn (1972/1973) only recovered 64 ciliates per ml of surface sediment in Loch Leven. Densities of L. striates decreased very rapidly in the fresh water lake sediment to zero between 2 and 3 cm, although other ciliates did survive down to 4 cm. In the brackish-water sand sediment, the densities decreased to 3 ciliates per 5.3 cm^3 of sand between 7 and 8 cm. Ciliates have been recorded down to 12 cm in a marine sediment (Agamaliev, 1970), however in the fresh water habitat of the Fulda River sand and gravel bottom, Lüpkes (1976) found 3 ciliates: Trachelophyllum sp., Haplocaulus sp. and Epistylis sp. as deep as 50 cm.

Chapter 4 Methods

Section A. Critique of methods used for the examination of protozoan and bacterial populations in aquatic benthic environments and their application to slow sand filters

4.1 Extraction of cores from aquatic sediments

In order to extract cores of sand from an operational filter bed, the sampling apparatus had to be designed such that following the removal of a sample, no reduction in the effective filter depth of sand occurred, i.e. a hole could not be left in the filter bed sand. The samples had to be extracted from beneath 1 to 1.5 m of water, in order to examine temporal changes in population density during a filtration run, and intact cores of sand were required to allow depth distributions of the protozoa present to be determined.

A variety of methods for removing cores from natural aquatic sediments have been described but none of these were practicable for the present study. Those described for sampling sand have generally been developed for marine or inter-tidal studies and consequently involve a corer which is pushed into the sand, the upper end is then sealed tightly with a cork or snap cover, the corer lifted and the bottom end sealed off similarly (Neel, 1948; Fenchel, 1966, 1967; Hartwig, 1973). A corer based on the same principle but operable in shallow water was described by Maitland (1969). All these sampling devices involve pushing a corer into the benthos.

This causes compaction, which could destroy many of the delicate ciliates, and possible transfer of material from the surface layers to lower depths in the extracted core, both of which disturb the vertical distribution of organisms within the core. These samplers also leave a hole in the sampled benthos.

To overcome the above problems, Lloyd (1973), studying the microfauna of slow sand filters, developed a sampler which was inserted into the filter bed sand while it was drained for cleaning. This consisted of two strips of picture glass spaced 1 mm apart and sealed together at the edges, such that an internal space 1.5 cm X 1 mm ran the length of the sampler column. A number of these were filled with clean, dry sand and pushed into the filter bed. After filtration had commenced a sampler could be removed from the bed and transferred directly to a microscope for examination. The main advantage of this technique is that no compaction of the sand occurred during manipulation of the sampler. However, there are disadvantages: firstly, the water flow through the sampler would be disturbed by the narrow aperture between the glass strips, secondly, the examination technique would only view animals visible on the upper surfaces of the sand grains adjacent to the glass and animals in the centre of the column would easily be missed and thirdly, the high surface area to volume ratio of the sampler would alter biological development, the glass surfaces providing a relatively large surface area for colonisation.

A sampling device which overcame the problems of compaction and interference with vertical distribution of fauna present in a slow sand filter was designed by Lodge (1979). It could be used during a slow sand filter run, sampling from a boat without disturbing the filter in any way which might effect the quality of the water passing out of the filter. This sampling apparatus which was used for sampling the meiofauna of slow sand filters proved to be suitable also for the sampling of protozoa and bacteria in the present study. It is described in detail in Lodge (1979) and summarised in Section 4.4.2.

The apparatus consisted of a core 100 mm in diameter filled with sand, which was placed into the filter bed while it was drained for cleaning. It has been established (Ives, 1966) that any model filter should have a diameter of at least 50 times the largest grain size, in order to render any boundary effects of the apparatus negligible. As the largest sand grains found in the filter bed sand were about 5 mm across, the sampling cores should have been a minimum of 250 mm in diameter. Consequently there may have been some boundary effects in the cores used. However, with this in mind, subsamples from the cores were taken as near to the centre as possible.

This technique of sampling has many advantages; there is no compaction of the sand during any stage of sampling and consequently no risk of destroying many of the ciliates, which are by nature extremely delicate; samples of known age can be removed from the filter bed as required, and an accurate depth distribution of organisms

in the top 25 to 30 cm of sand may be determined. The main disadvantage of the sampling cores is that no horizontal movement is possible between sand inside and sand outside the core wall; however, as the filter bed functions vertically this may not be a particularly important problem, especially at the centre of the core from where the sub-samples were taken. This lack of potential for horizontal movement may be more important for the larger animals, the metazoa, as they are likely to move further autonomously than the smaller protozoa.

4.2 Removal of protozoa from aquatic substrates

It was necessary to remove the protozoa from the sand grains in order to count them, as a direct examination of a sub-sample of sand would result in most of the protozoa being hidden from view by the grains of sand. The attached ciliates were counted in this manner, as they extended about 200 μm from the grains to feed in the water surrounding them and could be easily seen. However, the unattached protozoa were either present in the interstitial water or in close association with the detritus surrounding the sand grains. A technique was required which would separate the detritus and interstitial water from the sand grains without damaging the protozoa, most of which are extremely fragile organisms.

The majority of sand substrates studied previously have been from marine environments and the sea-water ice extraction method of Uhlig (1964) has been widely used for such samples. Ice is placed on top of the sand sample in a funnel over a collecting dish. As the ice melts a

stream of saline water passes through the sand and both the downward streaming of the water and its salinity gradient strongly influence the interstitial protozoa, forcing them out of the sand into the dish of sea water (Uhlig, 1968). As freshwater protozoa are very sensitive to change in osmotic pressure around them, to adapt this method to suit them, fresh water ice would need to be used. The only factor influencing the extraction of protozoa would be the downward streaming of melt-water and possibly the slight temperature gradient. I found that better results could be achieved by pouring water over the sand, a method which was also less time consuming than the use of melting ice. Spoon (1972) encouraged the migration of meiofauna from the sediment in the bottom of a test-tube to the surface of the overlying water by bubbling nitrogen or methane gas into the sediment and then stoppering the tube tightly, a method which may well be toxic to some of the sensitive protozoa.

Richards (1974), studied the protozoan fauna of slow sand filters and effected their removal from the sand by shaking each sample with Chalkley's medium (Kudo, 1971) prior to culturing the supernatant for enumeration of the protozoa. The grinding action between sand grains during shaking would probably fragment a large number of these fragile animals and the dilution culture method also used by Mare (1942) gives rise to a number of errors. It assumes that all organisms present in the sample will give rise to individual populations and that no cysts were present in the sample which would later increase the number recorded in culture by their excystment and growth.

The technique finally adopted in this study was based on that of Utermöhl (1958) which he used for counting phytoplankton. The water sample containing algae was poured into a sedimentation chamber, left for a suitable length of time and the bottom plate of the chamber was then examined on an inverted microscope, and the cells counted. It was adapted for the present study by passing a gentle stream of water over the sand, which was held in a muslin cloth over a collecting vessel. The filtrate containing detritus and protozoa was then sedimented prior to examination. The method is described in detail in Section 4.5.2.

4.3 Enumeration of bacteria in suspension

To examine changes in bacterial density within the filter bed, a technique was needed which removed either all or a constant proportion of bacteria from the sand grains into suspension. As sand grains have extremely uneven surfaces, providing small cracks and niches for bacteria to colonise, and tend to become partially covered with slime matrices in which bacteria are enmeshed, it was more realistic to attempt the removal of a constant proportion of the bacterial population. Consequently a standardised technique of shaking the sand sub-sample with sterile water was used. Once in suspension, a number of methods were available for bacterial enumeration. These could be grouped broadly in to those which are direct counts and those which require cultures of the suspension. Spread plate cultures, the most probable number technique of Harris and Sommers (1968) and direct counts using

epifluorescence microscopy were evaluated in the present study and the latter technique was found to give the most reproducible results.

Culture techniques have been used widely for enumerating sediment bacteria (ZoBell and Anderson, 1936; ZoBell, 1938; ZoBell, 1946; Pearse et al, 1942; Boucher and Chamroux, 1976), however they suffer from the following disadvantages as far as the counting of whole populations is concerned: selectivity of media for certain bacterial or spore development, and the production of one colony by an aggregate of bacteria. Pour plates may adversely effect psycrophilic organisms, as 46°C is the gelling temperature of the agar, a temperature which they must be able to withstand, while on spread plates 'spreading colonies' overgrow the surface giving aberrant counts of other colonies (Collins, 1977). The main advantage of culturing is that the count represents the number of viable bacteria present in the sample and is, therefore, a more useful indication of microbial activity than a total count would be. By culturing, colonies can also be picked off and sub-cultured to grow sufficient numbers for identification of the bacteria. Direct counts only allow identification according to morphology.

The plate dilution frequency of Harris and Sommers (1968) is a modification of the most probably number (multiple tube) technique described by McCrady (1915) and has been found to be particularly suitable for estimating bacterial populations in sewage liquors (Pike and Carrington, 1972). Replicate microsamples are inoculated from a series of dilutions on to delineated areas on agar plates. The

plates are incubated and the occurrence of growth is recorded for each inoculated area. By referring to most probable number tables, the microbial population of the sample is estimated. This method is subject to the same disadvantages and advantages as the other culture methods, described above, but presence or absence of growth is much easier to record than the total number of colonies present, so estimation using this technique is more expedient.

Direct counts estimate the total numbers of bacteria present. This would be a disadvantage if using a direct count to indicate viable bacterial density in a fairly inactive deep sediment, however in a slow sand filter, which has the function of removing organic matter from a constant flow of water passing through it, an estimate of total bacterial density was assumed to include a high proportion of active bacteria.

The direct method of Conn (1918), involved adding soil to gelatin, mixing it and spreading 0.01 ml evenly on a glass slide to cover 1.0 sq. cm. This was dried, stained and counted. Jones and Mollison (1948) modified this technique slightly using a haemocytometer, and Collins and Kipling (1957) concentrated waterbourne bacteria into glycerol, and stained and examined them in flat glass capillaries of known volume. However, these and the various counting chambers including Neubauer, Helber and Petroff-Hauser all require cell concentrations greater than 10^7 per ml in order for the count to be at all accurate (Collins and Kipling, 1957), and this magnitude of bacteria was not generally present in the bacterial suspensions produced in

the present study.

The above methods involve counting all stained, bacterial-shaped objects. Strugger (1948) found that by staining a soil sample with acridine orange, the bacteria fluoresced green when viewed under blue light, giving an excellent contrast against the background detrital particles. Acridine orange stains DNA and is the most common fluorochrome used for direct counts of aquatic bacteria (Hobbie et al, 1972; Francisco et al, 1973; Jones, 1974). A procedure which was based on that described by Jones and Simon (1975) was adopted for counting the bacteria extracted from the slow sand filter samples in this study. It is described later in this chapter (Section 4.7.2).

Section B Methods of sampling and analysis

4.4 Sampling

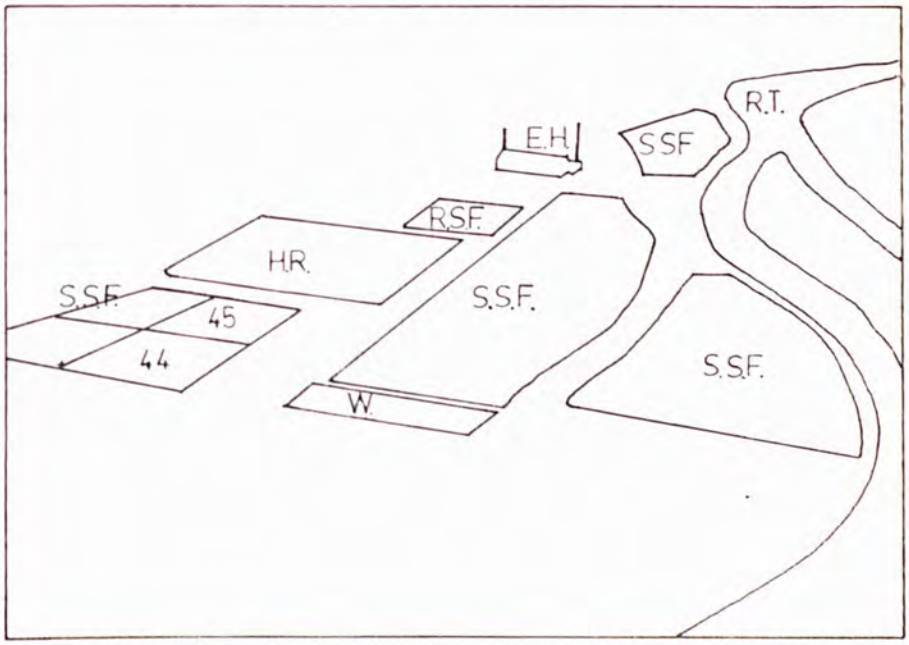
4.4.1 Slow sand filter beds used for sampling

The slow sand filter beds used in this study were Beds 44 and 45 of the Thames Water Authority Water Treatment Works at Hampton (Plate 4.1). These beds are in a block of four relatively small sand filters (6,400 m^2), and bed 45 has been used for research purposes prior to this study by the Metropolitan Water Board, Thames Water Authority and recently by Denise Lodge of Royal Holloway College. Throughout the period of the present study, bed 44 was run at a flow rate of 8" or 0.2M per hour and bed 45 at 16" or 0.4M per hour.

PLATE 4.1. HAMPTON WATER TREATMENT WORKS

courtesy of Thames Water Authority

E.H. Engine house
H.R. Holding reservoir
R.S.F. Rapid sand filters
R.T. River Thames
S.S.F. Slow sand filters
W. Washing bay

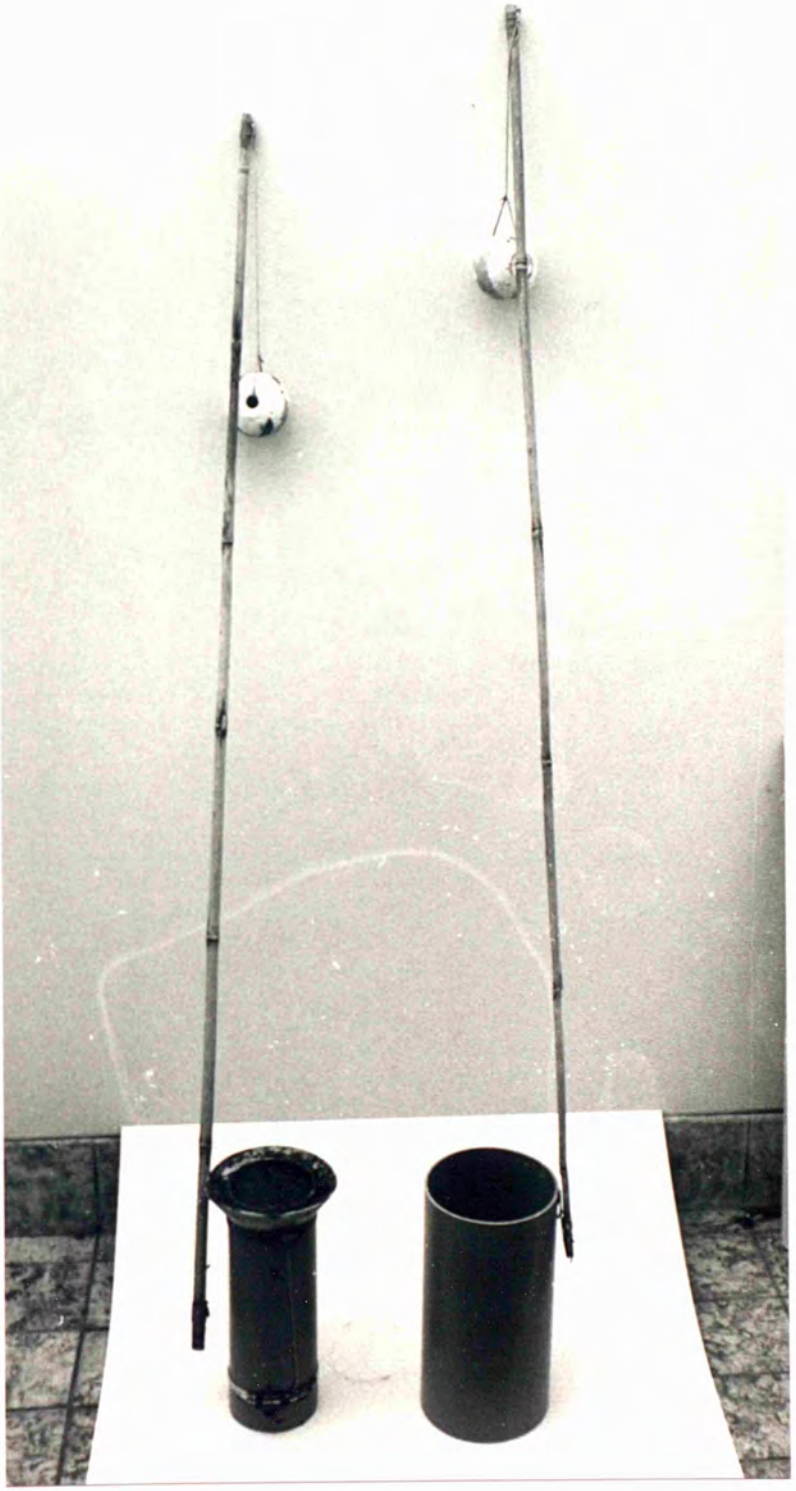


4.4.2 Sampling apparatus

As mentioned earlier the sampling apparatus and procedure used in this study was that used by Lodge (1979) for examining the meiofaunal populations of slow sand filters. It was found to be equally useful for protozoan sampling initially and at a later date was also used for studying the bacterial populations.

The apparatus (Plate 4.2) described briefly here is reported in detail in Lodge (1979). It consisted of two parts, an inner core and an outer core. The inner core made from a 31 cm length of grey PVC drainpipe had an inner diameter of 100 mm and was sawn longitudinally in half. A base plate, made of clear perspex and drilled with holes, was attached to one half, and this kept the sand in the core while allowing the water to flow through without impedence. The outer core consisted of a 31 cm length of PVC drainpipe with an inner diameter of 150 mm. The two halves of the inner core were held together with two jubilee clips and the split between the halves covered with plastic adhesive tape. A piece of nylon cord attached to opposite sides of the lower jubilee clip passed inside the upper jubilee clip and extended as a loop above the core. A plastic collar, made by cutting the top off a plastic filter funnel, fitted tightly over the top of the core and rested on the top jubilee clip. The inner core when placed inside the outer core, fitted such that the plastic collar completely sealed off the gap between the two cores allowing no water to pass down between them.

Plate 4.2 Sampling apparatus : inner and outer cores with floats attached



A polystyrene float was attached to each core by means of a length of cane and labelled with a letter either in red (inner core) or yellow (outer core). These floats were markers to enable location of the cores under the 1 to 1.5 m of water above the sand during a filter bed run.

4.4.3 Core placement

The inner cores were assembled and filled with washed sand obtained from the sand storage bay at Hampton. The full cores were placed into an empty tank and back-charged slowly by gradually filling the tank with water from a hose. This caused the sand to settle, so more sand was added until no further settling occurred. The cores were then transported to the filter bed. In order to ensure that the cores were positioned randomly in relation to one another, a system of random co-ordinates from 2 fixed points was used. An area of 250 m^2 was chosen in the centre of the filter bed, in order to avoid any flow effects or colonisation from the side walls (Lodge, 1979). Pairs of random co-ordinates for each core position were taken from random number tables and the distance of the core site from two fixed datum points was calculated using Pythagoras's theorem. Tape measures were tied to poles pushed into the bed at each datum point and each core position was then located in the bed.

An outer core was dug in at each site such that its top edge was level with the filter bed surface, and its respective inner core was placed inside it so that its top was also level with the sand surface of bed. The float belonging to the inner core was then attached to the

loop of nylon cord with a paper clip. The filter bed sand surface was smoothed over to remove all foot prints and the bed was then ready for backcharging at the beginning of the next run. Up to 12 cores were dug into the bed before each filtration run (Plate 4.3).

4.4.4 Sampling procedure

In order that a hole should not be left in the sand bed when a sample core was removed, reducing the effective filter depth at that point by 30 cm, an identical core was inserted into the outer core to replace that removed for sampling. The replacement core was taken onto the filter-bed in a flat bottomed boat, the sample core located by the floats and the use of a diving mask, and pulled up through the water using a hook on the end of a pole, which hooked under the loop of nylon string attached to the inner core. In the boat the float (attached by a paper clip) was transferred to the replacement core which was then lowered into the outer core (Plate 4.4). Cores were collected in this manner at intervals varying from daily to fortnightly, the daily sampling being necessary for bacterial monitoring at the start of a run. Protozoa were sampled at three to four day intervals initially, but after a few weeks this interval could be lengthened to weekly and then fortnightly sampling.

At the end of a filtration run, the bed was drained for cleaning. All the cores were removed before cleaning could commence and the holes filled with clean sand from the storage bays. The apparatus was then dismantled cleaned and repaired if necessary. The time interval

Plate 4.3 Cores dug in to the central area of the filter bed at random sites

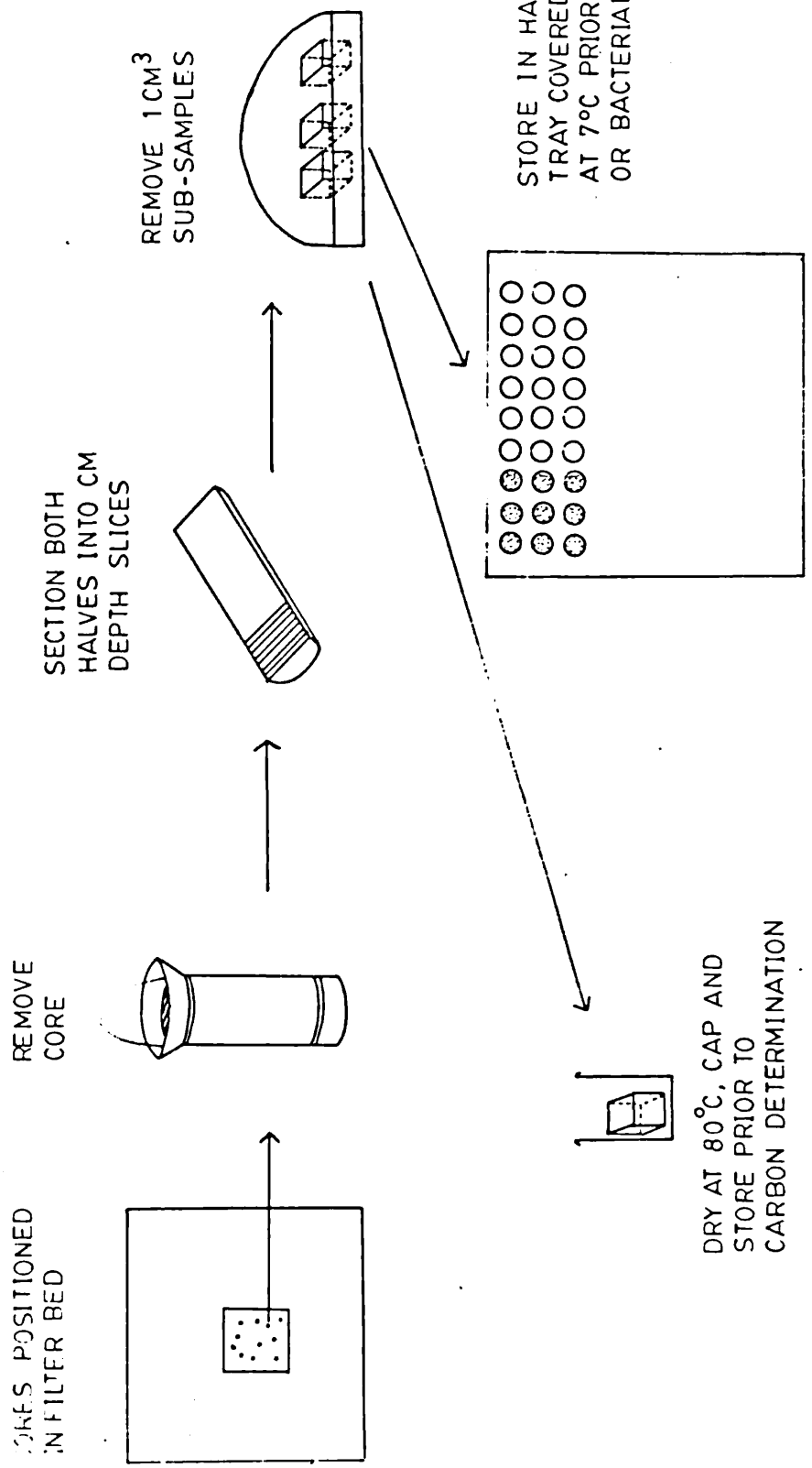
Plate 4.4 Core in position during a filter bed run

between the end of one run and the beginning of the next was usually two to three days.

4.4.5 Treatment of sample (Figure 4.1)

The inner core was taken to the side of the filter bed and sectioned immediately. The collar and jubilee clips were removed and two perspex hemicircles placed on the sand surface on each half of the core to prevent sand spillage. The core was then placed on its side in two wooden supports, the adhesive tape removed and the core cut in to halves using a cheese wire which was pulled through the slits in the plastic core. The halves were then put into a frame and sectioned one at a time. A rectangular piece of perspex (30 X 12 X 0.6 cm) with slits drilled in it at 1 cm intervals was placed over the half core. Aluminium slicers could just pass through these slits when pushed vertically downwards. Both halves of the core were cut in this way (Plate 4.5) into cm sections by the side of the bed so that no vertical migration could subsequently occur within the sand. Each sliced half core was placed in the bottom of a tank covered with polythene and a damp cloth and transported rapidly back to the laboratory. On arrival, the cm sections were further subsectioned using a brass corer of 1 cm^2 cross section giving sub-samples of 1 cm^3. The sub-samples were taken as near to the centre of the core as possible to avoid any edge effects caused by faster flow down the sides of the core. Three subsamples from each depth were taken for particulate organic carbon (P.O.C.) determinations, one from the following eight depths: 1 cm; 2 cm; 4 cm, 7 cm, 10 cm, 15 cm, 20 cm and 25 cm was counted

FIG. 4.1 PROCEDURE FOR SAMPLING PROTOZOA, BACTERIA AND CARBON



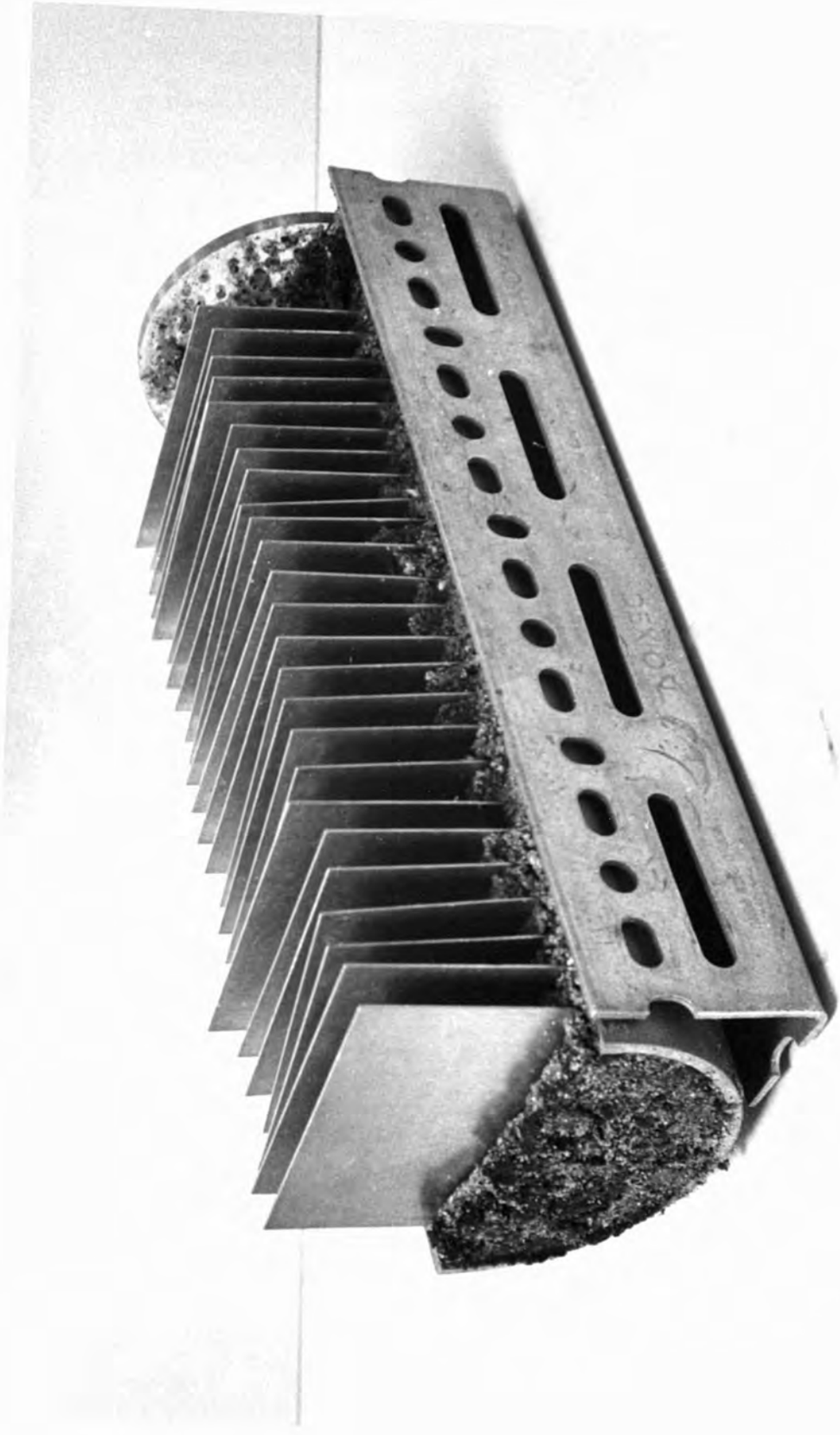


Plate 4.5 A half core sectioned with aluminium slicers (perspex cutting frame removed).

for protozoa, and for bacterial determinations two replicates at each of five depths : 1 cm, 5 cm, 10 cm, 20 cm and 25 cm were counted. The sub-samples for protozoan and bacterial analyses were kept in a haemocytometer tray covered with a sheet of parafilm, to prevent dessication, and stored in a 7°C refrigerator for the short period prior to analysis. The subsamples for P.O.C. determination were dried in glass vials at 80°C for 24 hours, they were then capped and stored until analysed.

4.5 Examination of protozoa

Direct counts of live protozoa were made within 24 hours of sampling; this minimised errors caused by replication, predation or natural mortality. The protozoa were counted live as they are more easily identified live than when fixed, fixed specimens tending to lose their characteristic shape, and many may not even survive the fixation procedure intact. Further advantages of counting live animals are that their movement aids the differentiation between the animal and the detritus with which most are associated, and many also have a very characteristic type of movement which helps in identification.

A technique was required which would extract the protozoa from the sand grains without agitation of the sand, as this could well destroy many of the fragile organisms, but which would remove all unattached protozoa from around the grains and concentrate them for counting. Utermöhl's method of examining sedimented cells by means of an inverted microscope (Utermöhl,1958) satisfied these requirements

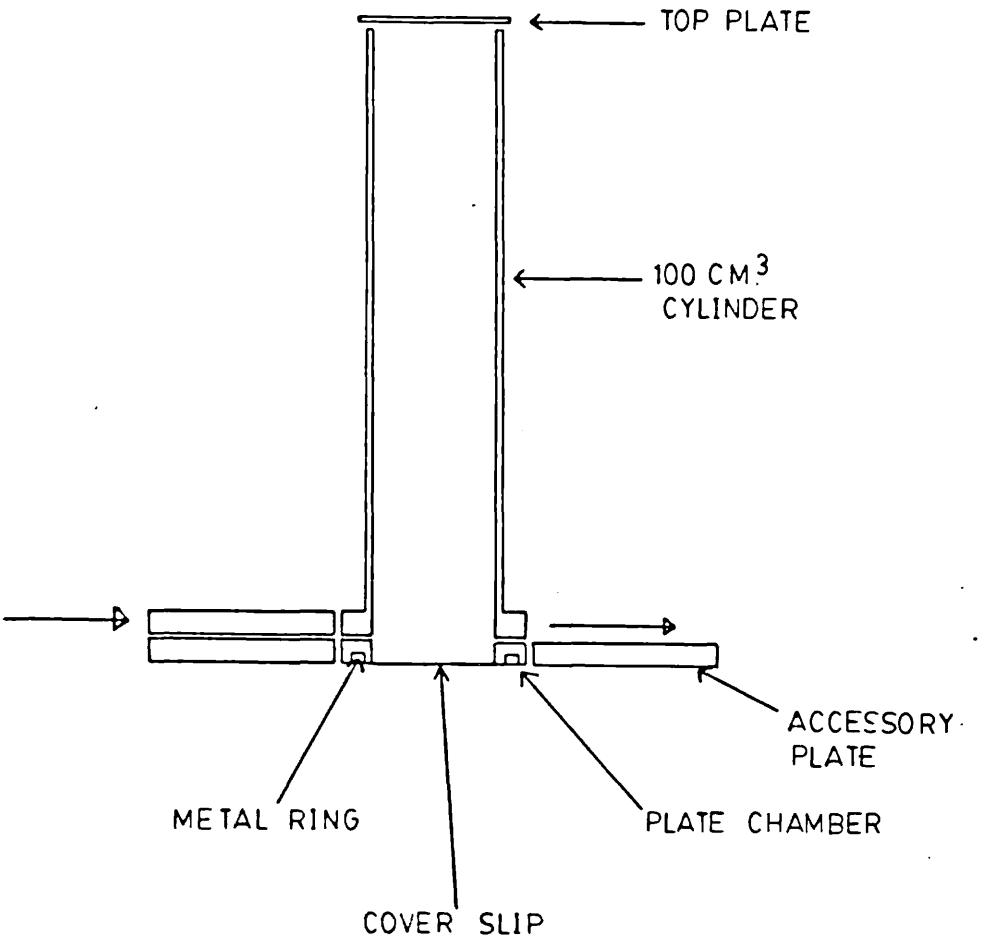
and was therefore adopted. A large sedimentation chamber (100 ml) was needed so that the sand sub-sample could be thoroughly washed with 100 ml of protozoa-free water which was then allowed to sediment. The ciliates which remained attached to the sand grains were counted separately under a binocular microscope.

4.5.1 Sedimentation apparatus

The sedimentation apparatus (Fig. 4.2) is marketed by E. Leitz Ltd. for the concentration of plankton, prior to counting with a Wild M40 'Inverted Biological Microscope'. It was found to be useful in the present study, for counting live protozoa. It is constructed in perspex and consists of a sedimentation chamber with a capacity of 100 ml, around the base of which a square base plate is bonded, and a plate chamber having the same dimensions as the base plate, with a hole of 28 mm diameter in the centre. The plate chamber received the settled organisms. A coverslip (30 mm diameter) is held into the bottom of the plate chamber by a metal ring which screws into the perspex wall of the chamber. This seal was made watertight by smearing soft paraffin wax on the edge of the coverslip before screwing the ring into place. Any excess wax on the coverslip was removed with alcohol. The join between the sedimentation chamber and plate chamber was also made watertight using paraffin wax.

In order to examine the sedimented organisms under an inverted microscope, the sedimentation chamber had to be removed from the plate chamber without disturbing or mixing the contents of the two. This was achieved by sliding the sedimentation chamber onto an accessory plate,

FIG. 4.2 THE SEDIMENTATION CHAMBER



→ DIRECTION OF SLIDING FOR REMOVAL OF THE SEDIMENTATION CYLINDER FROM THE CHAMBER

a plate of solid perspex having the same dimensions as the plate chamber, while a second accessory plate was slid over the plate chamber from a third plate on the opposite side of the chamber (Fig. 4.2). All joins between the accessory plates, and chambers were sealed with paraffin wax so that no water could escape from the sedimentation chamber and the contents of the plate chamber were undisturbed. The chamber was then transferred directly to an inverted microscope for examination and counting of the sedimented organisms.

4.5.2 Sedimentation procedure

A 1 cm^3 sample of sand was removed from the haemocytometer tray, in which it had been stored for a maximum of 24 hours after sampling, and placed on a double thickness of muslin cloth in a filter funnel, over an 100 ml measuring cylinder. 100 mls of protozoa-free sand filter water, were poured gently over the sand, so that a continuous stream of water passed through it, leaving the filter funnel at a rate of about two drops per second. (The water had been collected from the filter bed at the time of sampling and prefiltered through a Whatman GF/C glass fibre filter to remove all protozoa). The filtrate was immediately transferred to the sedimentation apparatus, covered and left to stand for 5 hours at 7^0C.

A few of the very fine grains of sand were washed through the muslin and these remained in the measuring cylinder during the transfer of water to the sedimentation chamber. These grains were then washed into a petri dish using prefiltered filter bed water. The washed sand in the muslin

was also transferred to this petri-dish which was then examined under a Nikkon binocular microscope, at a magnification of X 40, for sessile ciliates and any free-swimming protozoa which occasionally escaped the washing treatment.

After five hours, the protozoa in the plate chamber were counted under a Wild M40 inverted microscope at a total magnification of X 235. The whole area of the coverslip was covered systematically, traversing backwards and forwards using the mechanical stage. Ciliates were all identified to genus level and some of the more common species were identified using Kahl (1930 - 1935). The length and width of the first five individuals encountered of each species were measured using a micrometer eye piece. This counting process took from $1\frac{1}{2}$ to 2 hours per sample. All counts were expressed as no. cm^{-3} of sand. In some samples, one or two species were extremely common, i.e. $> 100\ cm^{-3}$; in such cases these species would only be counted in half the chamber, and would be ignored while counting the second half. Pairs of counts from both halves of the chamber were tested using rank correlation for a number of different species and no significant difference was found between them ($p = 0.01$).

4.5.3 Efficiency of sedimentation procedure

The washing procedure removed most of the detritus from the sand sample and in a few sub-samples, with high densities of detritus, it proved necessary to dilute the filtrate prior to sedimentation and counting. Detrital material was the main source of inefficiency in the

counting of protozoa, but it was necessary to wash the detritus into the filtrate, as many of the cilates lived in close association with it, and would otherwise have been overlooked. The efficiency of the washing technique was tested by washing the same sample of sand with successive 100 ml aliquots of filtered sand water, and sedimenting and counting these. After the third washing, the sand was examined thoroughly for any remaining protozoa. The extraction efficiency of the first washing was shown to be 93% for the ciliates and 72% for the flagellates (Table 4.1).

The duration of sedimentation was kept to 5 hours as this was the longest time possible in order for the eight sub-samples to be sedimented within 24 hours of sampling, using the three sedimentation columns available and allowing two hours for each chamber to be counted. The efficiency of the sedimentation period was tested by setting up a sedimentation as described above and leaving it for five hours. The sedimentation chamber was then transferred to a second plate chamber for a further two hours, then to a third for two more hours and finally to a fourth for a further thirty-six hours, by which time all the protozoa were assumed to have sedimented out. The results of this test are shown in Table 4.2. The efficiency of ciliate sedimentation as a whole was 81% over the first five hours, however the free swimming species, Cyclidium heptatrichum, was only removed with 59% efficiency, while the species associated with the detritus were removed to a far greater degree e.g. 84% for Cinetochilum margaritaceum. The low sedimentation

efficiency of the amoebae may be due to thigmotaxis against the side of the sedimentation chamber.

Taking into account both washing and sedimenting processes, the overall counting efficiency for ciliates was 75%.

Table 4.1 Test of efficiency of the washing technique

Protozoa	NO. OF INDIVIDUALS COUNTED AFTER EACH WASH		
	1st	2nd	3rd
Glaucoma sp.	4		
Cyclidium heptatrichum	15		
Pleuronema sp.	7		
Cinetochilum margaritaceum	32	6	3
Litonotus spp.	7		1
Spathidium spp.	1		
Tachysoma pellionella	90	3	
Aspidisca costata	7		1
Oxytricha spp.	6		
Stylonichia mytilus	3		
Vorticella spp.	1		
Heliozoa	9	1	
Flagellata	1,362	298	226

Washed sand - No protozoa

Efficiency of 1st Washing : ciliates 93%

flagellates 72%

Table 4.2 Test of efficiency of the sedimentation technique

SEDIMENTATION	1ST	2ND	3RD	4TH
TOTAL SEDIMENTATION TIME	5 HOURS	7 HOURS	9 HOURS	45 HOURS
PROTOZOA				
Cyclidium heptatrichum	13	5	1	3
Pleuronema sp.	2	-	-	-
Cinetochilum margaritaceum	90	8	4	5
Litonotus spp.	4	-	-	-
Oxytricha spp.	3	1	-	-
Vorticella spp.	4	1	-	-
Heliozoa	-	1	-	-
Amoebae	6	1	2	1

Flagellates were not counted but were present in all four sedimentations

Efficiency of 1st sedimentation : Ciliates 81%
Amoebae 60%

4.6 Examination of the replicability of the sampling programme

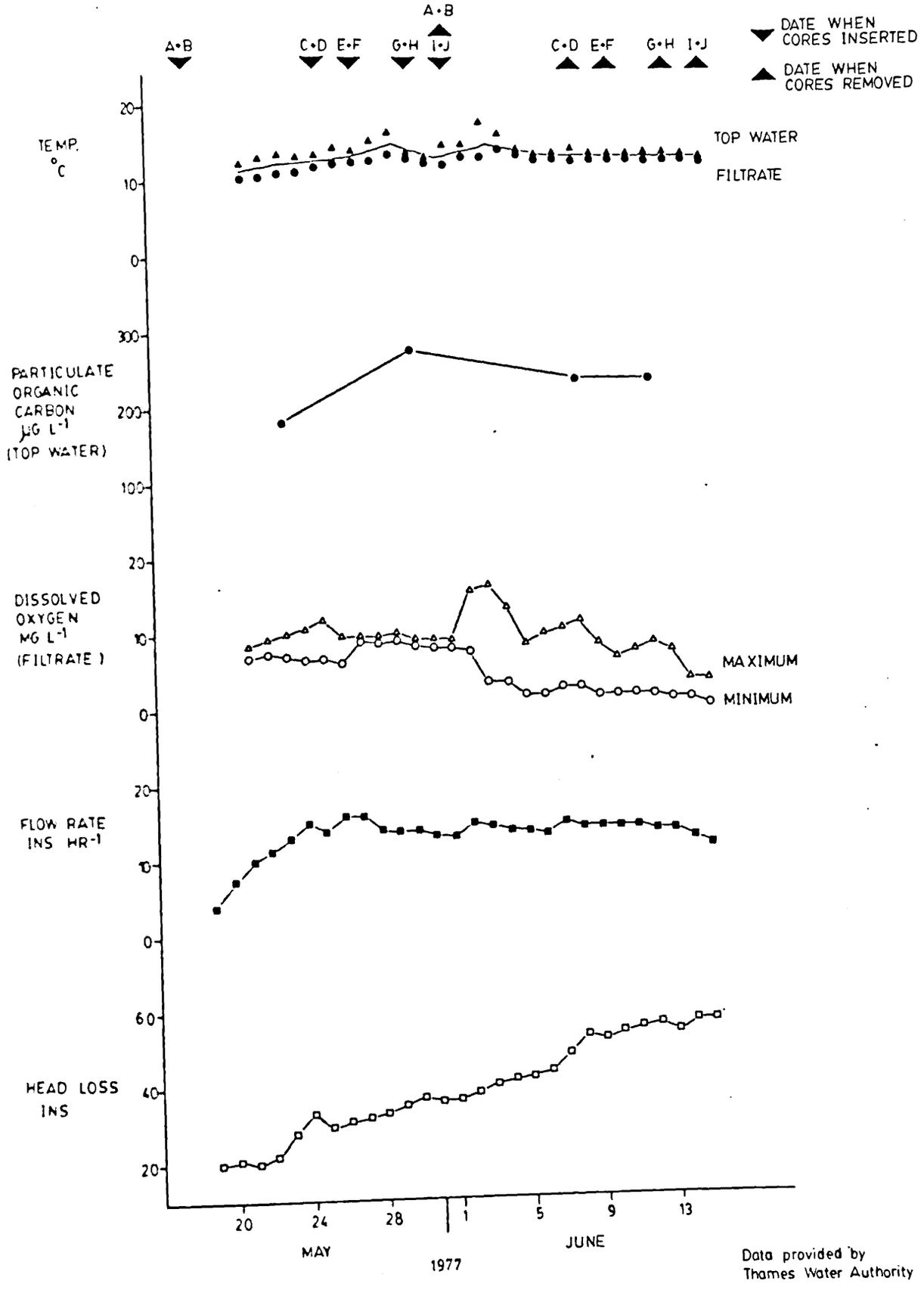
During the series of runs examining the spatial and temporal distribution of protozoa, the sedimentation time of 5 hours and the counting time of $1\frac{1}{2}$ to 2 hours involved in processing each cm^3 sub-sample, made it possible to examine only 1 sub-sample per core-depth in one core of sand removed per sampling day. It was therefore necessary to determine whether the data recorded in the one sub-sample analysed, was representative of the other cores present in the filter bed. There was no possibility of testing the replicability of normal sampling by counting 10 cores taken simultaneously,

because of the time involved in processing these, so it was necessary to devise a special sampling programme for this purpose and it was decided to replicate 10 cores of the same age (14 days) but taken on different dates, to allow for the handling and counting time. Two cores were taken on each date and three replicate sub-samples taken from the centre of each core were counted at one depth only, 10 cm; this enabled both within core and between core variability to be tested. Three replicates at the 10 cm depth in each core were also analysed for organic carbon to test for within and between core carbon variability.

The sampling programme was organised in the following way:- 10 initial cores were inserted into the bed at the start of a run and these were replaced by new initial cores over a period of two weeks, so that 14-day-old cores could be removed for counting at intervals, allowing sufficient time for each count (Fig. 4.3).

Although all the cores were 14 days old, the dates of removal and so the conditions of the bed over each two week period were different, subjecting the samples to an unavoidable extra variability. The temperature remained fairly constant around 13^{o}C but the flow rate, head loss, dissolved oxygen level, source of supply water and the degree of algal cover on the bed varied. Initially the bed received half of its water from the King George VI reservoir and half from the Queen Elizabeth II reservoir, but by the final sampling date 40% of the supply was still from the Queen Elizabeth II, but the remaining 60% was from Wraysbury reservoir. Changing the supply source

FIG. 4.3 ENVIRONMENTAL PARAMETERS RECORDED IN BED '45 DURING THE TEST OF REPLICABILITY OF THE SAMPLING PROGRAMME



Data provided by Thames Water Authority

to the bed could have affected the carbon inflow, however the values of dissolved carbon recorded weekly during the sampling period, by the Thames Water Authority, remained steady around 240 μg C l^{-1} (Fig. 4.3). Cladophora grew unevenly over the bed, such that cores A, C, E, F, G and H were covered with a dense algal skin, while cores B, D, I and J had very little algal growth on the sand surface.

The numbers of ciliates counted in the ten cores are recorded in Appendix 4.1. Means and variances were calculated for each core (n = 3) and for each sampling day (n = 6). These statistics were calculated for the whole ciliate population and individually for the seven species which were numerically dominant:- Cyclidium heptatrichum, Cinetochilum margaritaceum, Litonotus sp., Oxytricha sp., Tachysoma pellionella, Aspidisca costata, and Vorticella convallaria (Table 4.3).

In most cases $s^2 > \bar{x}$ indicating a clumped or contagious distribution. It was necessary to normalise the data in order to compare the core populations using analysis of variance and the transformations used to do this were calculated using Taylor's Power Law (Elliott, 1977). The regression lines of $\ln s^2$ on $\ln \bar{x}$ were calculated for the whole ciliate population and the individual species in each core (core J was omitted from the calculations). Each count (x) was then replaced by x^P where $P = 1 - b/2$ and b is the slope of the regression line (Table 4.4).

Table 4.3 Means and variances for the whole ciliate population and individual species

(i) In cores (n = 3)

		A	B	C	D	E	F	G	H	I	J
CILIATES	$\bar{X}$	549	209	313	171	220	232	245	189	125	1.33
	S^2	8379	3475	1675	2641	2370	1905	1332	1726	1132	0.56*
CYCLIDIUM HEPTATRICHUM	$\bar{X}$	59.3	24.7	54.3	4.3	18.7	4.3	5.3	10.7	25	0
	S^2	409.3	14.3*	456.3	10.3	1.3*	9.3	0.3*	6.3*	468	0
CINETOCHILUM MARGARITACEUM	$\bar{X}$	149	88.3	70.3	58.7	110.3	120.7	41.3	38.7	47.7	0.67
	S^2	1518.7	660.3	116.3	57.3*	1066.3	322.3	149.3	424.3	9.3*	2.25
LITONOTUS SP.	$\bar{X}_2$	15	4	6.7	1.7	3.7	4.3	11.7	3.3	3	0
	S^2	7*	3*	66.3	4.3	6.3	6.3	4.3*	2.3*	12	0
OXYTRICHA SP.	$\bar{X}_2$	25.7	13.3	6.3	5.7	6.7	10.3	19.3	14.3	1.7	0
	S^2	121.3	10.3*	14.3	6.3	22.3	25.3	72.3	33.3	0.3*	0
TACHYSOMA PELLIONELLA	$\bar{X}_2$	245	57.3	90.3	41	47.3	28.3	41.7	12	24.7	0
	S^2	69.1*	329.3	310.3	61	305.3	2.3*	276.3	16	129.3	0
ASPIDISCA COSTATA	$\bar{X}_2$	13.3	10.3	11.7	2.3	2.7	15.3	9	8.7	0.3	0
	S^2	124.3	57.3	74.3	4.3	2.3	22.3	21	1.3*	0.3*	0
VORTICELLA CONVALLARIA	$\bar{X}_2$	6	2	42.3	16.3	17.7	26.3	67.3	57	12.7	0
	S^2	13	1*	156.3	212.3	42.3	158.3	330.3	61	60.3	0

* $S^2 \leq \bar{X}$

Table 4.3 (contd)

(ii) In Sampling days (n = 6)

		DAY 1 (A + B)	DAY 2 (C + D)	DAY 3 (E + F)	DAY 4 (G + H)	DAY 5 (I + J)
CILIATES	$\bar{X}_2$	379	242	225.7	217	62.3
	S^2	39421.6	7775.6	1753.1	2186.8	5115.5
CYCLIDIUM HEPTATRICHUM	$\bar{X}_2$	42	29.3	11.5	8	12.5
	S^2	530	936.7	65.9	11.2	374.7
CINETOCHILUM MARGARITACEUM	$\bar{X}_2$	118.7	64.5	115.5	40	24.2
	S^2	1975.9	110.3	587.5	231.6	666.6
LITONOTUS SP.	$\bar{X}_2$	9.5	4.2	4	7.5	1.5
	S^2	40.3	35.8	5.2	23.5	7
OXYTRICHA SP.	$\bar{X}_2$	19.5	6	8.5	16.8	0.8
	S^2	98.3	8.4	23.1	49.8	1
TACHYSOMA PELLIONELLA	$\bar{X}_2$	151.2	65.7	37.8	26.8	12.3
	S^2	12605.8	878.7	231.4	381	234.3
ASPIDISCA COSTATA	$\bar{X}_2$	11.8	7	9	8.8	0.2
	S^2	75.4	57.6	58	9	0.2*
VORTICELLA CONVALLARIA	$\bar{X}_2$	4	29.3	22	62.2	6.3
	S^2	10.4	350.3	102.8	188.6	72.3

* $S^2 \leq \bar{X}$

Table 4.4 Transformations derived from Taylor's Power Law used to normalise ciliate counts

($\ln S^2 = \ln a + b \ln \bar{x}$)

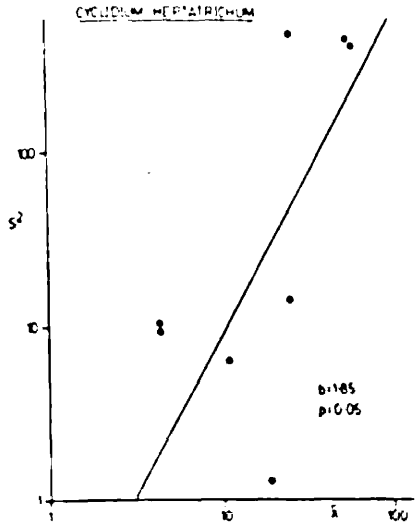
Species	Slope (b)	df	F	p	$P = 1 - b/2$
Total Ciliates	1.024	7	6.93	0.05	0.488
Cyclidium sp.	1.848	7	7.28	0.05	0.076
Cinetochilum sp.	2.025	7	4.44	0.1	-0.013
Litonotus spp.	0.308	7	0.33	0.75	0.847
Tachysoma sp.	0.783	7	1.30	0.5	0.608
Oxytricha spp.	1.952	7	46.88	0.001	0.024
Aspidisca sp.	1.327	7	13.58	0.01	0.337
Vorticella sp.	1.385	7	22.41	0.005	0.307

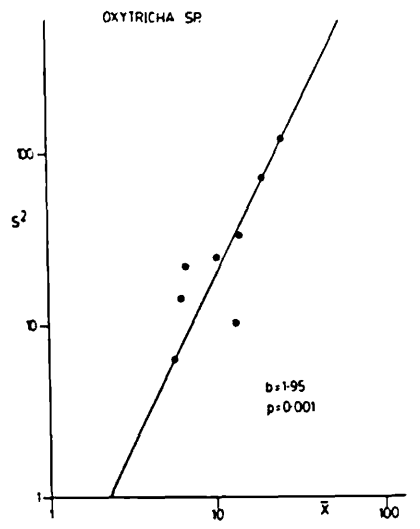
The above analysis, shown graphically for Cyclidium, Oxytricha, Aspidisca, Vorticella and the total ciliates in Fig. 4.4, indicates that the ciliate population as a whole was distributed in a close to random pattern ($b = 1.02$, $p = 0.05$) while the individual species were more clumped in distribution, Aspidisca sp the least ($b = 1.33$, $p = 0.01$) and Oxytricha spp. the most clumped ($b = 1.95$, $p = 0.001$).

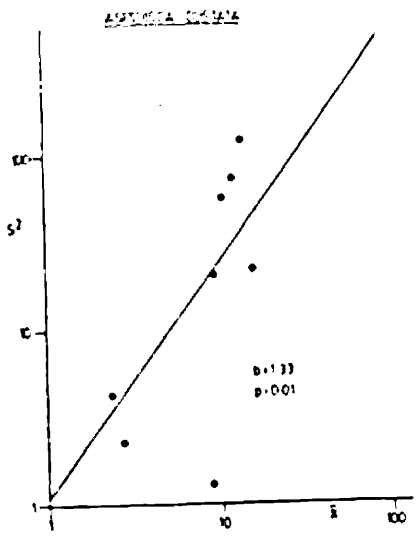
Species giving significant regressions were tested using analysis of variance (ANOVA) for differences between and within dates, and between and within cores. The ANOVA tables which are presented fully in Appendix 4.2, are summarised in Table 4.5.

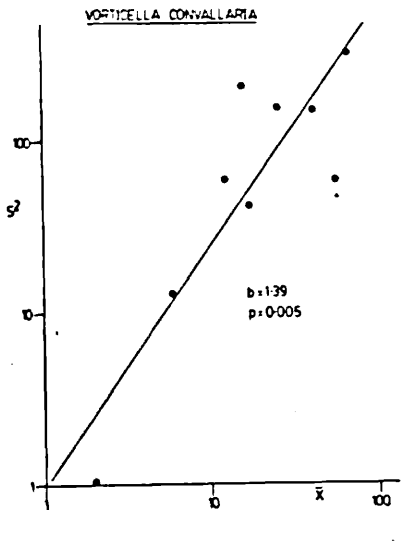
FIG 4.4 LN REGRESSION OF S^2 ON $\bar{x}$ TO DETERMINE TAYLORS POWER LAW INDEX OF DISPERSION (b)

b=0 max regularity, b=1 random, b=∞ max contagion









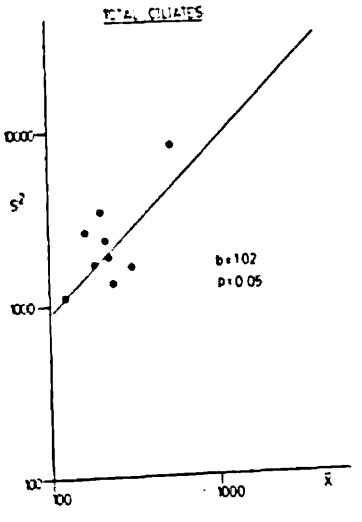


Table 4.5 Summary of analyses of variance - p values (Cores I and J are omitted for analyses of dates and cores A, I and J are omitted for analyses of cores - see text)

	Between Dates	Within Dates	Between Cores	Within Cores
Total Ciliates	0.25	0.5	0.1	0.75
Cyclidium sp.	*0.025	0.25	0.75	*0.001
Oxytricha sp.	*0.01	0.75	0.75	0.1
Aspidisca sp.	0.75	0.75	0.75	0.1
Vorticella sp.	*0.001	0.05	0.75	*0.001

* A low p value ($p < 0.05$) indicates that the probability of the differences in counts between samples being due to random effects is low and hence the samples clearly represent different populations.

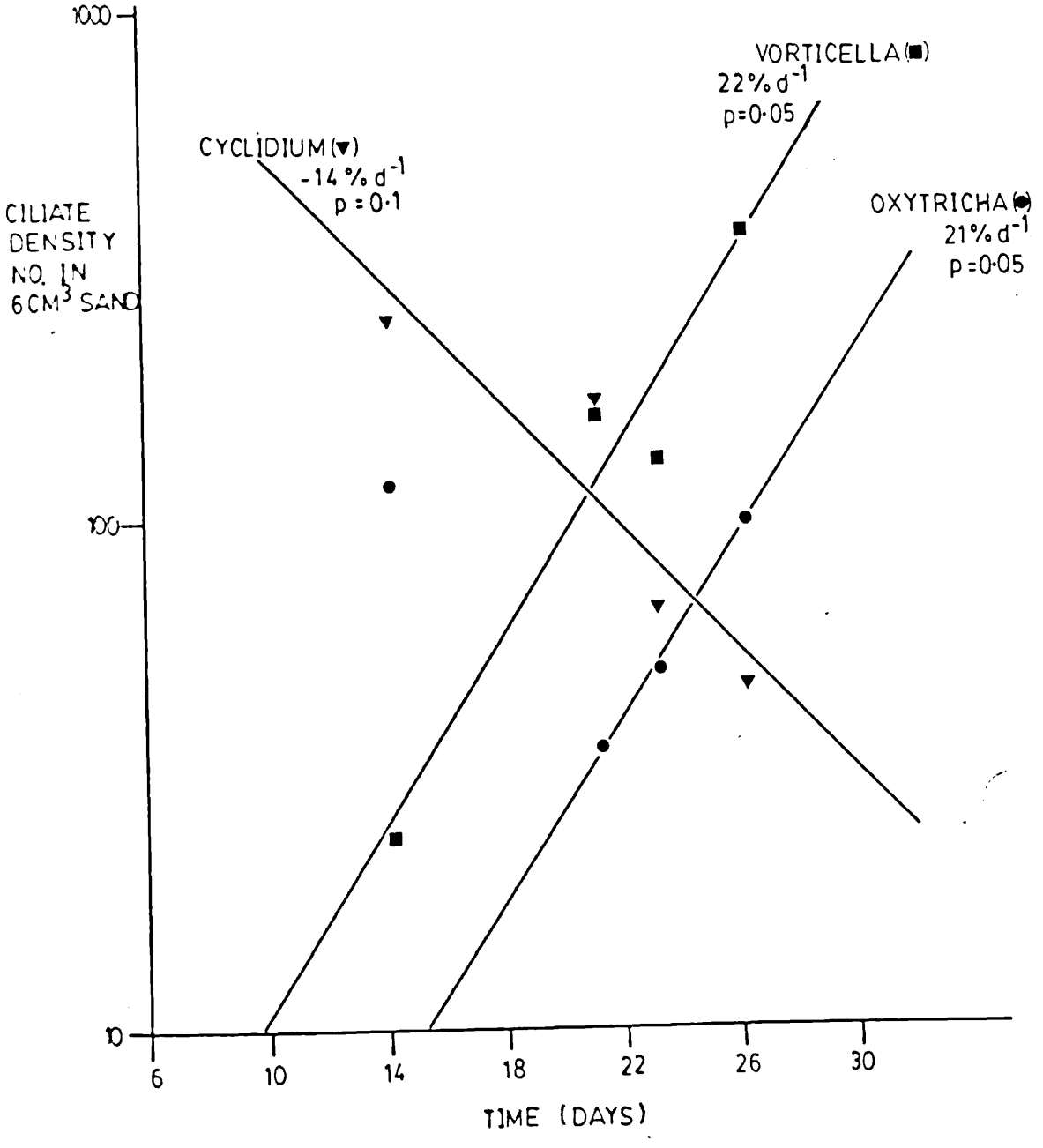
Since the cores were collected on five different dates, the first analysis examined the differences between and within dates for the ciliates in total, both of which proved to be significant (Appendix 4.2.1). However, core J had unusually low ciliate densities (Appendix 4.1) so all the data for this date were omitted from further analyses (Appendix 4.2.2). The ciliates as a whole and the individual species of Cyclidium, Oxytricha, Aspidisca and Vorticella were then examined for differences between and within dates over the first four sampling days. Within dates there was no significant variability but Cyclidium sp., Oxytricha sp. and Vorticella sp. showed significant differences in population size between dates while the total ciliates and Aspidisca sp. showed no significant differences ($p = 0.25$ and $p = 0.75$ respectively). Of the three species showing non-random variability, Vorticella sp. increased significantly at a rate of

10.3% d^{-1} ($p = 0.05$) over the whole test period, and *Oxytricha sp.* showed a similar trend (14.2% d^{-1}, $p = 0.05$) over the last three sampling days (Fig. 4.5). *Cyclidium sp.* decreased in number over the test period at a rate of 3.2% d^{-1} ($p = 0.1$), but the probability value is just too high for this decrease to be accepted as significant.

The rate of increase for *Vorticella convallaria* lies between the values recorded for this species during 2 runs performed at 12°C of 8.8% d^{-1} and 13.2% d^{-1} suggesting that the increase recorded is merely reflecting the general change in population numbers of the filter bed.

The second analysis examined the differences in total ciliate counts between and within cores using cores A to H, each with three replicates. This showed random variability within cores, but significant differences between cores (Appendix 4.2.3). Core A, which yielded higher densities of ciliates than the rest was omitted from the final analyses which examined differences between and within cores of the individual ciliate species in addition to the total ciliate count (Appendix 4.2.4). Random variability was apparent between cores in all the analyses (Table 4.5) and for all except *Cyclidium sp.* and *Vorticella sp.* within cores; these two species showed significant differences between replicate sub-samples within the core samples ($p = 0.001$). *Vorticella sp.* tends to grow gregariously possibly explaining the observed uneven distribution within cores; *Cyclidium sp.* however, is a small free-swimming ciliate and its lack of random variability within cores is difficult to account for. The lack of replicability between sub-samples suggests that the sampling technique used was not suitable for accurately monitoring the distribution of these two species.

FIG. 4.5 EFFECT OF AGE OF THE FILTER BED RUN ON THE DENSITIES OF THREE CILIATE SPECIES



The concentration of particulate organic carbon detected in the ten cores is recorded in Appendix 4.3. Means and variances were calculated for each core (n = 3) and for each sampling day (n = 6), and these data are presented in Table 4.6.

In all cases with the exception of core A, $S^2 > \bar{X}$, indicating a contagious distribution. In order to compare the samples using ANOVA, it was necessary to normalise the data and for this a ln transformation was used. The transformed data are recorded in Appendix 4.4. Means and variances were calculated for the transformed data (Table 4.7) and in all cases $S^2 < \bar{X}$.

Independence of the variances and the means was tested both for the days (df = 1,3) and for the cores (df = 1,8) by attempting a regression plot of S^2 v $\bar{X}$. In both cases the regression lines calculated were insignificant, p = 0.25 for the cores and p = 0.75 for the days. Also the sums of squares for the ln-transformed data were additive, hence the ln-transformed carbon data was normally distributed and could be used in analyses of variance to examine within and between date and core carbon content variations. The analyses, recorded in Appendix 4.5 and summarised in Table 4.8, show that there was possibly significant variability between the sampling days (5 dates with 6 replicates) and between the cores (10 cores with 3 replicates). However, both within cores and within dates the sub-samples showed no significant differences in their particulate organic carbon content.

Table 4.6 Means and variances for the carbon content

(i) In cores (n = 3)

CARBON μgcm^{-3}		A	B	C	D	E	F	G	H	I	J
	$\bar{X}$	147.33	146	191.67	114.67	196	174.33	190.33	225	171	164
	S^2	54.22	258.77	304.22	934.89	2318	860.22	749.56	284.67	968.67	400.67

(ii) In sampling days (n = 6)

CARBON μgcm^{-3}		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
	$\bar{X}$	146.67	153.17	193.17	207.67	167.5
	S^2	156.89	2101.81	1541.81	817.56	696.92

Table 4.7 Means and variances of the ln transformed carbon content

(i) In cores (n = 3)

ln carbon		A	B	C	D	E	F	G	H	I	J
	$\bar{X}$	4.99	4.98	5.25	4.71	5.25	5.15	5.24	5.41	5.12	5.09
	S^2	0.003	0.012	0.009	0.070	0.055	0.033	0.021	0.005	0.039	0.015

(ii) In sampling days (n = 6)

ln transformed carbon data		1.6.77 DAY 1	8.6.77 DAY 2	10.6.77 DAY 3	13.6.77 DAY 4	15.6.77 DAY 5
	$\bar{X}$	4.98	4.98	5.20	5.33	5.11
	S^2	0.007	0.114	0.464	0.021	0.027

Table 4.8 Summary of ANOVA analysis of carbon data

Source of Variance	
Between dates	$p = 0.05$
Within dates	$p = 0.25$
Between cores	$p = 0.05$
Within cores	$p = 0.5$

There is no good justification for omitting core A from the analyses of differences in counts between and within cores, except for the fact that using cores B to H one can say that, with the two exceptions already mentioned, there is random variability within and between cores and hence one sub-sample removed from one core is representative of the sand at that depth in the cores within the filter bed on the day of sampling. However, once core A is included in the calculations this no longer remains true.

Until a better sampling system is designed which permits the necessary replication, while avoiding the impossible counting load presented by these small ciliates, the errors inherent with the current system will remain, and should be appreciated when interpreting the counts from the regular monitoring of the filter beds.

4.7 Examination of bacteria

Three different techniques were used to examine the depth distribution of bacteria associated with sand grains in the top 30 cm of a slow sand filter bed; these were: direct examination of the sand grains by epifluorescence microscopy, direct examination by scanning electron microscopy and removal of bacteria from the grains, by

shaking with sterile water, prior to enumeration by epi-fluorescence microscopy. The latter was found to be the most practical method for repeated sampling and analysis and was, therefore, adopted to examine changes in bacterial density within the filter bed during the first ten days of a filtration run.

Direct examination of sand grains by epifluorescence microscopy

Grains of sand from 1 cm, 2cm and 4 cm depths of a 28 day old core were examined within 2 hours of sampling. Prior to analysis the samples were stored as described previously (Section 4.4.5) to prevent dessication, as this could cause the bacteria to slough off the grains. The grains were stained in filter and heat sterile[1], acridine orange[2] (10 mg/l) for 5 minutes and then washed in three consecutive aliquots of sterile water. They were transferred onto a microscope slide and viewed under an 100 X fluorescence water immersion objective (N.A. 1.20) with an overall magnification of 800 X, under incident ultra-violet light. The details of the microscopy are described later (Section 4.7.2). The acridine orange, which is taken up by bacteria into the DNA of the cell, absorbs blue light and emits light of a lower energy (longer wavelength), which is seen as fluorescent green. Care was required when changing the field of view in order to avoid damaging the objective on the uneven surface of the grain. Thirty fields of view were counted per grain and three grains were

1. All liquids to be sterilised were filtered through 0.22 μm Millipore filters before they were autoclaved.

2. Acridine orange - BDH, C.I.46005.

examined at each depth. The surface area of each grain was estimated by measuring the side of greatest area using a micrometer eyepiece under low magnification. The surface area measured was approximated to one or more geometrical shapes, the area calculated and then multiplied by two. Each sand grain was weighed so that the bacterial count could be expressed in terms of bacteria per unit surface area or bacteria per unit weight of sand.

The two main disadvantages of this technique were firstly that the bacteria were very unevenly distributed, tending to be localised in hollows and cracks as was also found by Meadows and Anderson (1966) and secondly, only the areas of the grain which were fairly free from 'slime' could be counted. Where the bacteria were embedded in this material, an overall fluorescent glow was seen which made counting individual bacteria an almost impossible task. Because of these disadvantages, and the danger of scratching the objective while examining sand grains, this method was not adopted.

Scanning Electron Microscopy

Sand grains from depths down to 25 cm in a 28 day old core were fixed in 4% glutaraldehyde in a 0.1M phosphate buffer (pH 7.4) within 1½ hours of sampling. They were fixed overnight at 4°C, washed in 0.1M phosphate buffer (pH 7.4) and finally dehydrated through a series of different concentrations of acetone from 30% up to 100% (C. Spurdon, Botany Dept. R.H.C., pers. comm) at which stage they were stored until microscope time became available. Before examination, the samples were critical-point-dried in carbon dioxide using a Polaron Critical Point Drier. There is a critical temperature at which a liquid and its vapour have the same specific gravity and will thefore mix with each other.

This property allows a liquid to be removed from the sample without a phase boundary passing through it, as that would disrupt fine structures (Cross, et al, 1977) and probably cause the bacteria to come off the sand grains. The sand samples in acetone were placed in the drying chamber and immersed in liquid carbon dioxide. The chamber was slowly heated to just above its critical temperature of 31.5°C at which its critical pressure is about 1,200 lb per square inch. The liquid disappeared and as soon as only carbon dioxide vapour was present in the chamber, the pressure was lowered to atmospheric pressure, while maintaining the temperature at 32°C. The samples were placed on adhesive coated stubs, sputter-coated with gold, using a Polaron E5100 Series 2 Cool Sputter Coater, to a thickness of 75-100 Å and viewed with a Cambridge S4/10 scanning electron microscope at an accelerating voltage of 30 KV.

4.7.1 Removal of bacteria from sand grains

Sand grains were found to be too coarse for a homogeniser, which has been used to effect removal of bacteria from sediments (Hobbie, pers. comm.). Shaking sand samples with water is of limited value in removing bacteria from the grains, as they seem to cling on with great tenacity (Pearse, et al, 1942). However, by accepting these limitations and standardising the shaking procedure to give comparable results, this technique has been used successfully (Pearse et al, 1942; Anderson and Meadows, 1969; Metropolitan Water Board Report No. 43, 1967/68). To improve the efficiency of bacterial removal, Babiuk and Paul (1970) and Fliermans and Schmidt (1975)

added various dispersal agents, e.g. 1% sodium metaphosphate, but these were found to be slightly toxic to certain bacteria even at low concentrations (Babiuk and Paul, 1970).

To remove bacteria from the filter bed sand, a 1 cm^3 subsample was shaken with 10 cm^3 of sterile distilled water for thirty minutes on a Gallenkamp flask shaker. The supernatant was used for bacterial enumeration. The efficiency of this shaking procedure was tested by removing some of the grains after they had been shaken and staining and viewing them as described earlier (p. 79). The supernatant was also stained and counted and the numbers in both cases were manipulated to estimate the total count of bacteria present in 1 cm^3 of sand. (p. 80 and 84). Approximately one third of the bacteria associated with the grain surface was found to be removed by shaking. However, as the procedure was strictly standardised it was assumed that all results were comparable, and that population changes measured in this way were accurate estimates.

4.7.2 Enumeration of bacteria in suspension

Spread plates and the plate dilution frequency technique of Harris and Sommers (1968) were tested using Collins CPS agar (Collins and Willoughby, 1962), and direct counts were also made in a Neubauer chamber, using the same bacterial suspension of stagnant pond water for all three techniques. The counts obtained by these three methods were vastly different ranging from 10^6 ml^{-1} to 10^{15} ml^{-1} and the variation within each method was

unacceptably high. Direct examination using acridine orange epifluorescence was found to be more reliable and the means of replicate counts did not differ significantly ($p > 0.05$, Table 4.9) so a procedure based on that described by Jones and Simon (1975) was used.

After the sand sample had been shaken for 30 minutes with sterile water, a ten-fold series dilution was prepared from the supernatant down to 10^{-3}. The dilution, found by trial and error to give no more than 30 counts per field was counted, this was generally the 10^{-1} or 10^{-2} dilution. 1 ml of sterile acridine orange (100 mg l^{-1}) was added to the bacterial suspension, giving a final concentration of 10 mg l^{-1} acridine orange. The tube was inverted once to mix the stain and suspension and left to stand for six minutes. During this time a Millipore 0.22 μm membrane filter which had been dyed black with Dylon No. 8 Ebony Black (Jones and Simon, 1975) was placed in a sterile Millipore filter holder, and covered with about 5 cm^3 of pre-filtered, sterile, diluent water. This soaked the membrane and facilitated even filtering of the bacterial suspension over the whole area of the membrane filter. After the six minute staining period, the suspension was filtered through the membrane filter and the retained bacteria and detritus washed with a further 20 cm^3 of sterile water. As the last of the water passed through the membrane it was transferred onto a drop of immersion oil on a microscope slide, immediately covered with more oil and viewed under incident ultra violet light. Thirty fields were counted per membrane, this was found to be the optimum number. When fewer fields were counted

there was a tendency to overestimate the numbers present, but no further accuracy was gained by counting more than thirty fields. A random choice of field was ensured by moving stepwise across the membrane using the mechanical stage and counting the number of bacteria present in the field of view after each pair of movements on the horizontal and vertical scales. Using a micrometer eye piece, the surface area of the field of view was calculated to be $4.16 \times 10^3 \mu^2$. The filtering area of the membrane filter was $2.01 \times 10^8 \mu^2$, so the number of bacteria present over the whole filter, i.e. shaken from 1 cm^3 of sand was estimated by:

$$\text{Total no. of bacteria shaken from 1 } cm^3 \text{ sand} = \text{Mean no. of bacteria per field of view} \times \frac{2.01 \times 10^8}{4.16 \times 10^3}$$

Microscopy

Counts were made on a Leitz Orthoplan microscope fitted with a 50-W ultra high pressure mercury lamp and a Pleomopak 2 fluorescence vertical illuminator. Excitation was through a 2 mm KG1 heat absorption filter, a 4 mm BG 38 red suppression filter, a K490 nm suppression filter and the dichroic mirror/suppression filter combination TK 510/K515. The image was viewed through a K510 nm suppression filter in the filter slide (Fig. 4.6).

Bacteria were counted through an NPl 100X oil immersion objective (N.A. 1.32) at a total magnification of 800 X. The mean exciting wavelength of acridine orange is 470 nm and its fluorescence maxima lie between 530 and 650 nm. Figure 4.7 shows the effect of the filter combination used to suppress the exciting radiation and transmit the fluorescent radiation only.

FIG. 4.6 DIAGRAM OF LIGHT PATH FOR EPIFLUORESCENCE MICROSCOPY

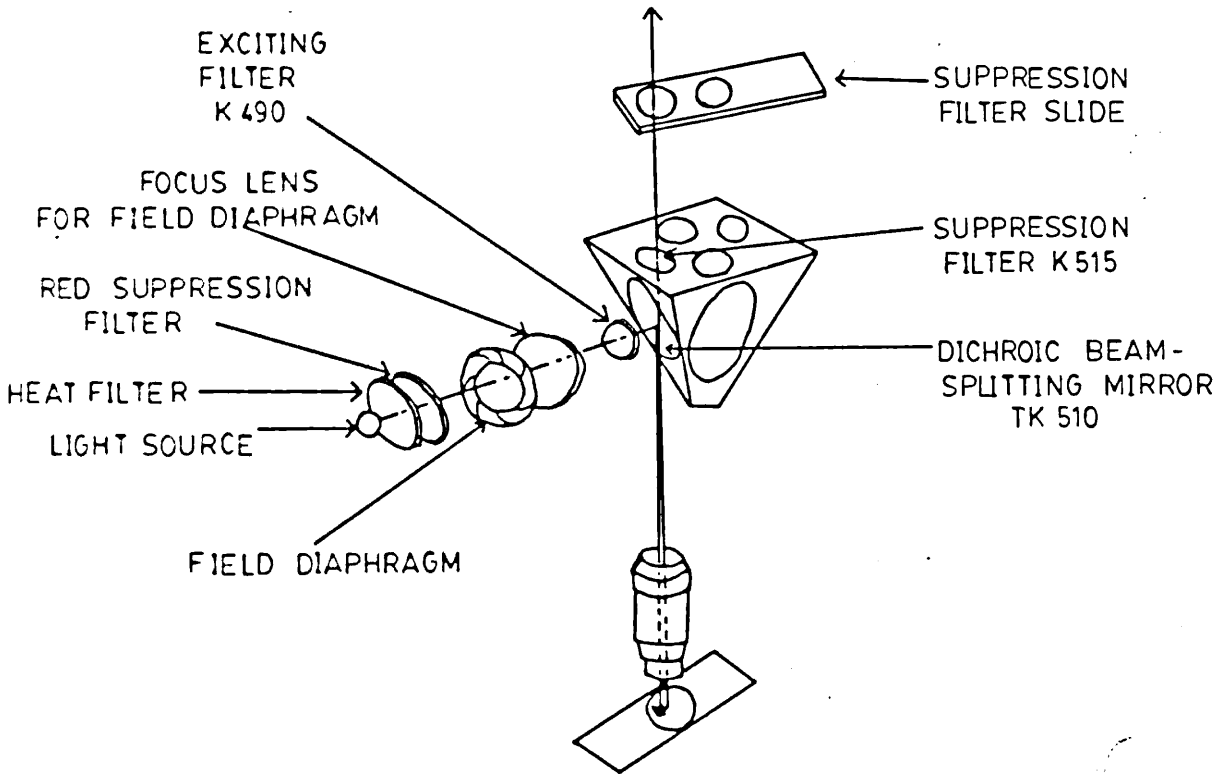
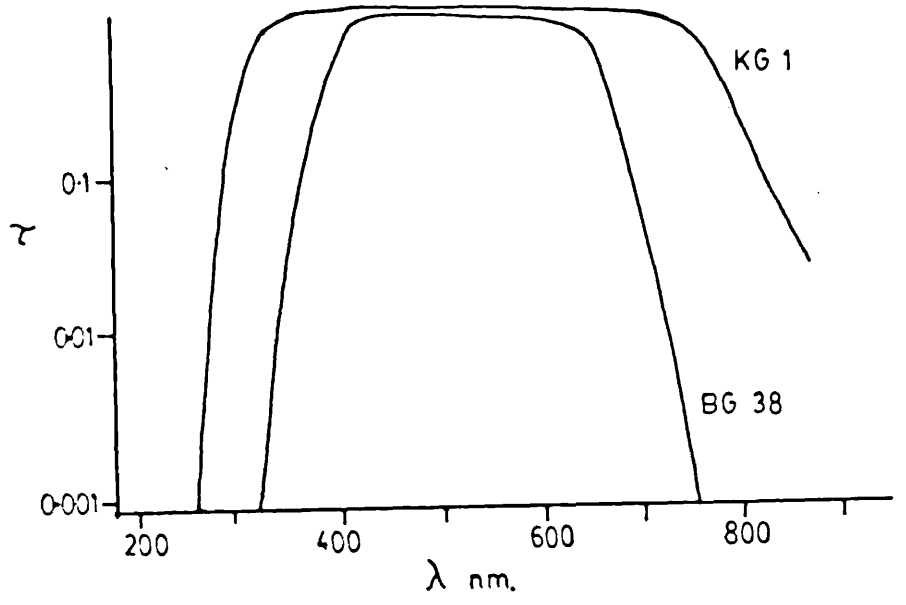
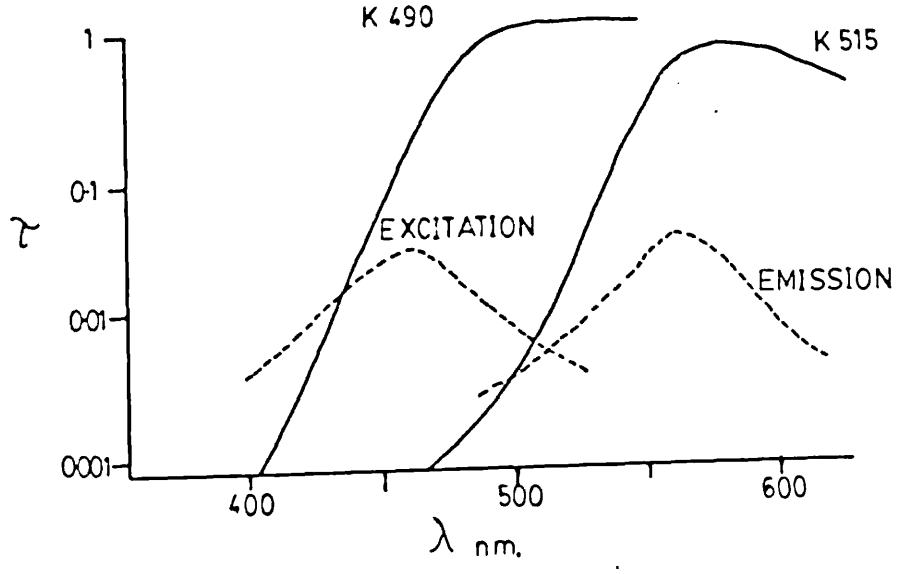


FIG. 4.7 THE EFFECTS OF FILTERS ON THE EXCITATION AND EMISSION MAXIMA OF ACRIDINE ORANGE





Tests on the sterile technique, conducted using a sterile water control in place of a bacterial suspension, suggested that a 0.2% error was introduced to the counts by the sterile water used as diluent, pre-filter diluent and wash water, the acridine orange solution and the black membrane filters.

Table 4.9 Replicate counts of bacteria stained with acridine orange

DEPTH (CM)	Mean number per field (30 fields)							
	Sample 1		Sample 2		Sample 3		Sample 4	
	Replicate		Replicate		Replicate		Replicate	
	1	2	1	2	1	2	1	2
1	2.93	4.33	3.07	1.87	2.00	2.13	2.93	5.83
2	1.57	3.70	1.33	1.50	1.87	2.23	3.43	3.17
3	3.63	7.13	2.43	5.83	1.93	5.17	3.63	1.80
4	4.47	3.53	1.46	2.40	2.63	2.03	4.33	3.10
5	2.70	1.87	0.97	2.93	4.13	2.00	2.80	1.93
$\bar{x}$	3.06	4.11	1.85	2.91	2.51	2.71	3.42	3.17
S	1.08	1.92	0.87	1.72	0.95	1.38	0.61	1.62
t	1.07		1.23		0.27		0.32	

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{1/n\,(S_1^2 + S_2^2)}}$$

Critical value of $t_{0.05}(8) = 2.306$

4.8 Organic carbon determination

Organic matter present in a slow sand filter consists of live microfauna, meiofauna and algae in addition to the decaying organic matter of both autocthonous and allocthonous origin. Consequently an overall measure of organic carbon does not give an accurate value of food availability for any particular organism but it does give an indication of food conditions, is easily determined and gives information which can be compared with other environments in addition to being used to examine seasonal and other time-related changes of food availability within the slow sand filter environment.

The method used to determine particulate organic carbon (P.O.C.) was one of wet dichromate oxidation followed by a potentiometric end point detection in a titration to determine the volume of potassium dichromate remaining after the oxidation process. This has been described in detail by Lodge (1979) and is summarised below.

4.8.1 Experimental procedure

The subsamples of sand used for carbon determination had been stored in capped vials after drying at 80°C for 24 hours on the day of sampling (Section 4.4.5). Analysis of 90 subsamples took two days, the first occupied by the oxidation procedure and the second by titrations.

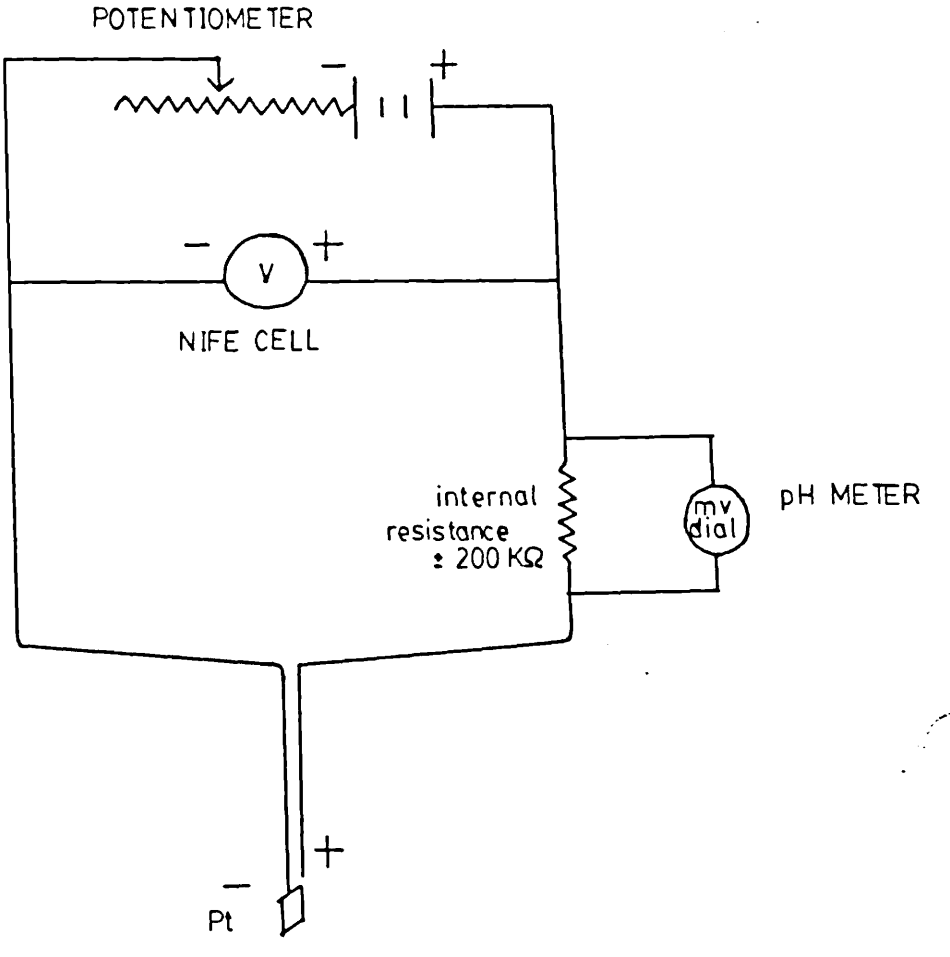
Five mls of distilled water were added to a 1 cm^3 sand sample and this was shaken for two hours. One ml of the supernatant was removed into a pyrex glass reaction vessel and to this one ml of 0.2N dichromate and four mls of concentrated sulphuric acid were added. The vessel was

swirled to mix the fluids and placed in an 120°C oven for three hours, and then allowed to cool. The reactants were diluted with twenty mls of distilled water, so that the platinum/calomel electrode used to detect the titration end point, could be immersed sufficiently, and titrated against a 2% acidic solution of 0.1N ferrous ammonium sulphate with continuous mixing by a magnetic stirer. The end point was determined potentiometrically.

The potential difference between a platinum indicator and a combined calomel reference electrode, with a resistance of 200 KΩ across it which reduced the voltage to a value detectable on the millivolt scale of a pH meter, was used to follow the titration. The platinum electrode was polarised by an applied potential of +1 volt, produced by a Nife cell in conjunction with a potentiometer, and the resulting low potential difference between indicator and reference electrode was corrected to zero on the mv scale of the pH meter by using the variable zero control (Fig. 4.8). A Metrohm EA234 combined platinum/calomel reference electrode was used and the potential difference measured by a Pye Dynacap pH meter.

The ferrous ammonium sulphate (FAS) titrant was added from a Metrohm E457 piston burette, which had a capacity of five ml. The normality of the FAS was chosen as 0.1N so that the volume of titrant used per titration was between one and two mls. FAS was added until all excess dichromate had been reduced and excess ferrous ions were present in solution. These caused the platinum electrode to become negatively charged, registering a potential difference on the mV scale:

FIG. 4.8 DIAGRAM OF CIRCUIT USED TO DETECT TITRATION END-POINT



$$Fe^{2+} \rightleftharpoons Fe^{3+} + e^-$$

A reading was taken immediately after the end point and four further readings were taken after successive additions of 3µl aliquots of FAS. The increase in potential registered was directly proportional to the volume of FAS added and so by extrapolation through these points back to zero mV, the end point value of FAS could be obtained (Fig. 4.9). Blanks to determine the carbon content of the distilled water used and controls to determine the normality of the FAS were also titrated.

4.8.2 Calculation of carbon content

After subtracting the average titrant value for three blanks from a sample titrant value, the resulting volume of FAS was equivalent to the volume of dichromate used in the carbon oxidation. From this value the carbon present in the 1 cm^3 sand subsample could be calculated:

Let V_B = Volume of FAS used to reach Blank end point (µl)
V_S = Volume of FAS used to reach Sample end point (µl)
N_F = Normality of FAS
V_W = Volume of water shaken with sample in vial (ml)
V_A = Volume of aliquot of suspended organic matter removed for reaction (ml).

The equivalent weight of an oxidising agent is that weight of the reagent which reacts with or contains 8.0 g of available oxygen (Vogel, 1955).

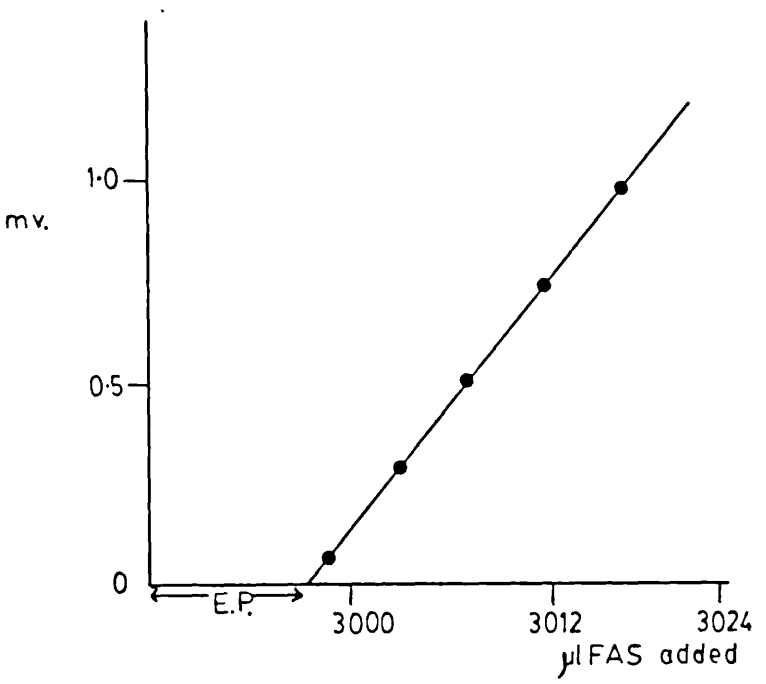
Hence 1 litre of 1 N $K_2Cr_2O_7 \equiv 8.0g\ O_2$

1.0 ml $N/8\ K_2Cr_2O_7 \equiv 1.0$ mg O_2

$\therefore$ 1.0 ml $N/8\ (NH_4)_2\ SO_4FeSO_4 \equiv 1.0$ mg O_2

$$(V_B-V_S)\ \mu l\ N_F\ FAS \equiv \frac{1.0 \times (V_B-V_S) \times 8 \times N_F}{1{,}000}\ mg\ O_2$$

FIG. 4.9 EXTRAPOLATION OF REGRESSION LINE, CALCULATED FROM TITRATION DATA, TO DETERMINE END POINT TITRE



E.P. = END POINT TITRE

This represents the titrant value for V_A, so in the whole subsample:

$$\equiv \frac{1.0 \times (V_B - V_S) \times 8 \times N_F}{1{,}000} \times \frac{V_W}{V_A} \text{ mg } O_2 \text{ cm}^{-3} \text{ sand}$$

If all the carbon present in the sample is assumed to be in the form of simple sugars, then:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

72 g equiv C 192 g equiv O_2

and 72 mg C $\equiv$ 192 mg O_2

$\therefore$ 0.3754 mg C $\equiv$ 1 mg O_2

$\therefore$ Volume of $K_2Cr_2O_7$ consumed in the reaction is equivalent to:

$$\frac{(V_B - V_S) \times 8 \times N_F}{1{,}000} \times \frac{V_W}{V_A} \times 0.3754 \text{ mg C cm}^{-3} \text{ sand}$$

$$= (V_B - V_S) \times 8 \times N_F \times \frac{V_W}{V_A} \times 0.3754 \mu\text{g C cm}^{-3} \text{ sand}$$

where $V_W = 5$ and $V_A = 1$ this simplifies to:

$$(V_B - V_S) \times N_F \times 15.0145 \mu\text{g C cm}^{-3}$$

To ease the lengthy processes of regression to determine V_B, V_S and V_C (the end point necessary for the determination of N_F), followed by the calculation of carbon present in the subsample, a computer programme was written in Fortran IV to perform all these tasks. On receiving the titration data input, the output listed the average control end point, the normality of the FAS, the average blank end point and the subsample titration regression

statistics, in addition to the carbon content of the sand expressed as μg C per cm^3. (Appendix 4.6).

4.9 Statistical analyses

4.9.1 Normal distribution parameters

Means quoted are arithmetic. 95% confidence limits of the means were calculated as:

95% confidence limits = mean $\pm$ tSE

where S.E. = Standard Error of the mean.

When the data did not fulfill the requirements of a normal distribution, it was necessary to transform it. Any such transformations are described where used in the text.

4.9.2 Numerical integration

Numerical integration was employed to estimate total population densities of organisms in a core of sand with a cross-sectional area of 1 cm^2, when only samples from eight depths, spaced down the core, had been examined. A Hewlett Packard Program Tape was used which accepted x(depth) and y (numerical abundance) data and calculated the area under the smoothest curve which could be described by the series of points entered in to the programme.

The integral obtained was then divided by the lowest depth examined to give an estimated average population density. Although this was not an accurate estimate for any given depth, as densities were always much higher at the surface of the sand, it did allow for comparisons between cores and between different filtration runs, as

during the earlier runs the lowest depth examined varied. Once a procedure had been established the lowest depth examined was 25 cm.

4.9.3 Linear regressions

Linear regression analyses were used to define the relationship between population density and time. The population density of the organisms was found to increase exponentially with time; described by the regression formula:

$$N_t = N_o e^{rt}$$

by taking the natural logarithms of both sides of the equation it becomes:

$$\ln N_t = \ln No + rt$$

Hence a regression plot of ln population density (N) against time (t) had a slope r which represented the instantaneous rate of numerical increase. The significance of the slope was determined by comparing the variance ratio of the regression equation at the required degrees of freedom with those tabulated in Rohlf and Sokal (1969).

4.9.4 Analysis of variance

Analysis of variance tests were used to compare the variation in a set of data which had been partitioned into components associated with the possible sources of variation. Two-way analysis of variance was used to compare matched groups of data and to determine whether variation was significant between and/or within these groups of data. Details of the calculations are given in Sokal and Rohlf (1969).

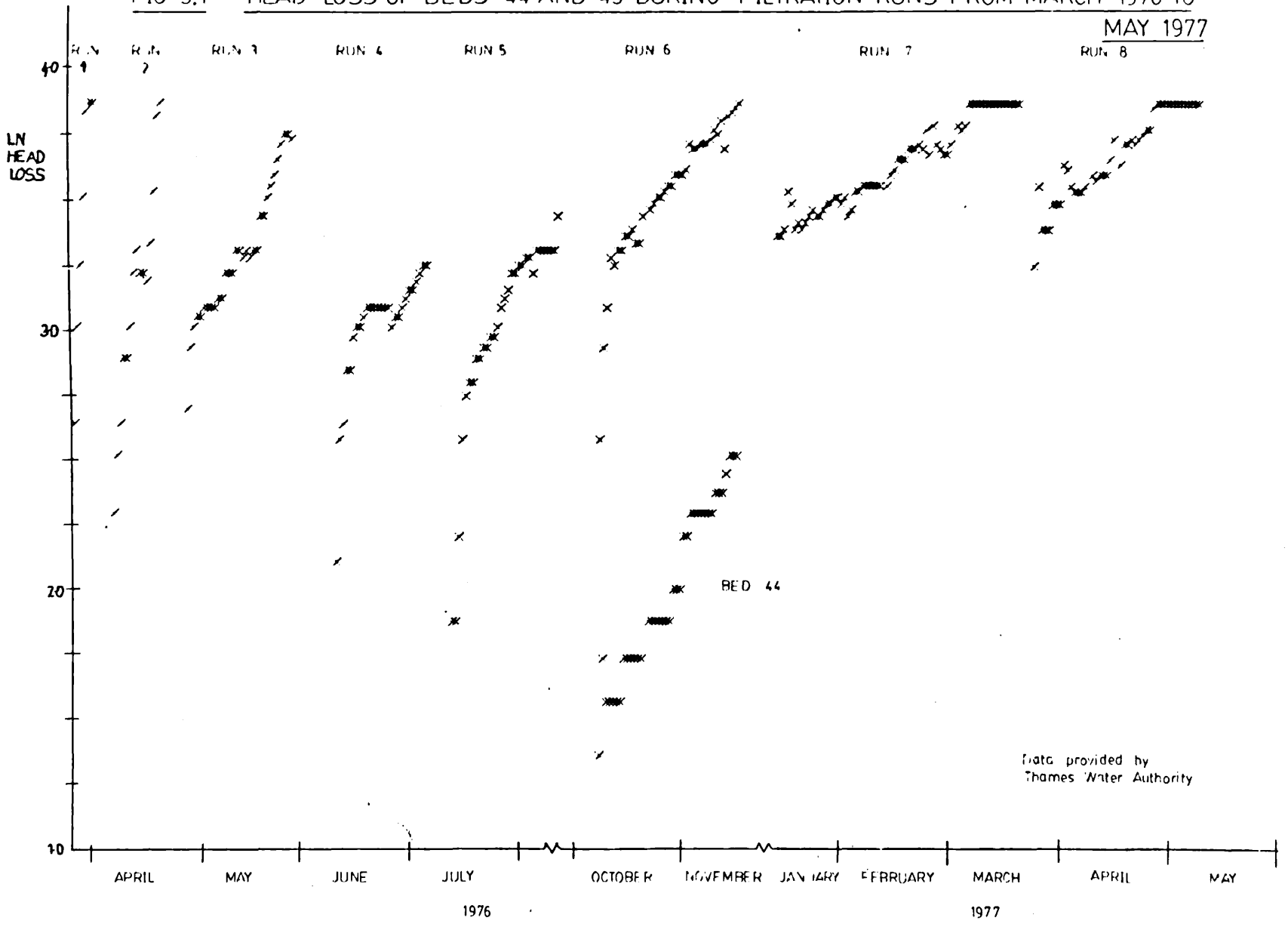
Chapter 5 Environmental Parameters

Certain physical and chemical parameters of the filter bed were monitored by the Metropolitan Water Division of the Thames Water Authority; these included head loss, flow rate and dissolved oxygen, which were recorded continuously, and particulate organic carbon content of the surface and filtrate waters, which were determined weekly. In addition to these, each sample core removed from the filter beds during the study, was analysed for particulate organic carbon content throughout its depth. Grain size and porosity of the filter bed sand in sampling cores were also determined. The surface water temperature was recorded on each sampling occasion and these temperature data are listed with the sampling programme in Appendix 5.1.

5.1 Head loss

During each filter bed run the head loss, or difference in head between raw and filtered water (described on Page 21) increased rapidly over the first few days (Fig. 5.1) resulting from the rise in filtration rate to the target value of 16" h^{-1} (0.4m h^{-1}) on bed 45 and 8" h^{-1} (0.2m h^{-1}) on bed 44 (Appendix 5.2). Following this initial sharp rise, the head loss usually continued to increase more gradually until the end of the run. However, during run 3, a second sharp rise occurred after an interval of two weeks. Observing the increase in head loss alone did not take into account the changing flow rate, which was gradually increased to the target value and maintained at this rate until the resistance of the

FIG 5.1 HEAD LOSS OF BEDS 44 AND 45 DURING FILTRATION RUNS FROM MARCH 1976 TO MAY 1977



bed increased to a value above which further head loss gain was accompanied by a decrease in filtration rate. The head loss data was corrected to the target filtration rate as follows:

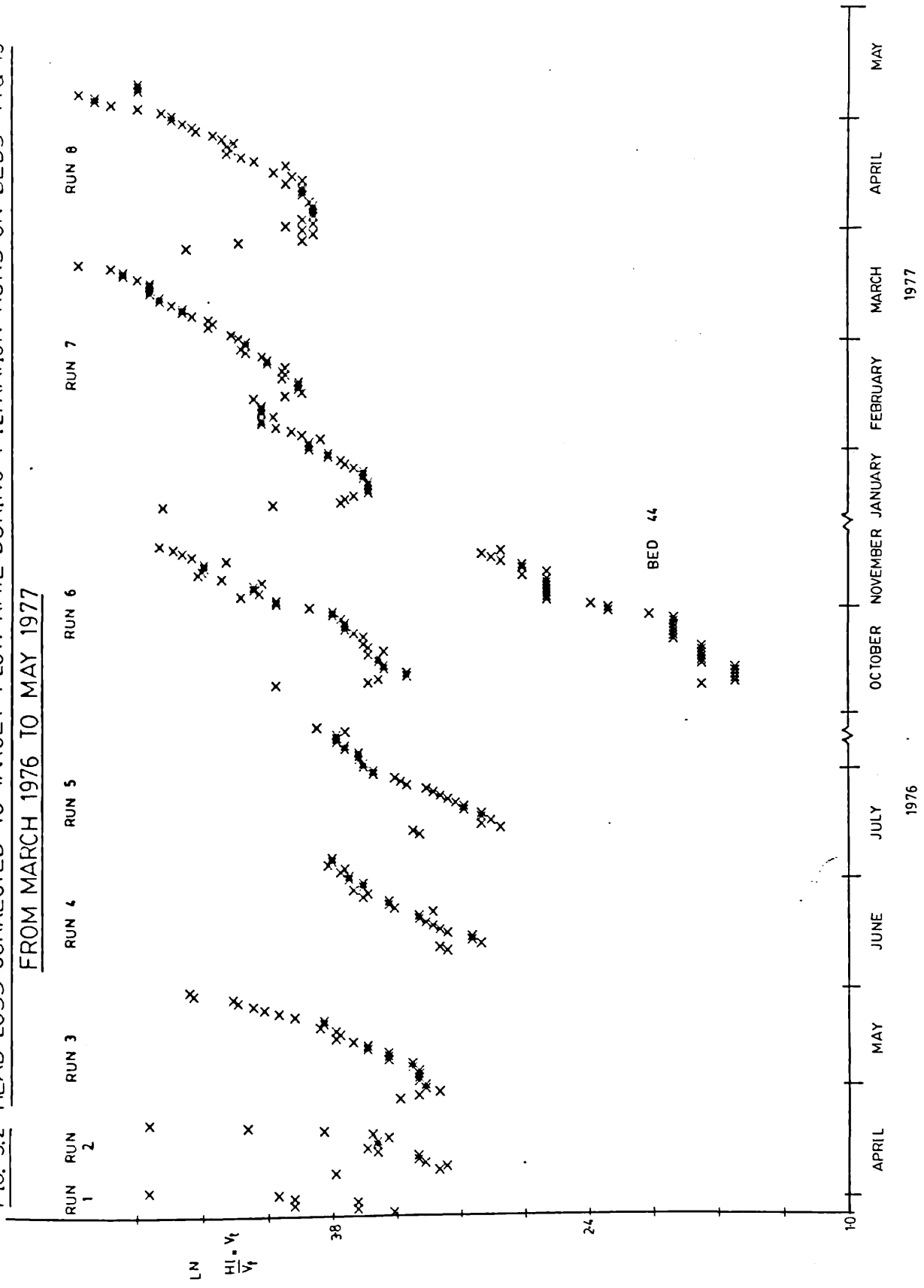
$$Ht = \frac{Hf}{Vf} \times Vt \text{ in.}$$

Where Ht = head loss at target filtration rate (in.)
Hf = head loss at actual filtration rate (in.)
Vt = target filtration rate ($in.h^{-1}$)
Vf = actual filtration rate ($in.h^{-1}$)

The increase of the corrected head loss with time (Appendix 5.3) was gradual over the first week or two of a run, but suddenly changed to a much more rapid increase after this period (Fig. 5.2). Variations from this characteristic pattern did occur; a rapid increase in head loss occurred throughout run 1, a very short run following the resanding of bed 45 in March 1976. During runs 4 and 5, the two summer runs, the low rate of gain in head loss was continuous throughout the length of the runs; a possible explanation of this is given later (p.100). During run 7, a very cold winter run with an average temperature of $4^{o}C$, the increase in head loss was again fairly constant throughout the run, apart from a drop in head loss during the fifth week. Head loss per unit flow increased constantly in bed 44 over the whole run, while at the same time in bed 45, with twice the flow rate, the rate of head loss gain doubled after 22 days.

Particulate organic carbon was determined in the 30 cm cores removed from the filter bed on each sampling occasion (Appendix 5.4). The carbon content of the top

FIG. 5.2 HEAD LOSS CORRECTED TO TARGET FLOW RATE DURING FILTRATION RUNS ON BEDS 44 & 45
FROM MARCH 1976 TO MAY 1977



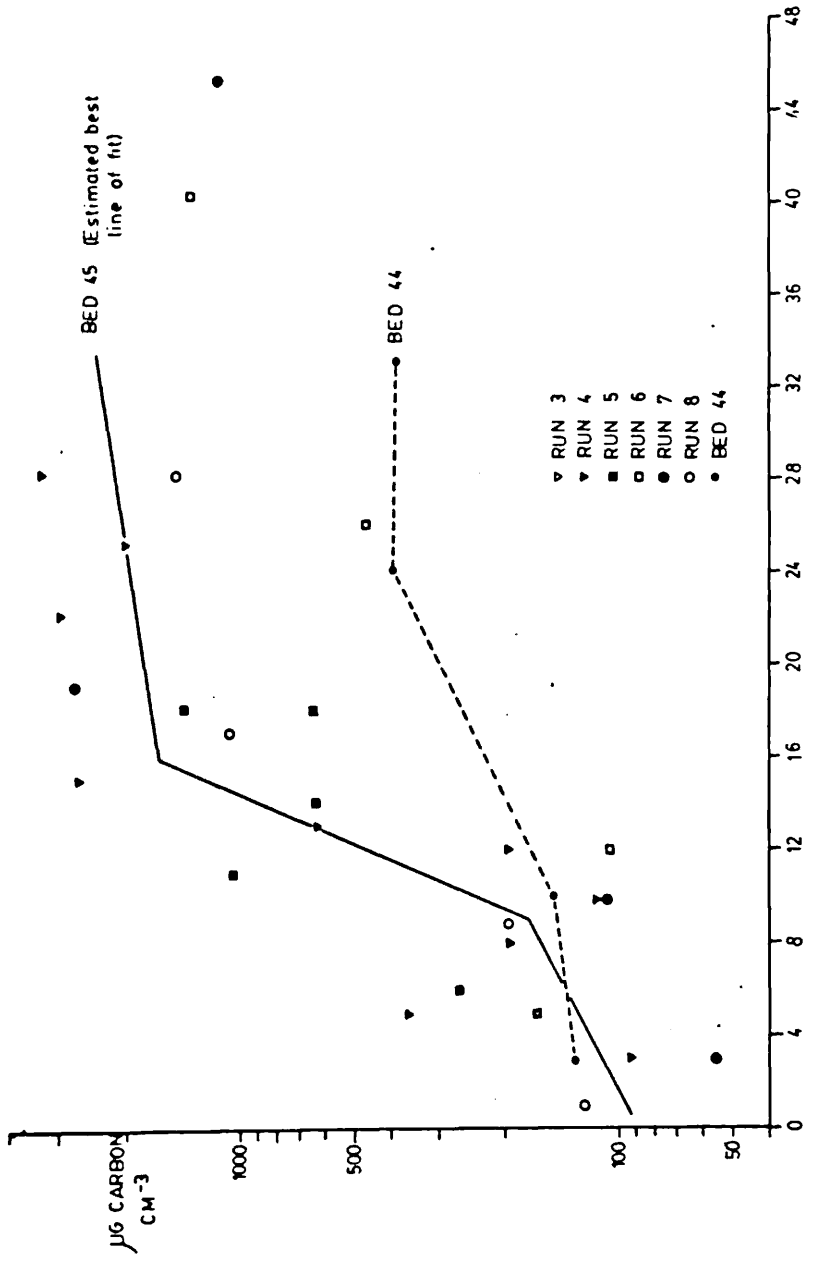
1 cm of sand is shown in Figure 5.3. During every run on bed 45, between day 10 and 15, a sudden accumulation of surface carbon occurred giving at least a 10-fold increase over that period. In bed 44 the increase was of a lower magnitude (about 3-fold) and occurred over fifteen days between days 10 and 25.

During runs 3 and 8, the two spring runs, this rapid carbon increase coincided with the change loss gain and in run 6, the change in head loss immediately followed the carbon increase. However, the rapid carbon accumulation during the 4°C winter run (run 7) had no apparent effect on the increase of head loss, and similarly during the two summer runs (runs 4 and 5) the rapid carbon increase did not effect the constant rate of gain in head loss. This constant rate of gain in head loss observed over the summer runs may have been due to the formation of a 'Schmutzdecke' over the filter skin. This reduces the suspended matter reaching the filter proper, provided that compaction of the Schmutzdecke does not occur onto the filter bed surface (Huisman, 1970). In run 5, the dissolved oxygen of the top water correspondingly increased from 5 mg l^{-1} to 12 mg l^{-1} over this period of the run from day 18 to 26 indicating higher primary productivity above the filter surface (Fig. 5.4a) which would concur with the presence of a Schmutzdecke.

5.2 Dissolved Oxygen

Limited data are available from the continuous monitoring of dissolved oxygen, as the recording unit was first introduced in July 1976 and was then run on an

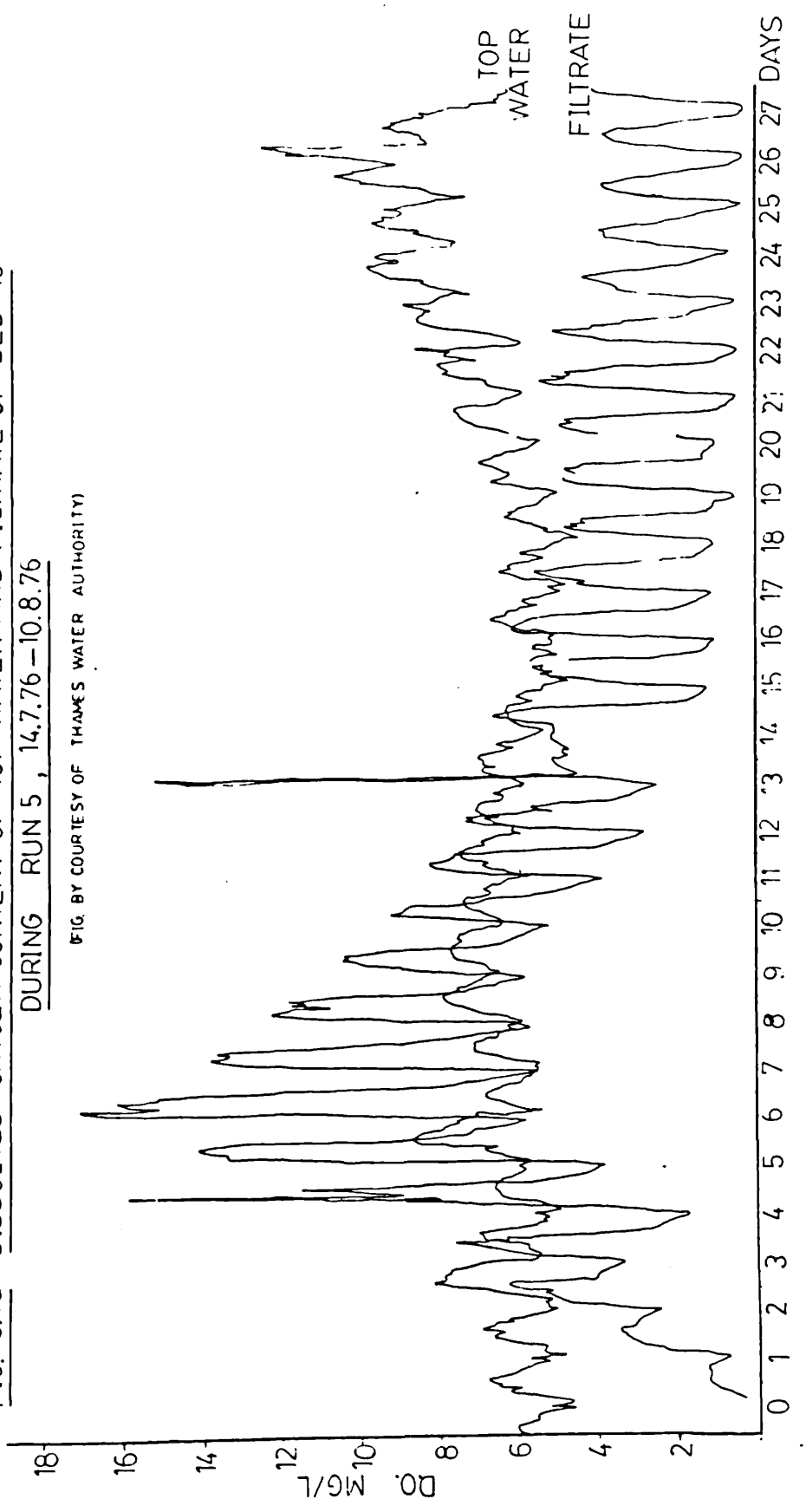
FIG. 5.3 CARBON CONTENT OF THE TOP CENTIMETRE OF SAND DURING EACH FILTER BED RUN



experimental basis only for the first few months (Personal communication, Thames Water Authority). However, general trends in the dissolved oxygen contents of the top water and filtrate of bed 45 can be seen in the data plotted in Figs. 5.4 a and b for runs 5 and 8 respectively. The dissolved oxygen in the top water remained steady, fluctuating daily by only 1 or 2 mg l^{-1}. At the end of the summer run (run 5) it increased, presumably due to increased photosynthesis by such plants as Cladophora which densely colonised the bed at this time. The dissolved oxygen of the filtrate exhibited large diurnal fluctuations throughout both runs. The daily peaks in the filtrate increased over the first 7 to 10 days, presumably due to oxygen production by the growing algal population with little demand from animal respiration; after about two weeks, these maxima declined and correspondingly the minima also decreased. The animal fauna developed strongly after about 10 days (Chapter 9) and their respiratory oxygen demand would probably account for these decreases, in addition to bacterial decomposer activity. In the summer months (Fig. 5.4a) these minima approached zero mg l^{-1}, while at other times of the year they rarely fell below 5mg l^{-1}. In the summer the filter skin would have been shaded by the Schmutzdecke above it in the latter week of the run. Photosynthesis at the filter surface was apparently then reduced, as the filtrate oxygen never rose above 5 mg l^{-1} while the top water oxygen rose steadily from 6 to 12 mg l^{-1}.

FIG. 5.4a DISSOLVED OXYGEN CONTENT OF TOP WATER AND FILTRATE OF BED 45 DURING RUN 5, 14.7.76—10.8.76

FIG. BY COURTESY OF THAMES WATER AUTHORITY)



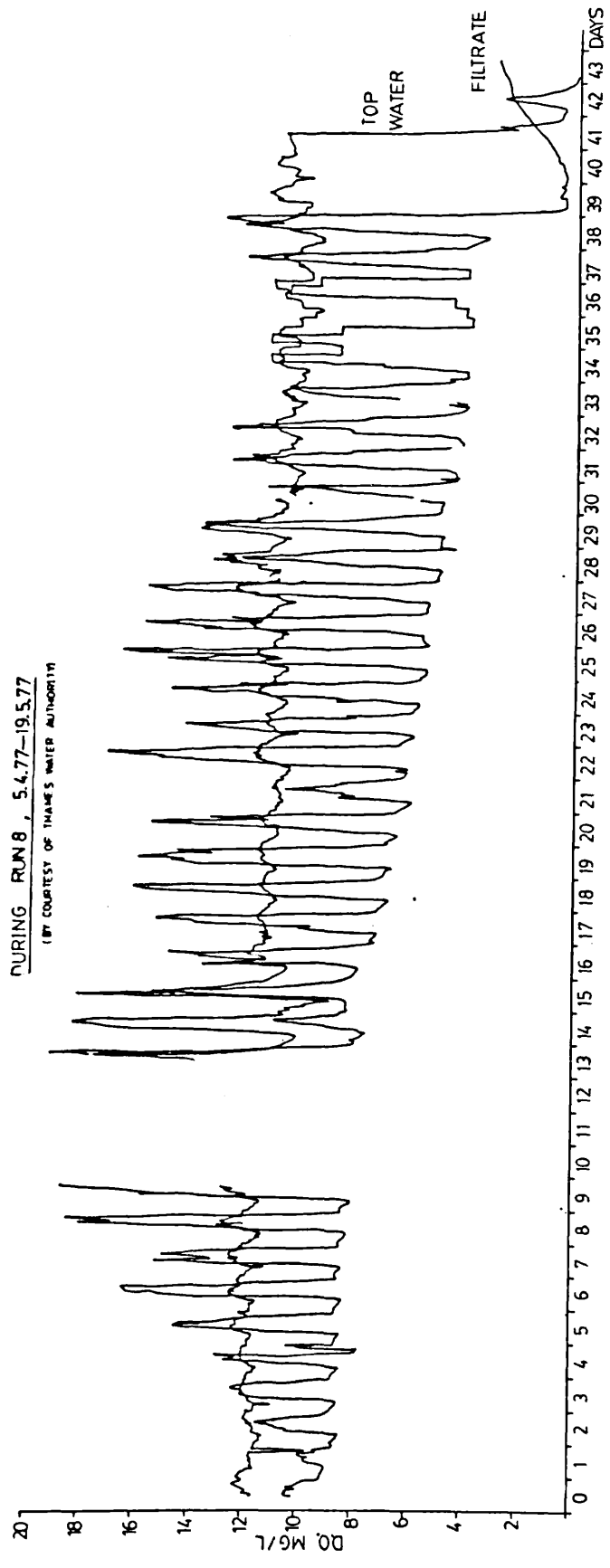


FIG. 54b DISSOLVED OXYGEN CONTENT OF TOP WATER AND FILTRATE OF BED 45
DURING RUN 8, 5.4.77–19.5.77
(BY COURTESY OF THAMES WATER AUTHORITY)

5.3 Particulate organic carbon

(a) Top Water and Filtrate

The particulate organic carbon (P.O.C.) content of the top water and filtrate, monitored by the Thames Water Authority, is represented in Fig. 5.5 (data in Appendix 5.5). The area between the two graphs represents the removal of P.O.C. by the filter bed. The removal efficiency apparently increased at the higher summer temperatures and also with time during the filter bed runs. This would correspond with increased biological activity both with temperature and with maturity of the bed during each run. Because of this variation in activity, despite the four-fold difference between summer and winter P.O.C. content of the top water, the filtrate levels remained similar throughout the year.

(b) Sand Cores

The particulate organic carbon content per cm^3 of sand in the sand cores was determined on each sampling occasion as described in section 4.8. The results of these analyses are tabulated in Appendix 5.4. Carbon accumulated mainly at the surface of the sand (Fig. 5.6) its concentration decreasing with depth to 10 cm below the surface and remaining fairly constant at depths below this to 25 cm. The data was ln-transformed to convert this exponential curve to a linear slope in order to assess the change in gradient with the age of a filtration run. Linear regressions of ln carbon content against depth were calculated (Appendix 5.6) and those giving significant gradients ($p \leq 0.05$) are summarised below in Table 5.1.

FIG 55 SURFACE WATER TEMPERATURE, AND PARTICULATE ORGANIC CARBON CONTENT OF TOP WATER AND FILTRATE OF BED 45 FROM MARCH 1976 TO MAY 1977

(Carbon data provided by Thames Water Authority)

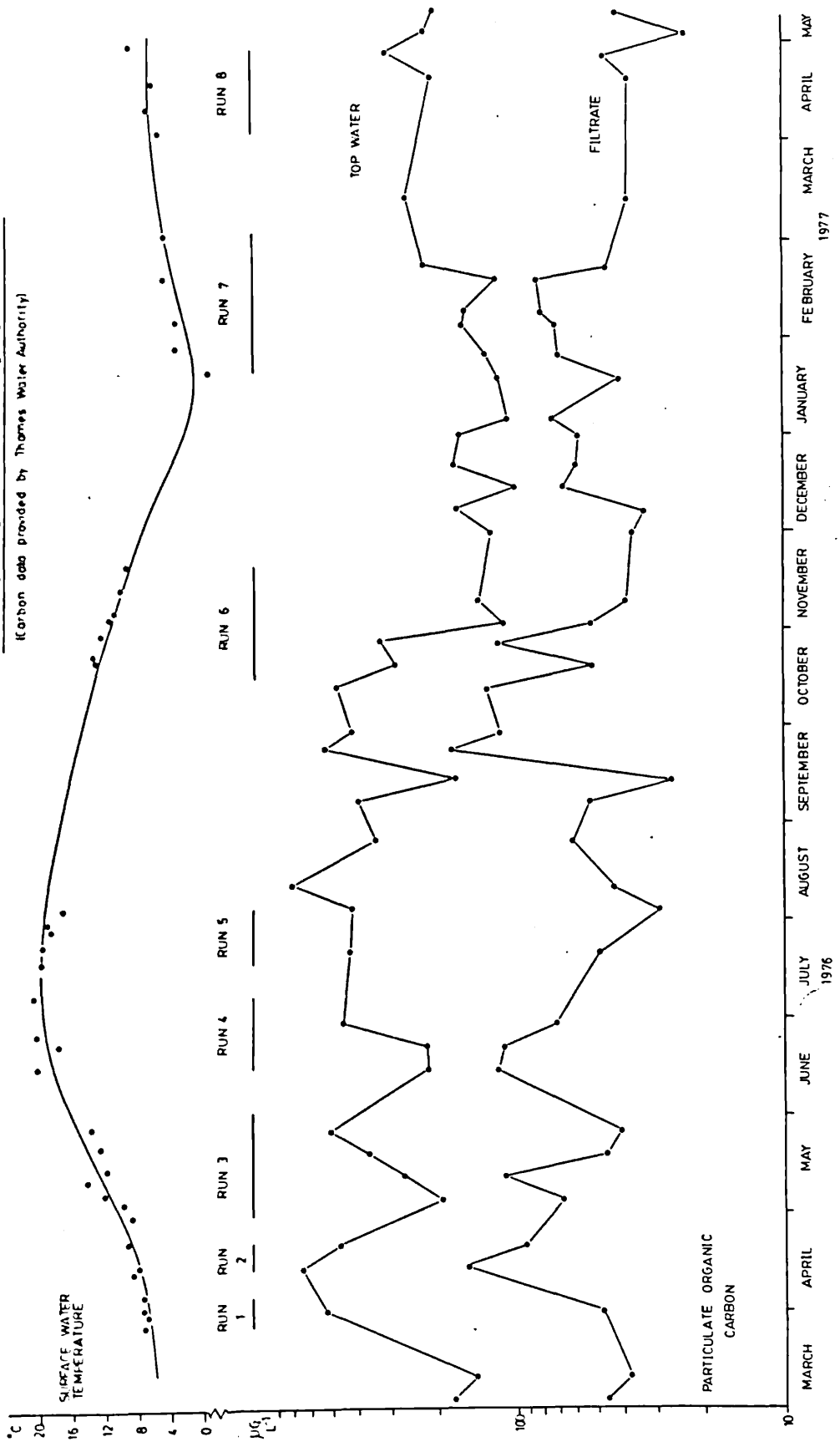


FIG. 56 DENSITY OF PARTICULATE ORGANIC CARBON IN SAND CORES DURING RUN 3 ON BED 45, APRIL/JUNE 1976, 12°C

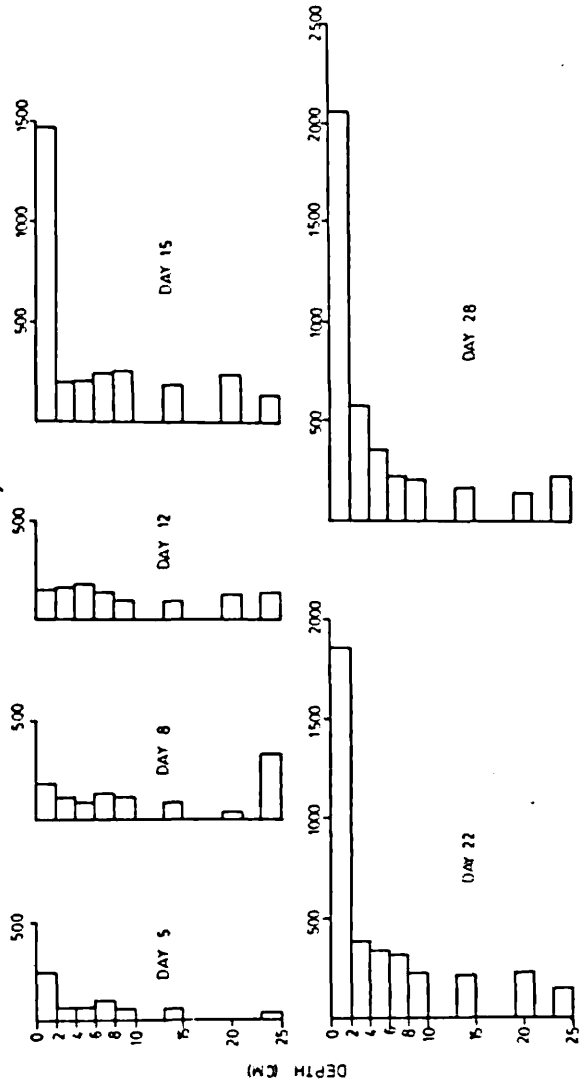


Table 5.1 Significant Gradients of Decreasing Particulate Organic Carbon Content (mg C cm^{-3}) with Depth

Month	Run	Day	Slope (r)	S.E. of Slope	Intercept (ln Co)	Variance Ratio	dF	p
V	3	15	-0.043	±0.015	5.911	7.884	1,23	0.01
		22	-0.065	±0.013	6.427	26.064	1,22	0.001
		28	-0.069	±0.017	6.436	16.039	1,21	0.001
VI - VII	4	3	0.058	±0.019	3.710	9.770	1,16	0.01
		10	0.030	±0.014	4.554	4.650	1,21	0.05
		13	-0.042	±0.020	5.333	4.398	1,23	0.05
VII - VIII	5	11	-0.058	±0.013	6.215	18.970	1,23	0.001
		13	-0.045	±0.019	5.625	5.901	1,23	0.025
X - XI	6	26	-0.028	±0.010	5.732	7.348	1,23	0.025
		40	-0.080	±0.009	6.290	77.011	1,23	0.001
I - III	7	45	-0.102	±0.027	6.079	14.057	1,13	0.005
III - V	8	17	-0.037	±0.017	5.682	4.946	1,13	0.05
	BED	28	-0.049	±0.017	5.854	8.650	1,13	0.025
X - XI	44	24	-0.021	±0.008	5.420	6.428	1,23	0.025
		33	-0.045	±0.007	5.673	45.679	1,23	0.001

Regression Equation : $\ln C_d = \ln Co + rd$

C_d = particulate organic carbon content ($mgcm^{-3}$) at depth d (cm)
Co = particulate organic carbon content ($mgcm^{-3}$) at surface (i.e. 0 cm)
d = depth (cm)
r = slope

An exponential gradient of carbon content does not seem to occur through the top 25 cm of sand until a certain length of time has elapsed, this being shorter in the summer runs (about 10 days) and longer in the winter (20 - 30 days). The positive gradients, showing an increase in P.O.C. content with depth during the first half of run 4 are difficult to account for, and do not appear to be related to any operational or biological properties of the filter bed at this time.

Figure 5.7 demonstrates that the gradient of the slope of the P.O.C. content against depth regression increased in magnitude (i.e. became more negative) with the age of a filtration run, regardless of the season ($p = 0.005$). The regression is summarised in Table 5.2. Bed 44 data were omitted from the calculation as the filtration rate effected the depth of penetration.

Table 5.2

Regression Table : Increase in P.O.C. Depth Gradient with Time in Bed 45

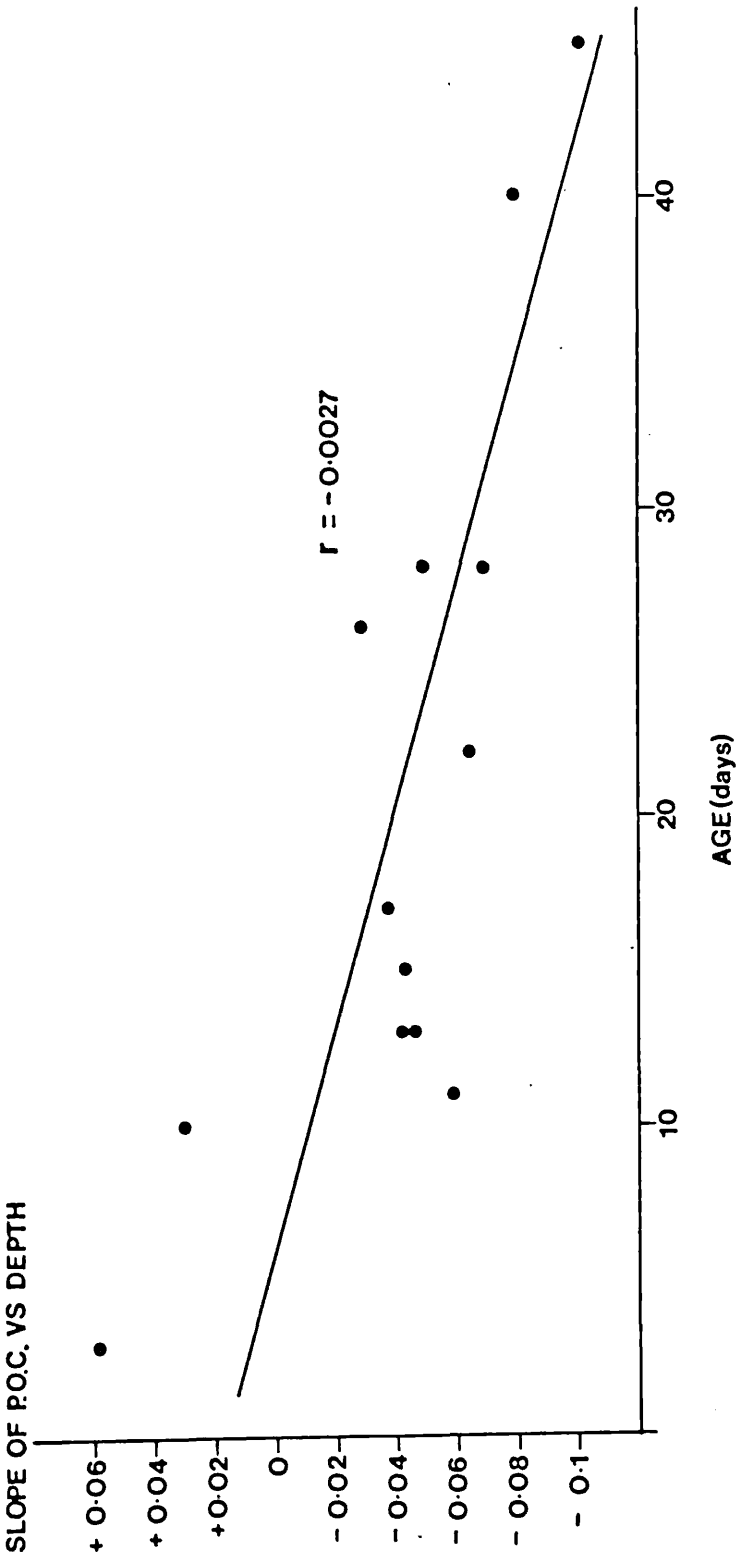
SLOPE b	S.E. OF SLOPE	INTERCEPT (a)	VARIANCE RATIO	df	P
-0.0027	±0.0007	0.0149	15.1735	1,11	0.005

$$Y = a - bx$$

$$\text{slope} = 0.0149 - 0.0027 \times \text{Age of filtration run}$$

The variation in total carbon content of the sample cores with age of a filtration run was examined by summing

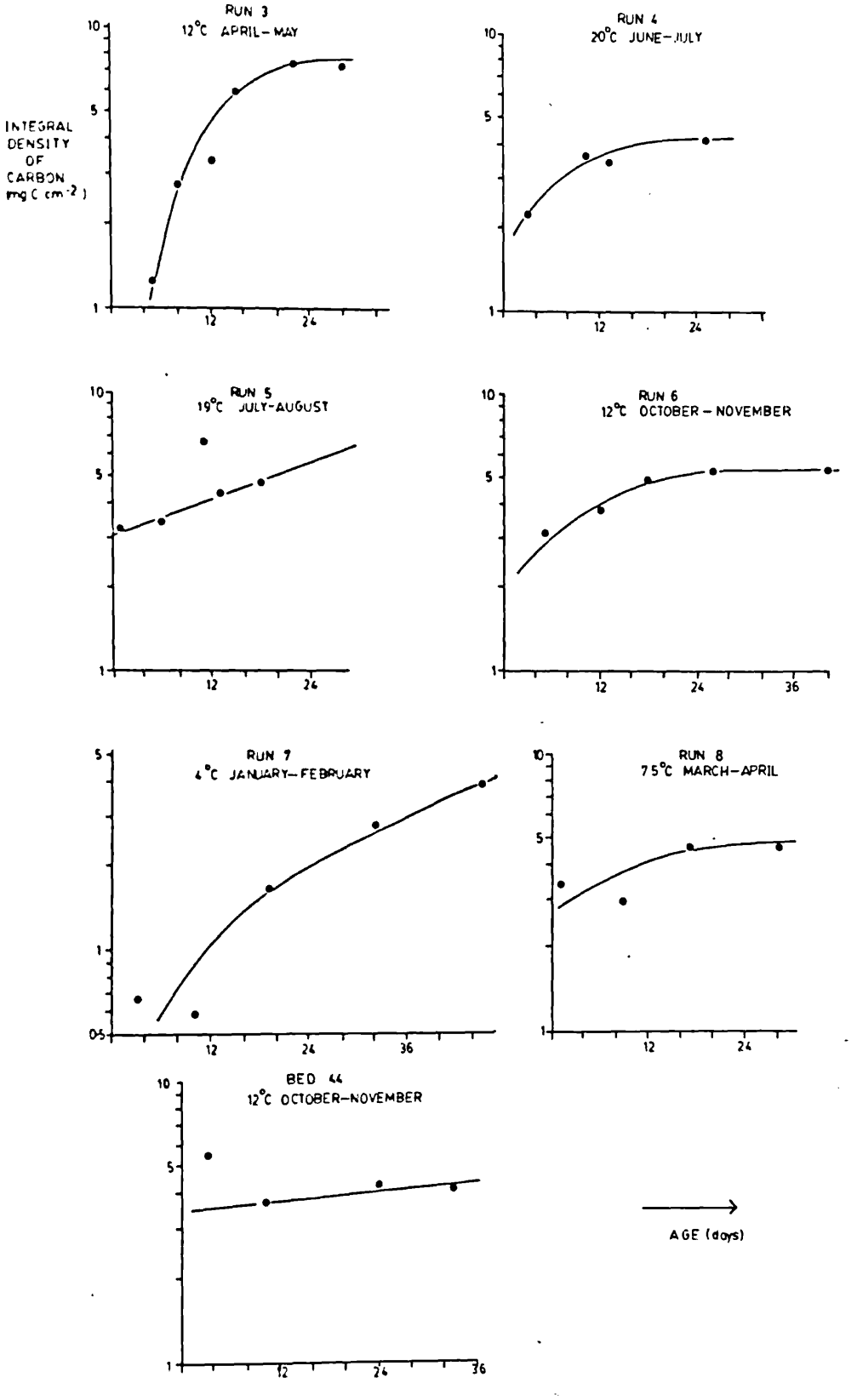
FIG. 5·7 DECREASE OF CARBON GRADIENT WITH AGE OF A FILTRATION RUN



all the depth values (mg C cm^{-3}) from 1 cm to 25 cm where recorded, and integrating the values obtained between 1 and 25 cm where not all depths were analysed (Appendix 5.7). These values were plotted against time for each run (Fig. 5.8). During most runs (3, 4, 6 and 8) a rapid increase in particulate organic carbon per cm^2 over the first two weeks was followed by a plateau, with no further change in carbon concentration. During runs 5, 7 and the run on Bed 44 the increase in carbon content was more or less constant throughout the time periods examined. The low temperature of run 7 ($4^{o}C$) and the slow flow rate of the Bed 44 run were probably the main contributory factors causing low accumulation of organic carbon. The low and constant rate of increase in carbon concentration during run 5 may have been caused by the primary screening effect of the Schmutzdecke overlying the filter bed surface.

As most of the carbon accumulation occurred in the top 10 cm of sand (Fig. 5.6) the data were divided into two parts: 2 to 10 cm and 11 to 25 cm. Regressions were then calculated of the carbon content against age of the filtration runs for each run (Appendix 5.8). The top cm. was not included in these calculations as it had a far greater content of carbon than the lower depths due to its associated algal growths and has consequently

FIG. 5.8 DENSITY OF PARTICULATE ORGANIC CARBON IN FILTER BED CORES



been considered separately, earlier in this chapter (5.1). The regression plots (Fig. 5.9) confirm that accumulation occurred mainly in the top 10 cm of sand throughout the run duration in runs 3, 6, 7 and 8, but only up to day 13 in run 4 and day 11 in run 5. Some accumulation occurred at the lower depths during the first half of the spring and summer runs (runs 3 and 5) and throughout the winter run (run 7). No pattern of accumulation was detectable in the lower depths during runs 4 and 6 nor in either of the depth levels of the bed 44 run.

5.4 Grain size and porosity of the filter bed sand.

Grain Size

The grain size of the sand was determined at seven depths in 3 replicate cores extracted from bed 45 in November 1976. These cores had been filled with sand from the sand bay at Hampton Treatment Works, in which sand was stored before being put on to the filter bed. The three cores were left in the bed for 25 days of a filtration run before they were removed. Samples of about 40 cm^3 were taken at depths of 1 cm, 2 cm, 5 cm, 9 cm, 13 cm, 19 cm and 25 cm down the cores. These samples were passed through a nest of sieves with pore diameters of 4.0 mm, 2.0 mm, 850 μm, 500 μm, 250 μm, 125 μm and 63 μm, by shaking in a mechanical shaker for 30 minutes. The different grades of sand were weighed for each sample and the results are tabulated in Appendix 5.9.

FIG. 5.9 REGRESSIONS OF P.O.C. DENSITY VS. TIME IN FILTER BED CORES

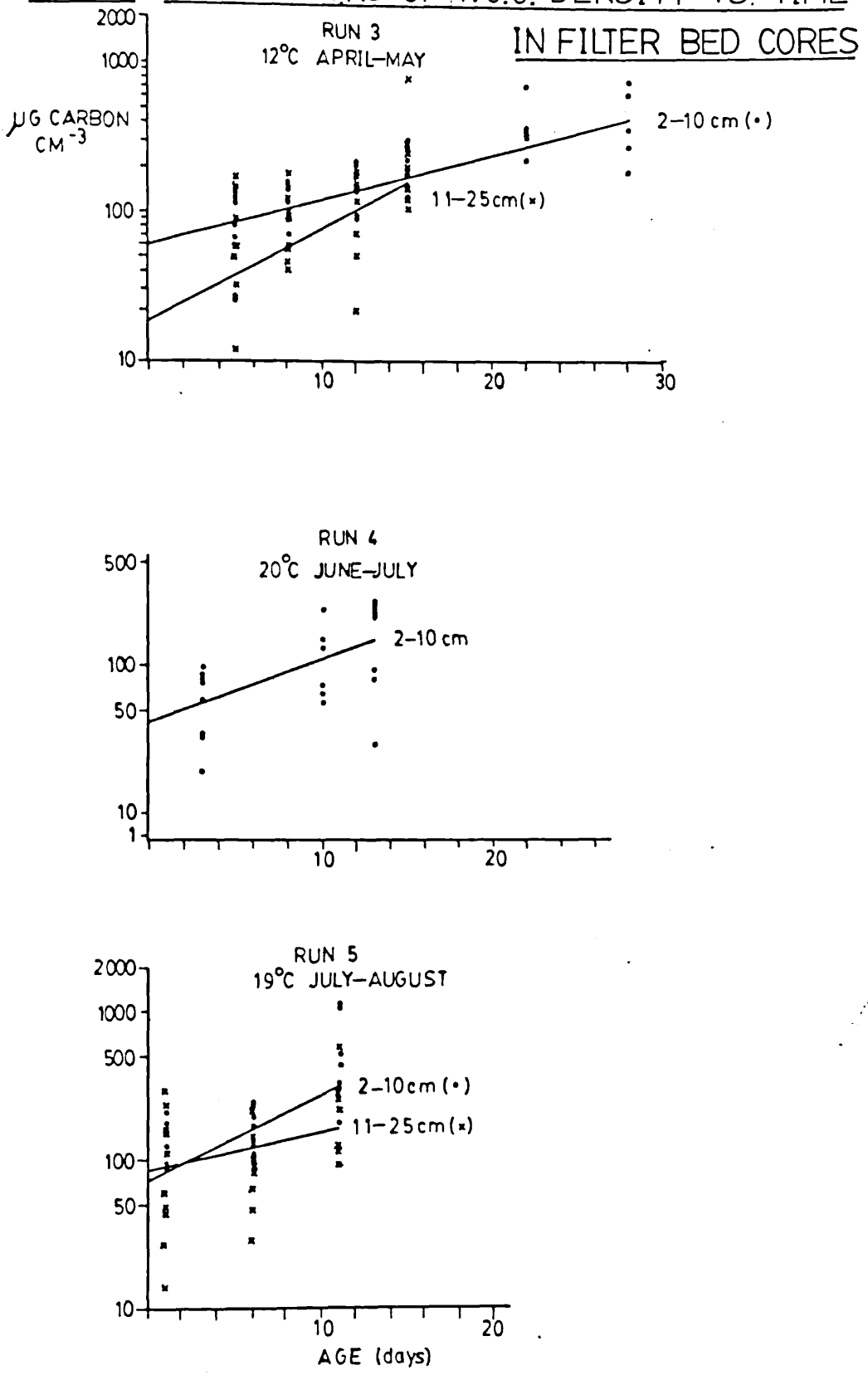


FIG. 5.9 Contd..

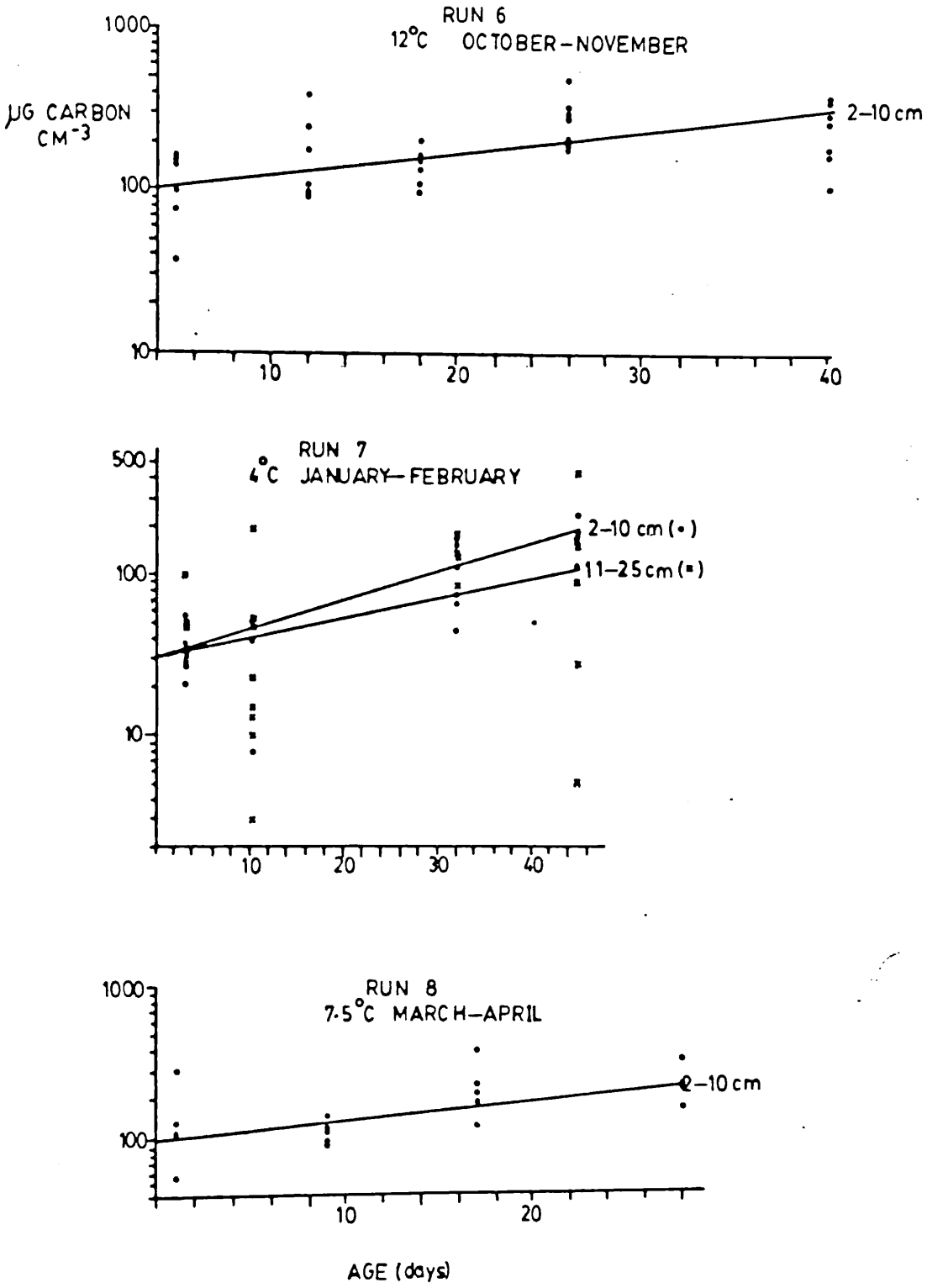

RUN 6
12°C OCTOBER–NOVEMBER
1000
100
10
µG CARBON CM⁻³
2–10 cm
10
20
30
40
RUN 7
4°C JANUARY–FEBRUARY
500
100
10
2–10 cm (•)
11–25 cm (▪)
10
20
30
40
RUN 8
7·5°C MARCH–APRIL
1000
100
2–10 cm
10
20
AGE (days)

Table 5.3

Average percentage of sand (by weight) from 3 cores, 25 days old, retained by each of a nest of sieves at various depths in the sand cores

Sieve Mesh Size	4.0 mm	2.0 mm	850μm	500μm	250μm	125μm	63μm
Depth (cm)							
1	2.89	5.35	14.65	27.15	46.13	3.83	0.006
2	1.88	4.25	13.59	26.40	49.48	4.38	0.03
5	1.73	3.32	9.63	24.36	55.39	5.56	0.006
9	1.36	2.58	8.73	23.83	57.57	5.93	0.006
13	1.78	3.51	11.88	24.67	53.54	4.60	0.013
19	0.92	3.52	10.17	25.25	55.34	4.79	0.017
25	2.11	3.44	9.91	25.00	54.40	5.13	0.013
Mean ± s.d.	1.81 ±0.61	3.71 ±0.87	11.22 ±2.21	25.24 ±1.16	53.12 ±3.95	4.89 ±0.71	0.013 ±0.009
Cumulative %	1.81	5.52	16.74	41.98	95.1	99.99	100.00

The average percentage compositions of grain size from the three cores are tabulated above (Table 5.3) and a cumulative curve for grain size, averaged over the depths, plotted in Fig. 5.10. The majority of the grains were between 250μm and 800μm in size. Little change occurred in composition with depth although the coarse sand accounted for about 10% more (by weight) at the surface than at lower depths while the medium sand was correspondingly 10% less at the surface (Table 5.4, Fig. 5.11).

FIG.5.10

CUMULATIVE CURVE OF THE GRAIN SIZE DISTRIBUTION OF SAND IN THE SAMPLE CORES

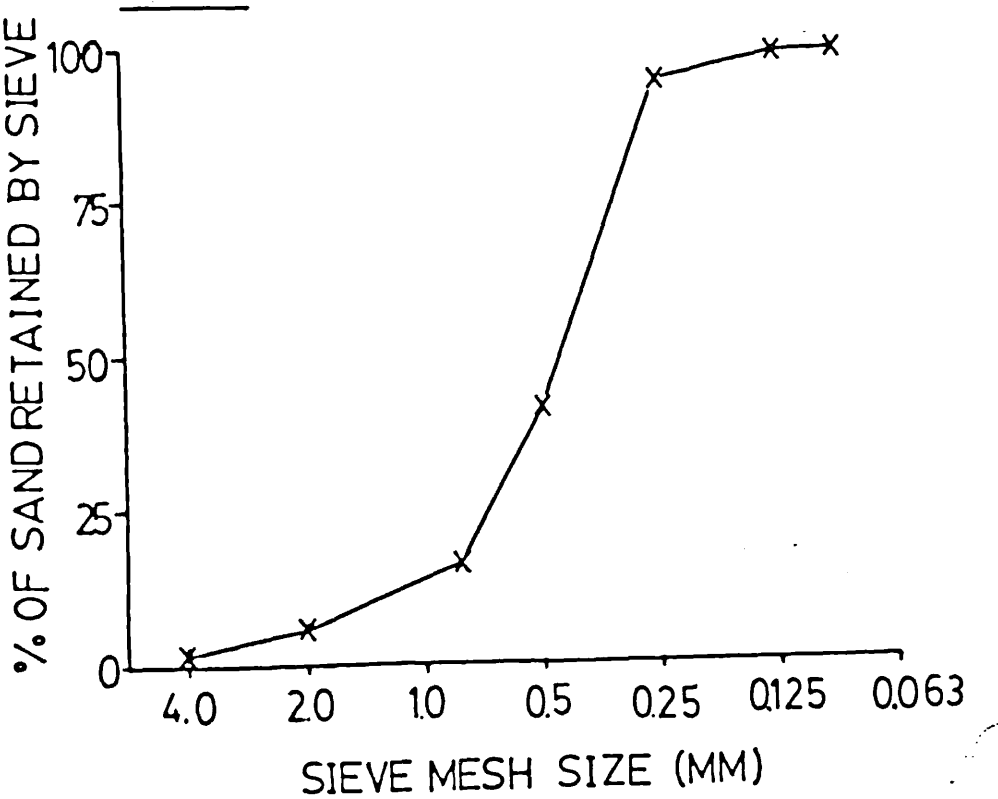


FIG.5.11
DEPTH DISTRIBUTIONS
OF GRAIN SIZES

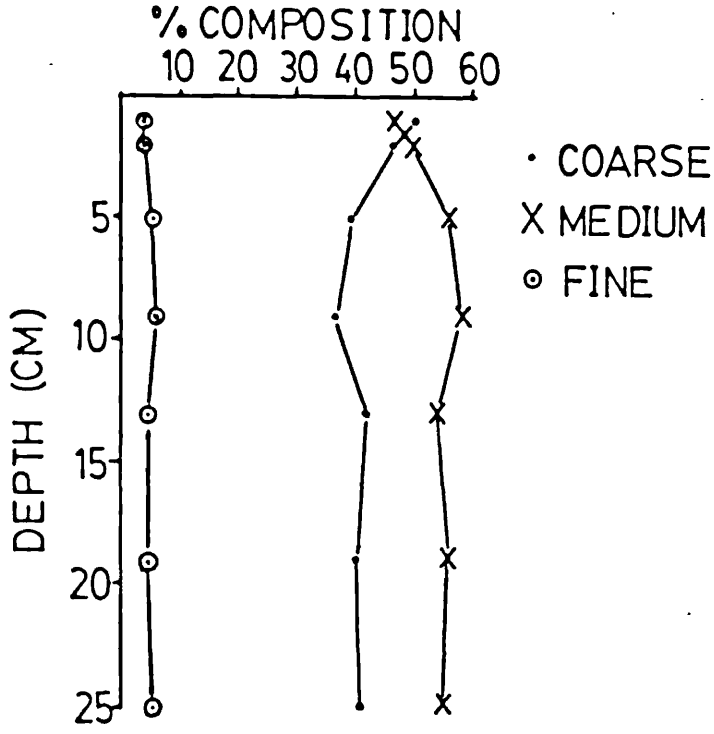


Table 5.4

Average percentage composition (by weight) of sand with depth in 3 replicate cores

DEPTH (cm)	COARSE 4.0 - 0.5 mm	MEDIUM 0.5 - 0.25 mm	FINE 0.25 - 0.063 mm
1	50.04	46.13	3.84
2	46.12	49.48	4.41
5	39.04	55.39	5.57
9	36.50	57.57	5.94
13	41.84	53.54	4.61
19	39.86	55.34	4.81
25	40.46	54.40	5.14

Porosity

The porosity was calculated using the following equation:-

$$\% \text{ pore space} = 100 - \frac{\text{apparent specific gravity}}{\text{real specific gravity}} \times \frac{100}{1}$$

The apparent specific gravity of the sand is its specific gravity including trapped air whereas the real specific gravity is the specific gravity of the sand particles alone.

Determination of real specific gravity

A specific gravity bottle full of distilled water was weighed at room temperature (= a gm). Half the water was poured out and air dried sand was added to the bottle which was then reweighed (= b gm). The mixture was boiled gently to release the trapped air, cooled to room temperature and the bottle filled with distilled

water before reweighing (= c gm). The increase in weight (c-a) was due to the difference between b gm of sand and the weight of an equal quantity of water. Therefore, the specific gravity of the sand particles was equal to:

$$\frac{b}{b - (c-a)}$$

a = 22.12366 g
b = 5.49704 g
c = 25.57219 g

$\therefore$ real specific gravity = 2.68343

Determination of the apparent specific gravity

A tube was weighed and filled to a pre-determined mark firstly with air dried sand and secondly with water. The apparent specific gravity is equal to:-

$$\text{a.s.g.} = \frac{\text{weight of sand}}{\text{weight of same volume of water}}$$

Values obtained for a.s.g. = 1.71874 g
1.54925 g
1.65792 g
mean a.s.g. = 1.64197

$$\text{Porosity} = 100 - \frac{1.64197}{2.68343} \times 100$$

$$= \underline{38.81\%}$$

Volume of Water Flowing through the sample cores of sand

Water passes through the filter bed (Bed 45) at 40 cm hr^{-1}

$\therefore$ volume passing through 1 cm^2 area = 40 x 24 $cm^3 d^{-1}$

= $\underline{0.96\ l\ d^{-1}}$

Surface area of core (diameter 10 cm) = 78.54 cm^2

$\therefore$ volume passing through core = 78.54 x 0.96 l d^{-1}

= 75.4 l d^{-1}

Chapter 6 Interstitial Ciliate Species Recorded in the Slow Sand Filter Beds

The system of classification adopted for the ciliates isolated from the sand samples removed from the filter beds, is that described by Corliss (1977). This scheme supersedes those of Kahl (1930 - 1935) and Fauré-Fremiet (1950) due to increased knowledge of ultra-structure brought about mainly by studies using the electron microscope. Table 6.1 lists the ciliates and their size ranges, and the more common species are shown in plates 6.1 to 6.10. The ciliates were generally identified to genus level although the dominant ones were identified to species level. Identification was aided by Kahl (1930 - 1935), Bick (1972), Curds (1970) and Kudo (1971). Amoebae, flagellates and helizoa were not identified but their size ranges are included in Table 6.1.

Most of the ciliates extracted from the filter bed sand were at the lower end of the size range reported in Kahl (1930 - 1935). It appears that rotifers removed from filter bed sand were also found to be smaller in general than those found elsewhere (R. Pontin, personal communication) and the recorded lengths of ciliates living in the periphyton of a freshwater stream (Pätsch, 1974) were similar to those of the present study. The effects which temperature, the rate of replication and the flow rate of water through the filter beds had on individual ciliate size are discussed in Chapter 8.

Table 6.1 Ciliates recorded in the slow sand filters

Classification (Corliss, 1977)	Authority	Size range µm		Plate No.
		Present Study	Kahl (1930-1935)	
Class Kinetofragminophora	de Puytorac et al, 1974			
Subclass Gymnostomata	Bütschli, 1889			
Holophrya sp	Ehrenberg, 1831	27 - 55	18 - 34	
Lacrymaria olor	O.F. Müller, 1776	69 - 275	Up to 1200	
Trachelophyllum sp	Claparede & Lachmann, 1858	33 - 137	250 - 400	
Homalozoon sp	Stokes, 1890	137 - 301	Up to 650	
Spathidium spp	Dujardin, 1841	33 - 164	70 - 180	
Dileptus sp	Dujardin, 1841	82 - 137	250 - 400	
Hemiophrys sp	Wrzesniowski 1870	27 - 137	130 - 800	
Litonotus spp	Wrzesniowski 1870	33 - 206	90 - 600	6.1
Loxophyllum spp	Wrzesniowski 1870	60 - 164	80 - 400	
Subclass Vestibulifera	de Puytorac et al, 1974			
Colpoda steini	Maupas, 1883	14 - 22	32 - 48	
Subclass Hypostomata	Schewiakoff, 1896			
Chilodonella spp	Strand, 1926	19 - 69	30 - 90	6.2
Subclass Suctoria	Claparède & Lachmann, 1858			
Podophrya fixa	Quennerstedt 1867		10 - 28 (Curds, 1969)	

Table 6.1 (contd)

Classification (Corliss, 1977)	Authority	Size range µm		Plate No.
		Present Study	Kahl (1930-1935)	
Sphaerophrya sp	Claparède & Lachmann, 1858	11 - 15	50 (Curds, 1969)	
Tokophrya sp	Bütschli, 1889	72	50 - 70 (Curds, 1969)	
Class Oligohymenophora	de Puytorac et al, 1974			
Subclass Hymenostomata	Delage & Hérouard, 1896			
Glaucoma sp	Ehrenberg, 1830	11 - 30	40 - 80	6.3
Cinetochilum margaritaceum	Perty, 1852	16 - 41	15 - 45	6.4
Pleuronema sp	Dujardin, 1836	33 - 96	70 - 120	
Cyclidium heptatrichum	Schewiakoff, 1893	16 - 36	25	6.5
Subclass Peritricha	Stein, 1859			
Carchesium polypinum	Linnaeus, 1758		80 - 140	
Vorticella companula	Ehrenberg, 1831	40 - 100	50 - 150	6.6
Vorticella convallaria	Linnaeus, 1758	33 - 69	50 - 95	
Vorticella picta	Ehrenberg, 1831		40 - 63	
Vorticella microstoma	Ehrenberg, 1830	70	35 - 83	
Vorticella monilata	Tatem, 1870		50 - 80	

Table 6.1 (contd)

Classification (Corliss, 1977)	Authority	Size range µm		Plate No.
		Present Study	Kahl (1930-1935)	
Vorticella communis	Fromentel, 1874	25 - 41	30	
Zoothamnium sp	Bory, 1824	14 - 41	40 - 60	
Epistylis sp	Ehrenberg, 1838	44 - 82	60 - 80	
Opercularia sp	Stein, 1859		50 - 100	
Vaginicola sp	Lamarck-Ehrenberg, 1830	49 - 88	50 - 60	
Class Polyhymenophora	Jankowski, 1967			
Subclass Spirotricha	Bütschli, 1889			
Stentor polymorphus	Muller, 1773	96 - 233	1,000 - 2,000	
Uroleptus sp	Claparède & Lachmann, 1858	41 - 219	60 - 400	
Oxytricha spp	Bory, 1826	41 - 164	70 - 250	
Stylonichia mytilus	Ehrenberg, 1838	96 - 219	100 - 300	6.7
Tachysoma pellionella	Muller-Stein, 1859	49 - 96	65 - 85	6.8
Halteria sp	Dujardin, 1842	19 - 55	20 - 50	
Aspidisca costata	Dujardin, 1842	14 - 36	25 - 40	6.9
Euplotes sp	Ehrenberg, 1831	40 - 82	70 - 150	
Non-testate amoebae		16 - 164		6.10
Euglypha ciliata		151		
Flagellata		5 - 22		
Heliozoa		11 - 55		

Plate 6.1 Litonotus sp. (x 1,000)

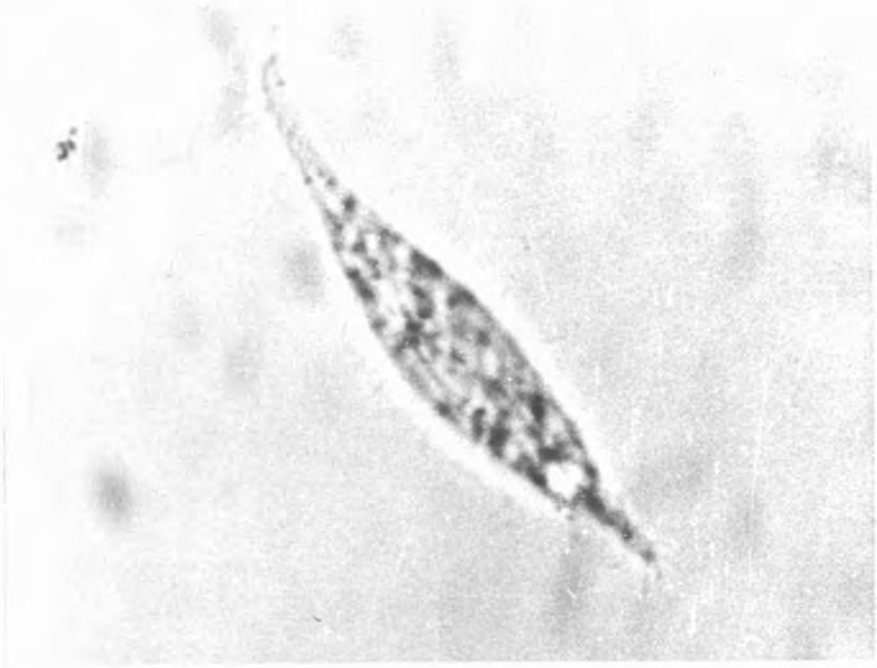


Plate 6.2 Chilodonella sp. (x 1,000)

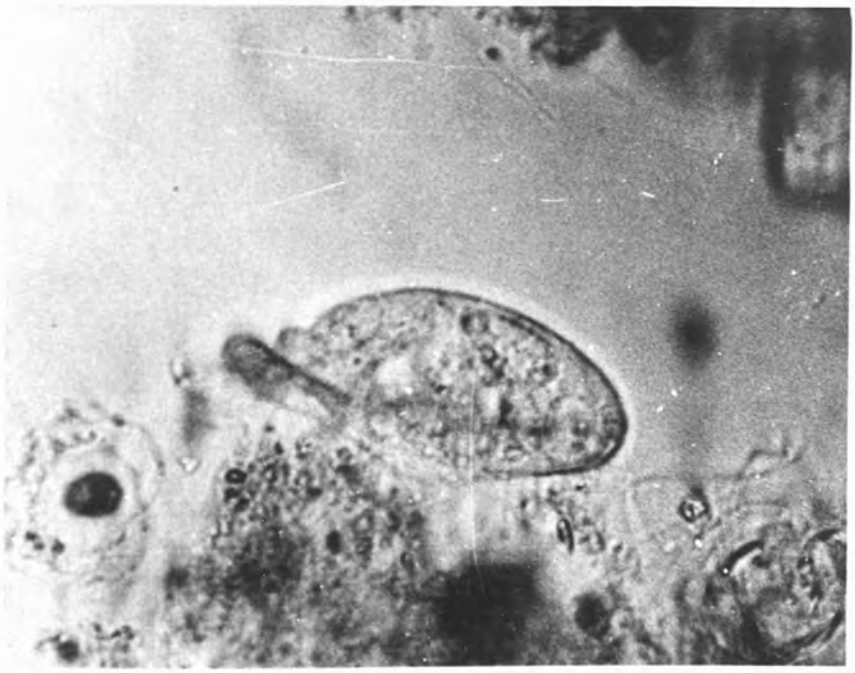


Plate 6.3 Glaucoma sp. (x 2,500)

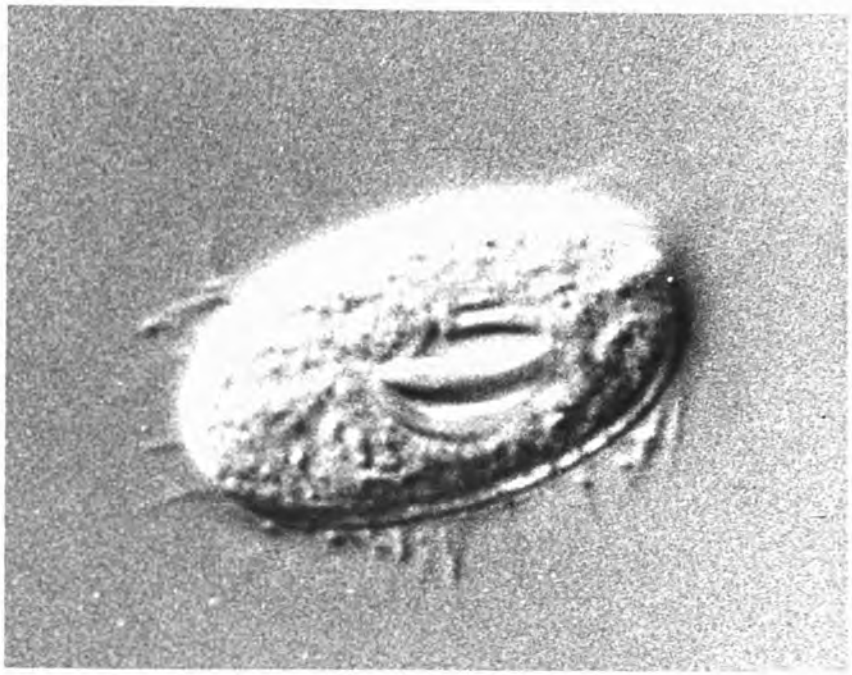


Plate 6.4 Cinetochilum margaritaceum (x 2,500)

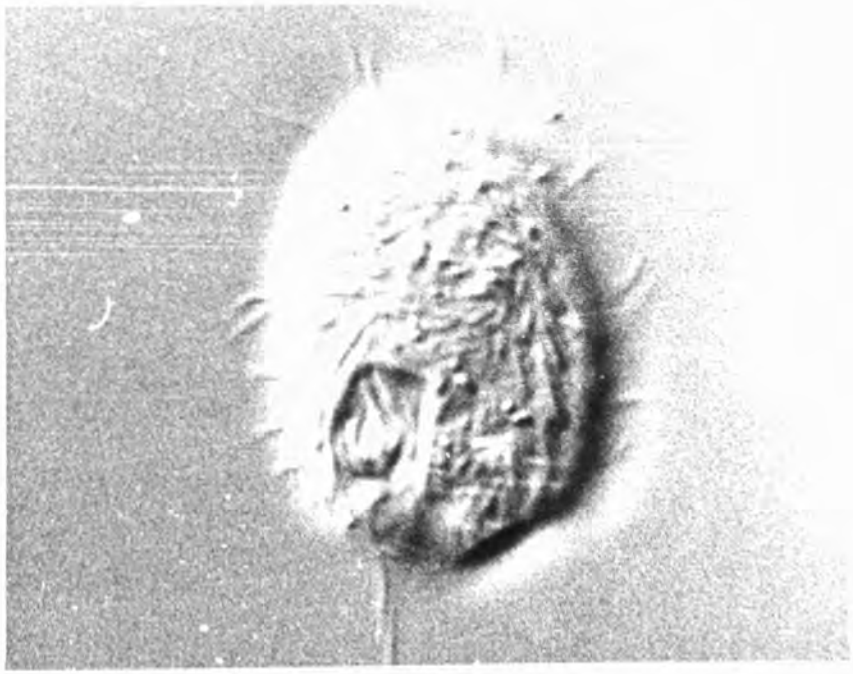


Plate 6.5 Cyclidium heptatrichum (x 2,500)

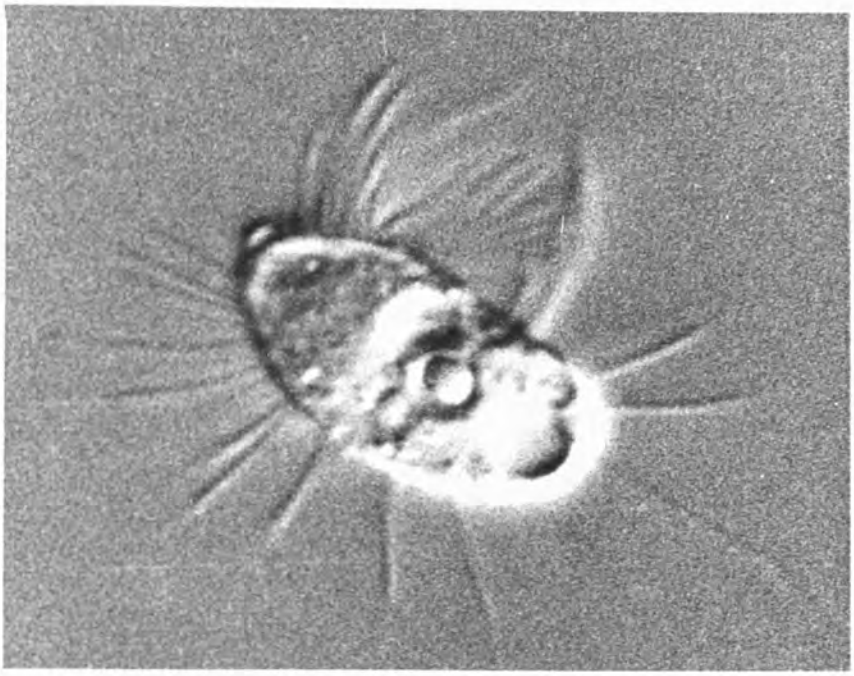


Plate 6.6 Vorticella campanula (x 500)

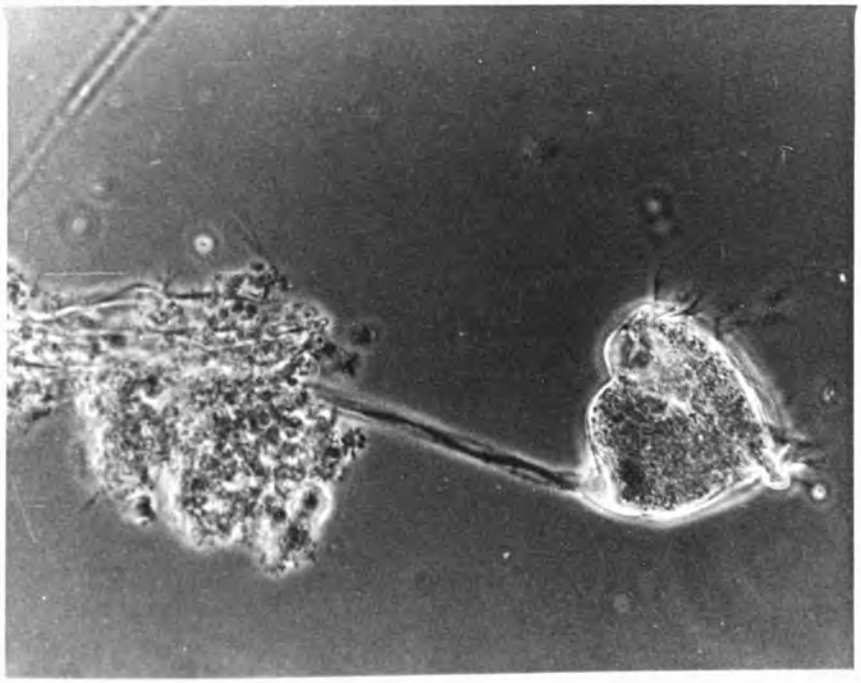


Plate 6.7 Stylonichia mytilus (x 700)

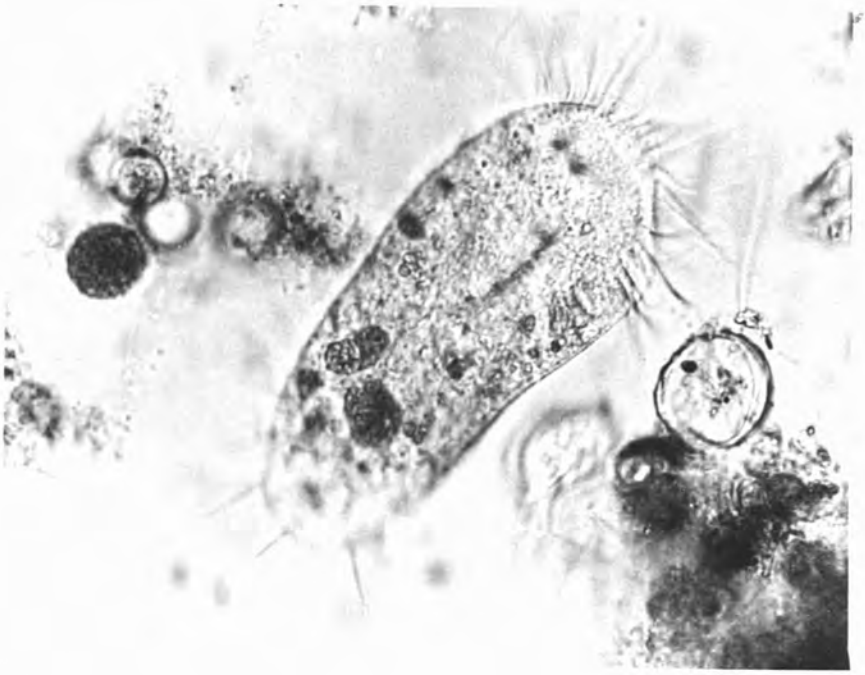


Plate 6.8 Tachysoma pellionella (x 1,500)

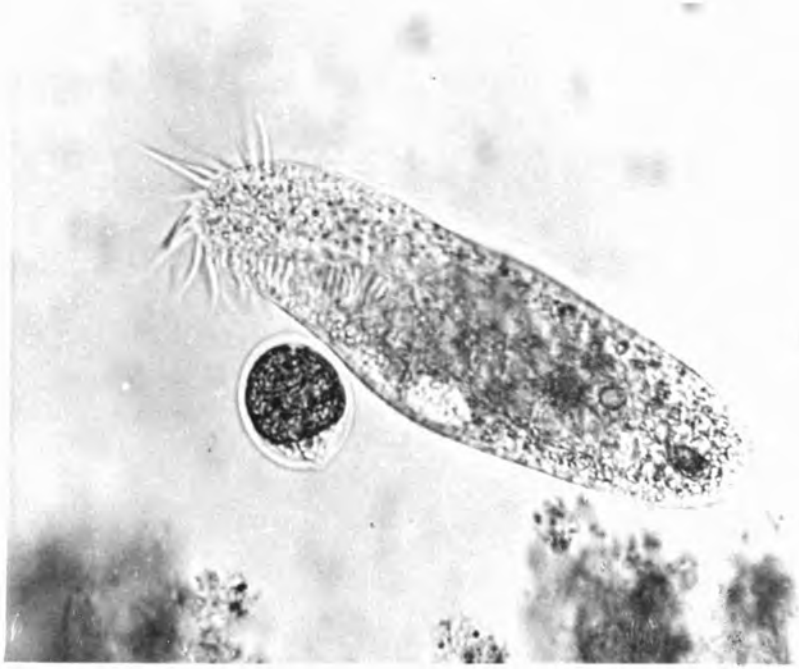


Plate 6.9 Aspidisca costata (x 2,000)

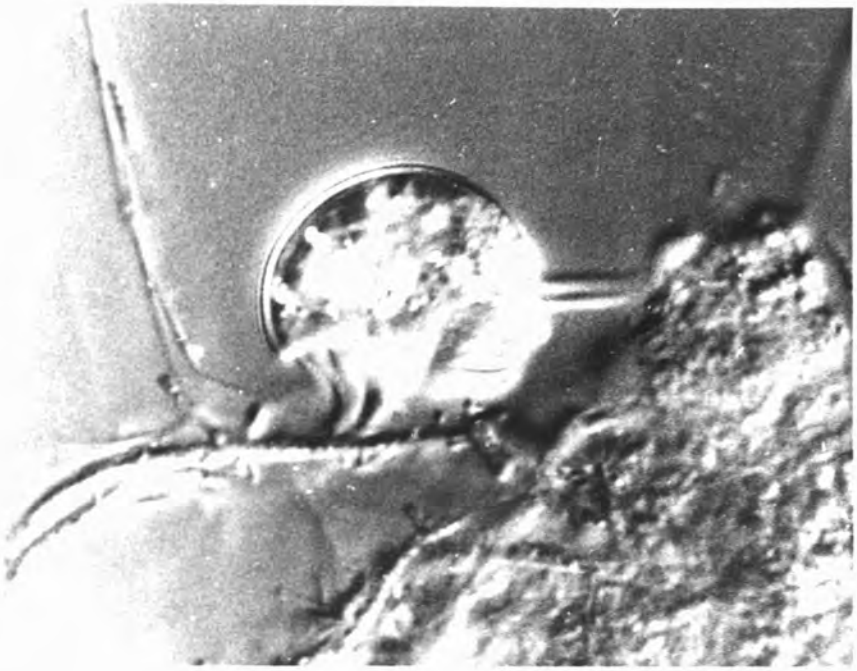
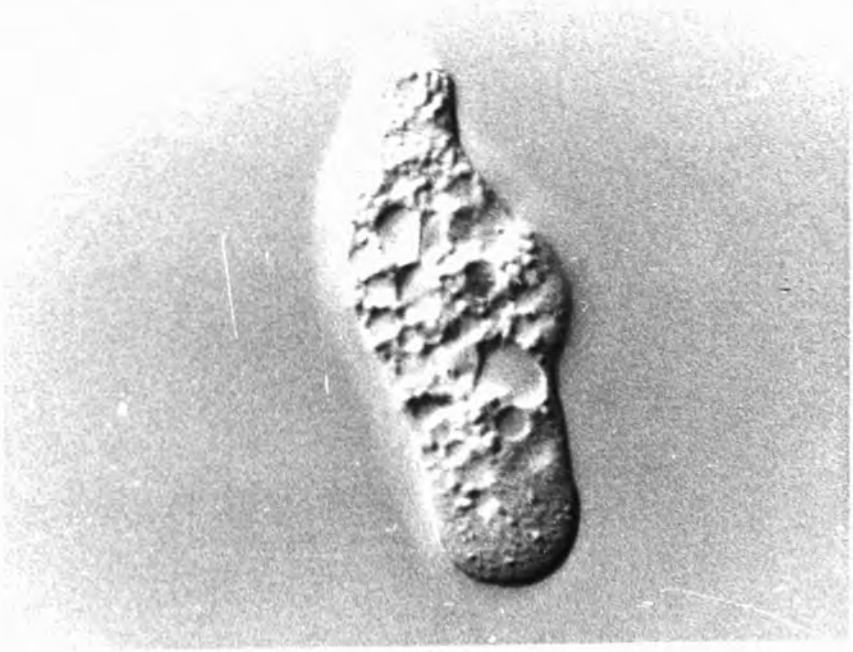


Plate 6.10 A non-testate amoeba (x 1,000)



Chapter 7 The Vertical Distribution of Ciliates and Flagellates in Filter Bed Sand

Introduction

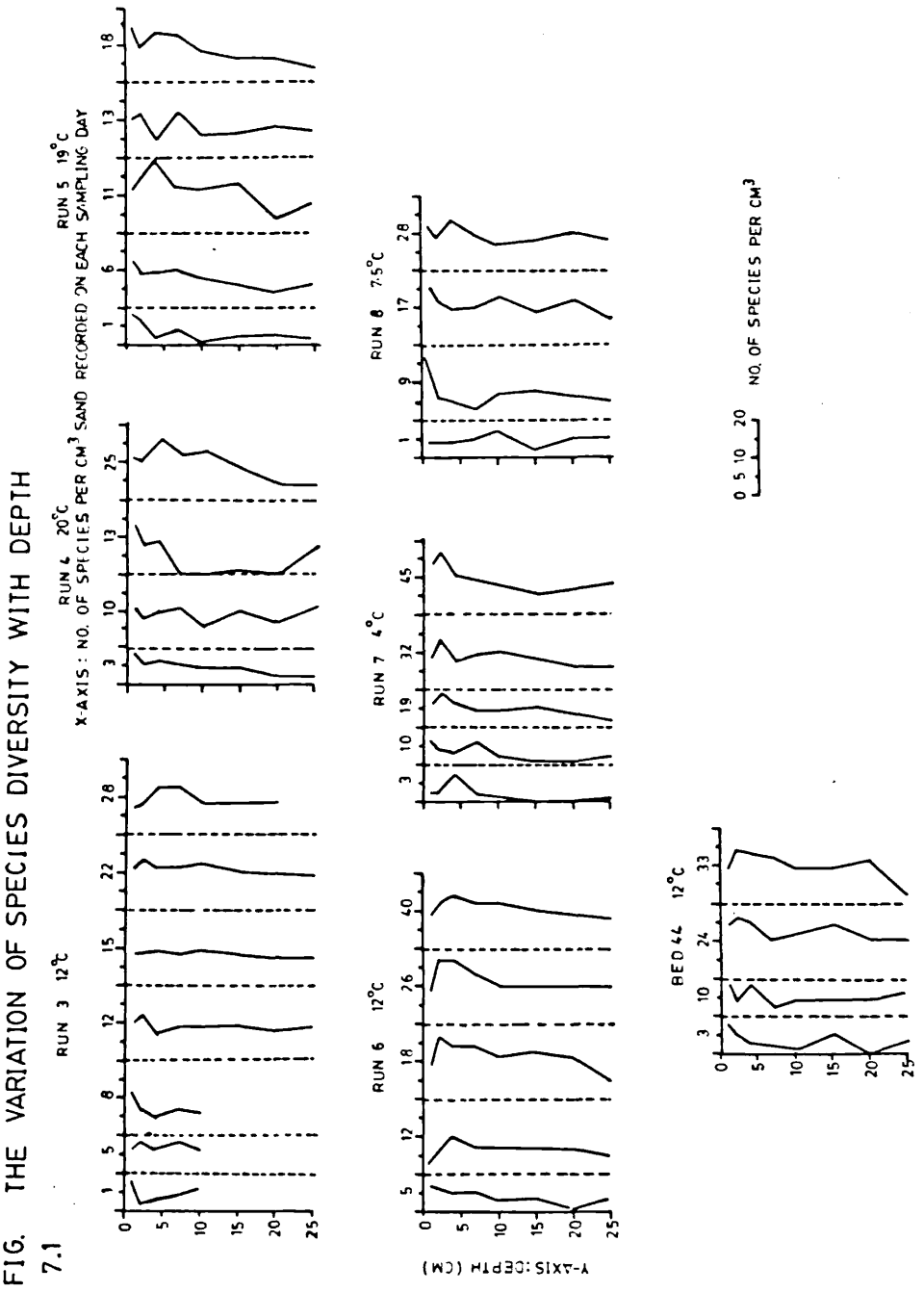
The depth distributions of the ciliates and flagellates were examined regularly during each filtration run (Appendix 5.1) by determining their densities per cm^3 sand as described earlier (p. 60) at eight depths between the sand surface and a depth of 25 cm. This data permitted the variations of species diversity, population density and cell volume of the ciliates, with depth and time during a run, to be analysed. Vertical and temporal variations in flagellate densities were examined and seasonal differences in the population densities of both ciliates and flagellates were studied. In addition, relationships between environmental variables, such as the filtration rate and particulate organic carbon concentration, and the population densities were assessed.

7.1 Species diversity

The variation of species diversity with depth in the top 25 cm of sand was examined in bed 45 during runs 3 to 8 only, as prior to this only depths down to 10 cm had been analysed. Figure 7.1 shows the change in numbers of ciliate species per cm^3 with depth and time during these runs (data are given in Appendix 7.1).

The source of ciliates present in the sand cores after just a few hours of filtration is of considerable importance, as their presence would greatly influence

FIG. 7.1 THE VARIATION OF SPECIES DIVERSITY WITH DEPTH



certainly the initial development of ciliate populations within the cores. A disadvantage of the sampling technique adopted was that no horizontal movement between the core and filter bed sand could occur. This may have been a major direction of travel within the filter bed itself although the dominant pressure would naturally have been in a downward direction. The ciliates, and flagellates were, however, able to enter the sand cores by any of the following routes:-

1. With the sand from the washing bay
   (a) as cysts in dry sand
   (b) as active cells and cysts in damp sand.
2. Carried in with the input water.
3. Washed in from the surrounding sand surface.
4. Migration upwards with the flow during back-charging.
5. Migration downwards in the direction of flow.
6. Migration upwards against the direction of flow.

The diagramatic representation in Figure 7.2 demonstrates three temporal phases in the depth distributions of ciliate species numbers in the cores, obtained from the data presented in Figure 7.1. The days on which these phases began in each run are shown in Table 7.1.

Figure 7.2 Colonisation of sand cores by the ciliates

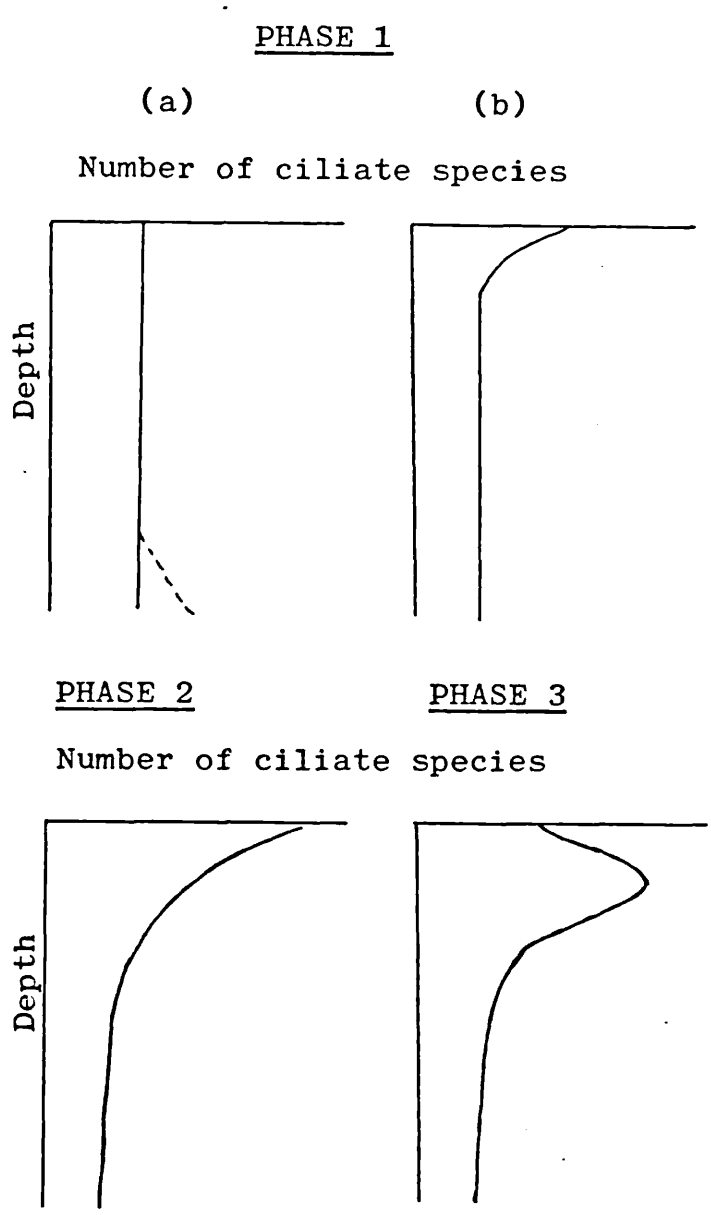


Table 7.1 Age of run at which changes in depth distribution of ciliate species occurred

RUN PHASE	Day marking the beginning of each phase						
	3	4	5	6	7	8	bed 44
1	1	-	1	-	-	1	-
2	8	3	6	5	10	9	3
3	28	25	11	12	19	28	24

The first phase, recorded only in runs 3, 5 and 8 when sampling was carried out on day 1, showed either a fairly uniform distribution of ciliate species numbers with depth, as in run 8, or a peak number of species in the surface sand with lower numbers at depths below (runs 3 and 5). The second phase was characterized by a large number of species at the surface, probably introduced via routes 2 and/or 3, and an exponential decrease with depth, while the third phase demonstrated surface avoidance, with the greatest number of species present just below the surface.

Data used to examine the replicability of the sampling programme (Section 4.6) were analysed for replicability of the numbers of species present in the cores at a depth of 10 cm. No significant difference was found in the number of species present in replicates within cores ($p = 0.75$) and although greater variability was found in replicates between the cores ($p = 0.05$), this was not very significant. (Data from 8 cores, each with three replicates were analysed).

Phase 1

Initial colonisation in runs 3 and 5 followed the distribution shown in Figure 7.2b, while that of run 8 was similar to Figure 7.2a. The species present on the first day of these three runs are given in Table 7.2 with their individual depth distributions.

Table 7.2 Depth Distribution of Ciliate Species in Sand Cores on the First Day of Runs 3, 5 and 8.

	RUN 3	RUN 5	RUN 8
Surface Colonisers	Litonotus sp Vorticella spp Oxytricha sp	Homalozoon sp Cinetochilum margaritaceum Pleuronema sp Uroleptus sp Tachysoma pellionella	Trachelophyllum sp Glaucoma sp Tachysoma pellionella
Ubiquitous species	Glaucoma sp Cinetochilum margaritaceum Cyclidium heptatrichum Tachysoma pellionella Halteria sp Aspidisca costata Euplotes sp	Chilodonella sp Cyclidium heptatrichum Oxytricha sp Aspidisca costata Euplotes sp	Litonotus sp Cinetochilium margaritaceum Cyclidium heptatrichum Oxytricha sp Aspidisca costata
Colonisers migrated from below			Chilodonella sp Euplotes sp

In Phase 1 or run 5, many of the ciliate species present as active cells in the core, were either at or just below the sand surface. It would appear that these had colonised from either the water above the sand or the filter bed sand surrounding the core.

The remaining species were distributed ubiquitously throughout the depth of the core, suggesting that they

were probably present as cysts in the sand before it was used to fill the cores.

The encysted form is a common occurrence providing protection against unfavourable conditions, e.g. drought or food deficiency. Not all species are able to form drought-resistant cysts, although some, e.g. Oxytricha spp cysts have been reported to survive four years of drought (Dawson and Mitchell, 1929). The sand used to fill the cores had been washed and drained but was always still moist. It is probable that the strength of the cyst wall was more important than its ability to withstand drought, as the mechanical agitation of the sand during the washing process and subsequent handling would be highly abrasive to any protozoa present.

Most ciliate species present in the first day's sampling of run 8 had probably been present as cysts in the washed sand, three species had possibly colonised from the surface and two species appeared to have migrated vertically upwards from the sand below the core. This migration of Euplotes sp and Chilodonella sp may have occurred with the back-charging of the bed before it was put back into operation.

No pattern was observed as to which species were present as cysts in the sand used to fill the cores at the beginning of a run, nor as to which species colonised the sand from above or below. The individual depth distributions of the common ciliate species are given for each run in Appendix 7.2.

Phase 2

The second type of distribution shows a diverse ciliate fauna present in the surface sand, with this diversity decreasing exponentially with depth. The phase occurred between days 5 and 10 in all runs except for run 4, in which it had occurred by day 3 (Table 7.1). The depths at which the individual common ciliate species were recorded during this second phase of colonisation are shown in Table 7.4.

Certain of the initial surface colonisers in Phase 1 appeared to have spread down into the sand while additional species also colonised, mainly in the surface layers. For example, during run 8, Glaucoma sp and Trachelophyllum sp descended further into the sand between days 1 and 9, while additional species such as Loxophyllum sp, Halteria sp, Lacrymaria olor, Vorticella campanula and Opercularia sp had also colonised the surface layers.

By day 6 of run 5, C. margaritaceum, Pleuronema sp and T. pellionella had spread further down into the sand since day 1, while additional surface colonisers included Litonotus sp, Spathidium sp and V. campanula. In both runs 5 and 8, V. convallaria had rapidly colonised the entire 25 cm depth in the few days between the first two samplings. There still appeared to be some colonisation from below, as L. olor was recorded for the first time during run 4 at 25 cm on day 3 (Appendix 7.2).

In order to migrate upwards the ciliate would have to overcome or avoid the flow rate of water downward through the interstices, which at a flow of 16" hr^{-1}

(40 cm hr^{-1}) would be about 1.75 cm min^{-1} (see Appendix 7.3 for calculation of interstitial flow). For a Lacrymaria olor of 300μm length this flow rate is equivalent to 60 body lengths per minute. However, by keeping very close to the sand grains the ciliate would avoid most of this flow of water (Page 27) .

Table 7.4 Depth Distribution of Ciliates at the Second Phase of Colonisation

Ciliate Species	Depths at which species were recorded					
	Run 3/ Day 8	Run 4/ Day 3	Run 5/ Day 6	Run 6/ Day 5	Run 7/ Day 10	Run 8/ Day 9
Lacrymaria olor	2	25				1
Trachelophyllum sp.						15
Homalozoon sp.		1	1			
Spathidium spp.	1 to 6		4	1 to 3		1 to 15
Hemiophrys sp.			4 to 25			
Litonotus spp.	1	7	1	1 to 2		1 to 2
Loxophyllum spp.						1
Chilodonella spp.	1 to 10	1 to 15	1 to 25	1		15 to 25
Glaucoma sp.	1 to 10	1 to 25	1 to 25	2 to 25	1 to 20	1 to 25
Cinetochilum margaritaceum	8	1 to 10	1 to 7	1 to 10	2 to 15	1 to 25
Pleuronema sp.			2 to 10	1 to 15		
Cyclidium heptatrichum	1 to 10	1 to 15	1 to 10		4 to 7	2 to 20
Vorticella campanula	1		1 to 7		1	1
Vorticella convallaria	1		1 to 25	5 + 10	7	1 to 25
Zoothamnium sp.	1	1				

Table 7.4 (contd)

Ciliates Species	Depths at which species were recorded					
	Run 3/ Day 8	Run 4/ Day 3	Run 5/ Day 6	Run 6/ Day 5	Run 7/ Day 10	Run 8/ Day 9
Opercularia sp.						1
Stentor polymorphus	1 to 6				1	
Uroleptus sp.			1			15
Oxytricha spp.	2 to 10	1 to 15	1 to 10	4 to 25	1 to 7	1 to 10
Stylonichia mytilus	2					1
Tachysoma pellionella	1 to 10	1 to 20	1 to 25	1 to 25	1 to 25	1 to 10
Halteria sp.			7 to 15			4
Aspidisca costata	1 to 10	1 to 25	1 to 25	1 to 25	1 + 25	1 to 25
Euplotes sp.		4	2			1 to 7

Phase 3

The third type of distribution which demonstrated surface avoidance by a large number of ciliate species occurred on days 25 and 28 in runs 4 (25), 3 and 8 (28), day 19 in run 7 and earlier, on days 11 and 12, in runs 5 and 6 respectively (Figure 7.1). The depth distributions recorded for each species on the above sampling days are summarised in Table 7.5 (detailed account in Appendix 7.2).

The ciliate species which constantly avoided the surface layers of sand, Lacrymaria olor, Trachelophyllum sp., Litonotus sp. and Loxophyllum sp. are all carnivorous, feeding on other ciliates (Sandon, 1932) as well as all being well adapted to life in the sand interstices

Table 7.5 Depth Distribution of Ciliates at the Third Phase of Colonisation

Ciliate Species	Depths at which species were recorded					
	Run 3/ Day 28	Run 4/ Day 25	Run 5/ Day 11	Run 6/ Day 12	Run 7/ Day 19	Run 8/ Day 28
Holophrya sp.		10	15			
Lacrymaria olor			4	4		4 to 25
Trachelophyllum sp.						7 to 20
Homalozoon sp.			4 to 10			
Spathidium spp.	4 to 15	2 to 10	4 to 15	20		1 to 4
Hemiophrys sp.			1 to 15			1
Litonotus spp.	2 to 20	1 to 25	4 to 25	4 to 25		2 to 25
Loxophyllum spp.		4 to 7	7 to 25			
Chilodonella spp.	1 to 20			15 + 20	1	1 to 25
Glaucoma sp.	1 to 20	1 to 10	4		2 to 25	1 to 20
Cinetochilum margaritaceum	2 to 4	4 to 25	1 to 25	1 to 25		1 to 25
Pleuronema sp		2 to 7	1 to 25	4 to 15		
Cyclidium heptatrichum	1 to 20	4 to 15	1 to 25	2 to 25	2	1 to 25
Carchesium polypinum						4
Vorticella campanula	1 to 15	1 to 15	1 to 20		1 to 10	1 to 4
Vorticella convallaria	1 to 20	1 to 15	1 to 15	1 to 10	1 to 10	1 to 4
Vorticella communis	2 to 4	4 to 10	4			1
Zoothamnium sp.		4 to 10	2 to 7		4	
Stentor polymorphus	2 to 10	2 to 15	1 to 4			1 to 15
Uroleptus sp		1 to 2	7			
Oxytricha spp	4 to 20	1 to 15	1 to 20	2 to 20	1 to 15	1 to 25
Stylonichia mytilus		1	2 to 4	4		

Table 7.5 (contd)

Ciliate Species	Depths at which species were recorded					
	Run 3/ Day 28	Run 4/ Day 25	Run 5/ Day 11	Run 6/ Day 12	Run 7/ Day 19	Run 8/ Day 28
Tachysoma pellionella	1 to 20	1 to 25	1 to 25	1 to 25	1 to 25	1 to 25
Halteria sp.			2			
Aspidisca costata	4 to 20	1 to 25	1 to 25	1 to 25	2 to 7	1 to 25
Euplotes sp.		1		7		7

through being particularly long and thin and extremely flexible. The remaining species which were able to withstand the surface conditions as well as living deeper in the sand cores were either omnivorous (*Stentor polymorphus*), bacteriovorous (*Vorticella spp.*, *Glaucoma sp.*, *C. margaritaceum*, *C. heptatrichum*, *Pleuronema sp.* and *A. costata*) or they consumed small particles such as small algae, detritus and flagellates (*Stylonichia mytilus*, *Uroleptus sp.*, *Oxytricha spp.*, *Euplotes sp.* and *T. pellionella*). The details of ciliate nutrition were obtained from Bick (1972) and Sandon (1932).

The obligate carnivores, which were also the larger ciliate species present, appeared to have moved to the sand depths below the region of maximum detrital density, where they could possibly move around more freely and where their prey density was still sufficiently high.

7.2 Population Density

The density-depth distributions of eleven common ciliate species are shown in Figures 7.3 to 7.13 (the data are tabulated in Appendix 7.2) for the filtration runs on bed 45. Figures 7.14 and 7.15 show the depth distributions of the ciliates in total and the flagellates respectively for the same runs, while Figures 7.16 and 7.17 illustrate the data obtained during the autumn 1976 filtration run on bed 44. (Note that the density scales of figures 7.14 and 7.15 differ from those of the other figures). When the ciliates were examined as a phylum (Figure 7.14), the maximum density always occurred at or just below the surface of the sand. This figure also demonstrates how the ciliates increased in density up to a certain day in each run, after which the population declined as the run proceeded. These population increases and decreases are examined in Chapter 8. In this section the vertical distributions of the dominant ciliate species are examined individually, with an attempt to explain why species differed in their distributions and why each species may have changed its depth of greatest density during the course of a filter bed run, and varied seasonally in its overall density.

Glaucoma sp. (Figure 7.3)

From the initial colonisation of the sand cores, until the end of a run, *Glaucoma sp.* was generally most abundant at the surface, decreasing to lower densities from 2 to 4 cm downwards. Maximum densities of between 150 and 250 individuals cm^{-3} were present at the surface throughout the year with generally less than 10 cm^{-3}

present below 5 cm.

Cyclidium heptatrichum (Figure 7.4)

Cyclidium heptatrichum generally avoided the sand surface throughout all the filtration runs, with maximum densities occurring just below it at 2 cm. During the autumn, winter and spring runs of 1976/77 (Runs 6, 7 and 8), the surface avoidance peaks progressed deeper into the filter bed as the runs proceeded. In run 6, the October/November run of 1976, very high densities were recorded; this may have been related to the quality of organic matter passing into the bed at that time, which would have been decaying green algae with its associated bacteria on which C. heptatrichum, being bacteriovorous, may feed. However, neither the carbon content of the input water nor the particulate organic carbon density in the sand were unusually high (Appendix 5.4 and 5.5). Maximum densities varied from 25, in the cold winter run (average temperature, 4°C) to 750 cm^{-3} at 12°C in run 6. Maximum densities were relatively low, 130 cm^{-3}, during the summer runs (20°C and 19°C) suggesting that C. heptatrichum has an optimum ambient temperature of about 12°C.

Cinetochilum margaritaceum (Figure 7.5)

Cinetochilum margaritaceum occurred at maximum densities normally either at or immediately below the surface. The highest populations occurred at the highest temperatures of runs 4 and 5 (20° and 19° respectively) where oxygen levels in the filtrate water ranged from near zero to about 6 mg l^{-1} (Figure 5.4a). During the

rest of the year, at lower temperatures and higher oxygen regimes, C. margaritaceum remained at much lower densities (maximum of about 150 cm^{-3}) relative to the maxima of up to 920 cm^{-3} attained during run 5. This seasonal variation corresponds well with the optimum values of 15°C and 0 - 6 mg l^{-1} oftemperature and dissolved oxygen put forward by Bick and Kunze (1971) for this ciliate.

Chilodonella sp. (Figure 7.6)

Chilodonella sp. was generally only present in significant densities in the latter half of a run and during this time it was most common at the surface, reaching high densities on only two occasions : 220 cm^{-3} in run 7 and 260 cm^{-3} in run 8, the late winter/spring runs of 1977. The active cells did not always survive to the end of the filter run, being absent at the ends of both runs 4 and 7. The temperature and dissolved oxygen (D.O.) content of the water were always well within its tolerances : 0 - 30°C, 0 - 12 mg l^{-1} dissolved oxygen (Bick, 1972), so some other factor such as competition, predation or an insufficient food supply must have been responsible for its seasonal variation in density.

Lacrymaria olor (Figure 7.7)

Lacrymaria olor was present in detectable numbers generally as the smaller ciliates began to decline in density and, whenever present it occurred in low densities throughout the 25 cm core of sand. Maxima of about 20 cm^{-3} occurred between 2 and 4 cm from the sand surface. The late colonisation of this species is possibly related to its carnivorous mode of nutrition, feeding on smaller ciliates.

Litonotus spp (Figure 7.8)

Litonotus spp. were also slow to reach detectable densities in most runs; these species also being carnivorous, feed on ciliates and flagellates. Surface avoidance was generally apparent, with peak densities occurring between 2 and 4 cm below the surface. However, on the last day of run 5 Litonotus spp. were present in particularly high numbers (130 cm^{-3}) at the sand surface. Higher temperatures favoured larger populations of Litonotus spp. with the maximum density in the winter ($4^{o}C$) run of 25 cm^{-3} compared with that of run 5 above ($19^{o}C$) and the $20^{o}C$ maximum (run 4) of 125 cm^{-3}.

Tachysoma pellionella (Figure 7.9)

During most filtration runs, Tachysoma pellionella increased in density fairly rapidly in the surface layers with little growth in population occurring below 4 cm. As the bed conditions became less favourable and the population density declined, the vertical distribution remained the same with higher densities occurring at the surface. The highest population maxima were recorded in both the spring filtration runs studied : 985 cm^{-3} in run 3 (spring 1976) and 560 cm^{-3} in run 8 (spring 1977), the other maxima ranged between 140 and 300 cm^{-3}. These high spring densities corresponded with the extremely high densities (940 cm^{-2}) found by Wilbert (1969) in the periphyton of a eutrophic pond during March and April of 1966.

Aspidisca costata (Figure 7.10)

A. costata was found predominantly at the surface throughout a filtration run. However, run 6 (October/

/November 1976) was a total exception, with A. costata exhibiting surface avoidance throughout the run with highest densities occurring between 4 and 25 cm below the surface. Population maxima ranged over the year from 150 cm^{-3} to 380 cm^{-3} with no obvious seasonal differences. Temperature and oxygen stati of the bed were well within the tolerance limits of A. costata throughout the year : 0 - 38°C and 0 - 22.4 mg l^{-1} D.O. (Bick and Kunze, 1971).

Oxytricha spp. (Figure 7.11)

Oxytricha spp. occurred in maximum numbers at the sand surface during the first half of a run, moving away from the surface by 2 to 4 cm as environmental conditions presumably deteriorated for them with the exception of run 8, where a surface peak remained on day 28. However, throughout run 6, as with A. costata, they exhibited surface avoidance, the population density peak descending from 2 cm on day 12 to 7 cm by day 40. Maximum population densities for each run varied from 40 cm^{-3} to 190 cm^{-3}, the higher numbers being present at temperatures below 12°C during the runs from January to the end of April 1977.

Stentor polymorphus (Figure 7.12)

S. polymorphus was generally detected only in the latter stages of a filtration run in the top 10 cm of sand. It was only ever present in low densities (maxima ranging from 5 to 80 cm^{-3}) and occurred mainly during the spring and early summer (runs 4 and 8). The water temperature fell below 4°C, the minimum temperature tolerated by S. polymorphus (Bick, 1972) during the early part of run

7 (Appendix 5.1), but had risen to 5·5°C by day 45, the age of the bed when this ciliate was recorded.

Vorticella spp. (Figure 7.13)

The Vorticella spp. began to colonise the cores after about 10 days. They were generally present in highest densities at the surface, although some surface avoidance occurred throughout the second halves of runs 3 (April to June, 1976) and 6 (October/November, 1976). The two summer runs (4 and 5) produced the highest densities of Vorticella spp. with as many as 494 cm^{-3} at the surface; maxima during the year ranged from 85 cm^{-3} in spring 1977 (run 8) to 494 cm^{-3}. Despite its sessile nature, Vorticella spp. were consistently found at depths down to 25 cm.

Flagellata (Figure 7.15)

The flagellates were generally far more numerous than the ciliates, being countable in thousands cm^{-3}, as opposed to the tens and hundreds cm^{-3} of the ciliates. Maximum numbers occurred at or just below the sand surface, with the highest densities of 7,400 cm^{-3} and 9,060 cm^{-3} occurring during the two summer runs (runs 4 and 5). The lowest maximum of 174 cm^{-3} was recorded during run 8, a run which was poorly populated by flagellates throughout the 28 days over which it was sampled. Run 6 also produced low flagellate densities (maximum of 916 cm^{-3}) and as with A. costata and Oxytricha sp., the flagellata also exhibited surface avoidance throughout the duration of this run.

The ciliate species able to thrive at the sand surface throughout a filtration run : Chilodonella sp.,

FIG. 7.3 DEPTH DISTRIBUTION OF GLAUCOMA SP. IN BED 45, 16 in hr^{-1}

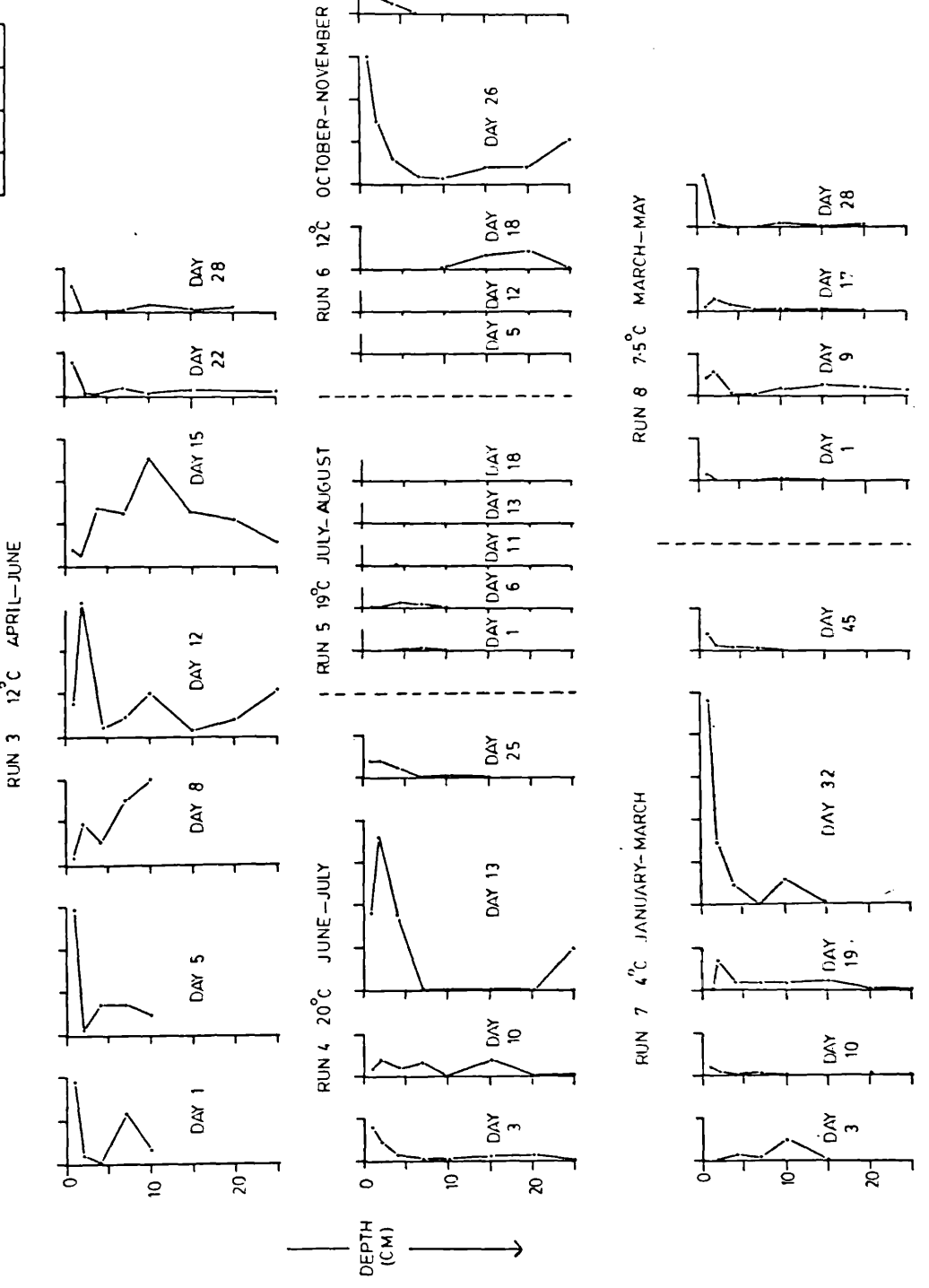


FIG. 7.4 DEPTH DISTRIBUTION OF CYCLIDIUM HEPTATRICHUM IN BED 45, 16 in. hr.$^{-1}$

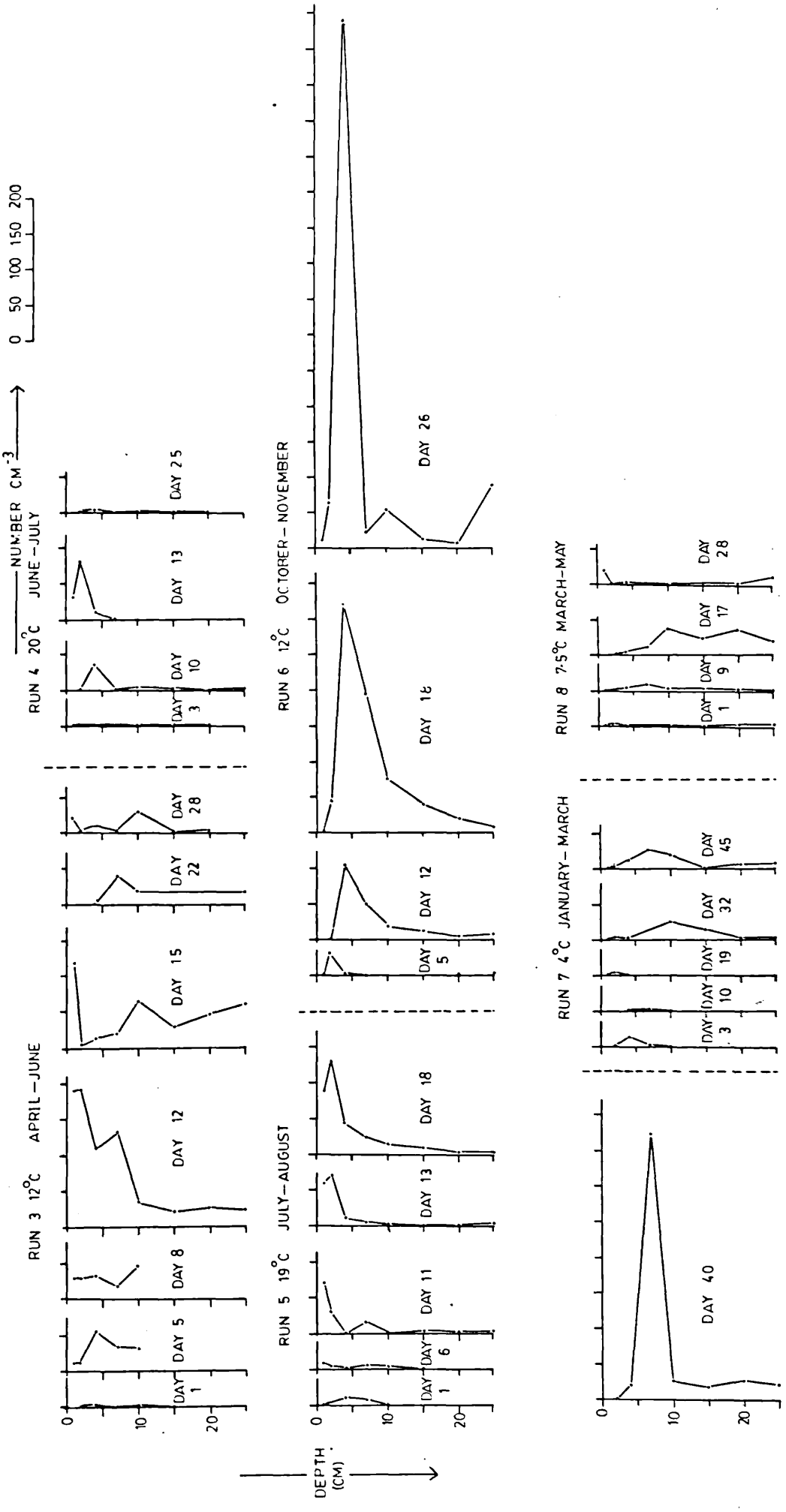


FIG. 75 DEPTH DISTRIBUTION OF CINETOCHILUM MARGARITACEUM IN BED 45, 16 in. hr.$^{-1}$

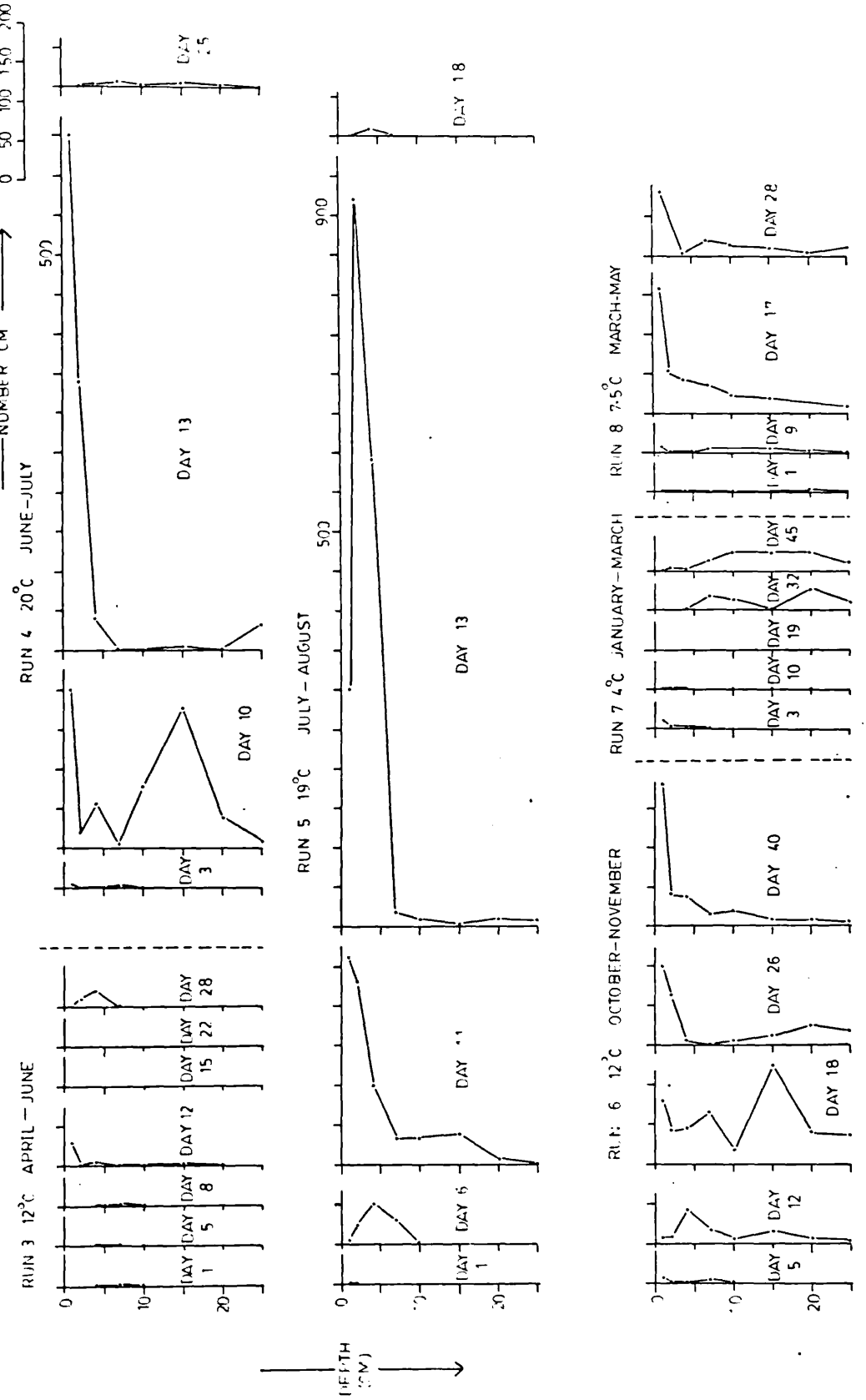


FIG. 7.6 DEPTH DISTRIBUTION OF CHILODONELLA SP IN BED 45, 16 in.hr.$^{-1}$

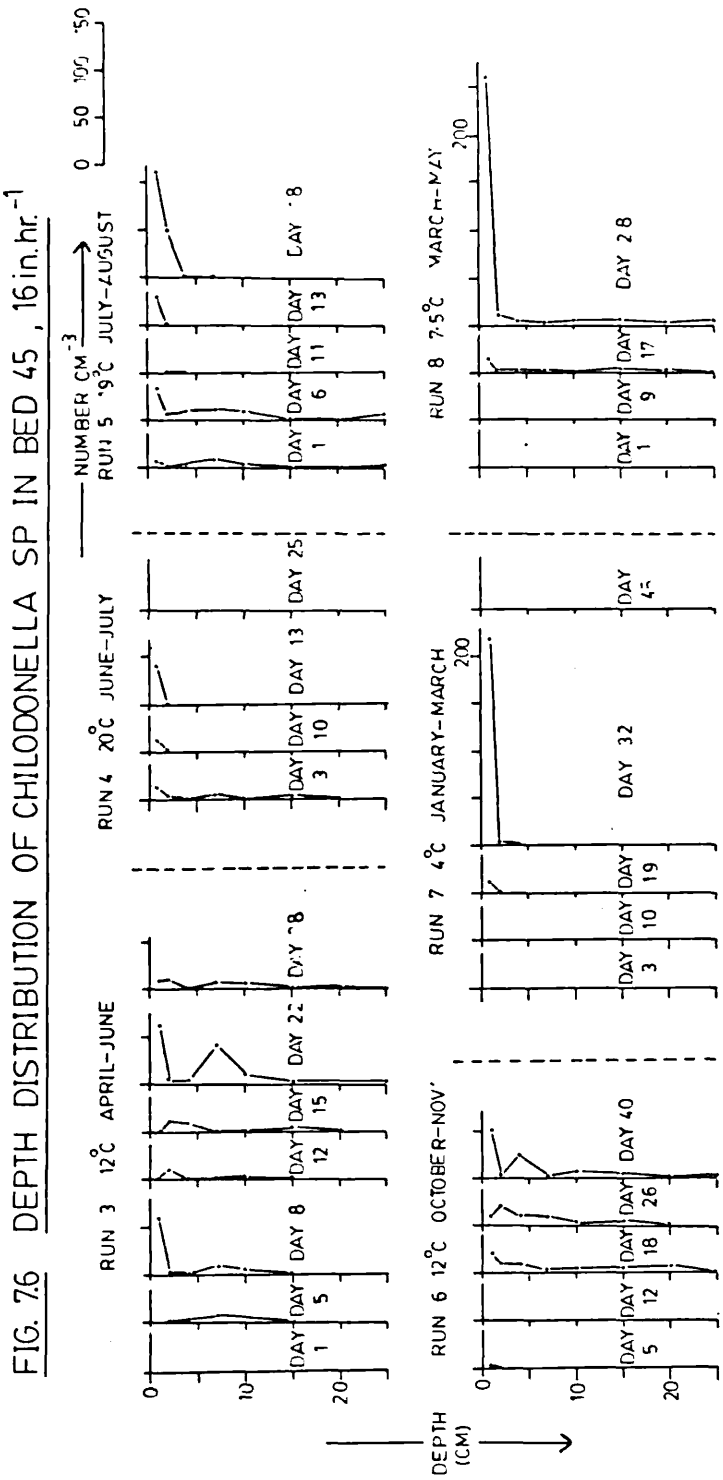


FIG. 7.7 DEPTH DISTRIBUTION OF LACRYMARIA OLOR IN BED 45

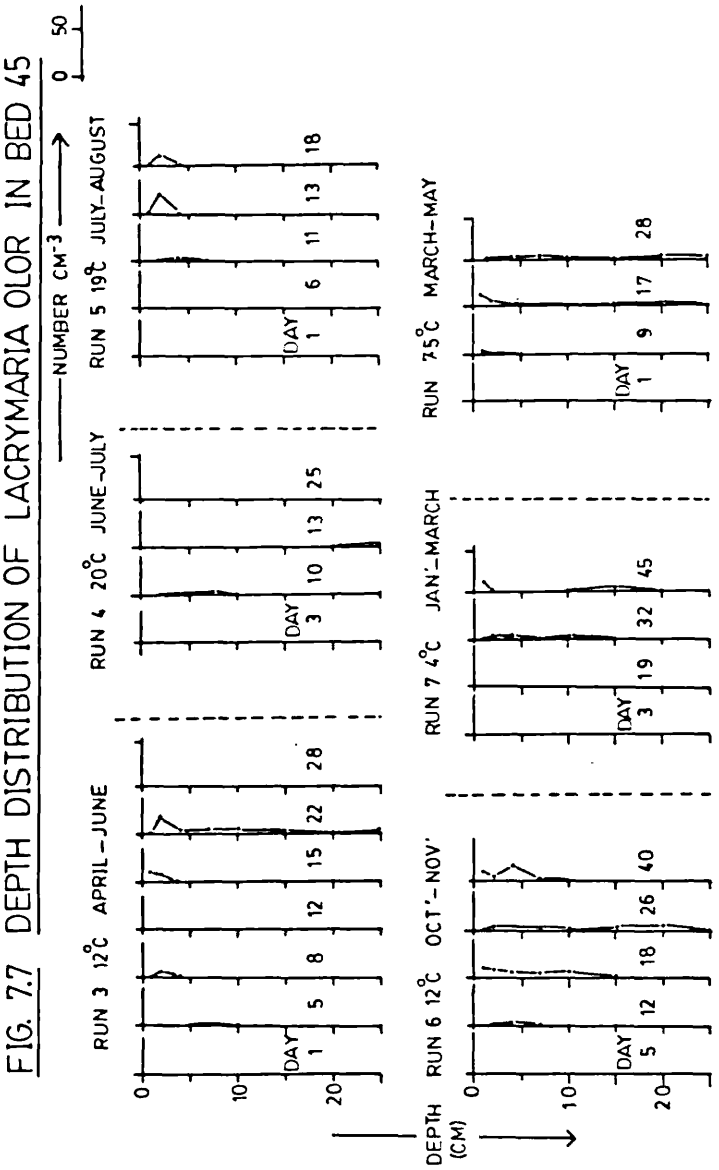


FIG. 78 DEPTH DISTRIBUTION OF LITONOTUS SPP IN BED 45, 16 in.hr.$^{-1}$

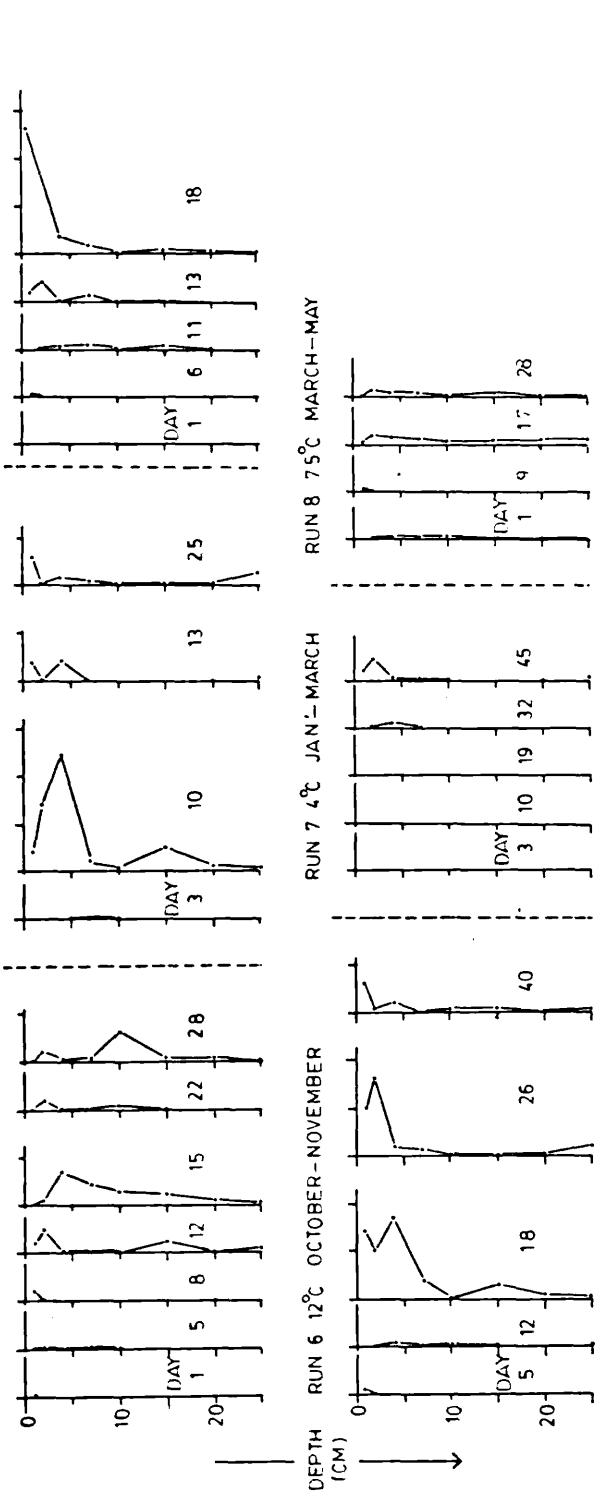


FIG. 7.9 DEPTH DISTRIBUTION OF TACHYSOMA PELLIONELLA IN BED 45, 16 in. hr.$^{-1}$

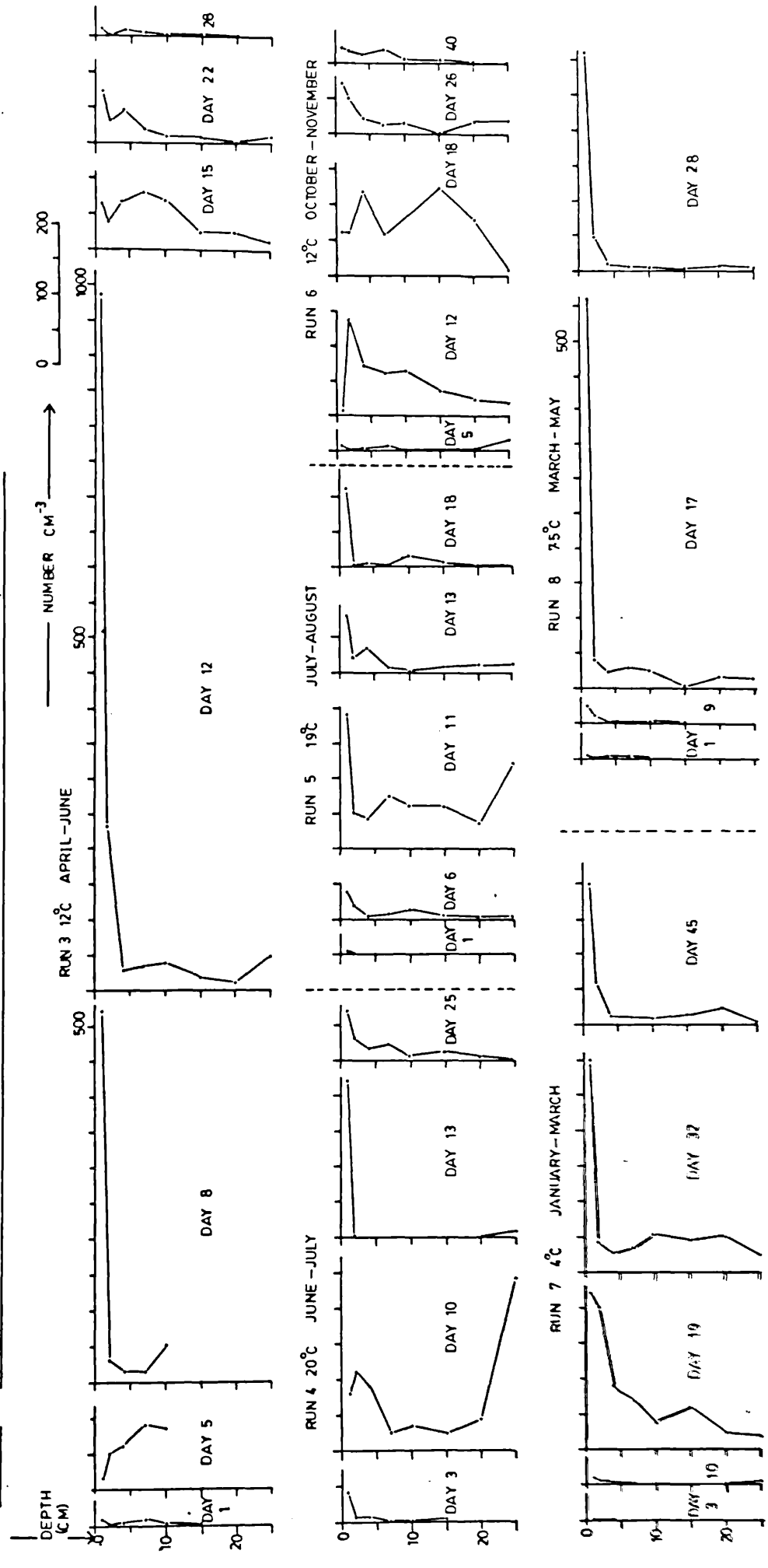


FIG. 7.10 DEPTH DISTRIBUTION OF ASPIDISCA COSTATA IN BED 45, 16 in.hr.$^{-1}$

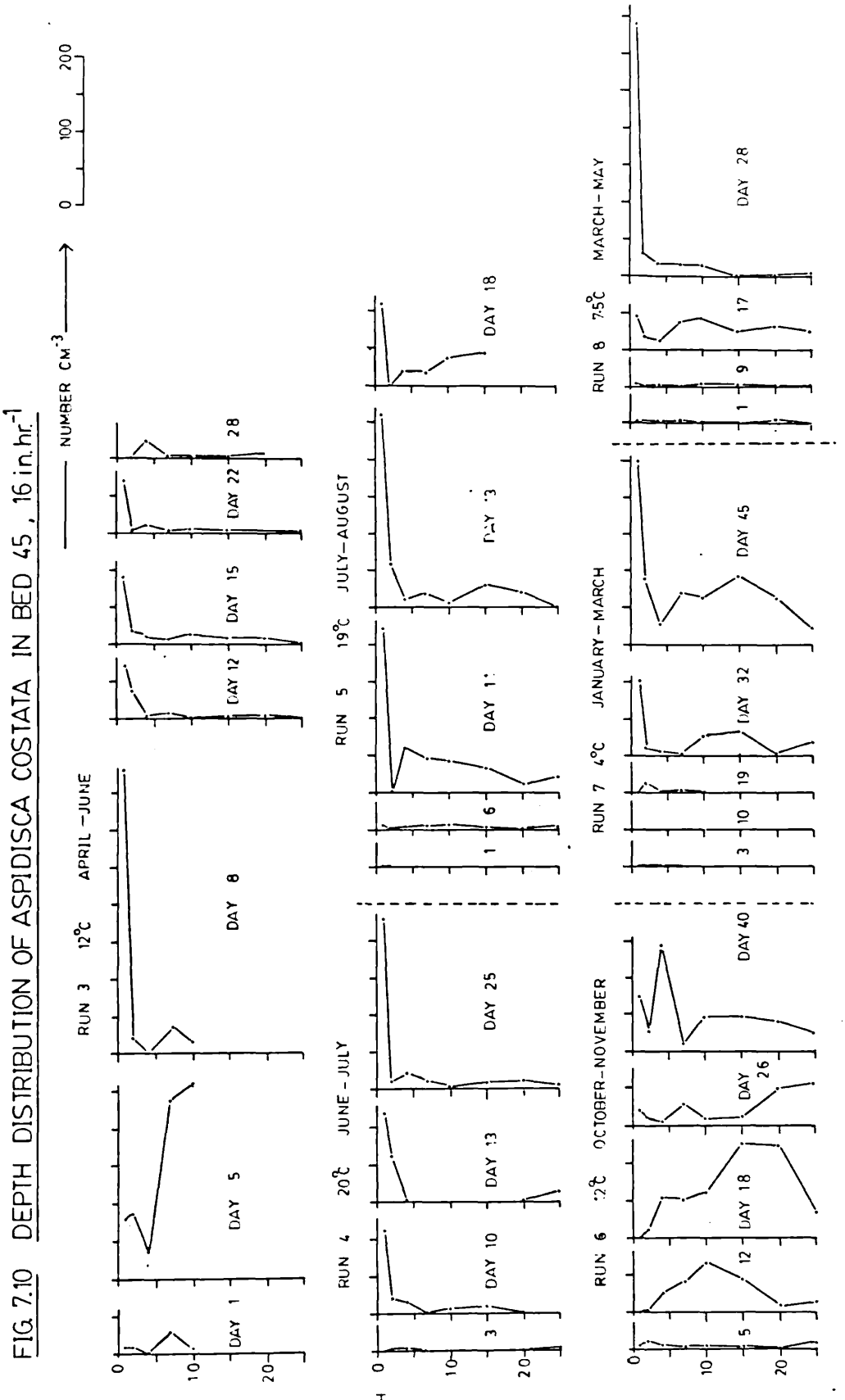


FIG. 7.11 DEPTH DISTRIBUTION OF OXYTRICHA SPP. IN BED 45, 16 in hr^{-1}

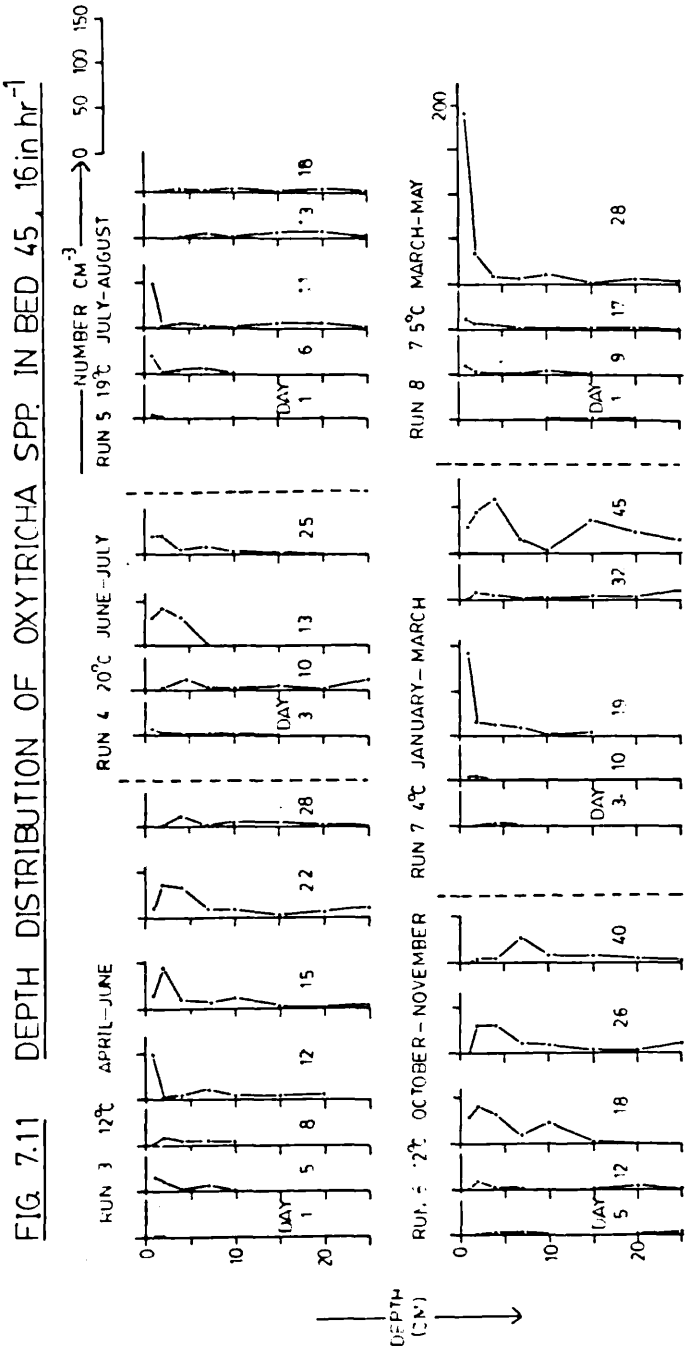


FIG. 7.12 DEPTH DISTRIBUTION OF STENTOR POLYMORPHUS IN BED 45, 16 in hr^{-1}

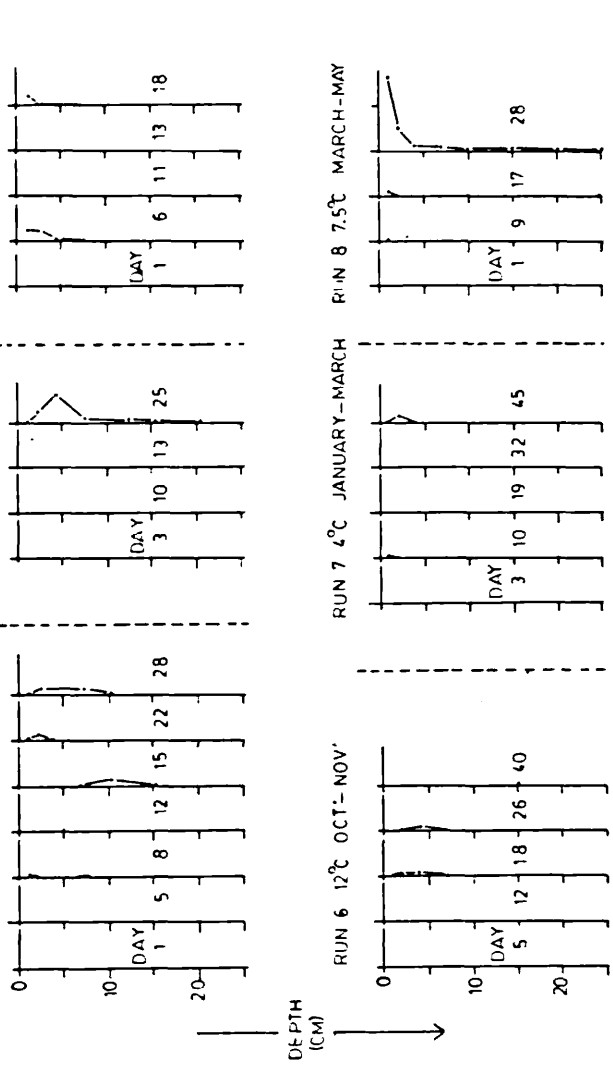


FIG. 7.13 DEPTH DISTRIBUTION OF VORTICELLA SPP IN BED 45, 16 in. hr^{-1}

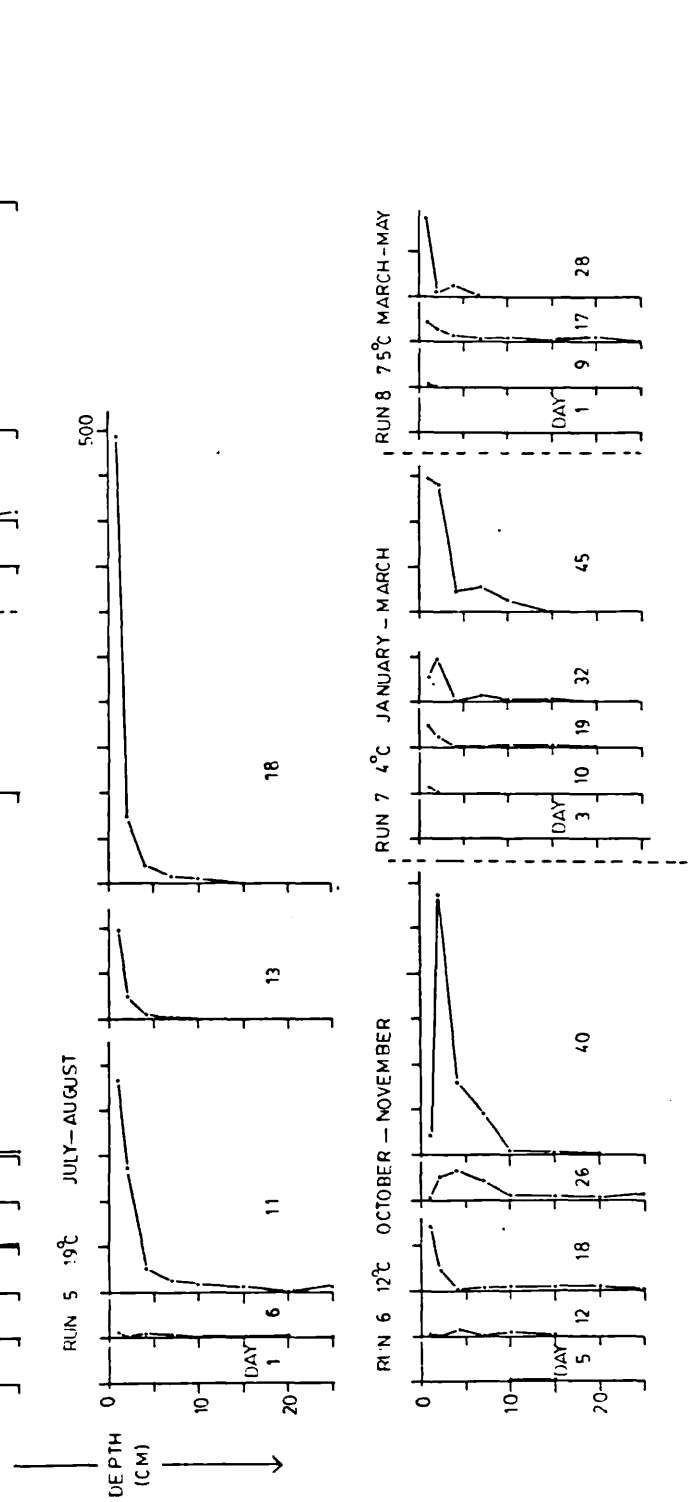
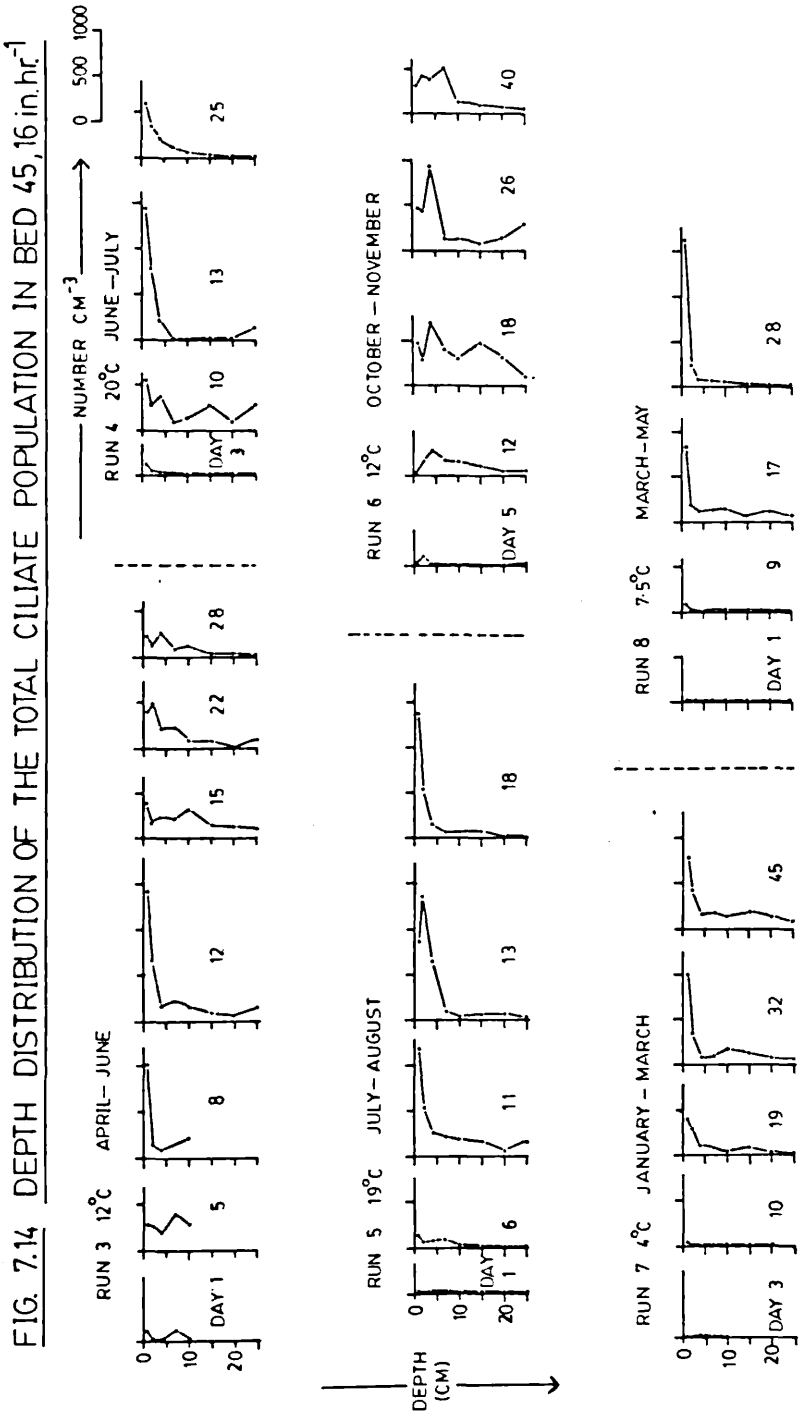


FIG. 7.14 DEPTH DISTRIBUTION OF THE TOTAL CILIATE POPULATION IN BED 45, 16 in.hr^{-1}



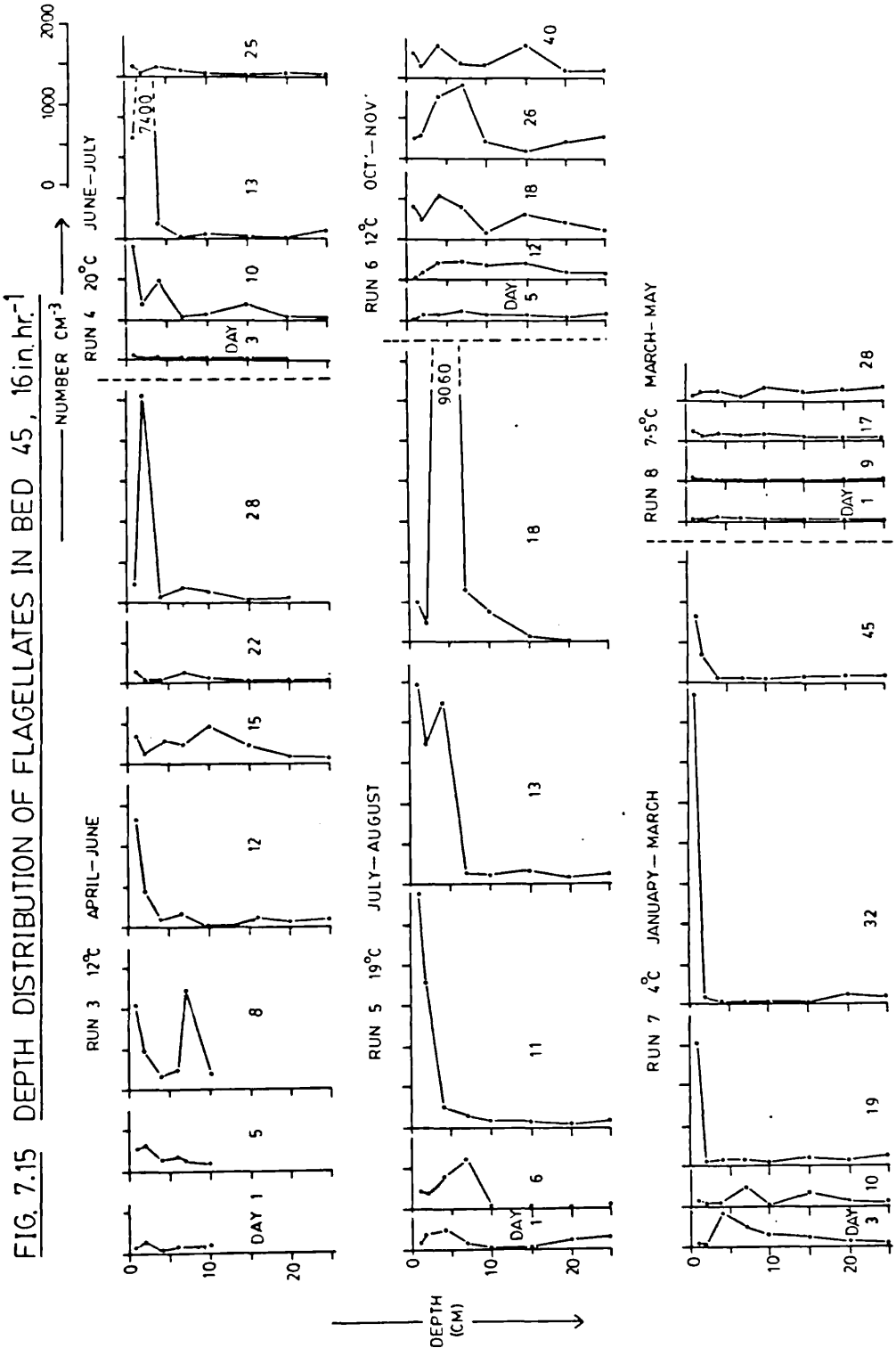


FIG. 7.15 DEPTH DISTRIBUTION OF FLAGELLATES IN BED 45, 16 in. hr.-1
NUMBER CM-3
0
1000
2000
RUN 3 12°C APRIL-JUNE
RUN 4 20°C JUNE-JULY
RUN 5 19°C JULY-AUGUST
RUN 6 12°C OCT.-NOV.
RUN 7 4°C JANUARY-MARCH
RUN 8 7.5°C MARCH-MAY
DAY 1
DAY
DEPTH (CM)
7400
9060

FIG. 7.16 DEPTH DISTRIBUTIONS OF CILIATES IN BED 44, 8 in.hr^{-1}, 12°C, OCT–NOV.

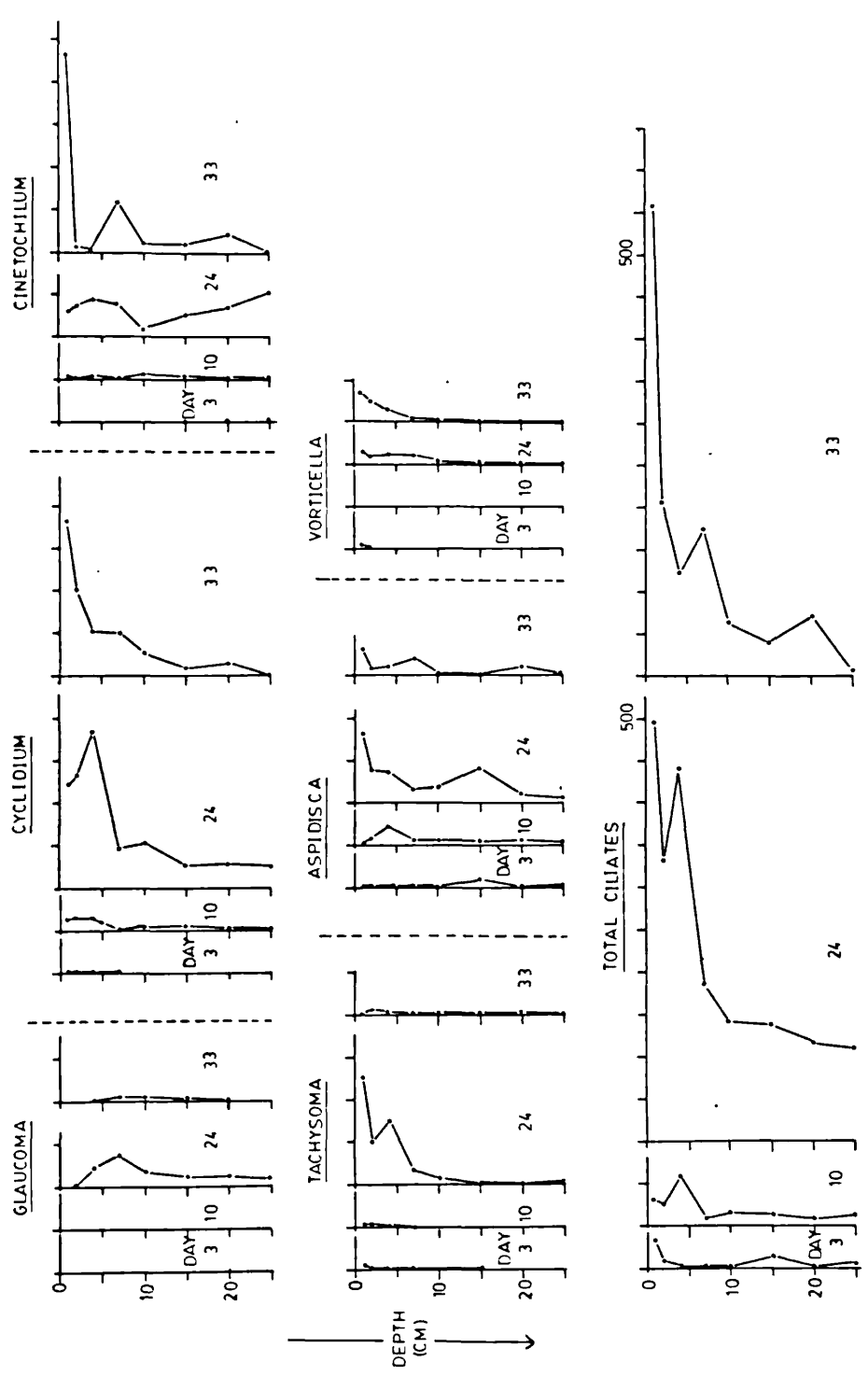
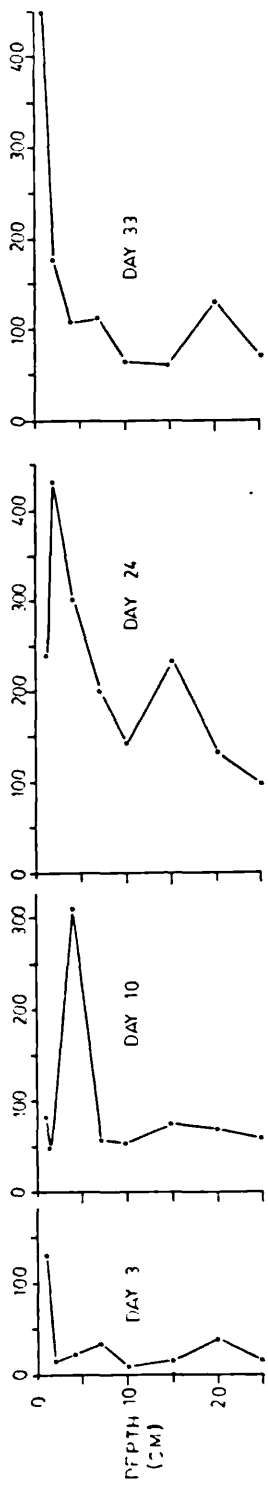


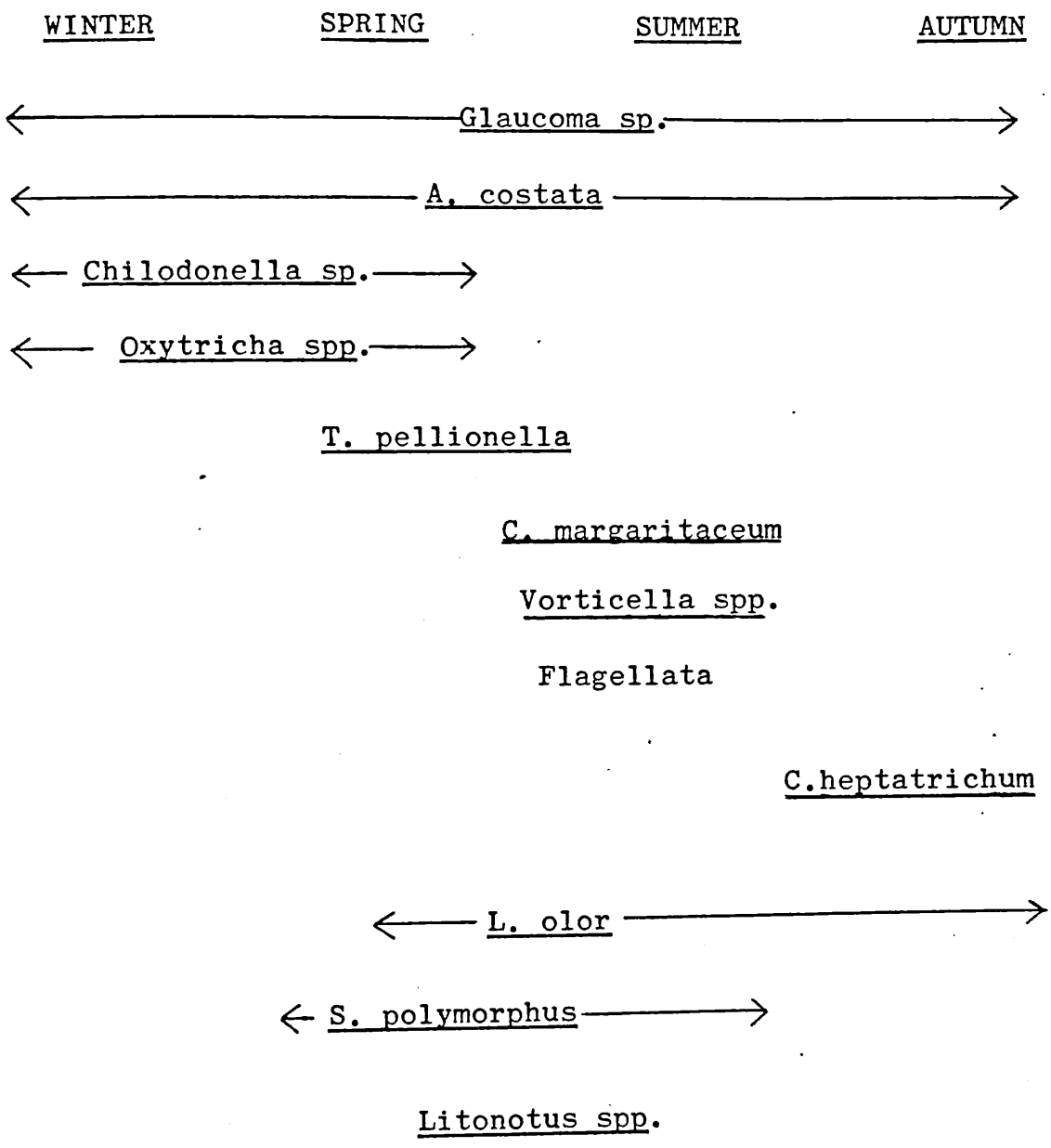
FIG. 7.17 DEPTH DISTRIBUTION OF FLAGELLATES IN BED 44, 8 in.hr^{-1}, OCT. NOV.



Tachysoma pellionella, Aspidisca costata, Vorticella spp., Cinetochilum margaritaceum and Glaucoma sp. all feed on bacteria, and the first two species, in common with the flagellata (Sandon, 1932), also feed on diatoms and algae (Bick, 1972). Noland (1925) found an association of algivorous ciliates with water of low free CO_2 content, as found in algal habitats where the CO_2 is taken up during photosynthesis. This may partially account for the surface avoidances of the other bacterivorous ciliates during the latter stages of a run when the surface algal populations became more dense. The other bacteria consuming ciliates : Cyclidium heptatrichum and Oxytricha spp. do not always seem to be able to survive the sand surface conditions but appear able to obtain sufficient nourishment and oxygen below the surface. During run 6 (October to November 1976) the unfavourable surface conditions appear to have extended to some depth in the sand, driving some of the ciliates downwards as far as 15 cm. What caused this effect is uncertain as neither the particulate organic carbon densities nor the head loss gain during the run were in any way unusual.

The seasonal occurrences of the maximum population densities of these ciliates is indicated below (Figure 7.18). Glaucoma sp. and A. costata colonised the sand surface all year round, while Chilodonella sp. a surface dweller and Oxytricha spp., which avoided the sand surface during the latter stages of the filtration runs, predominated from winter to spring. During the spring, T. pellionella was also present in high numbers at the surface.

Figure 7.18 Seasonal occurrences of the maximum population densities of the most common ciliates and the flagellates in bed 45



During the summer, when higher primary productivity at the surface of the bed would have indirectly (through decay) provided greater populations of heterotrophic bacteria, C. margaritaceum, Vorticella spp. and the flagellates co-existed at the surface with the other two perennial species; and in the autumn, C.heptatrichum thrived below the sand surface, while Glaucoma sp. and A. costata remained above them at the surface.

Stentor polymorphus, an omnivore, favoured the region just below the sand surface, as also did Lacrymaria olor and Litonotus spp. the most common carnivores in bed 45. Here they were presumably able to detect and consume their prey in the absence of high detrital densities. L. olor was present in highest densities from spring to autumn, while Litonotus spp. thrived only during the summer runs and S. polymorphus was most abundant in spring and early summer. Large ciliates were also found to be characteristic of the summer months by Finlay et al (1979) who studied the benthic ciliate communities of a eutrophic loch.

7.3 Individual cell volumes and total biovolume of the ciliates

The aim of this analysis was to determine whether the depth at which the ciliates occurred in the sand had any effect on either their individual cell size or their total biomass. Biomass is represented here by estimated biovolume, determined using equation 1 for all the ciliates which could be approximated to an ellipsoid shape. Biovolumes of species such as the Vorticella spp.

and Stentor polymorphus were estimated using the formula for the volume of a cone (equation 2), and the stalks of the Peritricha were measured separately, using the formula for the volume of a tube (equation 3).

1. $V = L \times W \times \frac{\pi}{4} \times C$

This equation should read: $V = L \times W \times \frac{\pi}{3} \times C$

Thus all biovolume data of ellipsoid ciliates are 25% low.

2. $V = \frac{1}{3}\pi r^2 L$

3. $V = \pi r^2 L$

Where L = length of ciliate (stalk in equation 3)

W = width of ciliate

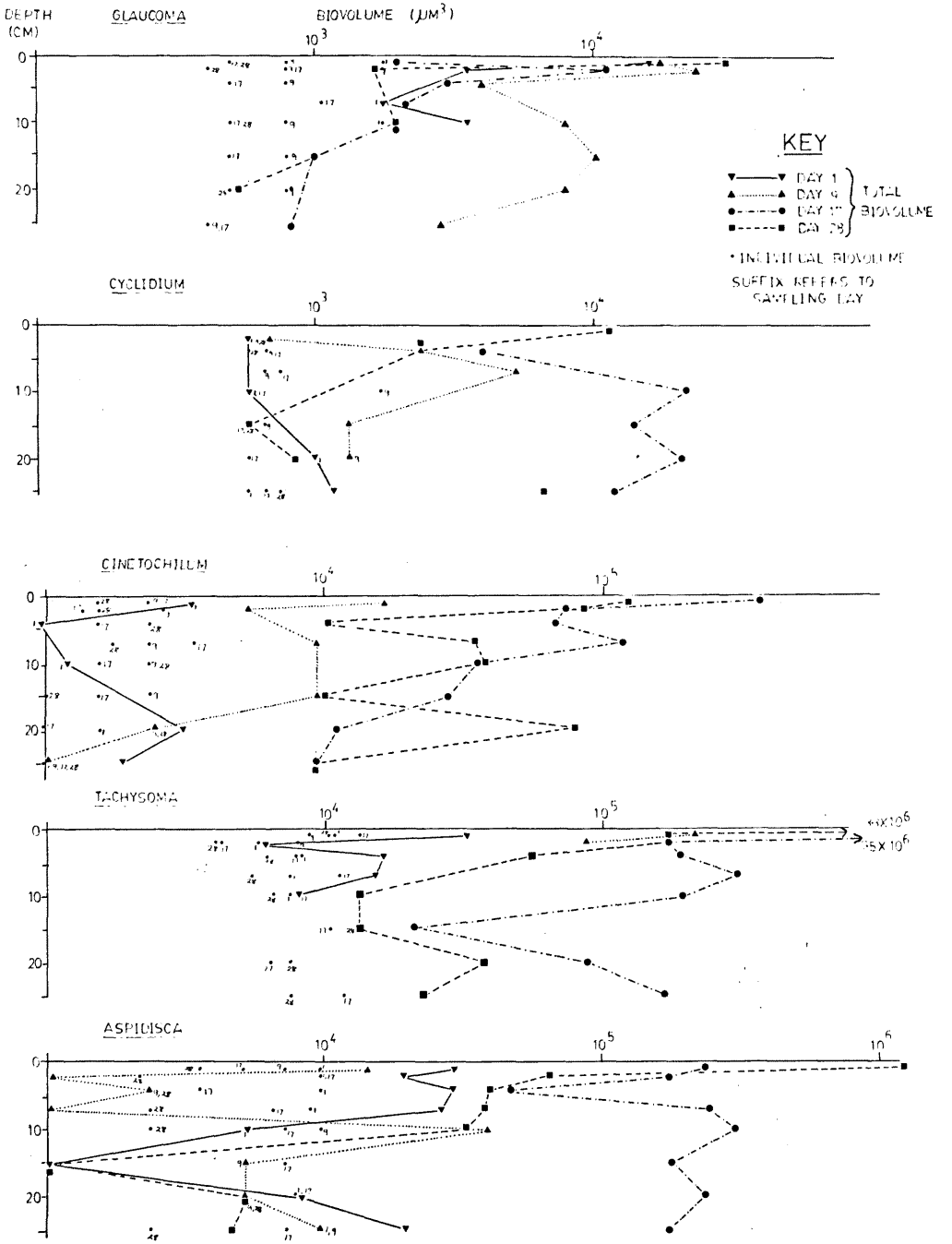
C = factor estimated to represent the depth of the ciliate ($C = \frac{1}{2}W$ or $\frac{1}{3}W$ depending on the species)

r = radius of peristome - equation 2

radius of stalk - equation 3

Measurements (L, W and r) of the first five individuals of each species, recorded when counting the ciliate densities, were averaged to give a mean cell size per depth on each sampling occasion. From these data the individual cell size and total biovolume of the dominant ciliate species were calculated; Figure 7.19 illustrates this data for run 8 (Appendix 7.4). The biovolume depth distributions were very similar to those of the population density, and individuals of each species did not vary significantly in size with depth. When measured under the microscope at a magnification of X 100, 1 division on the eye-piece graticule used, was equivalent to a distance of 2.74 μm on the specimen. The measurements are

FIG. 7.19 INDIVIDUAL AND TOTAL BIOVOLUMES OF 5 COMMON CILIATE SPECIES IN RUN 8, BED 45, MARCH–MAY 1977



therefore likely to be inaccurate by a maximum of ± 2.74μm. Cyclidium heptatrichum measured about 22 X 14 μm which gives it an estimated cell volume of 1.06 X 10^3μm. If the measurements are varied by a maximum of ± 2.74 μm, the range of the resulting cell volumes lies between 7.68 X 10^2 μm^3 and 2.22 X 10^3 μm^3. It is therefore impossible to interpret Figure 7.19 as showing that depth has any effect on individual cell size as the cell volumes did not vary greater than the error of the measurement.

During run 8, it was also apparent (Figure 7.19) that the age of the bed had no effect on the cell size of Cyclidium heptatrichum, Cinetochilum margaritaceum or Tachysoma pellionella. However, Glaucoma sp. decreased in cell size by about 40% between day 1 and day 9, corresponding with a rapid increase in total biovolume, while Aspidisca costata decreased in cell size by 42% between days 17 and 28, corresponding with an overall decrease in biovolume of the species below the sand surface. The decrease in the cell size of Glaucoma sp. was probably caused by a high rate of replication, while that of A. costata seems more likely to have been caused by worsening environmental conditions below the surface during this late part of the filtration run, with an associated general decline in the population.

7.4 Relationships between density depth distributions and filtration rate

This section compares the depth distributions of the ciliates and flagellates in bed 45 during run 6 (October/November, 1976) with those in bed 44 during the

same months when the filter beds were operating at 16" h^{-1} (0.4 mh^{-1}) and 8" h^{-1} (0.2 mh^{-1}) respectively. The depth distributions in bed 44 (Appendix 7.2) are shown graphically in Figures 7.16 (ciliates) and 7.17 (flagellates).

Very few ciliates were recorded before day 24 on bed 44 and maximum numbers of most of the dominant species were present at the surface on day 33 following an earlier period of surface avoidance. A comparison of the maximum densities recorded for the dominant ciliate species and the flagellates, at the depth at which they occurred, in beds 44 and 45, is given in Table 7.6.

Table 7.6 Comparison of the maximum ciliate and flagellate densities in beds 44 and 45 during October - November 1976.

	Bed 44 (20 cm h^{-1})		Bed 45 (40 cm h^{-1})	
	Maximum density ($No.cm^{-3}$)	Depth (cm)	Maximum density ($No.cm^{-3}$)	Depth (cm)
Glaucoma sp.	36	7	150	1
C. heptatrichum	186	4	740	4
C. margaritaceum	230	1	180	1
T. pellionella	125	1	136	2
A. costata	80	1	144	4
Vorticella spp.	38	1	291	2
Flagellata	450	1	916	7

In general, maximum densities were below the sand surface during run 6 of bed 45; this may have been due to adverse conditions at the surface but presumably could be due to the higher rate of filtration supplying sufficient

nutrients and oxygen deeper into the sand to support populations of far greater density than those which occurred in bed 44 at the surface. Very low population densities were recorded below 7 cm in bed 44.

7.5 Relationship between the depth distribution of ciliates and the concentration of particulate organic carbon in the sand.

As, in general, both the carbon and ciliate densities were greatest at or near the surface of the sand, decreasing fairly rapidly in the top few centimetres and to a lesser extent at greater depths, both densities were transformed using a ln transformation to convert these exponential changes of density with depth to a more linear relationship (a $\ln(n + 1)$ transformation was used for the ciliate densities as some zero values were recorded). To look for interdependence of ciliate density and particulate organic carbon content throughout the depth of the sand cores, linear regression analyses were performed on the transformed ciliate and carbon data from the same depths in the cores, for each sampling occasion, during each filtration run. Between 6 and 9 pairs of data were taken from each core (Appendix 7.5), this was restricted by the number of depths examined for ciliates (Section 4.5). Regressions significant at 95% or at a greater level of significance ($p \leq 0.05$) were accepted as showing a positive interdependence of ciliate density and carbon concentration. These regression analyses performed on the total number of ciliates present against carbon content (Table 7.7), indicate that only in the latter period of a filtration run

were the two densities interdependent throughout the depth of the core. The slopes (b) of all the significant regressions were positive, ranging from 0.323 to 2.354.

Analyses performed on individual ciliate species showed very little interdependence between numbers and carbon density; this was probably due to the low frequencies of individual animals. The flagellate frequencies were also found to be independent of the carbon density.

Table 7.7 The slopes and significance of regression analyses performed on the interdependence of ciliate frequency and carbon content with depth

RUN	DAY	b	p	RUN	DAY	b	p
3	5	-0.047	0.75	6	5	1.290	0.10
	8	2.408	0.50		12	0.604	0.75
	12	0.832	0.10		18	0.556	0.50
	15	0.323	0.05		26	1.331	0.025
	22	0.685	0.025		40	0.675	0.1
	28	0.522	0.05	7	3	1.507	0.5
4	3	-0.291	0.75		10	0.006	0.75
	10	-0.025	0.10		32	0.050	0.75
	13	1.921	0.25		45	0.423	0.01
	25	0.664	0.05	8	1	-0.249	0.5
5	1	0.155	0.75		9	1.259	0.25
	6	1.467	0.25		17	1.018	0.001
	11	0.814	0.05		28	0.673	0.10
	13	1.219	0.25	BED			
	18	1.076	0.05	44	3	-0.753	0.25
					10	0.083	0.75
					24	0.681	0.25
					33	2.354	0.01

b = slope of regression

p = probability that the points of the analyses differ significantly from the calculated regression

Chapter 8 Temporal Distribution of Ciliates and Flagellates

Introduction

The aims of this part of the study were to detect changes in cell sizes and densities of the ciliate and flagellate populations both during a filtration run and at different seasons of the year. It is convenient, where possible, to be able to express the rate of change by a single value, and the instantaneous rate, which was found to be most suitable (Section 4.9), has been used throughout this chapter. Doubling times of the dominant ciliate species and the flagellates were also calculated in order to compare this sand environment to other environments in which the growth rates of protozoa have been studied. The dynamics of the protozoan populations are effected by a number of variables including food abundance and availability, inter and intraspecific competition, temperature, dissolved oxygen conditions and flow rate. Only a few of these variables were monitored during this study, but an attempt has been made to relate these to the population changes.

8.1 Variations in individual cell volume

Differences in cell volume occurring seasonally were examined and related to effects of temperature, flow rate and carbon content of the sand. Cell sizes were calculated as described in Section 7.3, where no significant change in cell volume was found to occur either with depth on any one sampling occasion, or with time during a filtration run.

Variation of individual cell volumes of the most common ciliate species were calculated for all runs in which measurements were recorded (runs 4 to 8 and the bed 44 run), and these are shown in Table 8.1. The error in measuring cell sizes (Section 7.3) would be partly responsible for larger percentage variations in volume with the smaller species, e.g. Glaucoma sp. than with the larger species, e.g. Tachysoma pellionella. Differences in size between a ciliate immediately prior to replication and the resultant daughter cells would also account for some of the observed variations.

Table 8.1 Average Individual Cell Volumes of the Dominant Ciliate Species

Species	Cell Volume (μm^3) Mean ± 95% confidence limits (percentage deviation from mean)	
	Run 4*	Run 5
Glaucoma sp.	798 ± 243 (30)	1171 ± 514 (44)
C. heptatrichum	1045 ± 437 (42)	797 ± 117 (15)
C. margaritaceum	1885 ± 392 (21)	2644 ± 596 (23)
T. pellionella	6913 ± 590 (9)	10235 ± 1078 (11)
A. costata	1493 ± 321 (22)	3407 ± 752 (22)
	Run 6	Run 7
Glaucoma sp.	784 ± 92 (12)	1139 ± 197 (17)
C. heptatrichum	944 ± 108 (11)	942 ± 101 (11)
C. margaritaceum	1720 ± 323 (19)	2042 ± 399 (20)
T. pellionella	9515 ± 1075 (11)	8784 ± 835 (10)
A. costata	2450 ± 493 (20)	3512 ± 957 (27)
	Run 8	Bed 44
Glaucoma sp.	881 ± 239 (27)	827 ± 156 (19)
C. heptatrichum	885 ± 117 (13)	678 ± 83 (12)
C. margaritaceum	1825 ± 293 (16)	1896 ± 342 (18)
T. pellionella	8201 ± 1121 (14)	8538 ± 899 (11)
A. costata	4109 ± 712 (17)	3522 ± 731 (21)

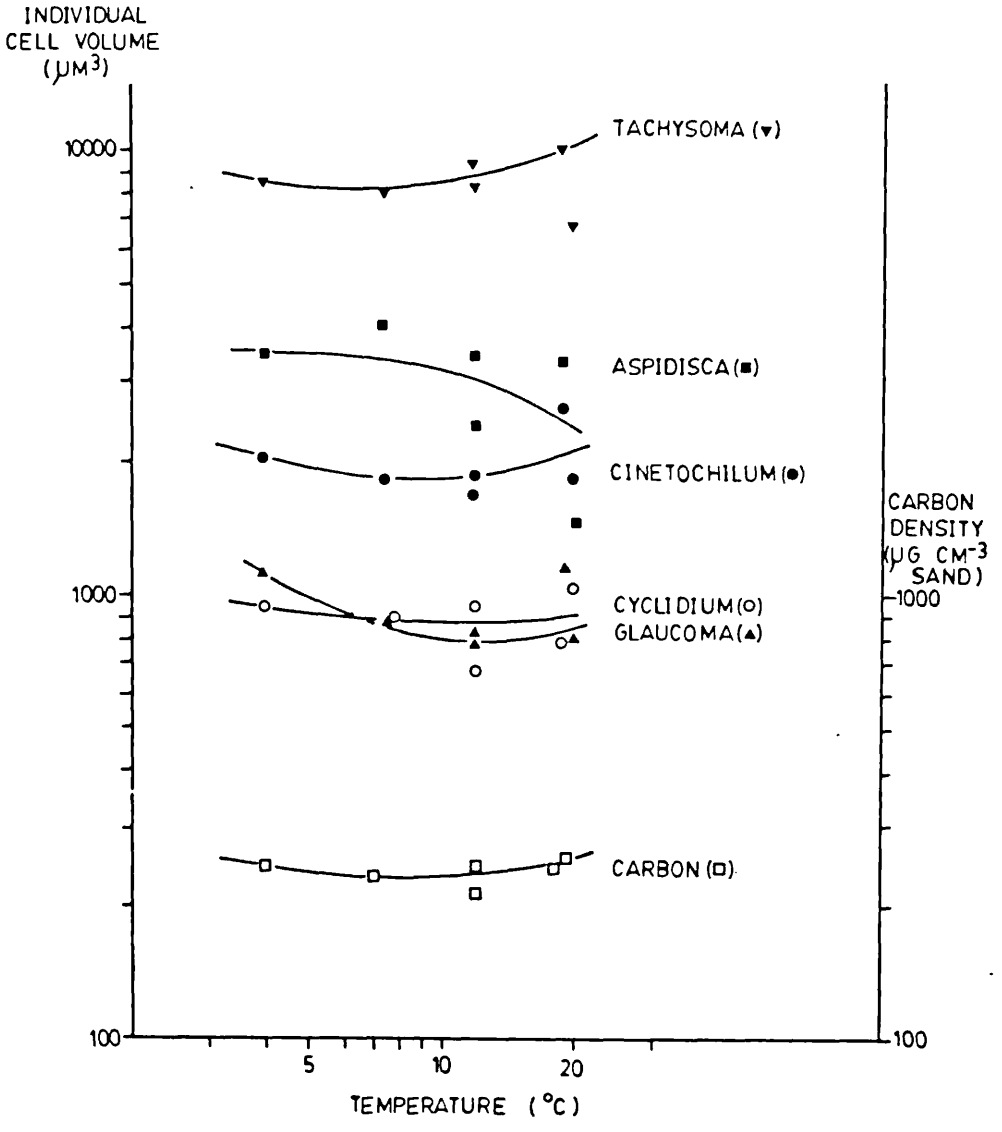
* Cell measurements, from which the volumes were calculated, were only recorded regularly from run 4 onwards.

The filtration rate had no apparent effect on the average individual cell size of the common ciliate species with the exceptions of Cyclidium heptatrichum and Aspidisca costata. The range of volumes within the 95% confidence limits of the means did not overlap with the former of these species between run 6 (the fast rate) and bed 44 (the slow rate). C. heptatrichum was considerably smaller in the slow rate bed while A. costata was much larger in the slow rate than in the fast rate bed. C. heptatrichum feeds on bacteria in the water and at a slower flow rate these would be less abundant. A. costata grazes on detrital flocs, dashing from one to another using its ventral cirri as 'legs' to propel it, hence a slower flow rate would enable it to move and graze more easily.

Temperature appears to have some effect on individual cell size (Fig. 8.1). For most of the common ciliate species a decrease in size occurred with increasing temperature up to about 12°C but further increases in temperature were accompanied by an increase in cell size. However, Aspidisca sp. showed a continuous decrease in size with increasing temperature, this effect being stronger above 12°C. These size differences may be related to growth and replication rates. The effect of temperature on these rates is discussed later in this chapter (Section 8.3.1).

The average carbon content of the depths analysed for ciliates showed a similar trend with temperature (Fig. 8.1), but the change in carbon was very little, varying only between 218 and 254 μg C cm^{-3}. To look for a relationship between carbon content of the sand and cell size of the ciliates, linear regression equations were calculated

FIG. 8.1 VARIATION OF CELL VOLUME WITH TEMPERATURE



for each species (ln cell volume v ln carbon density). All were insignificant, $p > 0.05$, suggesting that no direct relationship exists between individual cell size and particulate organic carbon density.

8.2 Changes in population density and biovolume

8.2.1 Density

The ciliates and flagellates were counted and measured in 1 cm^3 sand samples removed from eight depths of the sand cores as described in section 4.5. The frequency data obtained (Appendix 7.2) are plotted for the total ciliate and flagellate populations (Figs. 8.2 and 8.3); however, these give no tangible indication of changes in population density. From this data an estimate of the population density, in a volume of sand of 1 cm^2 surface area and as deep as the lowest sample counted, was calculated by numerical integration (Section 4.9), and this value was divided by the lowest depth counted, usually 25 cm, to give comparable values of density per cm^3 throughout the period of study (Appendix 8.1). These densities derived from the integrals are plotted for the total ciliates, the flagellates, Aspidisca costata and Vorticella spp. (Figs. 8.4 - 8.7) with the linear regressions calculated using all the original individual depth counts. The slopes of the regressions represent the instantaneous rate of increase or decrease in the population. A full regression table is given in Appendix 8.2 for the dominant ciliate species, the total ciliates and the flagellate population. Those regressions having significant slopes (p for regression ≤ 0.05) are summarised in Table 8.2.

FIG. 8.2 POPULATION DENSITIES OF CILIATES RECORDED IN THE SAND CORES DURING FILTRATION RUNS ON BED 45

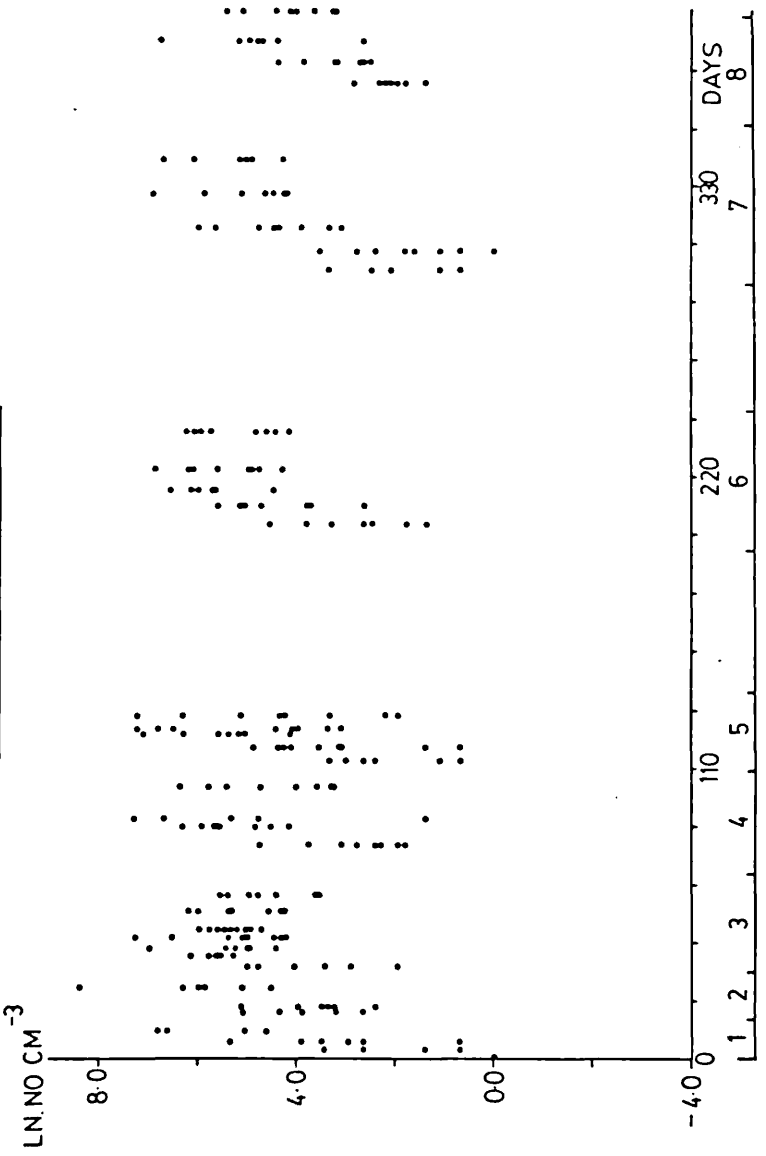


FIG 83 POPULATION DENSITIES OF FLAGELLATES RECORDED IN SAND CORES DURING FILTRATION RUNS ON BED 45

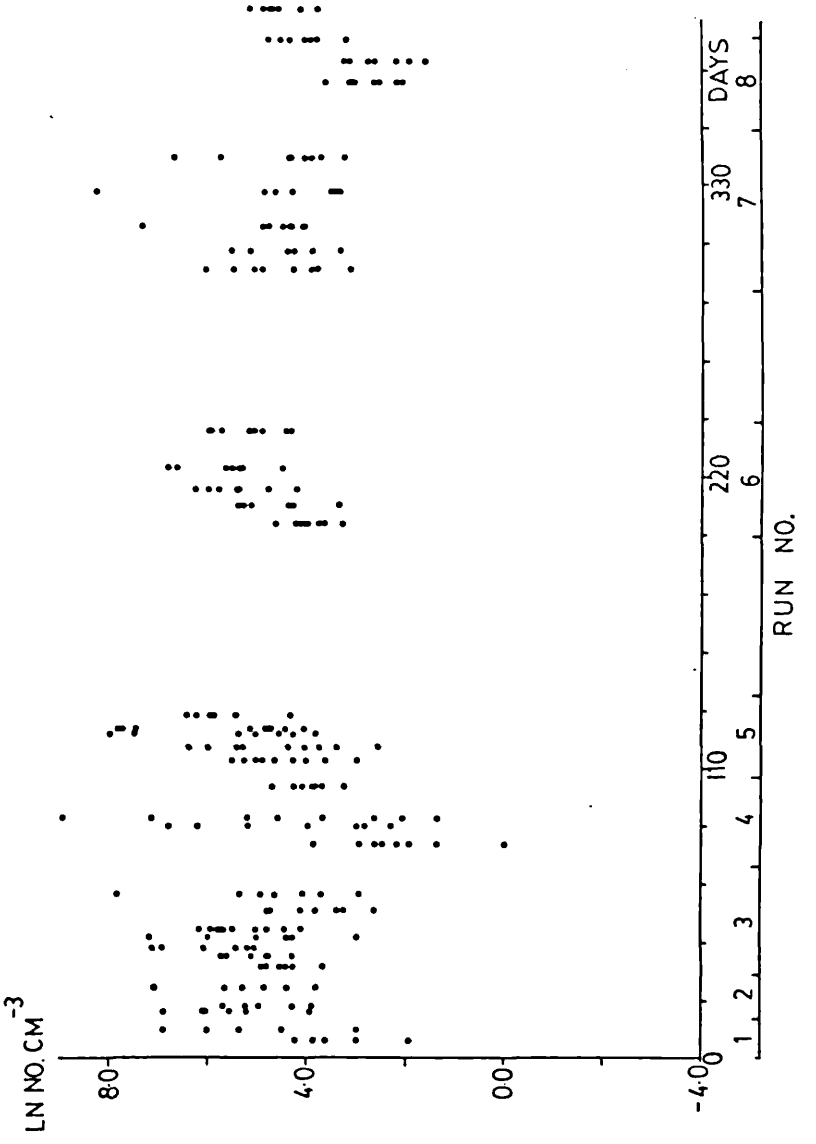


Table 8.2 Summary of ciliate and flagellate regressions having significant instantaneous rates of increase or decrease

SPECIES	TIME PERIOD (DAYS)	SLOPE	S.E. SLOPE
RUN 1			
Glaucoma	4 - 11	0.213	0.097
Tachysoma	4 - 11	0.576	0.211
Vorticella	4 - 11	0.332	0.078
Total Ciliates	1 - 11	0.541	0.095
Flagellates	7 - 11	0.513	0.188
RUN 2			
Tachysoma	3 - 12	0.199	0.059
Aspidisca	3 - 12	0.400	0.096
Total Ciliates	3 - 12	0.263	0.066
RUN 3			
Glaucoma	8 - 28	-0.119	0.025
Cyclidium	1 - 12	0.218	0.049
	12 - 28	-0.110	0.030
Tachysoma	1 - 5	0.584	0.113
	5 - 28	-0.097	0.025
Aspidisca	1 - 5	0.521	0.143
	5 - 28	-0.123	0.027
Vorticella	1 - 22	0.132	0.039
Total Ciliates	1 - 5	0.453	0.239
	5 - 28	-0.036	0.015
RUN 3 (Contd)			
Flagellates	1 - 8	0.212	0.050
	8 - 28	-0.062	0.028
RUN 4			
Glaucoma	3 - 13	0.190	0.061
Cyclidium	3 - 13	0.281	0.076
Cinetochilum	3 - 13	0.335	0.109
	13 - 25	-0.258	0.074
Tachysoma	3 - 10	0.371	0.086
	10 - 25	-0.123	0.040
Aspidisca	3 - 13	0.321	0.076
Total Ciliates	3 - 10	0.352	0.062
Flagellates	3 - 13	0.244	0.091
RUN 5			
Cinetochilum	1 - 13	0.242	0.104
Tachysoma	1 - 11	0.412	0.069
	11 - 18	-0.263	0.096
Aspidisca	1 - 11	0.431	0.063
Vorticella	6 - 11	0.463	0.166
Total Ciliates	1 - 11	0.366	0.055
Flagellates	1 - 18	0.108	0.035

Table 8.2 (Contd)

SPECIES	TIME PERIOD (DAYS)	SLOPE	S.E. SLOPE
RUN 6			
Cyclidium	5 - 18	0.172	0.071
Cinetochilum	5 - 18	0.254	0.034
Tachysoma	5 - 18	0.223	0.046
	18 - 40	-0.081	0.022
Aspidisca	5 - 18	0.199	0.038
Vorticella	5 - 40	0.062	0.023
Total Ciliates	5 - 18	0.241	0.035
Flagellates	5 - 26	0.085	0.015
RUN 7			
Glaucoma	3 - 32	0.054	0.026
Cinetochilum	3 - 45	0.047	0.015
Tachysoma	3 - 19	0.281	0.036
	19 - 45	-0.052	0.020
Aspidisca	3 - 45	0.110	0.014
Vorticella	10 - 45	0.073	0.031
Total Ciliates	3 - 19	0.183	0.040
RUN 8			
Glaucoma	1 - 9	0.195	0.058
	9 - 28	-0.072	0.024
Cyclidium	1 - 17	0.179	0.030
	17 - 28	-0.188	0.045
RUN 8 (Contd)			
Cinetochilum	1 - 17	0.204	0.030
	17 - 28	-0.088	0.039
Tachysoma	1 - 17	0.169	0.046
Aspidisca	1 - 17	0.166	0.030
	17 - 28	-0.094	0.046
Vorticella	9 - 28	0.080	0.023
Total Ciliates	1 - 17	0.182	0.021
	17 - 28	-0.078	0.036
Flagellates	1 - 28	0.079	0.011
BED 44			
Glaucoma	24 - 33	-0.115	0.047
Cyclidium	3 - 24	0.183	0.027
Cinetochilum	3 - 24	0.152	0.021
Tachysoma	3 - 24	0.127	0.032
	24 - 33	-0.210	0.081
Aspidisca	3 - 24	0.111	0.018
	24 - 33	-0.131	0.055
Total Ciliates	3 - 24	0.171	0.020
	24 - 33	-0.099	0.058
Flagellates	3 - 24	0.099	0.016

Most of the common genera showed a significant instantaneous rate of population increase in the initial stage of a filter bed run, reaching peak densities and subsequently decreasing. The decrease although significant on occasion was generally insignificant. These population changes are shown in Figs. 8.4, 8.6 and 8.7 for Aspidisca costata, one of the dominant ciliate species, the total ciliates and the flagellates respectively. However Vorticella spp., peritrichs which grow attached by their s t a l ks to the sand grains, always showed a different pattern of increase to the other ciliate species in that they developed later in the filtration run and continued to increase in density throughout the remainder of the run (Fig. 8.5). The other ciliate species did not always reach their peak densities on the same day although it was more usual for them to do so. The age of the filtration run at which these peaks were achieved varied between filtration runs and was found to be negatively correlated with temperature (Section 8.3.1).

There were deviations from this general pattern. Runs 1 and 2 (March - April 1976) were the first two runs following the resanding of Bed 45 in March 1976. As is normal for re-sanded beds, these first two runs were very short and the bed 'jacked-up' after 11 and 14 days respectively. (The term 'to jack-up' describes the state of a filter bed when the head loss per unit flow of water through the bed suddenly increases very rapidly). As discussed in Chapter 5 this rapid increase in head loss is generally associated with a rapid accumulation of surface organic carbon; however, after resanding, the

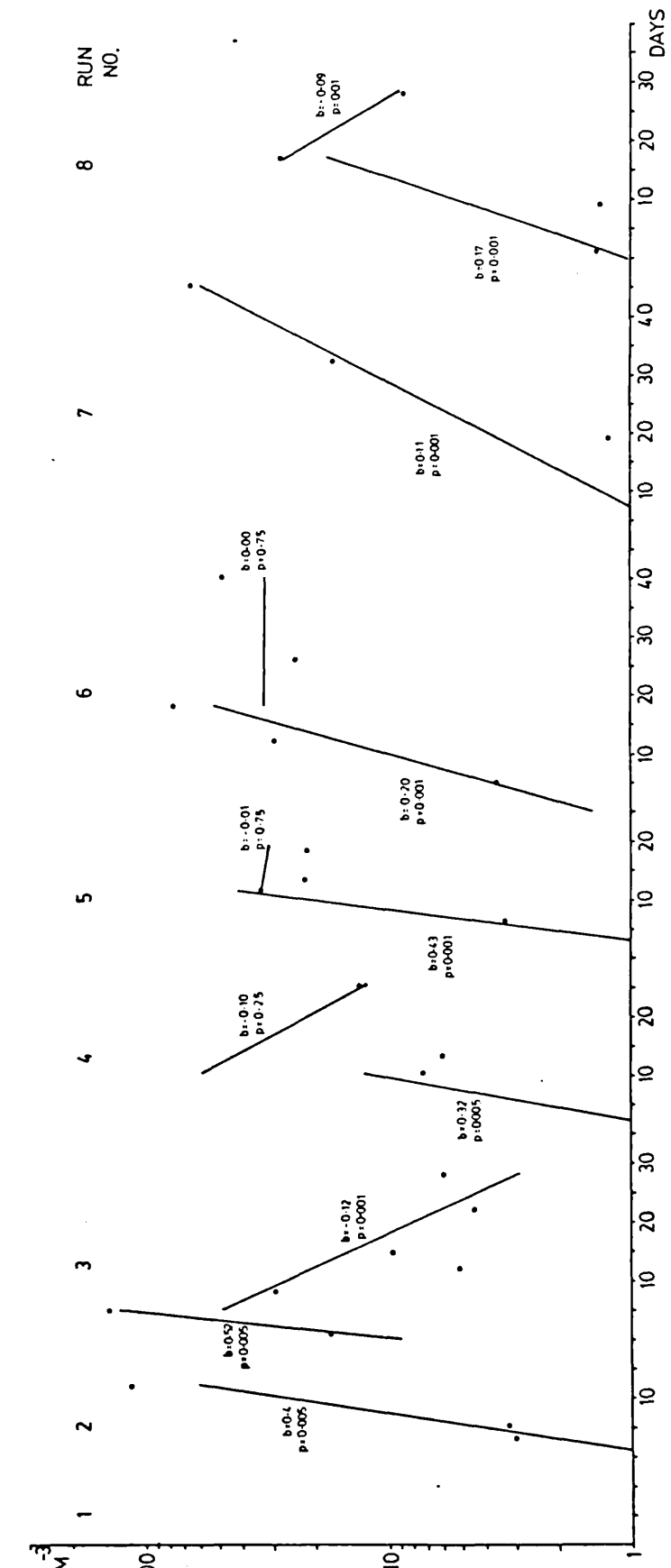


FIG. 8.4 REGRESSIONS OF CHANGE IN DENSITY OF ASPIDISCA COSTATA WITH TIME DURING FILTRATION RUNS ON BED 45

FIG. 8.5 REGRESSIONS OF CHANGE IN DENSITY OF VORTICELLA SPP. WITH TIME DURING FILTRATION RUNS ON BED 45

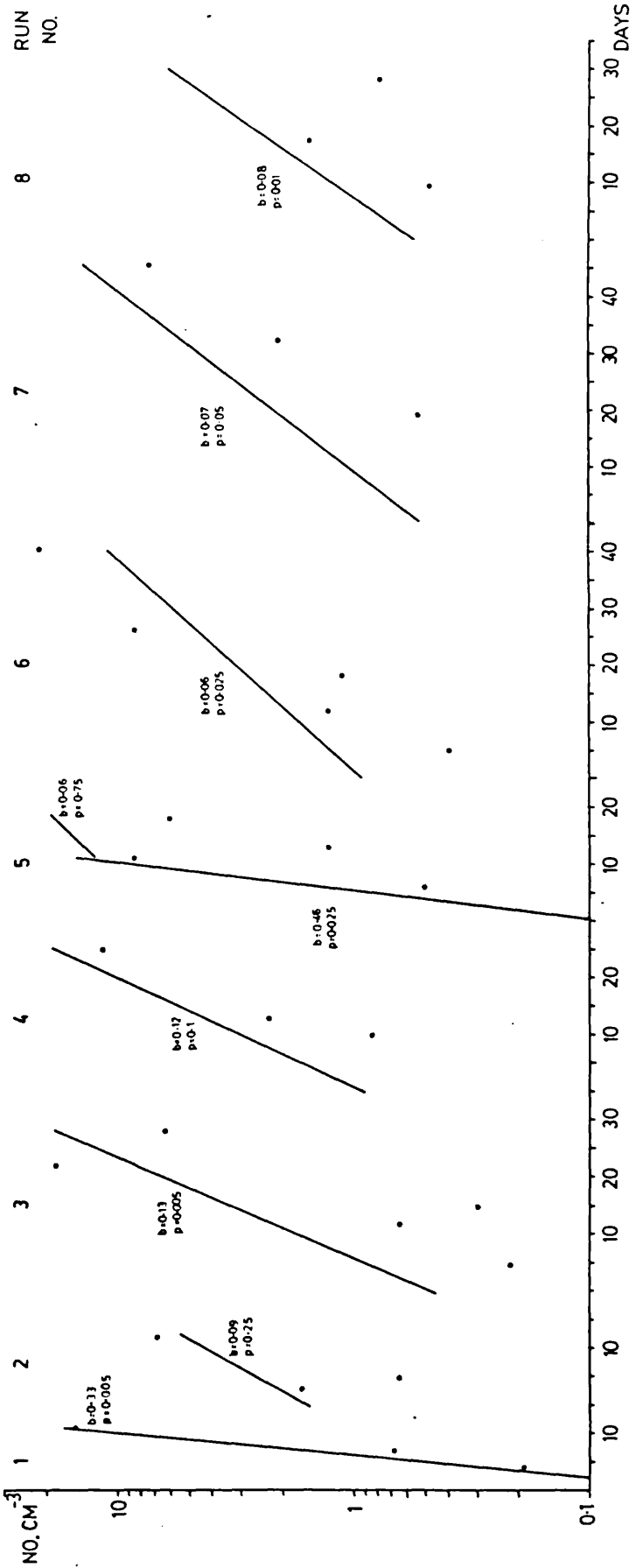


FIG. 8.6 REGRESSIONS OF CHANGE IN DENSITY OF THE CILIATE POPULATION WITH TIME DURING FILTRATION RUNS ON BED 45

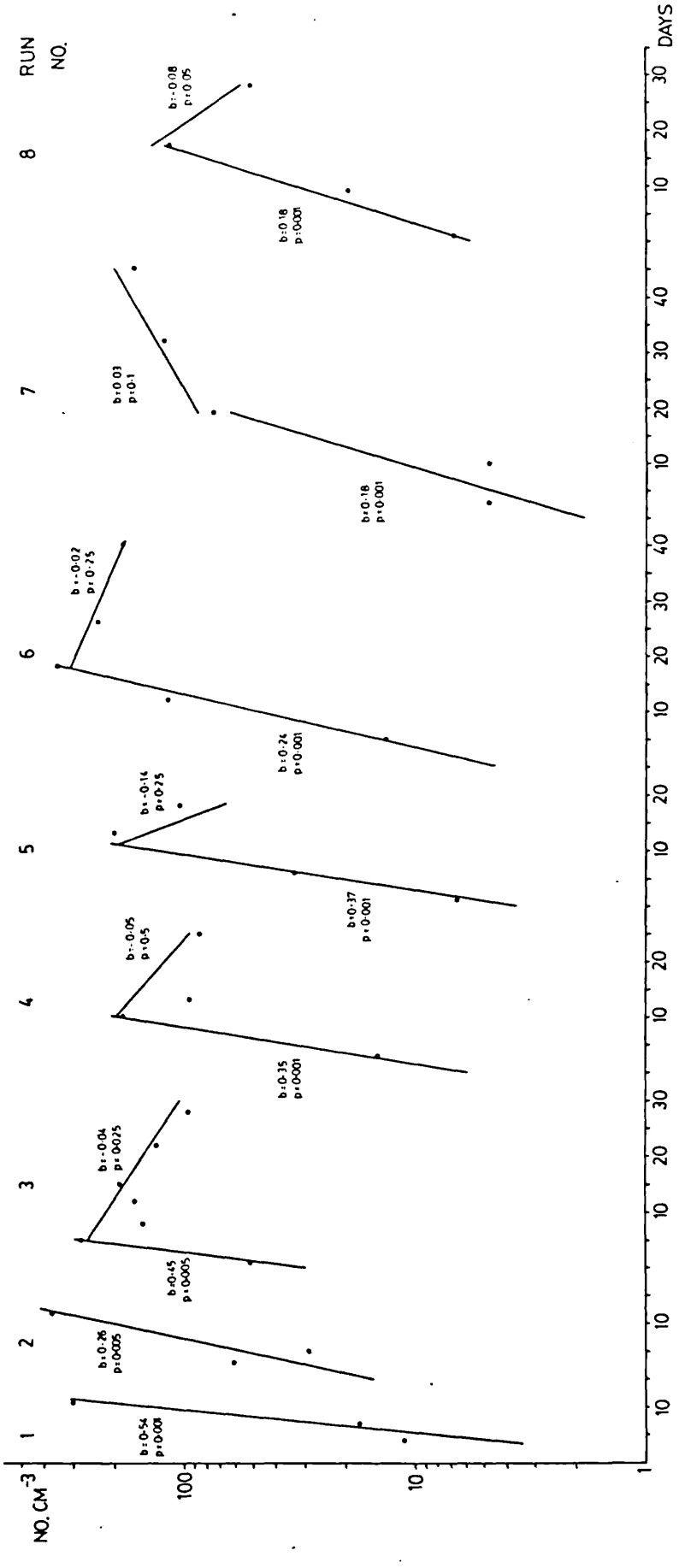
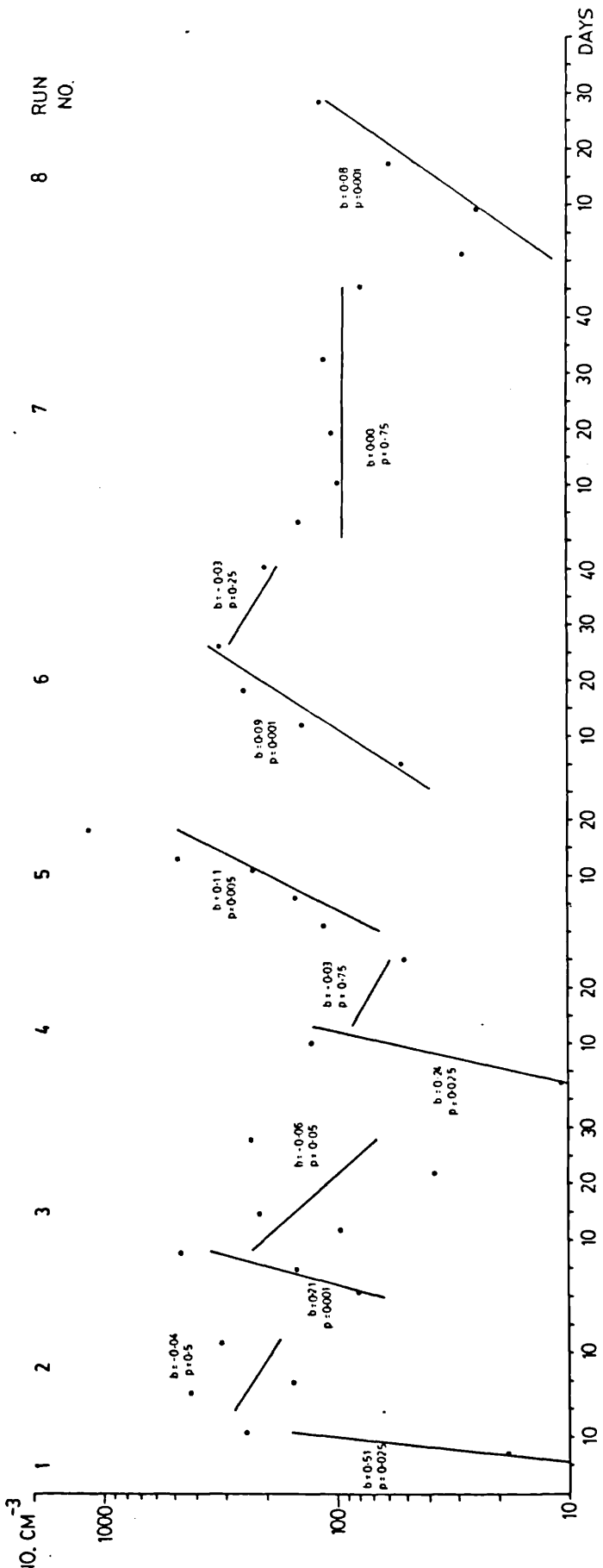


FIG. 87 REGRESSIONS OF CHANGE IN DENSITY OF FLAGELLATES WITH TIME DURING FILTRATION RUNS ON BED 45



bed is biologically immature and the interstices become blocked more rapidly, possibly due to the fact that there is little biological assimilation of allocthanous organic matter filtered from the water by the sand. As a result of this, these runs were short and the ciliates increased rapidly throughout them.

During the January/March run of 1977 (run 7), the temperature of the surface water averaged 4°C and a deviation from the normal pattern of change in population density again occurred. The ciliates when considered all together, increased slowly during the first 19 days of the run and then levelled off to an instantaneous rate of increase of 3.2% per day over the remaining days. Some individual species increased at a constant but slow rate over the entire run, e.g. Aspidisca costata and Cyclidium heptatrichum, while others reached a peak density and then declined, e.g. Tachysoma pellionella and Glaucoma sp. Of particular interest were the flagellates, which remained at the same density throughout the run.

Of the dominant ciliates, some species, e.g. T. pellionella and A. costata were often more numerous than others within the sand, and hence had a greater influence on the rates of change in abundance of the ciliates when treated all together. Table 8.3 lists the maximum densities recorded per cm^3 in each run for the common ciliate species, the total ciliates and the flagellates. (extracted from Appendix 7.2).

Table 8.3 Maximum densities of ciliates and flagellates

	Density ($No.cm^{-3}$)								
Run	1	2	3	4	5	6	7	8	BED 44
Species									
Glaucoma sp.	70	188	148	180	8	150	240	29	36
Cyclidium heptatrichum	516	200	192	80	130	740	26	36	186
Cinetochilum margaritaceum	-	3	30	650	920	180	26	157	230
Tachysoma pellionella	454	1020	984	220	190	136	300	557	125
Aspidisca costata	30	2840	380	230	260	144	250	45	80
Vorticella spp	34	35	345	212	494	291	148	20	38
Total ciliates	879	4222	1402	1425	1355	943	988	846	558
Flagellates	1000	1200	2540	7400	9060	916	3860	174	450

Although numerically abundant some of the smaller ciliates, e.g. C. heptatrichum may not exert as great a biological effect in the sand filter, by for instance, predation on bacteria, as the relatively uncommon larger species, e.g. Stentor polymorphus and Loxophyllym sp. Consequently the instantaneous rates of change in biovolume were also examined (Section 8.2.2).

Doubling Times

The significant instantaneous rates of growth (Table 8.2) have been converted to doubling times, in hours, (Table 8.4) in order to compare these values with the available literature concerning ciliate growth rates. The equation for this conversion is as follows:

$$T = \frac{\ln 2}{r} \times 24$$

Where T = doubling time in hours
r = instantaneous rate of increase per day.

Table 8.4 Ciliate and flagellate doubling times calculated from the regression slopes of Table 8.2

$T = \frac{\ln 2}{r} \times 24$ Where T = doubling time (hours)
r = instantaneous rate of increase (day^{-1})

Species	Temperature °C	r(day^{-1})	T(hours)
Glaucoma	4.0	0.054	308
	7.5	0.213	78
	7.5	0.195	85
	20.0	0.190	88
Cyclidium	7.5	0.179	93
	12.0	0.218	76
	12.0	0.172	97
	12.0(Bed 44)	0.183	91
	20.0	0.281	59
Cinetochilum	4.0	0.047	354
	7.5	0.204	82
	12.0	0.254	66
	12.0(Bed 44)	0.152	109
	19.0	0.242	69
	20.0	0.335	50
Tachysoma	4.0	0.281	59
	7.5	0.576	29
	7.5	0.169	98
	8.75	0.199	84
	12.0	0.584	29
	12.0	0.223	75
	12.0(Bed 44)	0.127	131
	19.0	0.412	40
	20.0	0.371	45
Aspidisca	4.0	0.110	151
	7.5	0.166	100
	8.75	0.400	42
	12.0	0.521	32
	12.0	0.199	84
Aspidisca (contd)	12.0 (Bed 44)	0.111	150
	19.0	0.431	39
	20.0	0.321	52
Vorticella	4.0	0.073	228
	7.5	0.332	50
	7.5	0.080	208
	12.0	0.132	126
	12.0	0.062	268
	19.0	0.463	36
Total Ciliates	4.0	0.183	91
	7.5	0.541	31
	7.5	0.182	91
	12.0	0.453	37
	12.0	0.241	69
	12.0 (Bed 44)	0.171	97
	19.0	0.366	45
	20.0	0.352	47
Flagellates	7.5	0.513	32
	7.5	0.079	211
	12.0	0.212	78
	12.0	0.085	196
	12.0 (Bed 44)	0.099	168
	19.0	0.108	154
	20.0	0.244	68

Very few records are available for naturally occurring protozoan growth rates, as most measurements have been made for ciliates grown in pure cultures of bacteria. The available data is tabulated below (Table 8.5).

Fenchel (1968) grew ciliates in pure cultures of food items varying from bacteria to diatoms and ciliates, depending on the phagous nature of the ciliate under study and recorded generation times between 2.4 hours for Uronema sp. and 46 hours for Condylostoma patulum. The lower generation time was for the smaller ciliate examined while Condylostoma patulum was the largest. No similar relationship was found between size and doubling time in this study.

Table 8.5 Doubling Times of Ciliates (Quoted from the Literature)

Species	Culture Conditions	Doubling Time (hours)	Authority
Spirostomum sp.	5 X 10^6 ml^{-1} Aerobacter aerogenes	115	Klekowski and Fischer (1975)
Dileptus cygnus	1 prey Colpidium colpoda/predator	312	
Dileptus cygnus	5 prey Colpidium colpoda/predator	74	
Dileptus cygnus	10 prey Colpidium colpoda/predator	230	
Colpidium campylum	Batch culture of A. aerogenes at 20°C	4 - 5	Taylor and Berger (1976)
Paramecium bursaria	Culture of A. aerogenes	20	Taylor (1978)
Glaucoma scintillans	Culture of A. aerogenes	4	

Table 8.5 (Contd)

Species	Culture Conditions	Doubling Time (hours)	Authority
Microphagous ciliates Carnivorous ciliates	Continuous culture on organic substrate	$2\frac{1}{2}$ 4	Legner et al (1976)

The doubling times recorded for the present study rangedfrom 29 hours to 308 hours spanning the whole range of values recorded elsewhere except for the very fast doubling times achieved under certain culture conditions. The slow sand filter environment was itself similar to a chemostat culture system, in that a constant supply of nutrientsand oxygen were available in the inflowing water and metabolites were constantly being removed by the downward flow of water. However, the food supply was obviously not as nutritive as that of a laboratory monoculture system.

8.2.2 Biovolume

Biovolumes obtained from cell measurements (Section 7.3) were analysed as representative of cell biomass, the two parameters differing only by a constant : cell density. The ciliates were not measured during runs 1 to 3 so no biovolume data are available for these runs. Individual cell sizes of the numerically dominant ciliates are listed in Table 8.1. As the individual cell sizes did not vary appreciably during each run (Section 8.1), the total biovolume of these species (individual cell size X numerical abundance) increased and

decreased at the same instantaneous rates as did their population densities (numerical abundance) during each filtration run. Consequently the ciliate phylum as a whole increased in biovolume concurrent with the numerical increase to peak density. However, as the population density subsequently declined, the biovolume continued to increase but at a lower rate. Figure 8.8 illustrates this for run 8 while Figure 8.9 shows the increase in total biovolume during the five filtration runs where cell volumes were recorded (these data are tabulated in Appendix 8.3). The further increase in ciliate biovolume may be explained by a succession from the smaller ciliate species to larger forms such as Lacrymaria olor, Loxophyllum sp. and particularly Stentor polymorphus, in addition to the continued increase in population density of Vorticella spp. This species succession is illustrated in Table 8.6 for filtration run 8.

Some seasonal variation occurred in the diversity of the larger ciliate species present in the filter bed towards the end of a run (Table 8.7) Euplotes sp. favoured the autumn and winter months while Loxophyllum sp. and Uroleptus sp. were present only in the summer and autumn. Stylonichia mytilus, Lacrymaria olor and in particular, Stentor polymorphus were, however, present at the end of each run regardless of the season, although their maximum biovolumes were far greater between late spring and early autumn than they were throughout the rest of the year. The lower filtration rate of bed 44 apparently discouraged the larger ciliates, as Stentor polymorphus was the only late coloniser of the run on this bed.

FIG. 8.8 CHANGES IN CILIATE DENSITY AND BIOVOLUME IN SAND CORES DURING RUN 8 ON BED 45, 7·5°C

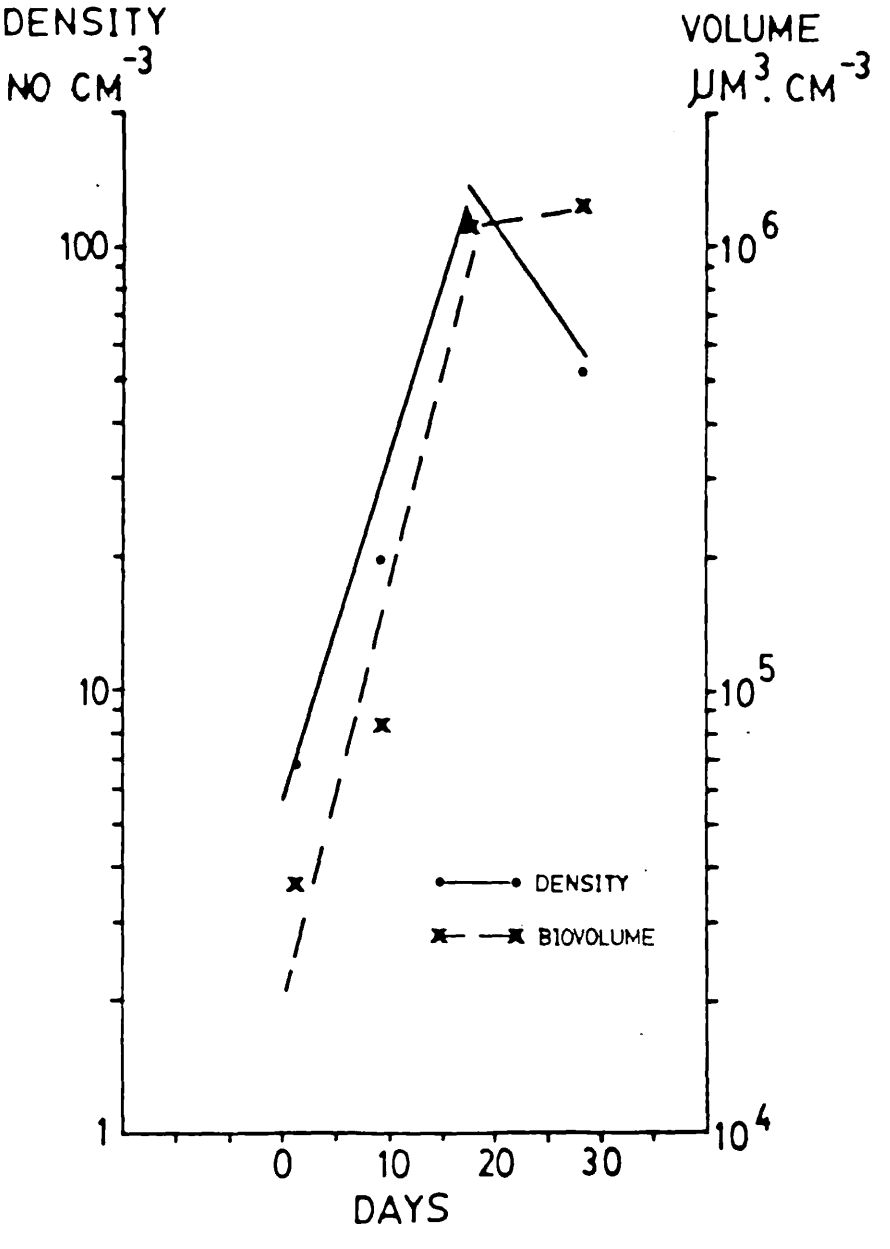


FIG 89 INCREASES IN TOTAL BIOVOLUME OF THE CILIATES DURING FILTRATION RUNS ON BED 45

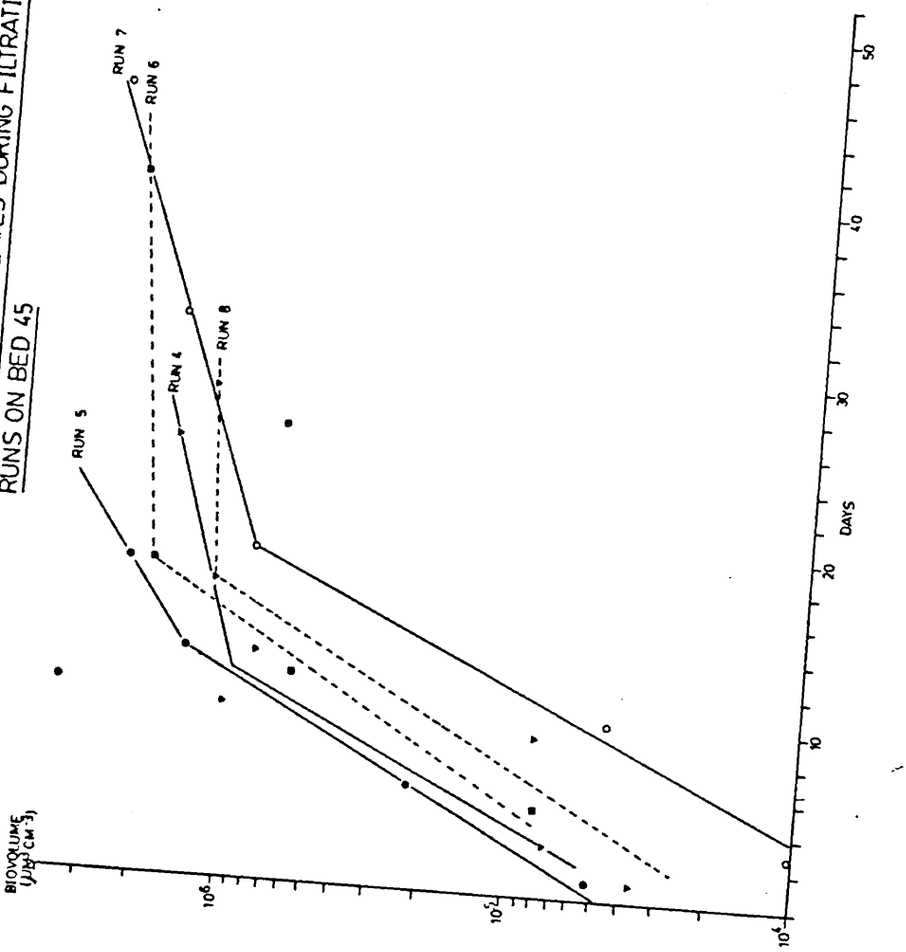


Table 8.6 Succession of ciliate species during run 8 (March - May, 1977)

Species	Mean Individual cell size (μm^3)	Period of Population Increase			
		Day 1	Day 9	Day 17	Day 28
Glaucoma sp.	833	←	→		
Cyclidium heptatrichum	680	←		→	
Cinetochilum margaritaceum	1226	←		→	
Litonotus sp.	1422	←		→	
Tachysoma pellionella	6151	←		→	
Aspidisca costata	7679	←		→	
Uroleptus sp.	17550	←		→	
Chilodonella sp.	3974	←			→
Euplotes sp.	14710	←			→
Vorticella spp.	12417		←		→
Lacrymaria olor	14048		←		→
Stentor polymorphus	129380		←		→
Loxophyllum sp.	14850			←	→

Table 8.7 Seasonal occurrence of the larger ciliate species

	Presence (✓) or Absence (X) on the final sampling day of the run (maximum total biovolume ($\mu m^3 cm^{-3}$) in parentheses)					
Run Months. Year / Species	Run 4 VI/VII '76	Run 5 VII/VIII'76	Run 6 X/XI '76	Run 7 I/III '77	Run 8 III/V '77	Bed 44 X/XI/'76
Euplotes sp.	X	X	✓(30,100)	✓(129,290)	✓(17,236)	X
Stylonichia mytilus	✓(381,800)	✓(187,800)	X	✓(215,484)	X	X
Lacrymaria olor	X	✓(323,050)	✓(551,036)	✓(7,962)	✓(13,475)	X
Loxophyllum sp.	✓(267,280)	✓(302,970)	✓(112,680)	X	X	X
Uroleptus sp.	✓(3,700,000)	✓(129,133)	X	X	X	X
Stentor polymorphus	✓(624,660)	✓(551,410)	✓(21,537)	✓(468,534)	✓(1,151,080)	✓(21,240)

8.3 Interrelationships between population changes and selected environmental variables

8.3.1 Temperature

Regression analyses performed to examine the dependence of the rate of numerical increase on temperature are summarised in Table 8.8 and Figure 8.10.

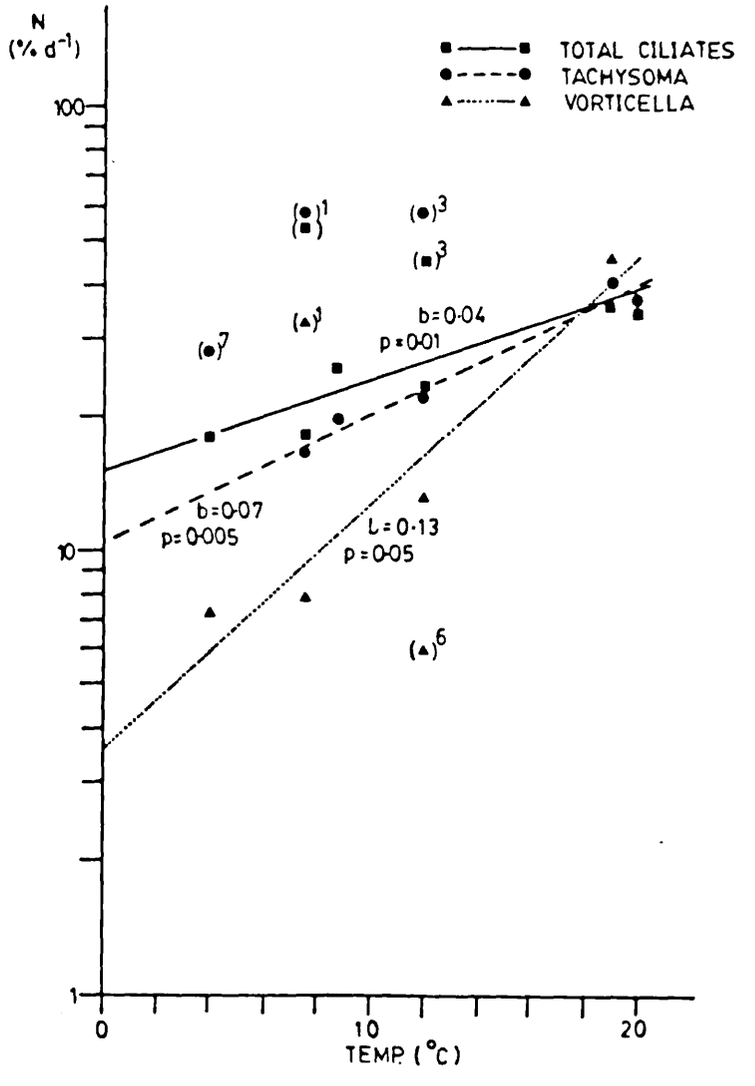
Table 8.8 Regression Table of ln instantaneous growth rates v. temperature

Species	Slope	S.E. Slope	Int.	Var. Ratio	dF	p
Tachysoma (1,3,7)* pellionella	0.067	0.007	2.350	85.44	1,3	0.005
Vorticella sp. (1,6)	0.127	0.023	1.268	30.55	1,2	0.05
Total Ciliates (1,3)	0.044	0.008	2.712	28.530	1,4	0.01

* Numbers in parentheses indicate the runs omitted from the regressions.

Only three regressions were significant, however the very low sample size ranging from n = 4 for Cyclidium sp. to n = 8 for Tachysoma sp. and the total ciliates (Table 8.2) is partly responsible for this, although a more complex relationship involving other external parameters, some of which are discussed later in this section, may also exist. However, the ciliate population as a whole was apparently influenced by temperature in its rate of numerical increase having a Q10 over temperatures between 4 and 20°C of 1.55. A few runs giving data which did not conform to the trend of the regressions were

FIG. 8.10 REGRESSION LINES OF INSTANTANEOUS GROWTH RATES OF CILIATES v TEMPERATURE



omitted from the analyses; these are indicated in parentheses in Table 8.8.

Figure 8.11 illustrates the relationship between temperature and the maximum densities attained by a few of the common ciliate species (Table 8.9).

Table 8.9 Regression table ; ln maximum density v. temperature

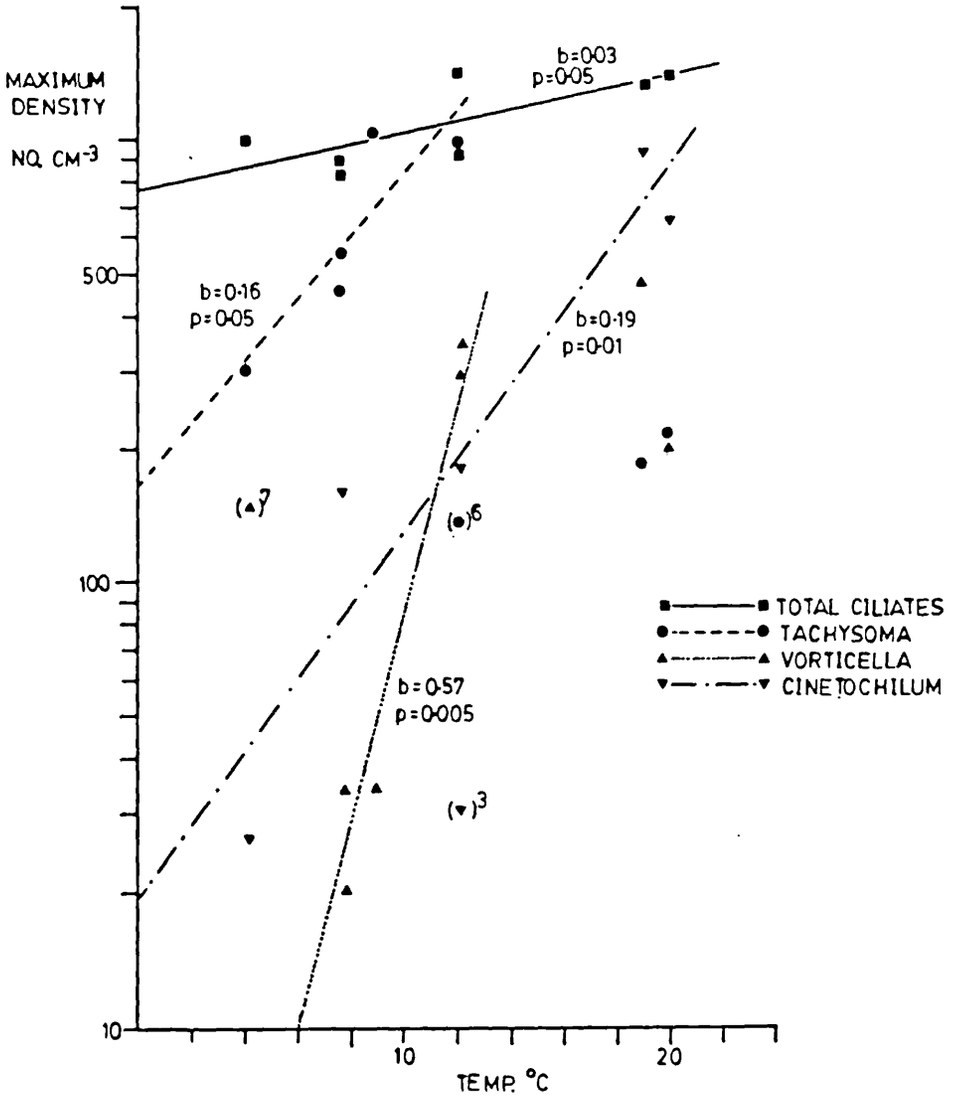
Species	Slope	S.E. Slope	Inter cept	Var. Ratio	dF	p	Temp. Range
Cinetochilum (3)*	0.191	0.037	2.979	26.491	1,4	0.01	4 - 20°C
Tachysoma (6)	0.161	0.048	5.112	11.094	1,3	0.05	4 - 12°C
Vorticella (7)	0.570	0.066	-1.126	73.83	1,3	0.005	4 - 12°C
Total Ciliates (2)	0.030	0.011	6.643	7.979	1,5	0.05	4 - 20°C

* Numbers in parentheses indicate the runs omitted from the regressions.

Significant increases in maximum density occured between 4 and 12°C for Tachysoma sp. and Vorticella sp., this being followed by a decline. However, the maximum densities of Cinetochilum sp. and the total ciliates continued to increase with temperature up to 20°C, the highest temperature recorded in this study.

Figure 8.1 demonstrated a trend for ciliate species to decrease in individual cell volume with temperature increases from 4°C to 12°C, this being followed by an increase in cell size at higher temperatures. This would correspond with the variation of maximum density with temperature recorded here for some species and the trend for the rate of replication to increase with temperature

FIG. 8.11 REGRESSION LINES OF MAXIMUM CILIATE SPECIES ABUNDANCE v TEMPERATURE



(Fig. 8.10). The general trend for the ciliate phylum was for the growth rate and maximum cell density to increase with temperature between 4 °C and 20°C at 4.4% and 3.0% respectively. However, there is by no means an empirical relationship between these four parameters (cell size, growth rate, maximum density and temperature) for all species, e.g. the individual cell volume of Cinetochilum sp. decreased with increasing temperature from 4°C to 12°C, but increased from 12°C to 20°C while the maximum density recorded in 1 cm^3 of sand increased from 4°C right through to 20°C, so an increase in cell size corresponded with an increase in density between 12°C and 20°C. Other parameters, e.g. greater food availability at higher temperatures, may have been involved in supporting larger populations of larger animals.

The days on which peak densities of ciliates were achieved (Appendix 8.2) during the filtration runs were found to be temperature related (Fig. 8.12, Table 8.10).

Table 8.10 Relationship between temperature and the age of the filtration run at which maximum abundance of ciliates were recorded

(a) Data

RUN	DAY OF PEAK ABUNDANCE	TEMP °C
3	5	12
4	10	20
5	11	19
6	18	12
7	45	4
8	17	7.5
BED 44	24	12

FIG. 8.12 REGRESSION OF DAYS ON WHICH PEAK CILIATE DENSITIES WERE RECORDED v TEMPERATURE

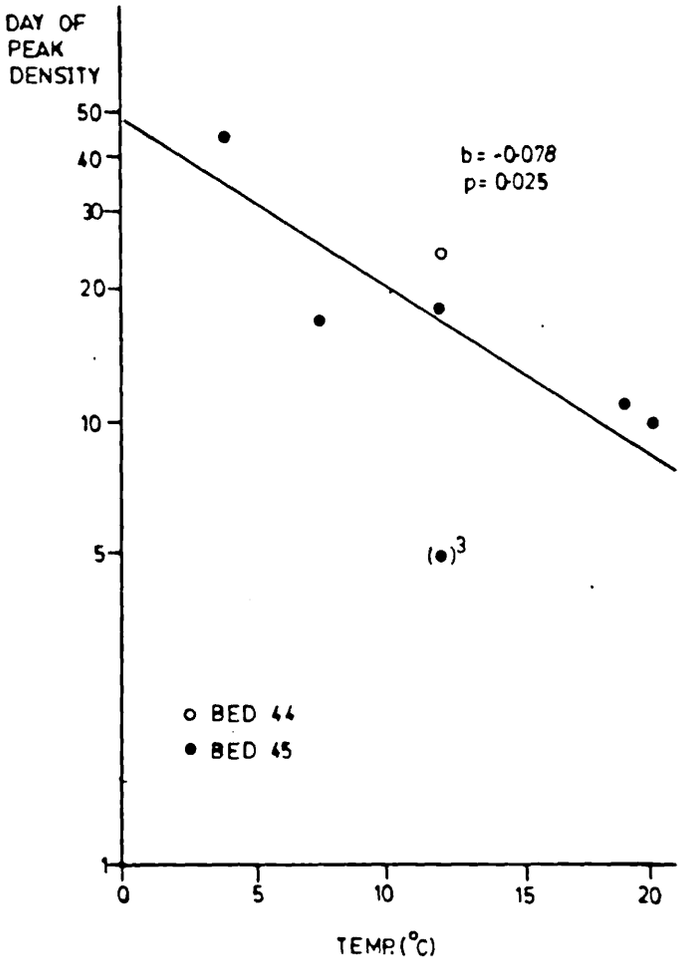


Table 8.10 (Contd)

(b) Regression Table (omitting run 3)

SLOPE	S.E. SLOPE	INTERCEPT	VAR. RATIO	dF	p
-0.078	0.020	3.867	14.476	1,4	0.025

Runs 1 and 2 were omitted from the regressions as they prematurely 'jacked-up' following the resanding of bed 45. Run 3 was also omitted. The day at which peak densities were achieved was inversely related to temperature. The gradient of this may be steeper than calculated here as the density of ciliates probably increased beyond day 45 of run 7 ($4^{o}C$), however no further samples were taken after day 45. At the lower rate of filtration the day on which the maximum ciliate population occurred was 6 days later than that in bed 45 (i.e. it took $1\frac{1}{3}$ times as long) corresponding to an instantaneous growth rate in bed 45 $1\frac{1}{3}$ times greater than that of bed 44 (Table 8.2).

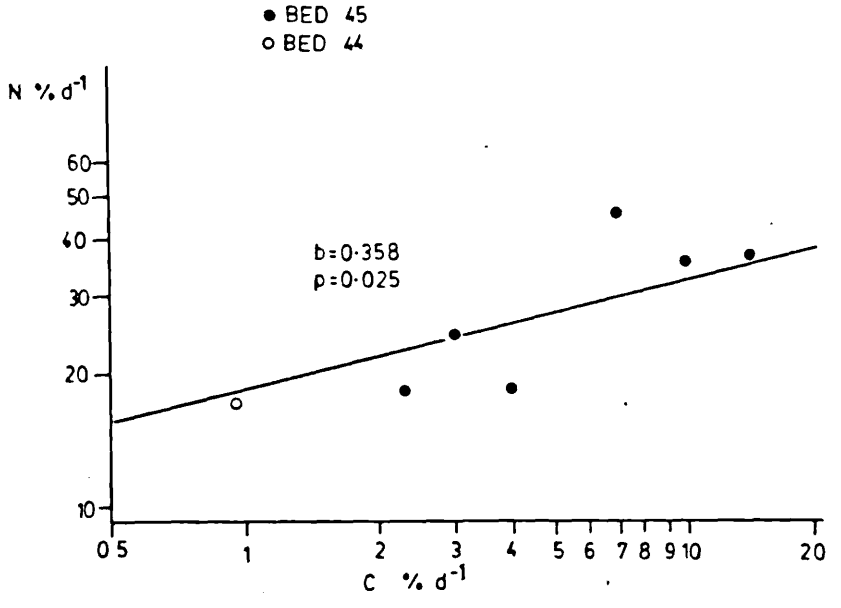
8.3.2 Carbon

The rate of numerical increase of the total ciliate population was found to be related to the rate of particulate organic carbon (P.O.C.) increase in the top 2 - 10 cm of sand during the period of high instantaneous growth rates (b = 0.358, p = 0.025). The regression is shown in Figure 8.13 and tabulated below(Table 8.11).

Table 8.11 Regression table of numerical increase against carbon increase

Slope(b)	S.E. Slope	Intercept	Variance Ratio	deg. freedom	p
0.358	0.107	2.739	11.223	1,5	0.025

FIG. 8.13 REGRESSION OF CILIATE NUMERICAL INCREASE v CARBON INCREASE



However, no valid relationship was found between maximum ciliate densities and the P.O.C. contents of the sand; nor between the day of peak population occurrence and the carbon content. However, Figure 5.8 demonstrates a change in gradient of carbon accumulation similar to that undergone by the total ciliate biovolume, Figure 8.9, suggesting that the two may be related or equally affected by a third or other factors.

8.3.3 Rate of filtration

In bed 44 (8" hr^{-1}) the ciliates and flagellates developed over a period 1 to 2 weeks longer than the time taken in the simultaneous run on bed 45 (16" hr^{-1}). The period of rapid growth lasted for 24 to 33 days in bed 44 compared with 18 to 26 days in bed 45. The instantaneous growth rates were generally about 10% per day higher in the faster flow rate with the exception of Cyclidium heptatrichum which had about the same growth rate in both beds but which, as mentioned earlier (Section 8.1) was significantly smaller in biovolume in bed 44 than it was in the faster flow rate of bed 45.

The maximum density achieved in total, in the slower flow rate, was about 60% for the ciliates and 50% for the flagellates of the densities achieved in bed 45 during this period of rapid growth. The individual species differences were, however, very varied. Vorticella spp. and Cyclidium heptatrichum, both bacterial filter feeders, were present in bed 44 at densities of 12% and 25% respectively of those recorded in bed 45, while detrital grazers such as

Cinetochilum margaritaceum and Tachysoma pellionella were present at very similar maximum densities in both beds.

8.3.4. Dissolved Oxygen

From Figures 5.4a and 5.4b, by subtraction of the area beneath the filtrate dissolved oxygen (d.o.) curve from that beneath the top water d.o. curve, figures were obtained for the oxygen balance of bed 45 during runs 5 and 8 (respectively the July/August 1976 and March/May 1977 runs). These are plotted in Figures 8.14 and 8.15. Initially, while there was a developing algal community on the bed surface the oxygen content of the filtrate was lower than that of the top water, i.e. respiration of the bed presumably by predominantly the bacterial population exceeded its surface photosynthetic production of oxygen. There then followed a period when photosynthetic production of oxygen exceeded respiratory uptake and finally once again respiration exceeded photosynthesis. Between the latter two stages there was a point at which photosynthetic production of the bed equalled respiratory uptake; this occurred on day 11 of run 5 and day 17 of run 8. In both runs these days were also those on which maximum ciliate densities were recorded. Following this balance, the total ciliate densities decreased while the respiratory uptake of the filter bed increased. This would suggest that the decrease in the smaller ciliates may have been due to a deficiency of oxygen which the larger ciliates were able to withstand.

FIG. 8.14 DISSOLVED OXYGEN BALANCE OF BED 45 DURING RUN 5, 19°C

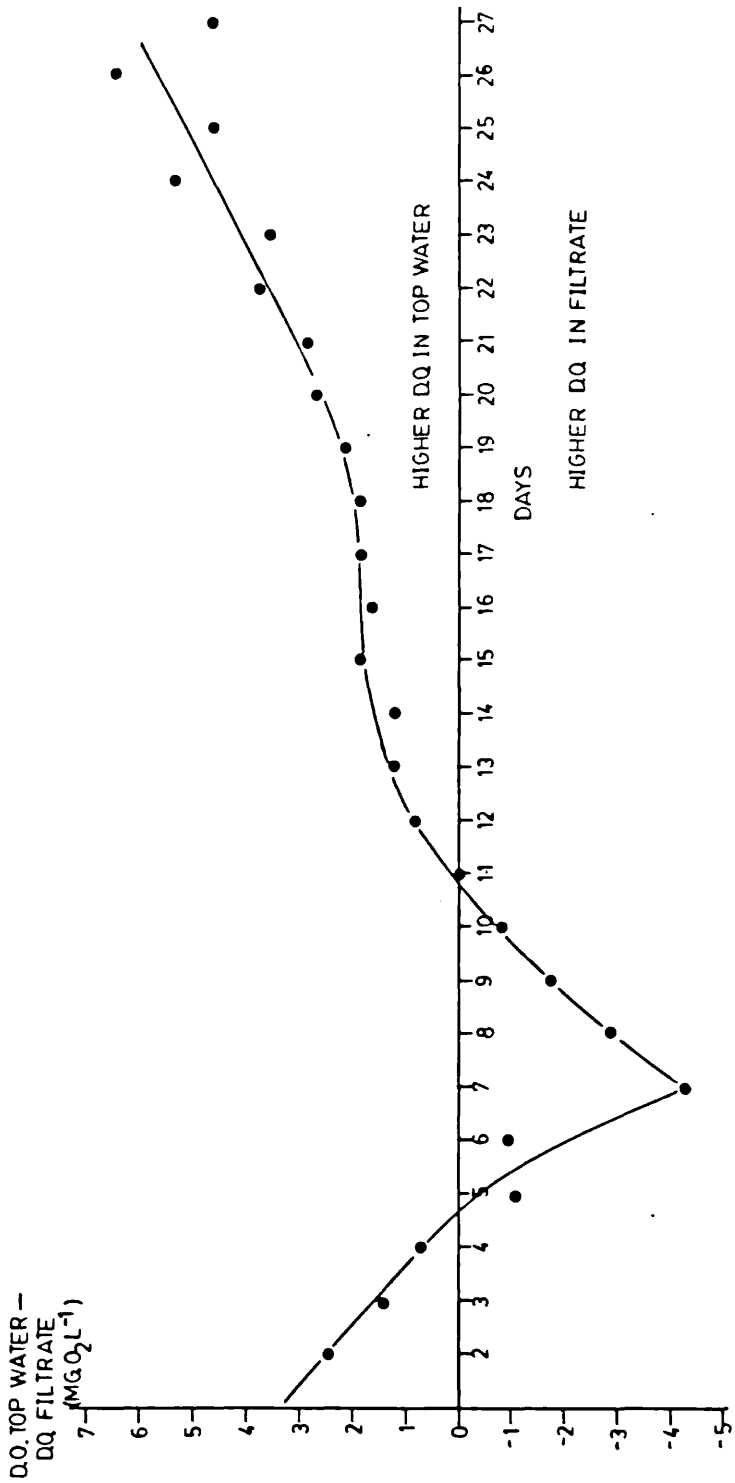
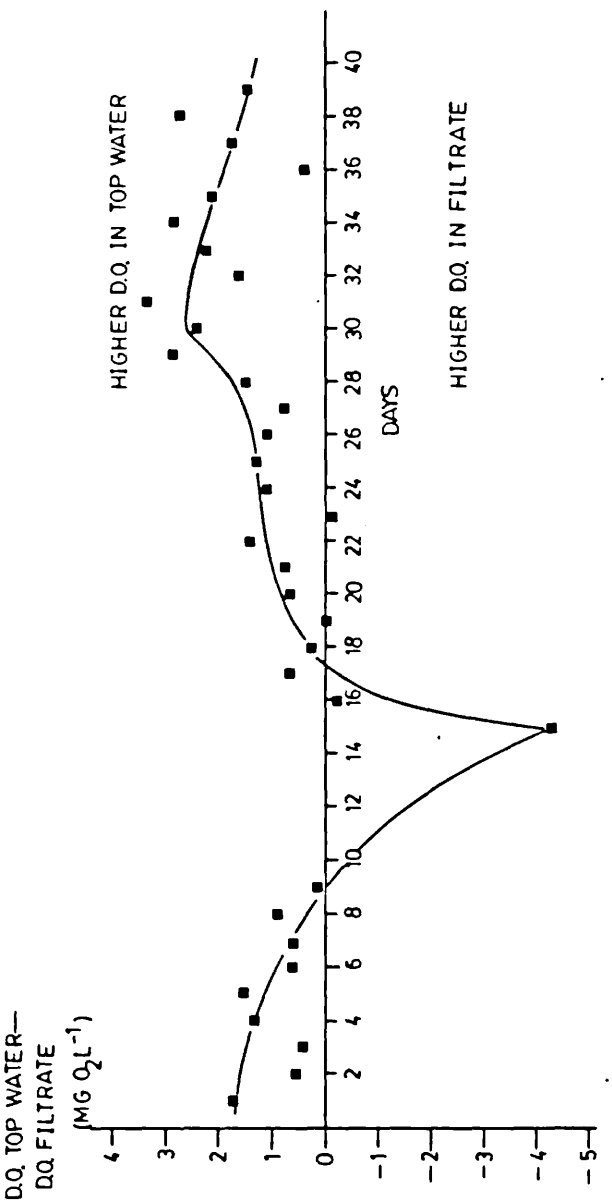


FIG. 8.15 DISSOLVED OXYGEN BALANCE OF BED 45 DURING RUN 8, 7·5°C



Chapter 9 The Depth Distribution of Bacteria

Introduction

In this part of the study an attempt was made to determine the speed at which bacterial populations developed within the filter bed and whether a constant population density was achieved after a certain length of time, in order to examine whether the ciliate colonisation bore any relationship to bacterial development within the filter bed.

Using the sampling cores, a filtration run on Bed 44 (February 1978, 3.5°C) was monitored for surface associated bacterial and total ciliate densities, and carbon concentrations for the first ten days of a run. Unfortunately the filtration rate was then decreased from 0.4m per hour to 0.2 m per hour. The relative abundance of surface associated bacteria at depths from 1 cm to 24 cm was also examined on a 'mature' 28 day old core using scanning electron microscopy, and the densities observed on the micrographs were compared with the densities obtained from the younger cores using the epifluorescence enumeration technique. The pilot-scale model filter column, designed and used by Lodge (1979) for the observation of depth distributions of dissolved oxygen in the interstitial water, was also used in this part of the study to monitor the interstitial bacteria. The development of bacteria in the interstitial water during the first week of a run was monitored in the column on two occasions, using first clean sand and then sand previously used for a filtration run, thus simulating conditions of both the sand cores

and the filter bed respectively. It was also possible to look for diurnal patterns in bacterial abundance using the filter column.

9.1 Bacteria associated with the sand grain surfaces

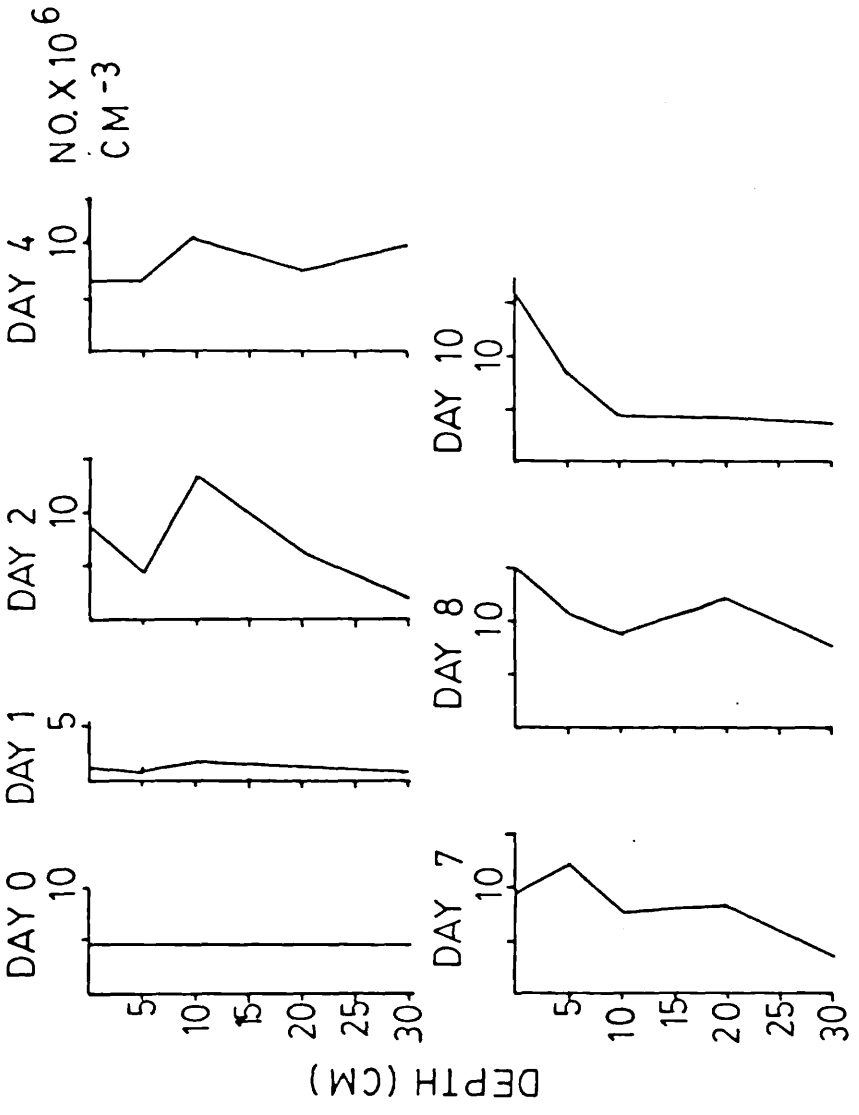
9.1.1 Enumeration of bacteria by epifluorescence microscopy

Estimates of the bacteria associated with the sand grain surfaces were made by shaking the sand in sterile water, staining the resulting supernatant with acridine orange, filtering the stained bacteria onto a black membrane and examining this by means of epifluorescence microscopy as described in Section 4.7. As explained earlier (Section 4.7.1) the shaking technique employed removed approximately $\frac{1}{3}$ of the bacteria, associated with the sand surface, into suspension. However, this standard procedure ensured comparable results although the actual population density of surface associated bacteria was probably in the order of three times that recorded.

During the ten day sampling period on Bed 44 in February 1978, the temperature averaged 3.5°C. Sample cores were analysed on days 0, 1, 2, 4, 7, 8 and 10 and the depth distribution data obtained for bacteria and ciliate densities, and particulate organic carbon content are presented numerically in Appendix 9.1 and graphically in Figures 9.1 to 9.3. (Due to lack of time for counting, no data are available for the ciliate densities on days 4 and 10).

Bacterial densities were uniform with depth until the second day, by which time highest numbers were recorded at a depth of 10 cm (Fig. 9.1). On day 7, the maximum

FIG.9.1 DEPTH DISTRIBUTION OF BACTERIA SHAKEN OFF SAND GRAINS FROM CORES IN BED 44, 04 M H^{-1} FEB. 1978, 3·5°C.



density was present at 5 cm and had reached the surface by day 8. An exponential curve was present on day 10, with maximum densities at the surface, decreasing rapidly down to a depth of 10 cm and thereafter remaining relatively constant.

The depth distributions of ciliate densities (Fig. 9.2) concurred with data recorded in bed 45 during the January to March run of 1977 which had a mean temperature of 4°C (Fig. 7.14). Maximum ciliate densities were always present at the sand surface, with an exponential decrease with depth developing by day 8. The distribution of particulate organic carbon (Fig. 9.3) also followed the general pattern recorded in bed 45 (Fig. 5.6) although little surface accumulation occurred and higher concentrations of carbon were more homogeneously distributed with depth.

The depth distribution data were integrated over the depth of sand analysed - 30 cm for bacteria and carbon, and 10 cm for the ciliates - to give an estimate of the densities present in a column of sand of 1 cm^2 cross section, and these integrals were then divided by the maximum depth analysed to give a comparitive density per cm^3 as described for the ciliates and flagellates (Section 8.2.1). The mean densities (Appendix 9.2) are plotted in Figure 9.4 with the regression lines of change in density with time which were derived from the raw data in Appendix 9.1.

Both ciliate density and particulate organic carbon concentration apparently increased at a constant but slow rate throughout the 10 day run, while the bacterial

FIG. 92 DEPTH DISTRIBUTION OF CILIATES IN BED 44, 0·4MH.$^{-1}$, FEB 1978, 3·5°C.

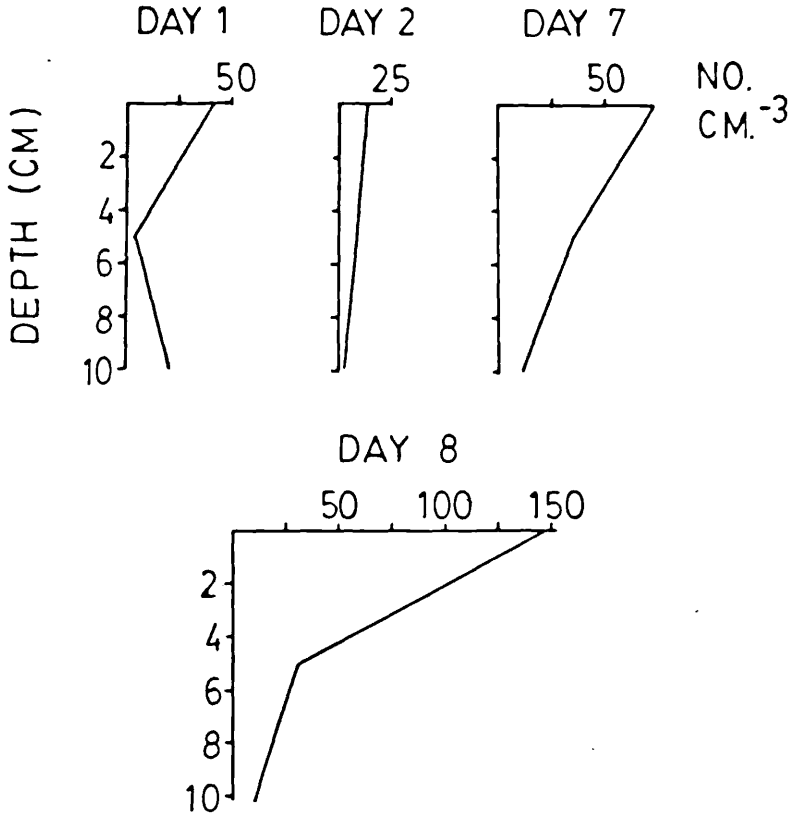
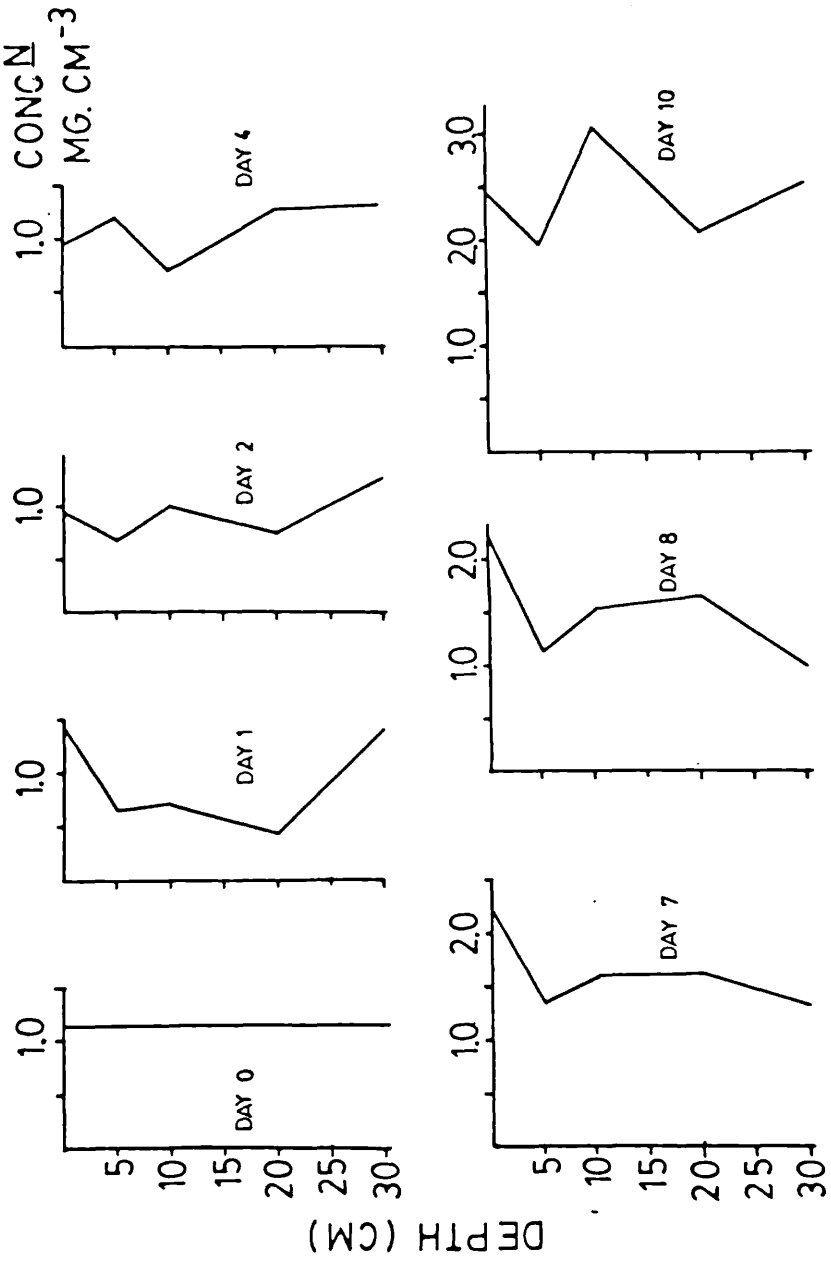


FIG. 9.3 DEPTH DISTRIBUTION OF PARTICULATE ORGANIC CARBON OF SAND CORES IN BED 44, 04 $M.H^{-1}$, FEB 1978, 3.5°C.



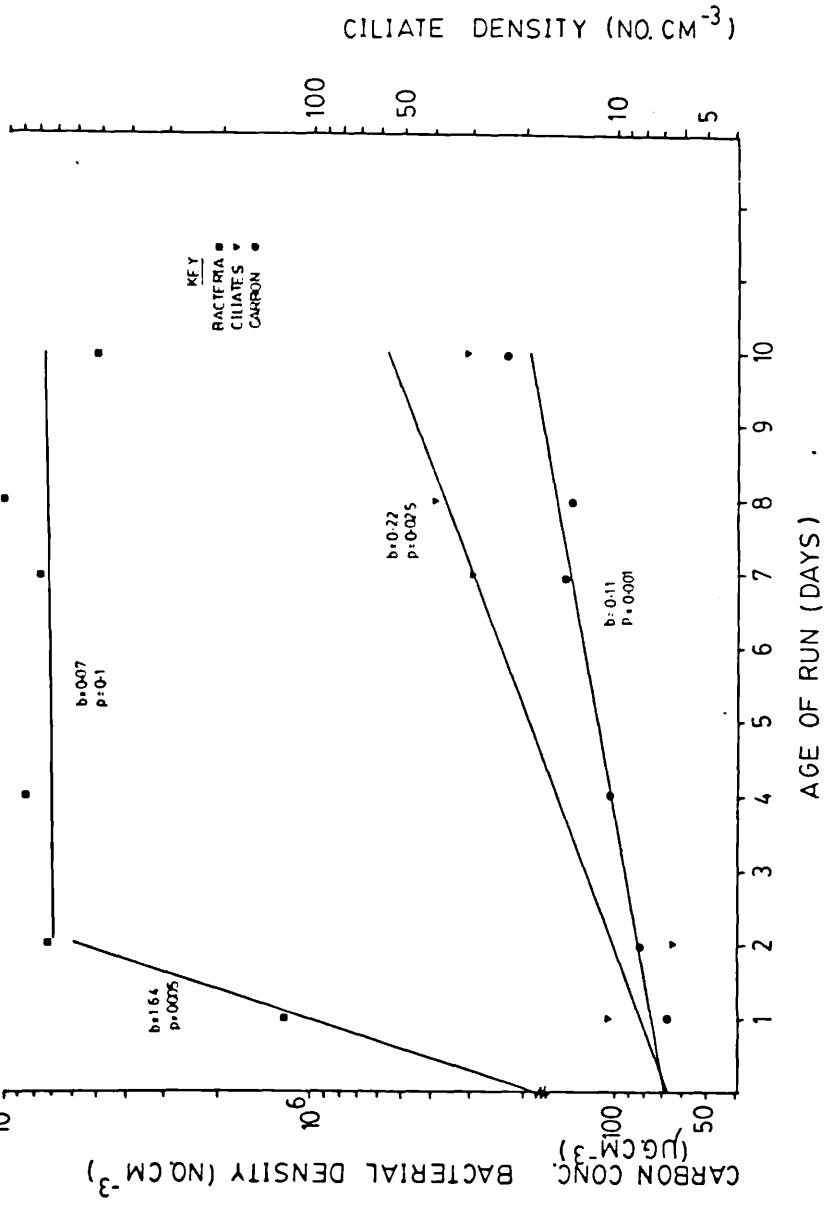


FIG. 9.4 REGRESSION LINES OF CHANGE IN BACTERIAL, CILIATE AND CARBON DENSITIES w TIME IN BED 44, 0·4 MH^{-1}, FEB 1978, 35°C.

population increased extremely rapidly over the first two days and thereafter remained at a similar density (Fig. 9.4). From the regression table (Table 9.1) the instantaneous rates of increase of bacteria, ciliates and carbon are seen to be highly significant.

Table 9.1 Regression table of changes in density and concentration respectively with time of bacteria, ciliates and particulate organic carbon (P.O.C.) in bed 44 sampling cores during a run at 0.4 mh^{-1} in February 1978. (Temp. 3.5°C)

	TIME PERIOD (DAYS)	SLOPE	S.E. SLOPE	INTER-CEPT	VAR. RATIO	dF	P(VAR. RATIO)
Bacteria	1 - 2	1.64	0.35	-1.52	22.34	1,8	0.005
	2 - 8	0.074	0.04	1.69	3.07	1,18	0.1
Ciliates	1 -10	0.22	0.08	1.90	6.99	1,11	0.025
P.O.C.	1 -10	0.11	0.02	4.23	27.81	1,57	<0.001

It is of interest to compare the instantaneous rates of increase for the ciliates and carbon recorded in this run, to those of run 7 which took place at the same time the previous year (1977), when the water was also at a similar temperature of 4°C. The comparative data (Table 9.2) are extremely similar, particularly for the ciliates : 18.3% per day in 1977 ($p < 0.001$) and 21.7% per day in 1978 ($p = 0.025$).

Although the instantaneous rate of increase of bacteria was only examined during this single run, it would appear that they develop very early in a filtration run and at a rate far greater than that recorded for the

ciliates or carbon. After this very short period of logarithmic increase (164% d^{-1}) the bacterial population continued to increase but very slowly (7.4% d^{-1}) until day 8, after which a decrease was recorded over the following 2 days. However, there is insufficient data to suggest that this decline represented a significant change in bacterial density and a coincident, apparent decrease in ciliate density was similary found to be statistically insignificant. Ommission of the day 10 ciliate data from the regression calculation also resulted in a decrease in significance of the instantaneous ciliate growth rate regression.

Table 9.2 Comparison of the instantaneous rates of increase of ciliate densities and carbon concentration during runs in January 1977 and February 1978.

	CILIATES		PARTICULATE ORGANIC CARBON	
	JAN 1977	FEB 1978	JAN 1977	FEB 1978
SLOPE	0.183	0.217	0.04	0.105
S.E. SLOPE	0.040	0.082	0.01	0.02
p(VAR. RATIO)	<0.001	0.025	<0.001	<0.001

Whether bacterial development would be different at higher temperatures and whether this steady population level of bacteria would remain after 10 days or after the eventual decline of the ciliate population is unknown. However, the generation time of bacteria in two reservoirs was found to be temperature independent by Straškrabová-Prokešová (1976). The growth rate of 164% per day corresponds to a doubling time of 10.14 hours. This value is a much larger

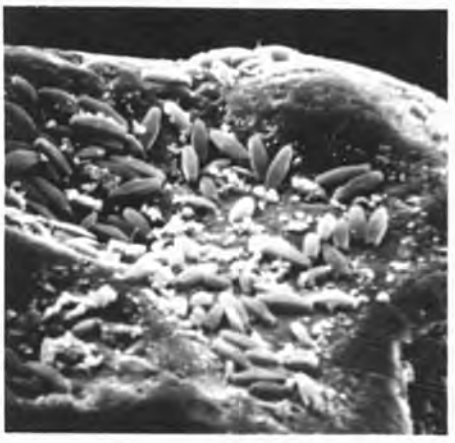
doubling time than those recorded for continuous culture systems of 0.2 to 1.4 hours (Curds, 1971b; Straškrabová, 1977), but is only twice the doubling time recorded by Bott and Brock (1970) of 5 hours for periphytic bacteria attached to glass slides in a cold sulphur spring.

9.1.2 Examination of sand grain surfaces by scanning electron microscopy

From the study of bacterial populations described above, it appeared that the bacteria associated with sand grains developed in greatest density at the surface after ten days (Fig. 9.1) but decreased rapidly with depth to 10 cm below which they remained at a constant level. Plates 9.1 and 9.2 are scanning electronmicrographs of areas of the sand grains (prepared as described in Section 4.7) both sheltered from and exposed to the water currents through the sand, at depths from 1 to 20 cm in a 28 day old core. From these micrographs it is apparent that the decrease in density recorded at lower depths may not accurately represent the change in population abundance, as the bacterial flora changes from predominantly short rod forms, probably pseudomonads, in the surface 10 cm to more filamentous forms deeper in the sand. Indeed, on exposed surfaces (Plate 9.2) bacterial densities apparently increased with depth. Plate 9.3 demonstrates clearly that at a depth of 24 cm on rough and exposed surfaces a high population density of bacteria may be found (Plate 9.3a) while on smoother more sheltered surfaces at the same depth a slime matrix may form (Plate 9.3d) and consequently no bacteria would be recovered from this surface by the

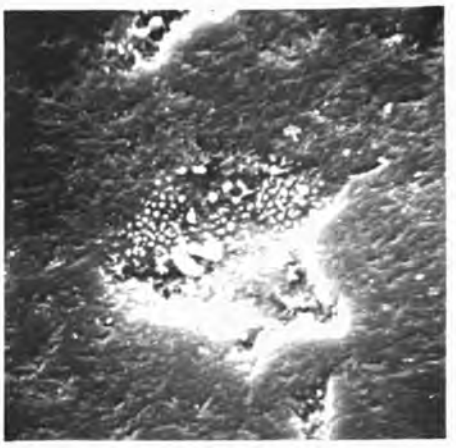
Plate 9.1 Sheltered surfaces of sand grains collected from a 28 day-old core

(a) 1 cm depth : High densities of diatoms and debris masking bacterial numbers.



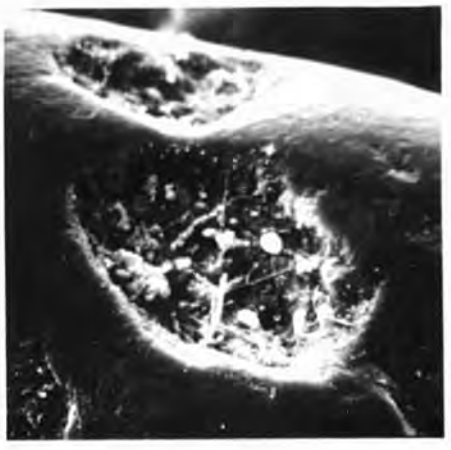
X 380

(b) 5 cm depth : Free from debris; mainly short rod bacteria and some filamentous bacteria.



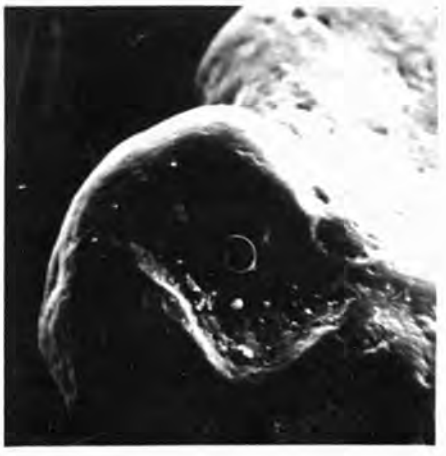
X 800

(c) 10 cm depth : Many filamentous bacteria and some short rods present in slime debris.



X 500

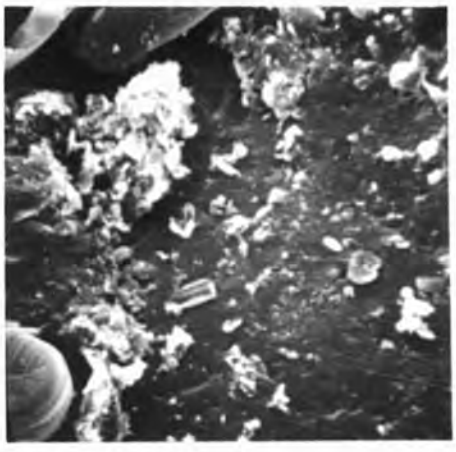
(d) 20 cm depth : Relatively free of filamentous forms but some short rods present.



X 215

Plate 9.2 Bacterial densities on surfaces of sand grains, from a 28 day-old core exposed to water currents

(a) 1 cm depth : Debris and diatoms.



X 3,850

(b) 5 cm depth : Some detritus but short rods and sheathed filamentous bacteria evident.

X 4,300

(c) 10 cm depth : Short rods and filamentous bacteria.

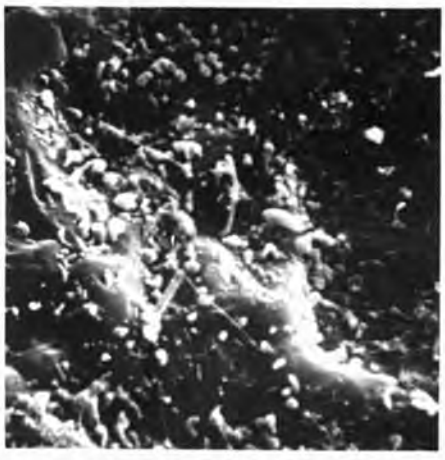
X 4,400

(d) 20 cm depth : short rods and filamentous bacteria with larger microbes also present.

X 4,900

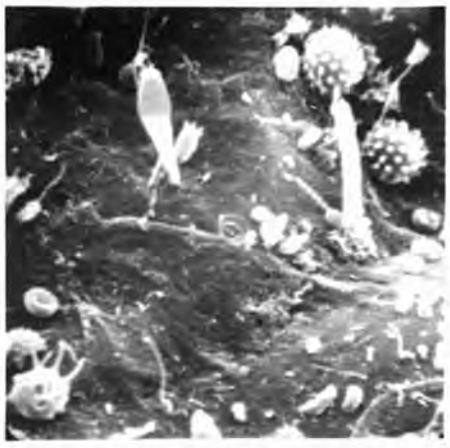
Plate 9.3 Bacterial types observed on the surface of sand grains collected from a 28 day-old core

(a) 24 cm depth : Mainly short rods, probably pseudomonads, with some filamentous bacteria, probably Sphaerotilus and Thiothrix.



X 2,000

(b) 10 cm depth : Caulobacter, Sphaerotilus and Microcyclus-type bacteria.

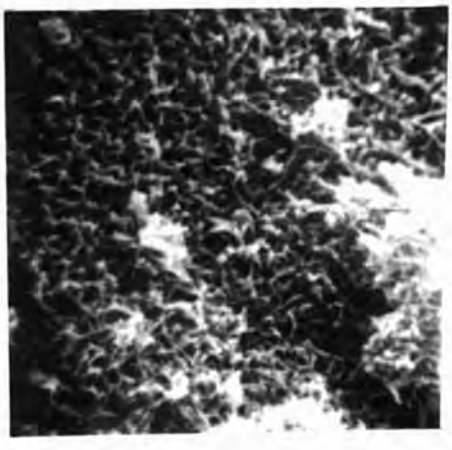


X 4,300

(c) 20 cm depth : Sphaerotilus and Thiothrix.

X 4,750

(d) 24 cm depth : Slime matrix in partly sheltered area.



X 9,750

shaking technique used for the enumeration of surface associated bacteria.(Section 4.7.1).

These micrographs indicate that the bacterial densities recorded in the first section of this chapter represent mainly the short rod bacteria on exposed and matrix free surfaces and as the relative covering of sand grain surface by slime matrices and filamentous bacteria increased with depth, a consequent apparent reduction in the rod density with depth was, perhaps erroneously, considered to represent a decrease in the bacterial population as a whole. However, such slime matrices as occurred in a 28 day old core, may not have developed by day 10, the final day on which bacteria were removed by the shaking technique.

9.2 Bacteria in the interstitial water

Enumeration of bacteria in the interstitial water of a slow sand filter bed poses serious sampling problems. However, a laboratory scale model (Plate 9.4) was designed and used by Lodge (1979) to examine the oxygen regime at various depths in a column of sand initially obtained from bed 45. The model, 13.9 cm inner diameter, had a perforated perspex base plate. Gravel was added to a depth of 10 cm above the base plate and sand, taken either from the washing bay or bed 45, was then added to a further depth of 60 cm. The column of sand was backcharged to drive air out from the interstices and was then drained down in order to place sampling and redox probes at the required depths. The column was then re-charged from the base and, once this was completed, downward flow through the model filter was

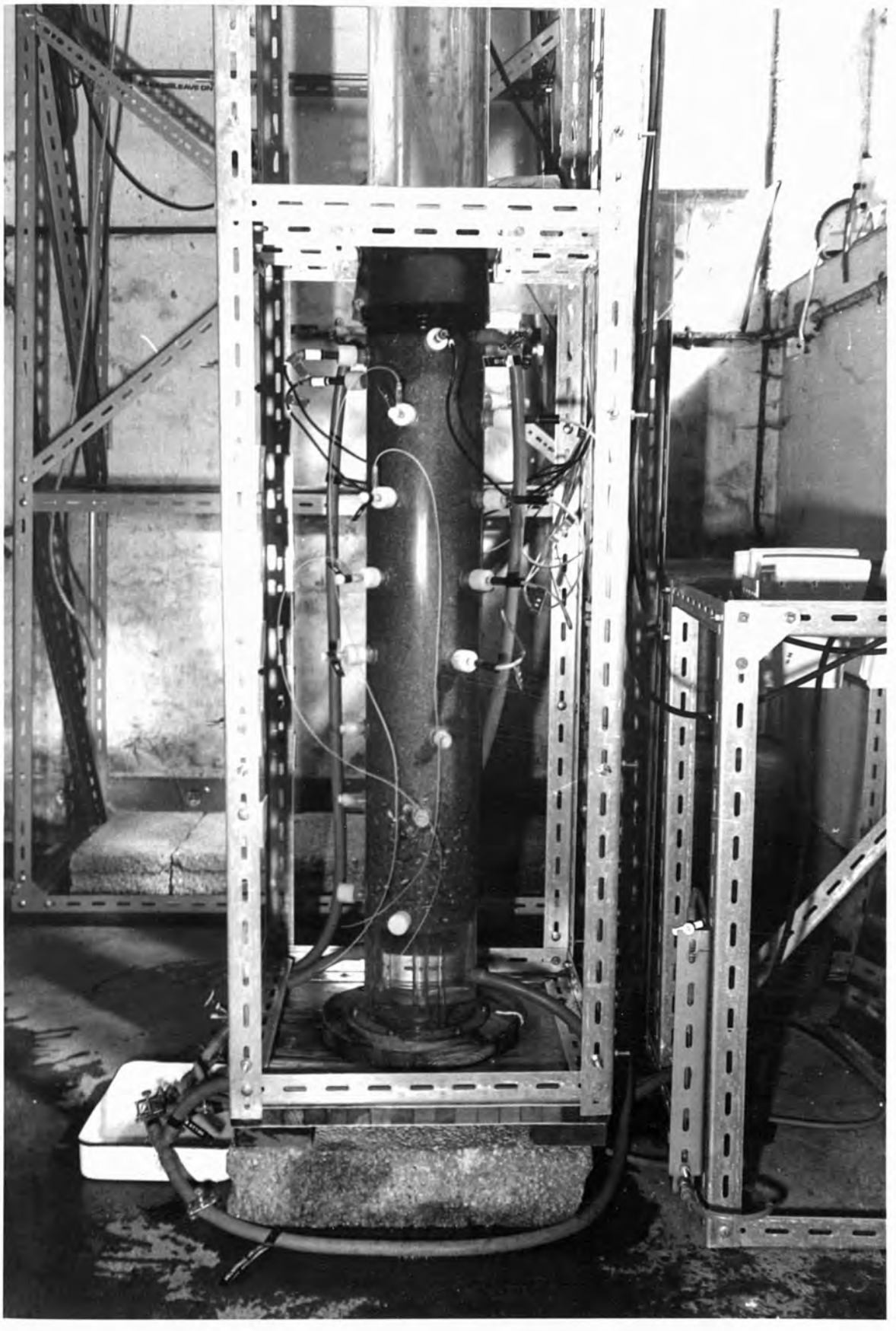


Plate 9.4 Laboratory scale slow sand filter column

(photograph by courtesy of Dr. D. Lodge, Royal Holloway College).

begun by a feed from above the column. The rate of flow was controlled by the effluent valve. Full details of the column design and operation are given by Lodge (1979).

The sampling probes, initially designed for the removal of water from the interstices for dissolved oxygen determination, were found to be equally useful for bacterial studies. The water flow into the sampling apparatus from the probes was controlled such that it equalled the rate of flow of water in the interstices within the model filter. Analysis of these samples (Section 4.7.2) was begun within minutes of sampling.

While samples were being taken in duplicate for bacterial analysis, the redox probes inserted at the same depths as the sampling probes were monitored in order to gain an estimate of the oxidising capacity of the water. Surface water temperature , head loss and dissolved oxygen in the supply and effluent water were also monitored on each sampling occasion.

9.2.1 Validation of the model filter column : a comparison of carbon concentrations and ciliate densities in the column and in sand cores of the filter bed

It was only possible to analyse sand from the model column for carbon concentration and ciliate densities after it had been drained down at the end of an experiment. Data are available for two such occasions (30.11.77 and 5.1.78) and these are compared below with data obtained from sand core analyses during run 8 on bed 45 (March to

May 1977). This run had a similar water temperature (7.5°C, the column feed water averaged 9°C), and data from sampling days closest in age of the filtration run to the column data have been used for the comparison. (Tables 9.3 and 9.4, Fig. 9.5). The data have been transformed from density per unit weight of sand (Appendix 9.3) to density per cm^3 for comparitive purposes using the conversion factor : 1 cm^3 = 1.598 g wet weight, determined from 34 replicate weighings of 1 cm^3 of sand.

Table 9.3 Carbon concentrations in sand extracted from the filter model compared with concentrations in sand cores from Run 8 Bed 45

CARBON (μg C cm^{-3})						
	COLUMN	CORE	RATIO	COLUMN	CORE	RATIO
DATE	5.1.78	6.4.77		30.11.77	25.4.77	
AGE	8 DAYS	9 DAYS		37 DAYS	28 DAYS	
DEPTH (CM)						
1	264	198	1.33	1,628	1,467	1.11
5	968	110	8.80	1,155	216	5.35
10	615	98	6.28	1,166	240	4.89
20	483	162	2.98	-	-	
30	487	154	3.16	-	-	

FIG. 9.5 COMPARISON OF PARTICULATE ORGANIC CARBON CONTENT IN THE COLUMN AND CORE SAND

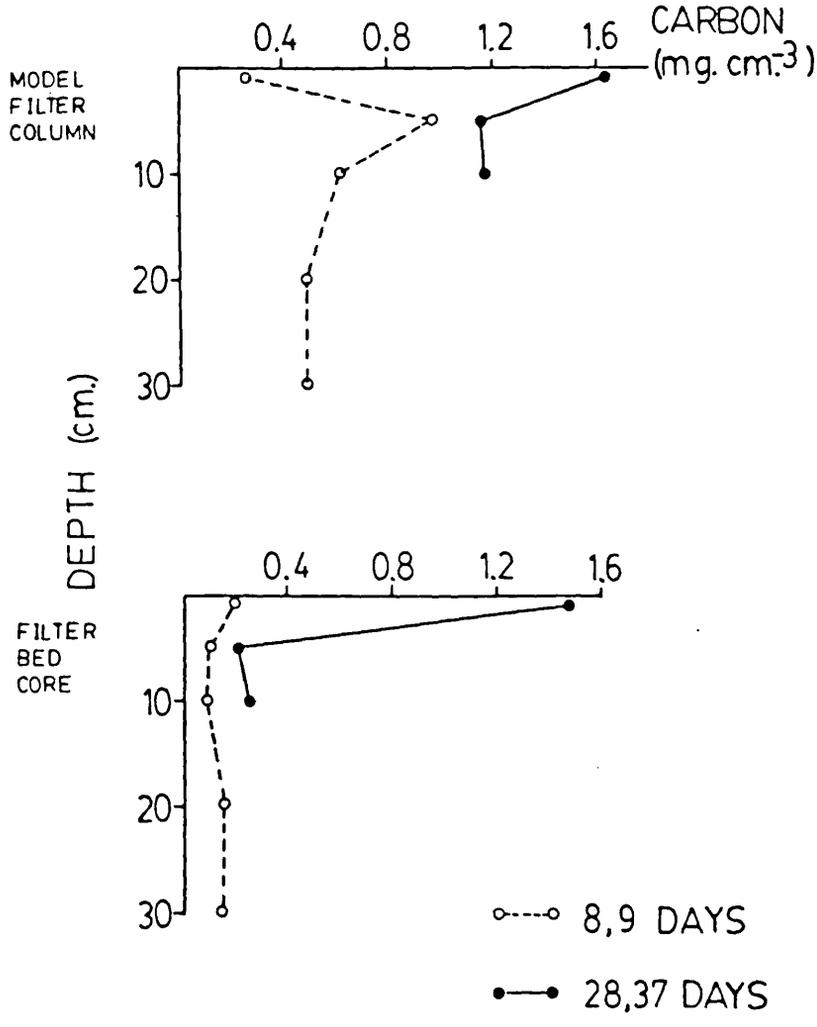


Table 9.4 Ciliate densities in sand extracted from the filter model compared with densities in sand cores from Run 8, Bed 45

	CILIATES (No. cm^{-3})					
	COLUMN	CORE	RATIO	COLUMN	CORE	RATIO
DATE	5.1.78	6.4.77		30.11.77	25.4.77	
AGE	8 DAYS	9 DAYS		37 DAYS	28 DAYS	
DEPTH (CM)						
1	31	79	0.39	117	162	0.72
5	205	12	17.08	-	-	
10	171	24	7.13	-	-	

Both carbon and ciliate densities in the model sand column after eight days were far in excess of those recorded in the cores at the same age of a filtration run, the ratios varying from 1.33 to 8.80 for carbon and 0.39 to 17.08 for the ciliates. These differences were somewhat reduced by days 37 and 28. The 8 day column sample was from a column run, operating with biologically mature sand, i.e. it had been used in a previous run, unlike the initially washed sand of the sample core with which it was compared. This would account for the more rapid development in the column sand relative to that in the core of similar age. The 37 day column sample was, however, from a column run operating initially with washed sand, from the sand bays at Hampton, and was consequently more directly comparable with the sample core of similar age. Figure 9.5 indicates that in comparing the two older samples (28 and 37 days), the model column may, for some reason, be more

productive below the surface layers than a filter bed core, despite the precautions taken to black out the sand below the surface to prevent light penetration (Lodge, 1979). The dissolved oxygen contents of surface and filtrate waters during both column runs (9 mg l^{-1} to 11 mg l^{-1}) did not vary from those recorded in the filter bed at the corresponding filtration ages.

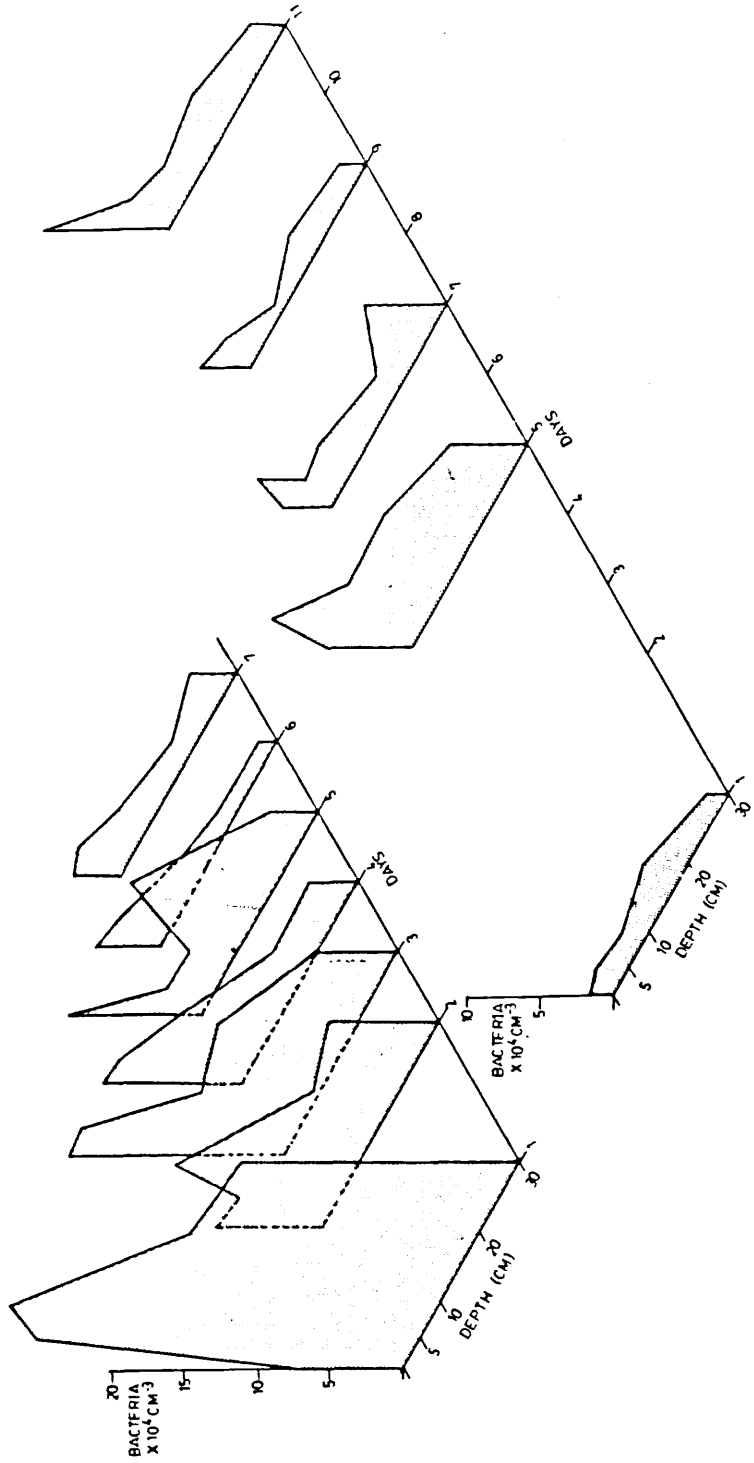
9.2.2 Development of the population of interstitial bacteria during a model filtration run

(a) Clean sand

The study of bacterial development in clean sand was performed in order to examine the colonisation of bacteria in the sand cores inserted into the slow sand filters and in the top 30 cm of the filter bed sand during the run immediately following resanding. The interstitial water was monitored in the top 30 cm of the model filter column sand over the first eleven days of a run in January 1978 which had an average temperature of 7°C. (Fig. 9.6, Appendix 9.4). All bacterial counts were performed on duplicate samples and the mean value taken. Low densities of about 2 X 10^4 bacteria per ml. occurred in the interstitial water throughout the monitored depth of sand after one day. This had increased after five days to a maximum density of 1.1 X 10^5 bacteria per ml. at 5 cm depth with half this number recorded at all other depths. By day 7, the bacterial densities had decreased, the maximum number of 6.5 X 10^4 ml^{-1} occurring still at 5 cm, but by day 9 the bacteria had begun to accumulate at the surface. After eleven days a maximum density of 8.5 X 10^4 ml^{-1} was recorded at the surface while below 5 cm, $\frac{1}{4}$ to $\frac{1}{2}$ this number were present.

FIG. 9.6 DEVELOPMENT OF INTERSTITIAL BACTERIA IN THE MODEL FILTER COLUMN

A. SAND ALSO USED IN A PREVIOUS MODEL FILTER RUN B. CLEAN SAND FROM HAMPTON



Total population estimates of bacteria, calculated by integration of the densities at each depth analysed (Section 4.9.2), increased from 6.9 X 10^5 $30ml^{-1}$ to 2.1 X 10^6 $30ml^{-1}$ by day 5 (Table 9.5, Fig. 9.7a) but subsequently decreased until day 9 (6.1 X 10^5 $30ml^{-1}$) after which the increase in density at the surface caused a second increase to occur, reaching 10^6 $30ml^{-1}$ by day 11. These integrals describe the bacterial density in the interstitial water of a column of sand where the volume of water sampled from the interstices equalled 1 ml at each cm depth examined between 1 and 30 cm.

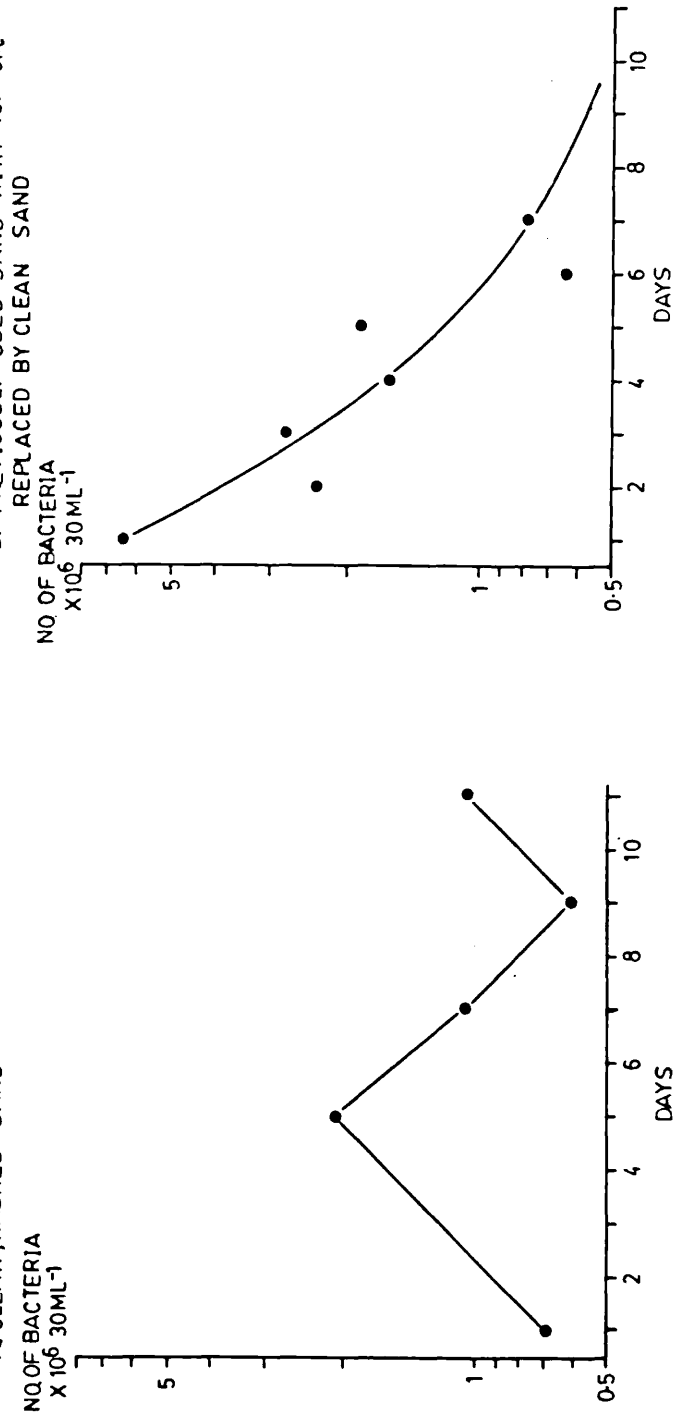
Table 9.5 Population densities of bacteria in the interstitial water of two model filter column runs estimated by integration of data in Appendices 9.4 and 9.5 (No. X 10^5 $30ml^{-1}$ interstitial water)

	DENSITY OF BACTERIA X 10^5	
DATE	12.1.78 - 23.1.78	29.12.77 - 4.1.78
DAY	RUN 1*	RUN 2 **
1	6.91	65.00
2	-	23.50
3	-	27.90
4	-	16.00
5	20.90	18.80
6	-	6.45
7	10.40	7.80
9	6.06	-
11	10.40	-

* Run 1 - column filled with clean sand obtained from washing bays at Hampton.

** Run 2 - column filled with sand used in a previous column run.

FIG 97 TOTAL POPULATION ESTIMATES OF INTERSTITIAL BACTERIA DURING MODEL FILTER COLUMN RUNS



(b) Sand used in a previous filter run

After the model filter column had been drained following an experimental run, the surface layer of detritus was removed, using a scraper which was lowered down from the top of the column, and fresh sand was added to bring the level back to the correct height for the probes. The sand was then re-charged from below and another run was started, eight days after the end of the previous one. The aim of this experiment was to examine the colonisation of bacteria in sand which had been previously used in a filtration run, that is, to simulate the colonisation of bacteria in a functional slow sand filter bed after it has been drained, cleaned and put back into supply. The feed water was at an average temperature of $9^{o}C$. After twenty four hours, very high densities of bacteria (2.9×10^{5} ml^{-1}) were present in the interstitial water at 10 cm depth (Fig. 9.6, Appendix 9.5) decreasing to about 2.0×10^{5} ml^{-1} at depths below this. The column of sand had been standing dry for eight days, consequently the bacterial slime associated with the sand grains would have dried out, so that once water started to flow through the interstices again, these bacteria were washed off into suspension. The presence of high bacterial counts in the filtrate following resanding, reported in the annual report of the Metropolitan Water Board (1971 - 1973), was also thought to be caused by this exposure of the sand to air instead of water. On the second day the densities in the interstitial water were much lower and by day four a distribution had developed with maximum densities (9×10^{4} bacteria ml^{-1}) in the

surface sand, decreasing to 4.0×10^4 ml^{-1} below 20 cm. This depth pattern remained over the rest of the sampling period, but the total bacterial population estimated by numerical integration of the depths examined, decreased over the seven day period from 6.5×10^6 to 7.8×10^5 $30ml^{-1}$ (Table 9.5, Fig. 9.7b).

To summarise this section concerning bacterial colonisation of the filter beds; assuming the model column is representative of an operational system, far greater densities of bacteria were found to be associated with the sand grain surfaces than were present in the interstitial water. Maximum population estimates were 3.1×10^8 $30\,cm^{-3}$ and 2.1×10^6 $30\,ml^{-1}$ for the surface associated and interstitial bacteria respectively in comparable core and model column (filled with clean sand) samples. As the technique for removing the bacteria associated with the sand grain surfaces was found to be about 30% efficient and as the porosity of the sand was about 39% (Section 5.4), the maximum density of the surface associated bacterial population was probably about 1,000 times that of the interstitial population in a unit volume of sand.

The depth of maximum density of surface associated bacteria was found at 10 cm on day 2 of the filter bed run but had gradually moved to the surface by day 10. The maximum density of interstitial bacteria also remained below the surface at 5 cm until day 9 of the column run with clean sand. The scanning electron micrographs of a 28 day old core indicate that slime matrices enmeshing the bacteria develop more with depth in a core of that age. If this

were also to occur gradually over the first ten days it could be responsible for the movement of maximum densities, either removed by shaking or dislodged by water currents, towards the surface.

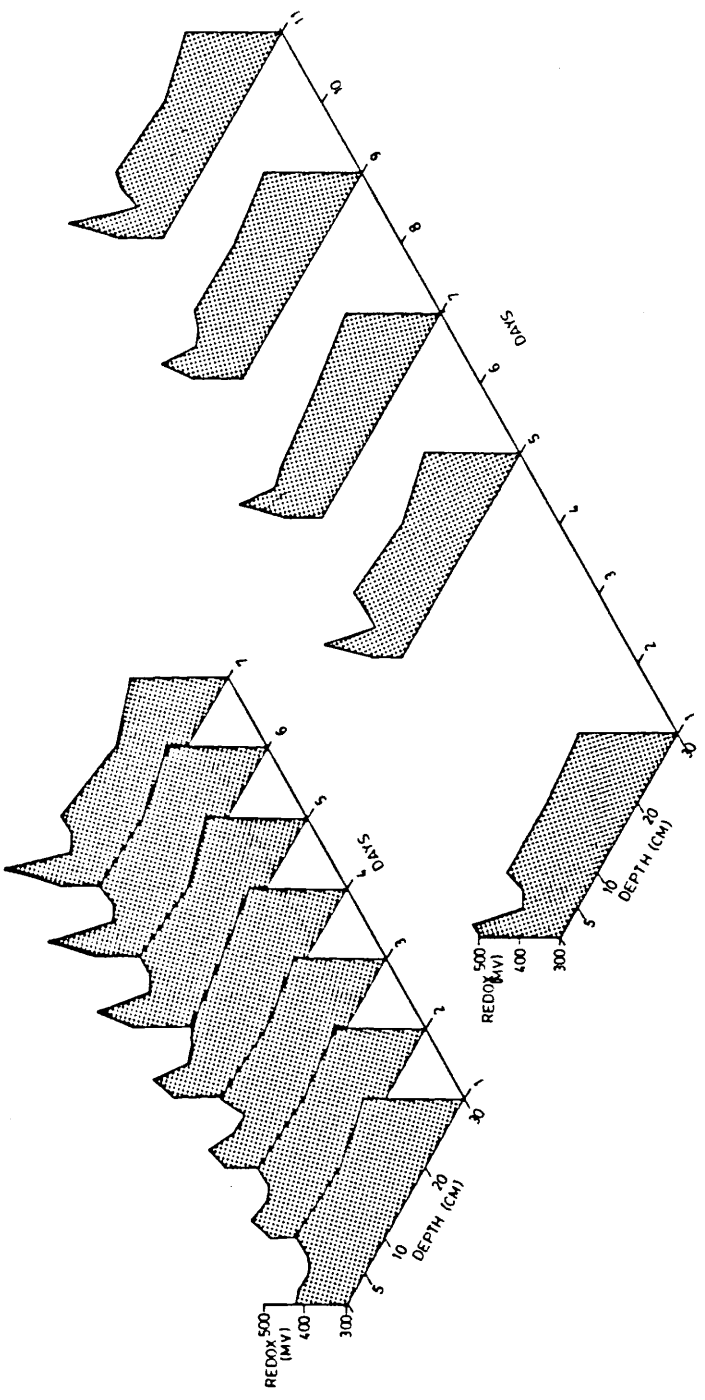
The very rapid instantaneous growth rate (164% d^{-1}) of surface associated bacteria recorded over the first 2 days was not apparently accompanied by an equivalent increase in interstitial bacteria, although a maximum density was achieved in the interstices by day 5. The subsequent decline of interstitial bacteria may be attributable to the development of filter feeding predators.

The changes in bacterial densities which may reflect more accurately the changes which occur in filter bed sand are those of the model filter run using previously used (mature) sand, where the surface sand and debris of the previous run had been replaced by clean sand. The interstitial bacteria decreased throughout the seven days of this run from 6.5×10^6 30 ml^{-1} to 7.8×10^5 30ml^{-1}.

9.3 Changes in oxidation-reduction (REDOX) potential during a model filter column run.

The redox potential did not fall below 350 mV at all depths monitored down to 30 cm, in the model filter column, throughout the experimental runs, demonstrating that oxidising conditions prevailed in the interstitial water of the sand column. During the run using washed sand from the Hampton Works, (Fig. 9.8b, Appendix 9.6) the redox potential varied only slightly with time, the depth profile remaining similar with a peak of 520 to 560 mV at 2.5 cm below the surface. The surface potential was lowest (370 to 410 mV) with a further low value consistently

FIG. 98 DEPTH PROFILES OF REDOX POTENTIAL DURING MODEL FILTER COLUMN RUNS



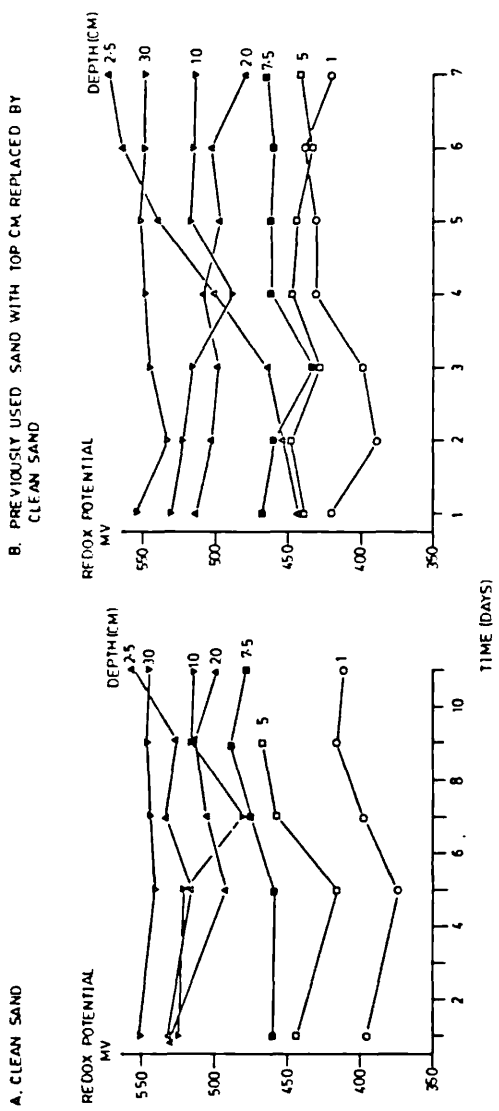
recorded at 5 cm. Between 5 and 10 cm the redox potential increased to a similar value to that at 2.5 cm, and below 10 cm a further gradual increase was recorded down to 30 cm.

The low surface values again occurred regularly in the run using sand which had already developed a biological flora in a previous run (Fig. 9.8a), with a peak at 2.5 cm developing after three days simultaneously with low values occurring at 5 cm. The lower depths, below 10 cm, remained around 500 mV throughout the run. This unexpected depth profile of Eh was also noted by Lodge (1979).

There was very little change of redox potential with time (Fig. 9.9) at any given depth, with the exception of the 2.5 cm values, which increased by about 125 mV over the 7 day run in previously used sand, and by 35 mV during the last days of the run with clean sand. However, as the latter was not continued past day 11, the recorded increase may be artificial.

The gradual increase of redox potential with depth is extremely puzzling. Oxygen produced at the surface by the benthic phytoplankton may easily pass straight into the sand producing good oxidising conditions just below the surface. One would expect this to be greater in previously used sand with a seed of phytoplankton, and also to increase as it did with the duration of the run. The gradual increase of potential with depth below 5 cm may have been caused by decreased bacterial activity due possibly to nutrient limitation. Although bacterial densities were generally lower below 10 cm (Sections 9.1 and 9.2) the difference was not as constant as the redox profiles

FIG. 9.9 VARIATION OF REDOX POTENTIAL DURING MODEL FILTER COLUMN RUNS



suggest that they should have been if they were the major cause of the observed variations in redox potential.

9.4 Diurnal variations in the interstitial bacterial density and redox profiles of the model filter column

9.4.1 Diurnal variation of bacterial density in the interstitial water

During the two twenty four hour experiments with the model filter column filled with biologically mature sand in January and February 1978, bacterial densities from duplicate samples and redox potentials of the interstitial water in the top 30 cm of sand, were recorded at intervals of four hours. The water temperature remained constant at 7.25°C and 4.5°C respectively during the two runs.

The relative densities of bacteria varied only slightly with depth during the 24 hour periods studied (Figs. 9.10, 9.11, Appendix 9.7) a maximum density generally occurring at a depth of 2.5 to 5 cm. At the lower temperature of 4.5°C the distribution was more eratic with a further increase occurring below 20 cm, between 16.00 and 24.00 hours. Integrals were calculated as described previously (Section 4.9.2) for both runs and were corrected to values per ml. in order to compare mean bacterial densities per ml at different times in each run and also between runs (Table 9.6, Fig. 9.12). In the second 24hour run, the mean densities calculated, indicated a gradual decrease in the overall bacterial population during the whole period

FIG. 9.10

DIURNAL VARIATION IN THE DEPTH DISTRIBUTION OF INTERSTITIAL BACTERIA OF THE FILTER COLUMN

RUN 1 (7·25°C)

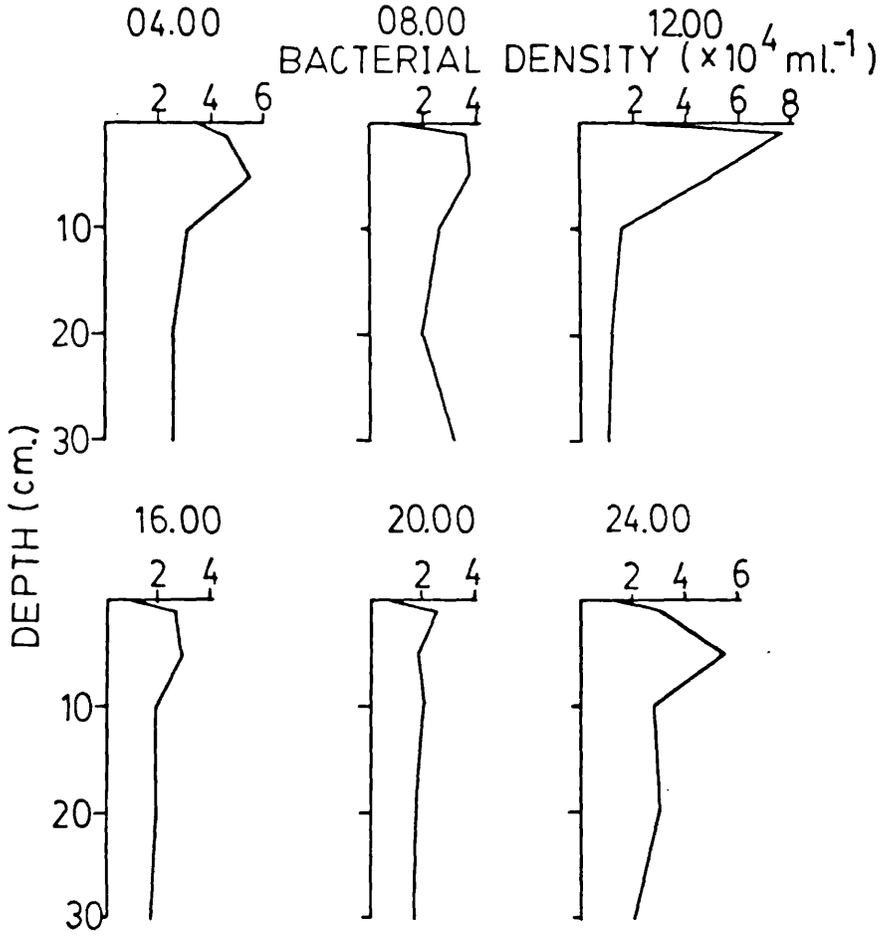
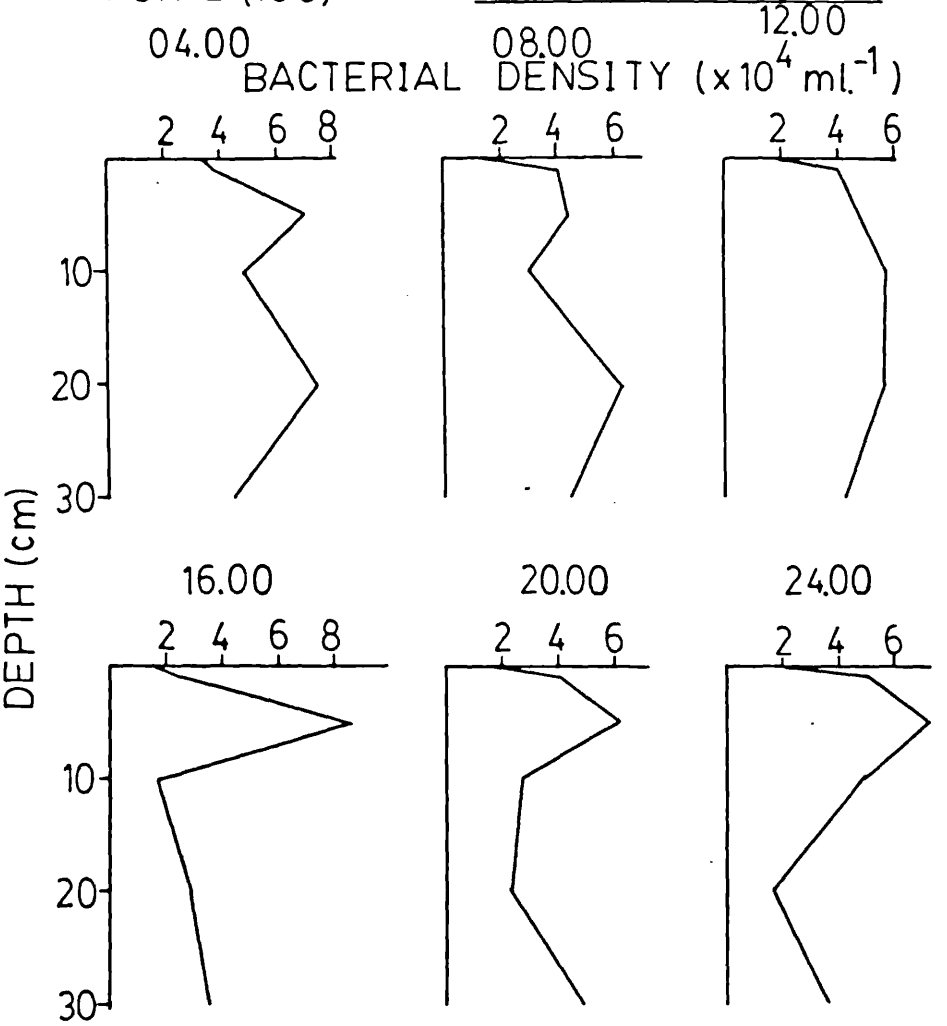


FIG. 9.11

DIURNAL VARIATION IN THE DEPTH DISTRIBUTION OF INTERSTITIAL BACTERIA OF THE FILTER COLUMN

RUN 2 (45°C)



(Fig. 9.12 b) and this was thought to be possibly due to the near freezing temperature of the feed water. However the bacterial density in the feed water was also found to vary considerably in both runs (Table 9.6, Fig. 9.12) and these densities were taken into account when interpreting the changes in density of the interstitial water. The feed water was extracted from immediately above the sand surface for these determinations.

Table 9.6 Bacterial density in the interstitial water of the model filter column during two 24-hour experiments

	RUN 1 (26.1.78, 7.25°C)		RUN 2 (11.2.78, 4.5°C)	
TIME	MEAN COLUMN DENSITY ML^{-1}	DENSITY IN FEED WATER ML^{-1}	MEAN COLUMN DENSITY ML^{-1}	DENSITY IN FEED WATER ML^{-1}
04.00	3.04×10^4	3.53×10^4	5.93×10^4	3.39×10^4
08.00	2.56×10^4	1.04×10^4	4.59×10^4	1.01×10^4
12.00	4.45×10^4	1.30×10^4	5.14×10^4	1.12×10^4
16.00	1.97×10^4	1.10×10^4	4.00×10^4	1.49×10^4
20.00	1.85×10^4	6.45×10^3	3.63×10^4	1.14×10^4
24.00	3.57×10^4	1.13×10^4	3.64×10^4	1.30×10^4

The absolute densities were higher in the second run than in run 1, corresponding with higher bacterial densities in the feed water during the second, colder run. When the densities of bacteria in the interstitial water of both runs were related to the bacterial density of the overlying feed water (Fig. 9.12) very similar changes in density over the 24 hour periods were apparent with a definite peak occurring between 08.00 and 12.00 hours,

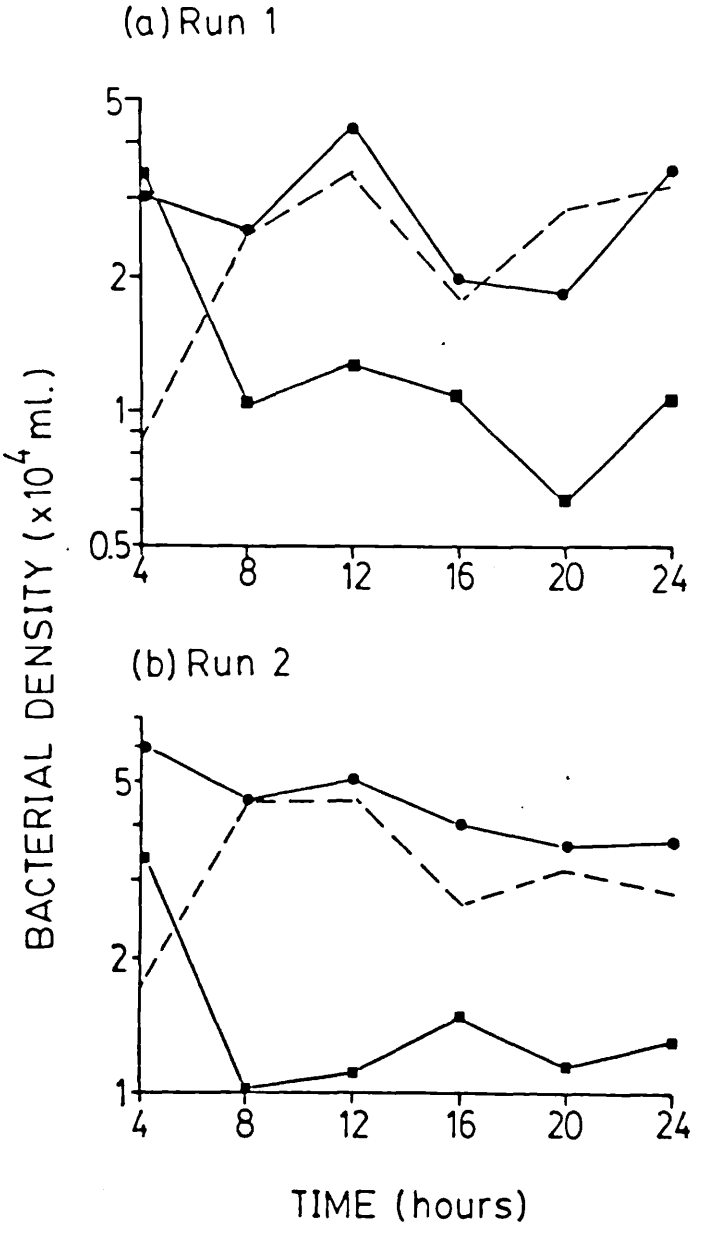
Key to Figure 9.12

Bacterial density of water in sand interstices ●——●

Bacterial density of input water to sand column ■——■

Ratio of bacterial density of interstitial water/ bacterial density of input water — — —

FIG. 9.12 DIURNAL VARIATION OF DENSITY OF INTERSTITIAL BACTERIA OF THE FILTER COLUMN WITH TIME

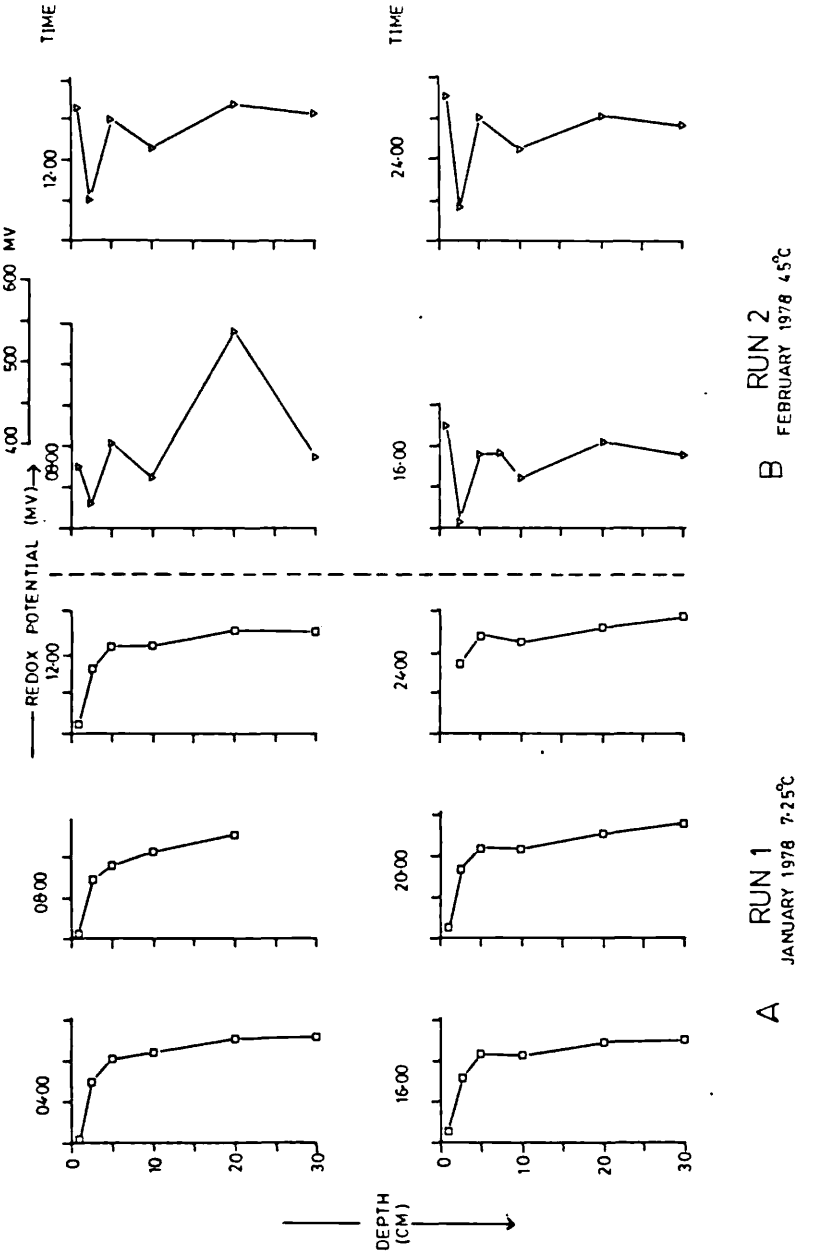


and a further peak at midnight recorded during the warmer run. The morning peak probably reflects an increase in primary productivity as the light intensity increases during that part of the day, but a further increase towards midnight is difficult to explain, unless it reflects increased decomposition of organic material. These findings suggest that the interstitial density of bacteria is dependent on the density in the feed water entering the sand, over a short term examination, rather than the bacterial growth associated with sand grains. Long term variations in the bacterial density of the interstitial water are more probably dictated by the biological activity within the filter bed.

9.4.2 Diurnal variations in redox potential

The redox profiles of the two twenty-four hour runs differed considerably (Fig. 9.13, Appendix 9.8). In the first run (Fig. 9.13a) it remained very similar throughout the day, with minimum values of about 400 mV recorded at the surface, and the depths between 5 and 30 cm remaining at potentials between 500 and 550 mV. The second run (Fig. 9.13b) was monitored only between 08.00 and 20.00. Apart from the 08.00 profile in which the redox potential appeared to increase irregularly with depth, at the other sampling times, the profiles were very similar, a maximum of 575 mV occurring at the surface, this decreasing rapidly in the 2.5 cm depth to around 450 mV and then increasing back to about 500 mV at 5 cm, and remaining at this level down to 30 cm.

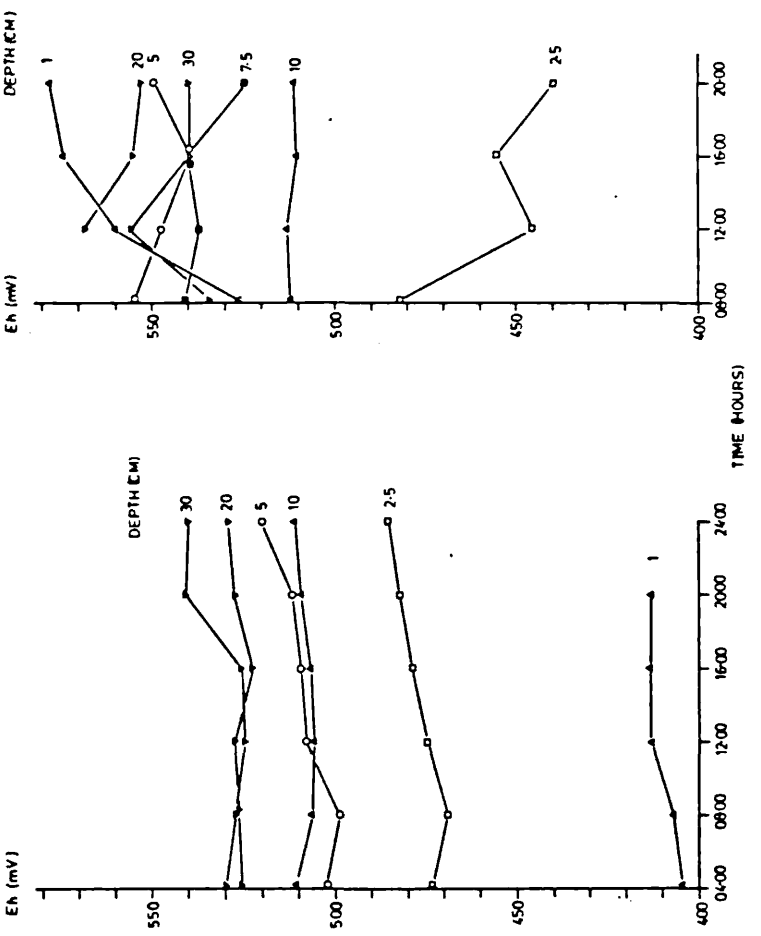
FIG 9.13 DEPTH PROFILES OF DIURNAL VARIATION IN REDOX POTENTIAL RECORDED IN THE MODEL FILTER COLUMN



The major differences between these runs occurred in the top 2.5 cm (Fig. 9.14) and may be explained by higher primary production occurring at the surface of the more 'mature' sand of run 2.

The redox potential was consistent at around 400 mV at the sand surface during run 1, but in run 2 there was a rapid and continuous rise in Eh at the surface between 08.00 and 20.00 from 526 mV to 578 mV, with a corresponding decrease in Eh at 2.5 cm, presumably caused by primary productivity at the surface and high microbial activity at 2.5 cm this being much reduced at the lower depths. High bacterial densities were recorded at 5 cm during this second run.

FIG. 9.14 DIURNAL VARIATION OF REDOX POTENTIAL AT DEPTHS BETWEEN 1 AND 30 CM. IN THE MODEL FILTER COLUMN SAND



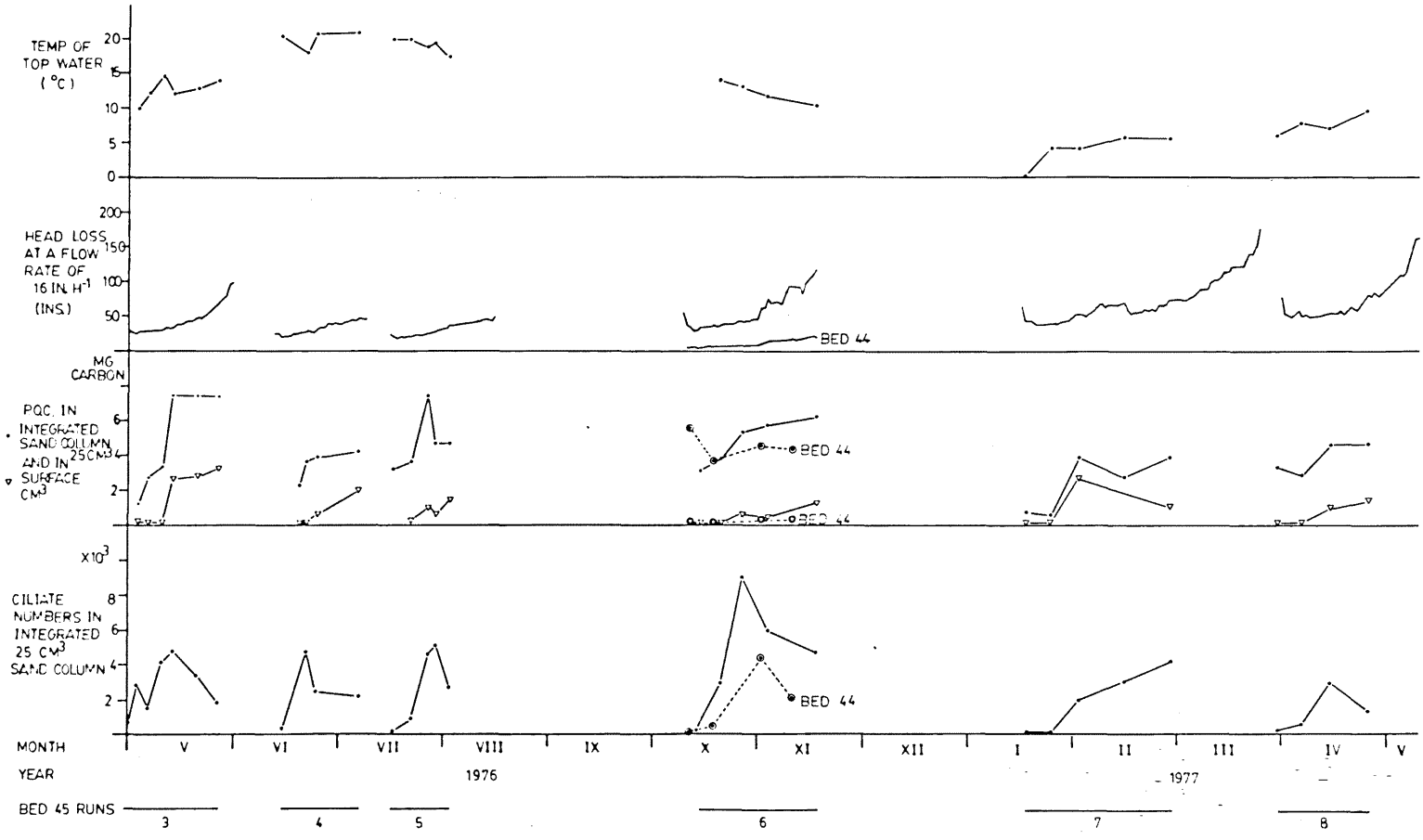
Chapter 10 Discussion

A graphical summary showing the concurrent changes of the physical, chemical and biological variables measured in slow sand filter bed number 44 and 45 is given in Figure 10.1. These include temperature, head loss, particulate organic carbon (for the surface and for the total of an integrated 25 cm column) and ciliate densities (for the total of an integrated column). The variation of these parameters with time is illustrated for runs 3 to 8, on bed 45 and for the bed 44 run, and these runs are placed in the calendar year, thus allowing seasonal changes to be seen. Bed 44 which was run at 8 in h^{-1} (20 cm h^{-1}) in contrast to the faster rate of bed 45 (16 in h^{-1} (40 cm h^{-1})) was studied during run 6.

Head loss is the means whereby the Water Industry measures the filtering efficiency of the slow sand filters; a rapid gain in head loss is taken to indicate loss in efficiency and at a head loss of 1.5 to 2.0 m, a bed is taken out of operation. Figure 10.1 allows the comparison of the changes in head loss with changes in particulate organic carbon and ciliate densities throughout a run, and at different temperature conditions and seasons. The slope of each of the graphs allows a rate of change to be calculated and, where the change is exponential, the rate calculated will be an instantaneous rate (e^{rt}) from which a doubling time can be derived, if desired (= 0.6931/r).

The pattern of head loss during a filtration run on bed 45 regularly demonstrated a dramatic gain after two to three weeks of operation. This can be seen in runs 3, 6, 7 and 8 (Fig. 10.1). Runs 4 and 5 of June, July and

FIG. 10.1 CHANGES IN TEMPERATURE, HEAD LOSS, PARTICULATE ORGANIC CARBON AND CILIATE DENSITIES IN BEDS 44 AND 45 DURING 1976 AND 1977



August proved an exception to this rule with a reduced rate of gain in head loss occurring throughout these two runs. During these summer months, dense growths of Cladophora developed two weeks or so after the beginning of a run, the period of colonisation by filamentous algae also recorded by Ridley (1971), and these may have been responsible for this reduced rate of gain in head loss. The Cladophora may pre-filter the water before it reaches the sand bed and thus reduce clogging of the interstices, while it itself is buoyed up above the sand surface by its own photosynthetic oxygen production. This phenomena was first described by Kemna (1899). At about the same time as the rapid increase in head loss, in all runs except run 6, the surface carbon density also increased by about 10-fold. During run 6 the surface carbon increased at a steady but much lower rate throughout the run. The depth distribution of ciliates in run 6 also differed from the other runs, and is discussed later.

Bellinger (1978) observed that the algae at the sand surface may be limited by low light levels caused by the shading by planktonic algal crops. Such planktonic algae, in the absence of Cladophora may have grown sufficiently to limit light penetration to the bed surface, resulting in the death of bottom-living algae and their subsequent decomposition. Such an increase of detrital matter may have caused the interstices to clog and at the same time be responsible for a rapid increase in the particulate organic carbon content of the surface sand. As this rapid accumulation of carbon at the sand surface

with a concurrent rapid increase in head loss was such a regular feature of the filtration runs, it would seem most likely to be caused by autochthonous organic products rather than the sudden arrival of allochthonous material, and the above theory seems a most likely explanation.

During the run on bed 44, which was operated at half the flow rate of bed 45, the rate of gain in head loss was fairly constant throughout the run, and showed a 3-fold accumulation of surface carbon occurring slowly over a two week period from day 10 to 24. The sudden increases in head loss gain and surface carbon concentrations characteristic of bed 45 (apart from runs 4 and 5) seems therefore to be associated with the more rapid flow rate of this bed. Algal crops in bed 44 were always much denser than those in bed 45. Consequently, a similar straining effect to that of Cladophora during the summer runs of bed 45, may well have been occurring throughout the run on bed 44 in October and November.

The removal of particulate organic carbon from the filter bed top water (Fig. 5.5 and page 105) increased with time during the filtration runs, and also increased seasonally with temperature, both these effects corresponding with increased biological activity. Logarithmic increases in carbon concentration occurred between 2 and 10 cm depths in the sand throughout all the filtration runs on bed 45 (Fig. 5.9) with the exception of the two summer runs 4 and 5. During these two runs, the logarithmic increase at 2 - 10 cm stopped early after 13 and 11 days respectively and thereafter no significant change in concentration was recorded. This

would also be attributable to the pre-filtering properties of the filter skin, predominantly Cladophora, suspended over the sand surface at this time.

Ciliate densities were also found to increase logarithmically over the first stage of each run, reaching maximum densities after 10 to 19 days on bed 45, the period of increase decreasing with rises in temperature from 4°C to 20°C (Table 8.10, Fig. 8.12). The maximum ciliate density generally coincided with the beginning of the more rapid increase in head loss, so that ciliate numbers declined as the head loss increased (Fig. 10.1). The three exceptions to this observation were the two summer runs 4 and 5 and run 7. During runs 4 and 5 the ciliate population declined after ten and eleven days respectively, although there was little change in the low rate of head loss gain throughout these runs. In run 7 the ciliate population continued to increase, but did so very slowly, throughout this cold, winter run (4°C). Maximum ciliate densities achieved during runs 3, 4, 5 and 7 were very similar, ranging between 4,100 and 5,050 in the 25 cm^3 column of sand sampled (Appendix 8.1) regardless of temperature, whereas the instantaneous growth rate did increase with temperature with a Q_{10} of 1.6. Runs 6 and 8 achieved more extreme ciliate densities of 8,950 and 2,968 respectively in the integrated 25 cm^3 sand column. It would seem that some limitation of the filter bed inhibited further numerical growth of the smaller ciliates but encouraged replication of the larger carnivorous and omnivorous forms. These colonised the bed at a time when the ciliate numbers were decreasing and as a result the total ciliate biomass

(measured as biovolume) still continued to increase even after maximal numbers had been achieved.

Run 6 was an unusual run in many respects, particularly in its pattern of carbon accumulation and ciliate depth distribution patterns. The much slower accumulation of surface carbon of run 6 was not associated with the rapid increase in head loss which began on day 21 of the run. However, the ciliate maximum of 8,950 $25cm^{-3}$ did coincide with the start of the period of high head loss gain. The increase of carbon content throughout the sand column in run 6 was much more marked than that occurring at the surface, and it is interesting to note that most ciliate species exhibited surface avoidance during this particular run (Fig. 7.4). Whereas in all other runs the ciliate maximum was associated with the high surface carbon levels, during run 6 maximum numbers were generally found between 5 and 10 cm.

Ciliate density and carbon content can not be directly related, as if this was so, the carbon content of the sand in run 6 would have been more than twice the usual level, corresponding with the particularly high maximum ciliate density, which it was not. The carbon is more probably representative of the bacterial densities present in association with the detrital flocs and the slime matrices on the sand grain surfaces. As the ciliate decline in numbers was associated with rapid increases in carbon levels and head loss, it is possible that the cause may have been unfavourable conditions of dissolved oxygen. Once bacteria became well established in the filter beds,

living in the accumulated detritus, their high respiratory demand may have rendered conditions unfavourable for the smaller ciliates, enabling the larger species to survive at their expense. This hypothesis is compounded by the observations of the oxygen balance of the filter bed during the two filtration runs where oxygen data for top water and filtrate were available (runs 5 and 8). Once an algal population had developed above the sand surface the photosynthetic production of oxygen at the sand surface exceeded respiratory uptake (Fig. 8.14 and 8.15). This occurred between days 5 to 11 and 9 to 17 in runs 5 and 8 respectively. On days 11 and 17 of these two runs, the two dissolved oxygen levels were balanced, and following this the respiratory demand of the bed exceeded the photosynthetic production. This resulted in an oxygen deficit in the filtrate. These days of oxygen balance coincided exactly with the days on which maximum ciliate densities were recorded for these two runs (Table 8.10, Page 201). Brink (1967) studied a biological sand filter through which primary settled sewage was passed and he found similarly that ciliates were recorded in greatest densities in places where organic matter had accumulated. However, what he assumed to be over-accumulation of the organic matter resulted in the inhibition of further ciliate growth.

Up until the day on which maximum ciliate densities occurred, the rate of numerical increase was also found to be related to the rate of particulate organic carbon accumulation throughout the top 10 cm of the sand cores, excluding the surface content. Ciliate growth may therefore have been dependent to some extent on the nutrient status

of the filter bed. As the majority of ciliate species present during the initial phase of exponential numerical increase, were bacterial feeders, a complementary study was undertaken to examine the development of the bacterial population over these first few days of a filtration run. The bacteria were present both in suspension, providing food for filter feeding ciliates, and also associated with detrital flocs and sand grain surfaces, providing food for the grazing ciliates. The latter were examined in sample sand cores extracted from the bed. A model filter column was used to sample the interstitial bacteria, as well as the oxidising capacity of the sand by means of redox probes.

Before accepting the results given by the model filter, it was necessary to establish how representative it was of sand in the sand cores or sand in the filter beds. This was attempted by comparing both ciliate densities and carbon concentrations from samples obtained from both the filter bed cores and the model column. The column appeared to be slightly more productive in carbon than the filter bed (Fig. 9.6), but the depth distribution patterns were very similar, and consequently the column was assumed to be adequately representative of the sampling cores. Lodge (1979) had reservations that the top water of the column did not truly represent the filter bed top water. The model filter was installed in a laboratory and consequently the top water, which she obtained weekly from bed 45, did not receive so much light as that of the filter bed during the summer months. This would have resulted in lower algal production and a consequent variation in particulate organic carbon and dissolved oxygen content of the model filter sand relative

to that of the operational bed. However, in the present study, the model filter was used in December, January and February, the darker months of the year. Also, as the column was run at 16" h^{-1}, water was obtained from bed 45 every 4 days as opposed to weekly. The differences between the model filter and operational bed top water may therefore have been reduced. Indeed, the artificial lighting of the model filter apparently resulted in a slightly greater organic production than that of bed 45.

The column was used to study the development of interstitial bacteria in both clean sand, representing the sample cores and 'biologically mature' sand, representing the filter bed.

This study showed that maximum bacterial densities of 1.5 X 10^7 cm^{-3} were found associated with the sand grain surfaces at the sand water interface on day 10 of the filter bed run, which was the final day of sampling. Densities may therefore have increased further. This figure represents only about 30% of the surface associated population as this was the estimated efficiency of the technique employed for enumeration of these bacteria (Section 4.7.1). Densities as high as 5 X 10^8 bacteria per cm^3 have been recorded for freshwater deposits using a culture technique for enumeration (Henrici and McCoy, 1938), however sands have been generally found to support smaller bacterial populations than finer sediments (ZoBell, 1946; Dale, 1974). Maximum densities of interstitial bacteria (8 X 10^4 ml^{-1}) were achieved after eleven days also at the surface in the experimental simulation of the sample cores. As before this was the final sampling day

and densities may have increased further. This density of suspended bacteria is low relative to those achieved under culture conditions (Porter et al, 1979), where densities of greater than 10^6 ml^{-1} were reported to be necessary for growth and reproduction of protozoans. The reason why the bacterial feeders and in particular the filter feeders, were smaller than those reported in the literature (Table 6.1) may therefore be related to nutrient limitation. Although the surface associated bacterial counts were relatively high, they were achieved after thirty minutes of vigorous shaking and may not therefore represent the bacteria easily available for the grazing ciliates.

During a column run simulating the filter bed by using 'biologically mature' sand, the interstitial bacteria were relatively abundant on the first day, at a depth of 10 cm(2.9×10^5 ml^{-1}). This was because they sloughed off from the sand grains after they had dried out following drainage of the column for removing the sand surface. However, by day 7 the density had declined to a maximum of 3.5×10^4 ml^{-1} which occurred at the sand surface.

The bacterial population associated with the sand grain surfaces increased very rapidly at a rate of 164% per day, to 7×10^6 cm^{-3} over the first two days of the filter bed run. After this initial rapid increase, further development was slow with a maximum population of 1×10^7 cm^{-3} recorded after 8 days. Romanenko (1979) found a similar pattern of population increase with bacteria from the Rybinsk reservoir cultured in distilled water which had a particulate organic carbon content of 1.5 mg l^{-1}, about 5 times as much as that in the slow sand filter top water.

The reservoir bacteria increased rapidly to about 1.2 X 10^6 ml^{-1} over the first 2 to 3 days and subsequently increased very slowly to 1.75 X 10^6 ml^{-1} after 40 days. The high initial instantaneous rate of increase recorded in the present study, corresponds to a doubling time (0.6391/r) of 10 hours which is at the lower end of the range of 10 to 20 hours recorded by Fenchel and Harrison (1976), for the initial bacterial colonisation of detrital particles and is also lower than the values recorded (21 and 39 hours) for bacterial development at two estuarine sites by Meyer-Reil (1977). However, Aizaki (1979) recorded a doubling time of 10.3 hours for bacteria grown on roughened polyvinyl plates submerged in a river during January, with a temperature range of 4°C to 9°C, which is similar to the temperature of 3.5°C at which the present study was performed.

The relatively short doubling time of 10 hours indicates that the bacterial population was highly active during the first two days of the run. The supply of oxygenated water and nutrients to the filter bed must render conditions highly favourable for such rapid replication, before the onset of colonisation by competitors and predators. The rapid instantaneous growth rate of these surface associated bacteria during the first 2 days of the run, was not accompanied by an equivalent increase in interstitial bacteria although a maximum integral density of 2.1 X 10^6 30ml^{-1} was achieved by day 5. The subsequent decline to 6 X 10^5 30 ml^{-1} may have been attributable to the development of filter feeding predators.

The order of ciliate colonisation of the filter bed was apparently dependent on their holozoic nature. Glaucoma sp., Cyclidium heptatrichum and C. margaritaceum were the only common members of the subclass Hymenostomata to colonise the sand, and they were exclusively early colonisers. They all have an undulating membrane on the right of the buccal cavity (Kudo, 1971) and it is most pronounced with C. heptatrichum, enabling it to feed on suspended interstitial bacteria. The other two hymenostomes predominantly graze on settled bacteria, but may also feed on suspended bacteria. Two small members of the subclass Spirotricha, Tachysoma pellionella and Aspidisca costata, were also regular early colonisers. The spirotrichs have a well developed adoral zone of membranelles which winds clockwise to the cytostome (Kudo, 1971) and they were seen to graze on detrital flocs. Both these spirotrichs always initially colonised at the surface, where the first detrital flocs accumulated. The only representative of the Hypostomata identified in the filter bed sand, Chilodonella sp., colonised after about a week and also grazed from flocs of detritus. The Peritricha and the Gymnostomata were also late colonisers in addition to the larger Spirotricha, e.g. Euplotes sp. and Stylonichia mytilus. The peritrichs filter feed on suspended bacteria but they must first become attached by a stalk to the sand grains and being relatively large they would require a higher bacterial density in order to thrive than the smaller filter feeders, e.g. C. heptatrichum. The gymnostomes are carnivorous and consequently require a healthy population of flagellates and small ciliates in order to thrive and the larger spirotrichs are omnivorous, feeding

on algae, flagellates and small ciliates.

The ciliates which colonised initially were those which are small and consequently require less food, and which also feed on suspended bacteria. In the model column experiments, the suspended, interstitial, bacteria were found to be largely dependent on the densities of bacteria in the top water (Fig. 9.12) whereas the surface associated bacteria, although found to colonise very rapidly, may have taken a while to become established in flocs on which the grazers could feed. The omnivores, e.g. Stentor polymorphus and the carnivores were limited by lack of food initially, but after 2 to 3 weeks once their prey of small ciliates and flagellates had become abundant they began to establish themselves. The late colonisation of such large species as Loxophyllum sp., Litonotus spp. and S. polymorphus maintained the level of ciliate biomass while total ciliate numbers declined. This dependence of size of the ciliate population on the food availability has also been reported by Noland (1925) and Fenchel (1969). Goulder (1974), who studied benthic ciliate populations of a lake, found larger populations at deeper sites where the sediment was flocculant and thought to provide both shelter and food.

Seasonal variations in species diversity were noted during this study and these were compared with similar observations reported in the literature. Only two of the common ciliate species were present in high numbers all year round: Glaucoma sp. and Aspidisca costata (Fig. 7.18). The temperature and dissolved oxygen (D.O.) tolerance limits for these species of 0^{o} - $35^{o}C$, 0 - 14.2 mg l^{-1} D.O. and

0^{o} - 30^{o}C, 0 - 22.4 mg l^{-1} D.O. respectively (Bick and Kunze, 1971) were well outside the corresponding ranges of 4^{o} to 20^{o}C and 0.5 to 12.0 mg l^{-1} D.O. recorded in the filtrate water over the annual cycle studied. Tachysoma pellionella was most abundant during the spring, a phenomenon also recorded by Wilbert (1969) and Cinetochilum margaritaceum was most abundant during the summer, when the dissolved oxygen content of the filtrate was low ranging from 0.5 to 6 mg l^{-1} (Fig. 5.4a), the optimum dissolved oxygen conditions for this ciliate according to Bick and Kunze. The large carnivore, Stentor polymorphus, was found to be characteristic of the late spring to early autumn runs. Finlay et al (1979) noted a similar periodicity of large ciliates in the benthos of Loch Airthrey, a eutrophic loch. Lacrymaria olor, a large carnivore, was also most abundant from spring to autumn, and was relatively infrequent at the lower temperatures in winter.

The depth distribution of protozoa was also found to vary seasonally, higher production of organic detritus in the summer apparently providing a greater population of bacteria at the sand surface for grazers, e.g. C. margaritaceum and also for filter feeders, e.g. Vorticella spp. and the flagellates. However, algal feeders such as Chilodonella sp. and T. pellionella were found at the sand surface throughout the year.

A further seasonal effect was recorded relating temperature to individual ciliate cell size. For most species, decreases in cell volume occurred with an increase in temperature from 4^{o}C to 12^{o}C (Fig. 8.1), corresponding

with a gain in their instantaneous rate of numerical increase between these temperatures (Fig. 8.10) but thereafter the individual cell size increased with further rises in temperature to 20°C. Between 12°C and 20°C this size increase was also accompanied by a rise in instantaneous growth rate. However, Aspidisca costata decreased in cell size with increasing temperature between 4°C and 20°C, the effect being greater above 12°C, so this species was not able to grow in cell size simultaneously with an increasing rate of numerical growth at the higher temperatures.

The depth distributions of the ciliates examined during this study were generally of the pattern described for marine sands (Fenchel, 1966), with maximum densities occurring at the surface and decreasing rapidly in the top few centimetres. However, this could not be related to an oxygen gradient from a surface oxidised zone to a redox discontinuity layer and then to an anaerobic zone, as was the parameter found to be responsible for the vertical distribution of ciliates in marine sand (Fenchel, 1967; Fenchel, 1969). The sand filter water remained oxidised throughout the year with the exception of a few summer nights when the dissolved oxygen level fell to zero. With the exclusion of these and anaerobic microzones which apparently occurred at the surface of some sand grains sheltered from the main water flow (Plate 9.3), no de-oxygenation occurred throughout the filter bed. The anaerobic microzones were implied from the presence of Sphaerotilus sp. and Leptothrix sp. on some of the sand grain surfaces examined by scanning electron microscopy. Studies on the model filter column demonstrated an increase in redox potential with sand depth

over the first 2 to 3 centimetres (Fig. 9.8), below which it remained fairly steady and high, at about 500 mV down to 30 centimetres, the lowest depth examined. This type of depth profile was first recorded by Lodge (1979) in the model filter column. The depth distribution of particulate organic carbon was very similar to that of the ciliates, with accumulation mainly occurring at the surface, the concentration decreasing with depth to 10 cm, below which it remained fairly constant. This would suggest that the ciliate distribution was, not unexpectedly, dependent on food availability assuming that particulate organic carbon is representative of this. Maximum densities of bacteria associated with the sand grains were detected at 10 cm just after the start of a run, but by the tenth day they had moved to the surface, the vertical distribution following that described above for carbon. It would appear that bacterial development was initially restricted, possibly by predation, in the top few centimetres, but once the bacterial population became established, the higher density of floc at the surface naturally encouraging the development of a high population of decomposer bacteria, the population of predator ciliates began to develop more strongly. This association of higher bacterial and protozoan activity at the surface also correlates with a higher consumption rate of oxygen per gm carbon recorded by Lodge (1979) at the sand surface relative to that below 5 cm. She recorded consumption rates of 25 and 1.25 mg O_2 $g^{-1}C$ h^{-1} respectively for these two depth zones.

The localised distribution of bacteria on the sand grains was examined more closely using scanning electron

microscopy (Plates 9.1 to 9.3). Sheltered areas on the grain surfaces were found to favour colonisation as has also been reported by Meadows and Anderson (1966) and Weise and Rheinheimer (1978), while Hossell and Baker (1977) found a similar selection of sites for colonisation on submerged macrophytes. In a 28 day old core, short rod bacteria were found to predominate at the surface, being accompanied in the deeper sand by larger, attached and filamentous forms including Caulobacter sp. and Sphaerotilus sp. At depths of 20 to 25 cm matrices of fibrous appearance were observed. These have been described by Costerton et al (1978) and have also been noted by Corpe (1970), Geesey et al (1977) and Weise and Rheinheimer (1978) as means of facilitating both surface attachment for the bacteria and also microcolony development. The morphology of the short rods found near the surface, make them a far more suitable food source for protozoa than does that of the filamentous bacteria, and this may partially account for the noted depth distribution of the ciliates and flagellates. Güde (1979) similarly reported a reduction of zooflagellates which coincided with a succession from single celled bacteria to spiral shaped and filamentous forms, in an activated sludge culture. The interstitial bacteria, monitored in the model filter column were all of the short rod form and they demonstrated a very similar depth distribution to that of the sand associated populations, with the population maximum rising from 5 cm to the surface by the ninth day of a run. The highest densities were however recorded on day 5, the subsequent decrease coinciding with increased predation by the development of the protozoan population, both bacteria and ciliates finally accumulating at the sand surface.

One might have expected ciliates at the surface to have had larger individual cell sizes than those found at the lower depths. However this did not occur, indeed most cell sizes recorded in this study were lower than those recorded in the literature (Table 6.1 Page 123) possibly due to limitations imposed by the rate of replication and the relatively poor nutrient levels available in slow sand filter beds when compared with those of laboratory cultures or activated sludge aeration tanks, from which the comparitive cell size data were obtained.

Figure 10.2 is a composite diagram of the depth distributions of ciliates, carbon and bacteria from runs during similar periods of the year, in order to show the interrelationships of these three within the sand filter beds, as described above.

The instantaneous rates of numerical increase, from the initial low ciliate populations of the core sand placed into the filter bed, to the maximum densities achieved, was examined in relation to a number of factors. Maximum integral densities of between 4,100 and 5,050 individuals were regularly recorded during each run on bed 45, however the rate of numerical increase to achieve this maximum density, increased with temperature, and consequently the period of exponential growth decreased with increasing temperature. The rate of numerical increase of the ciliate population as a whole ranged from 18% per day at $4^{o}C$ to 36% per day at $20^{o}C$. The corresponding Q_{10} was calculated to be 1.55 between 4^{o} and $20^{o}C$. This value is similar to that of 1.5 recorded for Tetrahymena sp. under culture conditions,

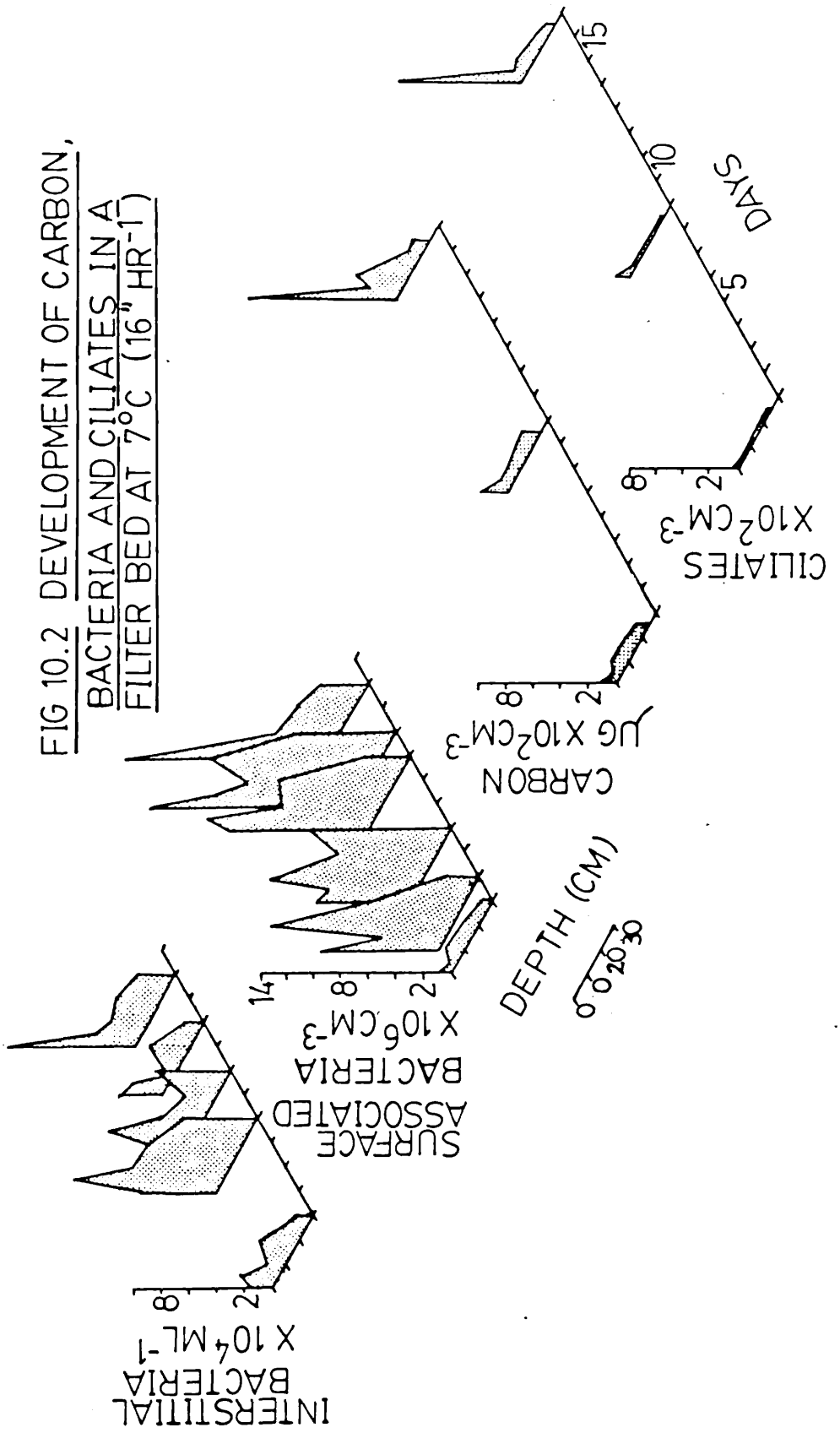


FIG 10.2 DEVELOPMENT OF CARBON, BACTERIA AND CILIATES IN A FILTER BED AT 7°C (16° HR^{-1})

between 20°C and 28.6°C (Phelps, 1946) but is much lower than those recorded in the literature for a similar temperature range to that of the present study: 2.5 and 2.3 for Uronema sp. and Litonotus sp. (Fenchel, 1968) and 2.9 for Tetrahymena sp. (Phelps, 1946). However these values obtained under well-fed culture conditions are not strictly comparable with field studies as the former provides ideal conditions for growth with no other limitations to the rate of growth than that of temperature.

In general, the flagellates replicated at a slower rate, achieving a maximum of 24% per day at 20°C. Replication rates of different ciliate species varied: Vorticella spp., attached peritrichs, had a generally slow replication rate of around 8% per day, while the spirotrich Tachysoma pellionella had a maximum growth rate of 58% per day. Vorticella spp., found mainly at the filter bed surface, did not reach a peak in density during any run, but maintained their slow rate of replication throughout each run. During the winter run at 4°C, the rates of numerical increase were low for the flagellates and for all the ciliate species. Although the ciliate population as a whole did not achieve a peak density during this run, some species, e.g. Glaucoma sp. and T. pellionella did so, after 32 and 19 days respectively.

Fenchel (1968) found that in pure culture systems, small ciliates had a lower doubling time and hence a greater rate of replication than the larger ciliates. No similar relationship was found during the present study, possibly due to the relatively low nutrient levels of the sand filter system. Indeed the doubling time of the

individual ciliate species ranged from 29 hours for T. pellionella (6,150 μm^3) to 308 hours for Glaucoma sp. (835 μm^3). These values span the whole range reported in the literature for ciliates (Table 8.5 Page 190) except for the very short doubling times achieved under certain culture conditions (Legner et al, 1976; Taylor, 1978).

The rate of flow of water passing through the filter bed was found to have a considerable effect on the biology of the bed. During the filtration run on bed 44 (October/ November, 1976), the flow rate was half that of bed 45: 8" h^{-1} relative to 16" h^{-1} in the latter. As mentioned earlier, the rates of carbon accumulation and head loss gain were much lower in bed 44 than in bed 45 which was monitored simultaneously with this run. An effect on the vertical distribution of the ciliates was also noted, with few individuals occurring below the 10 cm depth in bed 44 and peak densities of most species being located in the surface sand of the bed. The higher flow rate on bed 45 resulted in a deeper penetration of the ciliates and at relatively high densities down to 25 cm, the lowest depth regularly examined in the sample cores. This effect may have been purely one of the increased rate of flow physically transporting the ciliates further into bed 45 than they were transported in bed 44, or it may be that populations were able to survive and replicate deeper in the sand of bed 45 due to a greater supply of nutrients and bacteria and/or dissolved oxygen in the higher flow rate of water. No oxygen data were available for the top water and filtrate of bed 44 during the run examined, however the particulate organic carbon content, measured as an indication of nutrient

status, in the sand below 10 cm was no less than that recorded in bed 45 over the same period of time.

In general, no variation in ciliate cell size was noted between the fast and slow flow rates. However, two species: Cyclidium heptatrichum and Aspidisca costata did vary considerably, the former was smaller and the latter larger in bed 44 than in bed 45. C. heptatrichum feeds on bacteria which it filters from the water using a large undulating membrane. It would consequently ingest fewer bacteria over the same period of time in the slower flow of water than in the faster flow assuming that the bacterial density of the influent water was the same in both beds. This is a reasonable assumption, as both beds were fed from the same water source, although the greater retention time of the top water in bed 44 may have had some effect on its bacterial population. A. costata however, grazes on detrital flocs, dashing from one to another using its ventral cirri as 'legs' to propel it. The slower flow rate would enable it to move and graze more easily without the risk of being swept away.

The flow rate also affected the instantaneous rate of numerical increase of both ciliate and flagellate populations. At the slower flow rate, they developed at a rate generally 10% lower than in the fast rate bed and reached maximum densities about 2 weeks later, these densities being on average 60% and 50% of those recorded in bed 45 for the ciliates and flagellates respectively. The peak ciliate density achieved was 4,475 per cm^2 which lies within the range of maxima achieved throughout the

year on bed 45, except for run 6 which was unusual (Fig. 10.1) yielding a maximum of about 9,000 ciliates per cm^2 of core sample surface area.

Filter-feeding ciliates achieved much lower densities in bed 44 than the grazers, probably due to the difficulty of obtaining suspended bacteria in sufficient quantities.

Suggested Future Work

A useful continuation of this study would be to examine in more detail the development of both surface-associated and interstitial bacteria in the sand filter environment, extending the period of examination passed the time at which maximum protozoan densities are recorded. Examination of algal and in particular diatom development in relation to the late colonisation of omnivorous ciliates in the filter beds may also be of considerable interest.

Further examination of the oxygen balance between the top water and filtrate could also prove very useful, particularly if in association with this the respiratory oxygen demand of the component microfauna were also determined.

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Appendix 4.1 The numbers per cm^3 of ciliates at 10 cm depth in ten 14-day old cores collected on different dates from bed 45 at Hampton

Age of bed when core extracted (days)		14		21		23		26		28	
Date when core was 14 days old		1.6.77.		8.6.77.		10.6.77.		13.6.77.		15.6.77	
SPECIES \ CORE / REP		A	B	C	D	E	F	G	H	I	J
Glaucoma sp.	1	-	-	1	57	2	5	1	1	1	-
	2	-	-	-	41	-	4	1	-	1	-
	3	-	-	4	12	-	1	1	-	-	-
Cyclidium heptatrichum	1	72	22	42	8	18	1	5	11	49	-
	2	36	29	79	2	20	7	5	13	7	-
	3	70	23	42	3	18	5	6	8	19	-
Cinetochilum margaritaceum	1	142	74	75	62	90	107	28	41	51	1
	2	191	118	78	64	93	114	44	58	45	1
	3	114	73	58	50	148	141	52	17	47	-
Pleuronema sp.	1	4	-	15	-	1	1	-	5	1	-
	2	2	4	15	-	1	1	-	2	-	-
	3	8	6	8	1	-	1	-	5	-	-
Lacrymaria olor	1	2	-	1	-	1	1	14	1	-	-
	2	-	-	1	1	-	1	12	2	1	-
	3	1	-	-	-	-	3	8	6	-	-
Chilodonella sp.	1	11	3	1	1	1	4	-	-	-	-
	2	7	3	6	4	3	10	-	-	1	-
	3	7	5	3	-	8	9	-	1	-	2
Litonotus sp.	1	14	2	16	4	6	7	14	2	1	-
	2	18	5	3	1	1	2	11	5	7	-
	3	13	5	1	-	4	4	10	3	1	-
Tachysoma pellionella	1	306	38	109	45	62	27	24	12	34	-
	2	170	74	88	46	28	28	57	16	12	-
	3	259	60	74	32	52	30	44	8	28	-
Aspidisca costata	1	26	77	21	4	1	10	8	8	1	-
	2	5	19	4	3	4	17	5	8	-	-
	3	9	5	10	-	3	19	14	10	-	-
Oxytricha spp.	1	31	11	8	6	12	11	13	21	1	-
	2	13	17	9	8	3	5	16	11	2	-
	3	33	12	2	3	5	15	29	11	2	-
Stentor polymorphus	1	-	-	-	-	-	-	3	1	-	-
	2	-	-	-	-	-	-	1	1	-	-
	3	-	-	-	-	-	1	2	2	-	-
Vorticella convallaria	1	9	1	48	6	24	13	73	61	15	-
	2	2	3	28	33	11	28	47	62	4	-
	3	7	2	51	10	18	38	82	48	19	-
Others	1	25	1	11	2	11	6	26	27	6	-
	2	15	2	12	3	3	6	46	51	13	-
	3	25	3	15	1	7	12	34	27	5	-
Total Ciliates	1	642	159	348	195	229	193	209	191	160	1
	2	459	274	323	206	167	223	245	229	93	1
	3	546	194	268	112	263	279	282	146	121	2

Appendix 4.2 Analysis of Variance tables for transformed replication test data

1. Analysis of Variance of 5 dates with 6 replicates (A/B to I/J) of total ciliate counts

Source of Variation	df	SS	MS	F	p
Between dates	4	410.859	102.715	12.77	0.001
Within dates	5	113.321	22.664	2.82	0.05
Residual	20	160.870	8.044		
Total	29	685.051			

2. Analysis of Variance of 4 dates with 6 replicates (A/B to G/H)

(i) Total Ciliates

Source of Variation	df	SS	MS	F	p
Between dates	3	50.541	16.847	2.34	0.25
Within dates	5	42.588	8.518	1.18	0.5
Residual	15	108.038	7.203		
Total	23	201.116			

(ii) Cyclidium

Source of Variation	df	SS	MS	F	p
Between dates	3	0.088	0.030	4.28	0.025
Within dates	5	0.068	0.014	1.98	0.25
Residual	15	1.034	0.007		
Total	23	0.260			

(iii) Oxytricha

Source of Variation	df	SS	MS	F	p
Between dates	3	1.586	0.529	6.26	0.01
Within dates	5	0.186	0.037	0.44	0.75
Residual	15	1.267	0.085		
Total	23	3.039			

(iv) Aspidisca

Source of Variation	df	SS	MS	F	p
Between dates	3	1.168	0.389	0.82	0.75
Within dates	5	1.220	0.244	0.51	0.75
Residual	15	7.113	0.474		
Total	23	9.501			

Appendix 4.2 (contd)

(v) Vorticella

Source of Variation	df	SS	MS	F	p
Between dates	3	13.334	4.445	36.61	0.001
Within dates	5	1.933	0.387	3.18	0.05
Residual	15	1.821	0.121		
Total	23	17.089			

3. Analysis of Variance of 8 cores with 3 replicates (A to H) of total ciliate counts

Source of Variation	df	SS	MS	F	p
Between cores	7	168.435	24.062	10.442	0.001
Within cores	2	0.414	0.207	0.90	0.75
Residual	14	32.261	2.304		
Total	23	201.110			

4. Analysis of Variance of 7 cores with 3 replicates (B to H)

(i) Total Ciliates

Source of Variation	df	SS	MS	F	p
Between cores	6	30.723	5.120	2.413	0.1
Within cores	2	1.723	0.861	0.406	0.75
Residual	12	25.468	2.122		
Total	20	57.913			

(ii) Cyclidium

Source of Variation	df	SS	MS	F	p
Between cores	6	0.002	0.001	0.492	0.75
Within cores	2	0.165	0.028	13.674	0.001
Residual	12	0.024	0.002		
Total	20	0.191			

(iii) Oxytricha

Source of Variation	df	SS	MS	F	p
Between cores	2	0.072	0.036	0.505	0.75
Within cores	6	1.157	0.193	2.722	0.10
Residual	12	0.850	0.071		
Total	20	2.079			

Appendix 4.2 (contd)

(iv) Aspidisca

Source of Variation	df	SS	MS	F	p
Between cores	2	0.087	0.044	0.157	0.75
Within cores	6	4.849	0.808	2.910	0.1
Residual	12	3.333	0.278		
Total	20	8.269			

(v) Vorticella

Source of Variation	df	SS	MS	F	p
Between cores	2	0.089	0.045	0.306	0.75
Within cores	6	12.401	2.067	14.199	0.001
Residual	12	1.747	0.146		
Total	20	14.237			

Appendix 4.3 Concentration of carbon (μgcm^{-3}) at a depth of 10 cm in ten 14-day old cores collected on different dates from Bed 45 at Hampton

Age of bed when core extracted		14		21		23		26		28	
Date when core was 14 days old		1.6.77.		8.6.77.		10.6.77.		13.6.77.		15.6.77.	
CORE		A	B	C	D	E	F	G	H	I	J
CARBON	REP										
gcm^{-3}	1	148	130	204	81	173	192	192	208	192	139
	2	138	168	204	108	263	133	223	248	194	165
	3	156	140	167	155	152	198	156	219	127	188

Appendix 4.4 Concentration of carbon transformed using ln x transformation

CORE		A	B	C	D	E	F	G	H	I	J
ln Carbon	1	4.997	4.868	5.318	4.394	5.153	5.257	5.257	5.338	5.257	4.934
	2	4.927	5.124	5.318	4.682	5.572	4.890	5.407	5.513	5.268	5.106
	3	5.050	4.942	5.118	5.043	5.024	5.288	5.050	5.389	4.844	5.236

Appendix 4.5 ANOVA table for particulate organic carbon data

(a) Sampling Days (5 dates with 6 replicates)

Source of Variation	df	SS	MS	F	p
Within Dates	5	0.4060	0.0812	1.8256	0.25
Between Dates	4	0.5200	0.1300	2.9227	0.05
Residual	20	0.8896	0.0445		
Total	29	1.8157			

(b) Cores (10 cores with 3 replicates)

Source of Variation	df	SS	MS	F	p
Within cores	2	0.0597	0.0299	0.7399	0.5
Between cores	9	1.0298	0.1144	2.8364	0.05
Residual	18	0.7262	0.0403		
Total	29	1.8157			

Appendix 4.6 Fortran IV programme for calculating carbon concentration (μg C cm^{-3}) in sand from wet dichromate oxidation titration data

```
      PROGRAM REG (INPUT, OUTPUT, TAPE 1 = INPUT,
                                  TAPE 3 = OUTPUT)
      DIMENSION X (10)
      DIMENSION Y (10)
      DIMENSION AJUNK (6)

      PROGRAM FOR REGRESSION OF CARBON DATA

      READ (1,100 ) NREG
      FORMAT (I3)

      DO 500 J = 1,6

      WRITE (3,200)J
      FORMAT (24H REGRESSION PROBLEM NO. ,14,/)
      READ (1,300) NPR
      FORMAT (I3)

      READ (1,400) (X(I),I = 1,NPR)
      READ (1,400) (Y(I),I = 1,NPR)
      FORMAT (10F8.3)

      WRITE (3,401) (X(I),I = 1,NPR)
      FORMAT (5H X = ,10F8.3)
      WRITE (3,402) (Y(I),I = 1,NPR)
      FORMAT (5H Y = ,10F8.3)

      SUMX = 0
      DO2 I = 1,NPR
      SUMX = SUMX + X(I)
      CONTINUE

      XMEAN = SUMX/NPR

      SUMY = 0
      DO3 I = 1,NPR
      SUMY = SUMY + Y(I)
      CONTINUE

      YMEAN = SUMY/NPR

      SUMXY = 0
      DO4I = 1,NPR
      Z = X(I) *Y(I)
      SUMXY = SUMXY + Z
      CONTINUE

      SUMXX = 0
      DO5I = 1,NPR
      W = X(I)**2
      SUMXX = SUMXX + W
      CONTINUE
```

Appendix 4.6 (Contd)

```
SUMYY = 0
DO6I = 1,NPR
V = Y(I)**2
SUMYY = SUMYY + V
CONTINUE

SLOPE = (SUMXY-(SUMX*SUMY/NPR)/(SUMXX-((SUMX*SUMX)/NPR))

YINTCP = YMEAN-(SLOPE*XMEAN)

RXY = REGRESSION XY = (X-XMEAN)*(Y-YMEAN)
SUMRXY = 0
DO 41 I = 1,NPR
U = (X(I)-XMEAN)*(Y(I)-YMEAN)
SUMRXY = SUMRXY + U
CONTINUE
RXX = REGRESSION X SQUARED = (X-XMEAN)**2
SUMPXX = 0
DO 42 I = 1,NPR
T = (X(I)-XMEAN)**2
SUMRXX = SUMRXX + T
CONTINUE
RYY = REGRESSION Y SQUARED = (Y-YMEAN)**2
SUMRYY = 0
DO 43 I = 1,NPR
S = (Y(I)-YMEAN)**2
SUMRYY = SUMRYY + S
CONTINUE
SUMYBSQ= SUM Y BAR SQUARED
SUMYBSQ = (SUMRXY**2)/SUMRXX
WRITE (3,30) SUMYBSQ
FORMAT (35H EXPLAINED (REGRESSION) VARIANCE = ,F10.6)
SUMDYX = SUM OF D**2YX
SUMDYX = SUMRYY-SUMYBSQ
UNEVAR = UNEXPLAINED VARIANCE
UNEVAR = SUMDYX/(NPR-2)
WRITE (3,31) SUMDYX
FORMAT (21H UNEXPLAINED ERROR = ,F10.6)
WRITE (3,32) UNEVAR
FORMAT (32H UNEXPLAINED (ERROR) VARIANCE = ,F10.6)
TOTVAR = TOTAL VARIANCE
TOTVAR = SUMRYY/(NPR-1)
WRITE (3,33) TOTVAR
FORMAT (18H TOTAL VARIANCE = ,F10.6)
VARRAT = VARIANCE RATIO
VARRAT = SUMYBSQ/UNEVAR
WRITE (3,34) VARRAT
FORMAT (18H VARIANCE RATIO = ,F15.6)

DEGFRE = 1
BEGFRE = (NPR-2)
WRITE (3,35) DEGFRE
FORMAT (25H TOP DEGREE OF FREEDOM = ,F3.0)
WRITE (3,36) BEGFRE
FORMAT (26H SIDE DEGREE OF FREEDOM = ,F3.0)
```

Appendix 4.6 (Contd)

```
      RSQD = ((SUMXY-((SUMX*SUMY)/NPR))*(SUMXY-((SUMX*SUMY)/
                                              NPR)))/((
     CSUMXX-((SUMX*SUMX)/NPR))*(SUMYY-((SUMY*SUMY)/NPR)))

      XINTCP = (-YINTCP)/SLOPE

      WRITE (3,7)XMEAN,YMEAN
      FORMAT (9H XMEAN = ,F20.6,/,9H YMEAN = ,F10.6)

      WRITE (3,8)XINTCP
      FORMAT (23H INTERCEPT ON X-AXIS = ,F20.6)

      WRITE (3,9)YINTCP
      FORMAT (23H INTERCEPT ON Y-AXIS = ,F20.6)

      WRITE (3,10) SLOPE
      FORMAT (9H SLOPE = ,F10.6)
      WRITE (3,11) RSQD
      FORMAT (36H COEFF. OF REGRESSION (R-SQUARED) = ,F10.6)

      AJUNK(J) = XINTCP
      CONTINUE
      A = AJUNK(1)
      B = AJUNK (2)
      C = AJUNK (3)
      D = AJUNK (4)
      E = AJUNK (5)
      F = AJUNK (6)

      WRITE (3,435) A,B,C,D,E,F
      FORMAT (5H A = ,F12.6,5H B = ,F12.6,5H C = ,F12.6,5H D
                                              = ,F12.6,
     C/,5H E = ,F12.6,5H F = ,F12.6)

      READ (1,450) PDICHN
      FORMAT (F10.6)
      WRITE (3,451) PDICHN
      FORMAT (27H NORMALITY OF DICHROMATE = ,F10.6)
      ENDPT = (A+B+C)/3.0

      WRITE (3,440) ENDPT
      FORMAT (26H AVE. CONTROL END-POINT = ,F10.4)

      RFAS = (1000.0*PDICHN)/ENDPT
      WRITE (3,452) RFAS
      FORMAT (42H NORMALITY OF FERROUS AMMONIUM SULPHATE =
                                              ,F20.19)
      VBLANK = (D+E+F)/3.0
      WRITE (3,453)VBLANK
      FORMAT (29H VOLUME OF BLANK (MICRO-L) = ,F10.4)

      DO 600 J = 7,NREG

      WRITE (3,550)J
      FORMAT (24 H REGRESSION PROBLEM NO. ,14,/)
      READ (1,555) NPR
      FORMAT (I3)
```

Appendix 4.6 (contd)

```
      READ (1,560) (X(I),I = 1,NPR)
      READ (1,560) (Y(I),I = 1,NPR)
      FORMAT (10F8.3)

      WRITE (3,565) (X(I),I = 1,NPR)
      FORMAT (5H X = ,10F8.3)
      WRITE (3,570) (Y(I),I = 1,NPR)
      FORMAT (5H Y = ,10F8.3)

      SUMX = 0
      DO12I = 1,NPR
      SUMX = SUMX+X(I)
      CONTINUE

      XMEAN = SUMX/NPR

      SUMY = 0
      DO13 I = 1,NPR
      SUMY = SUMY + Y(I)
      CONTINUE

      YMEAN = SUMY/NPR

      SUMXY = 0
      DO 14I = 1,NPR
      Z = X(I)*Y(I)
      SUMXY = SUMXY + Z
      CONTINUE

      SUMXX = 0
      DO 15 I = 1,NPR
      W = X(I)**2
      SUMXX = SUMXX + W
      CONTINUE

      SUMYY = 0
      DO 16 I = 1,NPR
      V = Y(I)**2
      SUMYY = SUMYY + V
      CONTINUE

      SLOPE = (SUMXY-(SUMX*SUMY/NPR))/(SUMXX-((SUMX*SUMX)/
                                                    NPR))

      YINTCP = YMEAN - (SLOPE*XMEAN)

      RXY = REGRESSION XY= (X-XMEAN)*(Y-YMEAN)
      SUMRXY = 0
      DO 81 I = 1,NPR
      U = (X(I)-XMEAN)*(Y(I)-YMEAN)
      SUMRXY = SUMRXY + U
      CONTINUE
      RXX = REGRESSION X SQUARED = (X-XMEAN)**2
      SUMRXX = 0
      DO 82 I = 1,NPR
      T = (X(I)-XMEAN)**2
      SUMRXX=SUMRXX + T
      CONTINUE
      RYY = REGRESSION Y SQUARED = (Y-YMEAN)**2
      SUMRYY = 0
      DO 83 I = 1,NPR
```

Appendix 4.6 (contd)

```
      S = (Y(I)-YMEAN)**2
      SUMRYY = SUMRYY + S
      CONTINUE
      SUMYBSQ = SUM Y BAR SQUARED
      SUMYBSQ = (SUMRXY**2)/SUMRXX
      WRITE (3,60) SUMYBSQ
      FORMAT (35H EXPLAINED (REGRESSION) VARIANCE = ,F10.6)
      SUMDYX = SUM OF D**2YX
      SUMDYX = SUMRYY-SUMYBSQ
      UNEVAR = UNEXPLAINED VARIANCE
      UNEVAR = SUMDYX/NPR-2)
      WRITE (3,61) SUMDYX
      FORMAT (21H UNEXPLAINED ERROR = ,F10.6)
      WRITE (3,62) UNEVAR
      FORMAT (32H UNEXPLAINED (ERROR) VARIANCE = ,F10.6)
      TOTVAR = TOTAL VARIANCE
      TOTVAR = SUMRYY/(NPR-1)
      WRITE (3,63) TOTVAR
      FORMAT (18H TOTAL VARIANCE = ,F10.6)
      VARRAT = VARIANCE RATIO
      VARRAT = SUMYBSQ/UNEVAR
      WRITE (3,64) VARRAT
      FORMAT (18H VARIANCE RATIO = ,F15.6)

      DEGFRE = 1
      BEGFRE = (NPR-2)
      WRITE (3,65) DEGFRE
      FORMAT (25H TOP DEGREE OF FREEDOM = ,F3.0)
      WRITE (3,66) BEGFRE
      FORMAT (26H SIDE DEGREE OF FREEDOM = ,F3.0)

      RSQD = ((SUMXY-((SUMX*SUMY)/NPR))*(SUMXY-((SUMX*SUMY)/
                                                NPR)))/((
     CSUMXX-((SUMX*SUMX)/NPR))*(SUMYY-((SUMY*SUMY)/NPR)))

      XINTCP = (-YINTCP)/SLOPE
      WRITE (3,17) XMEAN, YMEAN
      FORMAT (9H XMEAN = ,F20.6,/,9H YMEAN = ,F10.6)

      WRITE (3,18)XINTCP
      FORMAT (23H INTERCEPT ON X-AXIS = ,F20.6)

      WRITE (3,19) YINTCP
      FORMAT (23H INTERCEPT ON Y-AXIS = ,F20.6)

      WRITE (3,20) SLOPE
      FORMAT (9H SLOPE = ,F10.6)
      WRITE (3,21) RSQD
      FORMAT (36H COEFF. OF REGRESSION (R-SQUARED) = ,F10.6)

      CARBON = (VBLANK-XINTCP)*RFAS*15.0145
      WRITE (3,498) CARBON
      FORMAT (34H CARBON (MICRO-G PER CUBIC CM.) = ,F20.6,//)

      CONTINUE

      STOP

      END.
```

Appendix 4.6 (Contd) Regression problems 1 to 3 and 4 to 6 determine 'control' and 'blank' end-points respectively

EXAMPLE

```
REGRESSION PROBLEM NO. 1

X = 1702.0  1705.0 1708.0  1711.0 1713.0
Y =     .260    .495   .710    .935  1.050
EXPLAINED (REGRESSION) VARIANCE = .412310
UNEXPLAINED ERROR = .000640
UNEXPLAINED (ERROR) VARIANCE = .000213
TOTAL VARIANCE = .103237
VARIANCE RATIO = 1931.629013
TOP DEGREE OF FREEDOM = 1.
SIDE DEGREE OF FREEDOM = 3.
XMEAN = 1707.800000
YMEAN = .690000
INTERCEPT ON X-AXIS = 1698.261053
INTERCEPT ON Y-AXIS = -122.843756
SLOPE = .072335
COEFF. OF REGRESSION (R-SQUARED) = .998449


REGRESSION PROBLEM NO. 2

X = 1699.0  1702.0  1705.0  1707.0  1710.0
Y =     .210    .550    .845    .995   1.140
EXPLAINED (REGRESSION) VARIANCE = .538090
UNEXPLAINED ERROR = .014640
UNEXPLAINED (ERROR) VARIANCE = .004880
TOTAL VARIANCE = .138182
VARIANCE RATIO = 110.263076
TOP DEGREE OF FREEDOM = 1.
SIDE DEGREE OF FREEDOM = 3.
XMEAN = 1704.600000
YMEAN = .748000
INTERCEPT ON X-AXIS = 1695.875717
INTERCEPT ON Y-AXIS = -145.400492
SLOPE = .085738
COEFF. OF REGRESSION (R-SQUARED) = .973513


REGRESSION PROBLEM NO. 3

X = 1707.0  1708.0  1710.0  1712.0  1714.0
Y =     .510    .620    .785    .955   1.095
EXPLAINED (REGRESSION) VARIANCE = .226723
UNEXPLAINED ERROR = .000807
UNEXPLAINED (ERROR) VARIANCE = .000269
TOTAL VARIANCE = .056882
VARIANCE RATIO = 843.303232
TOP DEGREE OF FREEDOM = 1.
SIDE DEGREE OF FREEDOM = 3.
XMEAN = 1710.200000
YMEAN = .793000
INTERCEPT ON X-AXIS = 1700.661900
INTERCEPT ON Y-AXIS = -141.393445
SLOPE = .083140
COEFF. OF REGRESSION (R-SQUARED) = .996455
```

Appendix 4.6 (contd)

REGRESSION PROBLEM NO. 4

X = 1616.0 1619.0 1621.0 1624.0 1626.0
Y = .285 .615 .785 1.035 1.165
EXPLAINED (REGRESSION) VARIANCE = .481338
UNEXPLAINED ERROR = .004142
UNEXPLAINED (ERROR) VARIANCE = .001381
TOTAL VARIANCE = .121370
VARIANCE RATIO = 348.596955
TOP DEGREE OF FREEDOM = 1.
SIDE DEGREE OF FREEDOM = 3.
XMEAN = 1621.200000
YMEAN = .777000
INTERCEPT ON X-AXIS = 1612.324845
INTERCEPT ON Y-AXIS = -141.155446
SLOPE = .087548
COEFF. OF REGRESSION (R-SQUARED) = .991468

REGRESSION PROBLEM NO. 5

X = 1574.0 1577.0 1579.0 1581.0 1583.0
Y = .375 .665 .825 .975 1.115
EXPLAINED (REGRESSION) VARIANCE = .327213
UNEXPLAINED ERROR = .001707
UNEXPLAINED (ERROR) VARIANCE = .000569
TOTAL VARIANCE = .082230
VARIANCE RATIO = 575.216715
TOP DEGREE OF FREEDOM = 1.
SIDE DEGREE OF FREEDOM = 3.
XMEAN = 1578.800000
YMEAN = .791000
INTERCEPT ON X-AXIS = 1569.140140
INTERCEPT ON Y-AXIS = -128.489426
SLOPE = .081885
COEFF. OF REGRESSION (R-SQUARED) = .994812

REGRESSION PROBLEM NO. 6

X = 1613.0 1616.0 1619.0 1621.0 1623.0
Y = .245 .585 .875 1.015 1.160
EXPLAINED (REGRESSION) VARIANCE = .527334
UNEXPLAINED ERROR = .005486
UNEXPLAINED (ERROR) VARIANCE = .001829
TOTAL VARIANCE = .133205
VARIANCE RATIO = 288.387739
TOP DEGREE OF FREEDOM = 1.
SIDE DEGREE OF FREEDOM = 3.
XMEAN = 1618.400000
YMEAN = .776000
INTERCEPT ON X-AXIS = 1609.904729
INTERCEPT ON Y-AXIS = -147.056646
SLOPE = .091345
COEFF. OF REGRESSION (R-SQUARED) = .989704

Appendix 4.6 (contd)

```
A = 1698.261053 B = 1695.875717 C = 1700.661900 D =
                                    1612.324845
E = 1569.140140 F = 1609.904729
NORMALITY OF DICHROMATE = .200000
AVE. CONTROL AND END-POINT = 1698.2662
NORMALITY OF FERROUS AMMONIUM SULPHATE =
                          .1177671658805592436
VOLUME OF BLANK (MICRO-L) = 1597.1232
RESULTS FROM PROCESSED SAND SAMPLES
REGRESSION PROBLEM NO. 7

X = 1516.0  1519.0  1522.0  1524.0  1526.0
Y =     .230    .545    .810    .985   1.145
EXPLAINED (REGRESSION) VARIANCE = .525691
UNEXPLAINED ERROR = .001339
UNEXPLAINED (ERROR) VARIANCE = .000446
TOTAL VARIANCE = .131757
VARIANCE RATIO = 1178.145248
TOP DEGREE OF FREEDOM = 1.
SIDE DEGREE OF FREEDOM = 3.
XMEAN = 1521.400000
YMEAN = .743000
INTERCEPT ON X-AXIS = 1513.253296
INTERCEPT ON Y-AXIS = -138.012532
SLOPE = .091203
COEFF. OF REGRESSION (R-SQUARED) = .997460
CARBON (MICRO-G PER CUBIC CM.) = 148.300099


REGRESSION PROBLEM NO. 8

X = 1522.0  1525.0  1527.0  1530.0  1531.0
Y =     .225    .575    .785   1.025   1.105
EXPLAINED (REGRESSION) VARIANCE = .504600
UNEXPLAINED ERROR = .004280
UNEXPLAINED (ERROR) VARIANCE = .001427
TOTAL VARIANCE = .127220
VARIANCE RATIO = 353.691589
TOP DEGREE OF FREEDOM = 1.
SIDE DEGREE OF FREEDOM = 3.
XMEAN = 1527.000000
YMEAN = .743000
INTERCEPT ON X-AXIS = 1519.313793
INTERCEPT ON Y-AXIS = -146.867000
SLOPE = .096667
COEFF. OF REGRESSION (R-SQUARED) = .991589
CARBON (MICRO-G PER CUBIC CM.) = 137.583837
```

Appendix 5.1 Sampling Programme and Temperature Data

BED 45

RUN NO.	SAMPLING DATE	AGE OF FILTRATION RUN (DAYS)	TEMP (°C)	MEAN TEMP FOR RUN (°C)
1	26.3.76	1	7.5	
	29.3.76	4	7.0	
	1.4.76	7	7.5	7.5
	5.4.76	11	7.5	
2	12.4.76	3	8.75	
	14.4.76	5	8.0	8.75
	21.4.76	12	9.5	
3	29.4.76	1	9.0	
	3.5.76	5	10.0	
	6.5.76	8	12.25	
	10.5.76	12	14.5	12.0
	13.5.76	15	12.0	
	20.5.76	22	13.0	
	26.5.76	28	14.0	
4	14.6.76	3	20.5	
	21.6.76	10	18.0	
	24.6.76	13	20.75	20.0
	6.7.76	25	21.0	
5	16.7.76	1	20.0	
	21.7.76	6	20.0	
	26.7.76	11	19.0	19.0
	28.7.76	13	19.5	
	2.8.76	18	17.5	
6	13.10.76	5		
	20.10.76	12	14.0	
	26.10.76	18	13.0	12.0
	3.11.76	26	11.5	
	17.11.76	40	10.0	
7	17.1.77	3	0.0	
	24.1.77	10	4.0	
	2.2.77	19	4.0	4.0
	15.2.77	32	5.5	
	28.2.77	45	5.5	
8	29.3.77	1	6.0	
	6.4.77	9	7.5	
	14.4.77	17	6.75	7.5
	25.4.77	28	9.5	

Appendix 5.1 (contd)

BED 44

SAMPLING DATE	AGE OF FILTRATION RUN (DAYS)	TEMP (°C)	MEAN TEMP FOR RUN (°C)
11.10.76	3		
18.10.76	10	13.75	12.0
1.11.76	24	12.0	
10.11.76	33	10.5	

Appendix 5.2 Flow Rate and Head Loss Data

(Raw data obtained from Thames Water Authority)

Vf = flow rate (ins hr^{-1})
H = head loss (ins)
Vt = target flow rate (16" hr^{-1}, Bed 45;
8" hr^{-1}, Bed 44)

BED 45

DATE	Vf	H	H/V_f X Vt	DATE	V_f	H	H/V_f X Vt
RUN 1				RUN 3 (Contd)			
30.3.76	8	16	32	19.5.76	12	32	43
31.3.76	10	24	39	20.5.76	12	33	44
1.4.76	9	31	55	21.5.76	11	33	48
2.4.76	17	41	39	22.5.76	13	38	47
3.4.76	17	58	55	23.5.76	13	38	47
4.4.76	16	60	60	24.5.76	12	41	55
5.4.76	8	60	120	25.5.76	11.5	43	60
RUN 2				26.5.76	11	45	65
				27.5.76	11	48	69
10.4.76	4	11	44	28.5.76	11	51	75
11.4.76	9	14	25	29.5.76	11	53	77
12.4.76	10.5	16	24	30.5.76	9	53	95
13.4.76	12.5	21	27	31.5.76	8.5	52	97
14.4.76	12	21	28				
15.4.76	12	24	28	RUN 4			
16.4.76	14	30	35	12.6.76	6	9	24
17.4.76	14	33	37	13.6.76	9.5	15	25
18.4.76	14	30	35	14.6.76	13	16	20
19.4.76	14	30	35	15.6.76	15	20	21
20.4.76	14	29	33	16.6.76	15	20	21
21.4.76	15	34	36	17.6.76	15.5	23	24
22.4.76	14.5	42	47	18.6.76	15.5	24	25
23.4.76	13	57	71	19.6.76	15	24	26
24.4.76	8	60+	120+	20.6.76	15	25	27
				21.6.76	15	26	28
RUN 3				22.6.76	15	26	28
1.5.76	9	17	31	23.6.76	14.5	26	26
2.5.76	12.5	22	28	24.6.76	13	26	32
3.5.76	15	24	25	25.6.76	12.5	26	33
4.5.76	15	25	27	26.6.76	12.5	26	33
5.5.76	15	25	27	27.6.76	11	26	38
6.5.76	15	26	28	28.6.76	10.5	24	37
7.5.76	15	26	28	29.6.76	10	25	40
8.5.76	15	26	28	30.6.76	10.5	25	38
9.5.76	15	26	28	1.7.76	11	26	38
10.5.76	15	27	29	2.7.76	10.5	27	41
11.5.76	15	27	29	3.7.76	11	28	41
12.5.76	14.5	30	33	4.7.76	10.5	28	43
13.5.76	14.5	30	33	5.7.76	11	29	42
14.5.76	14.5	30	33	6.7.76	10.5	30	46
15.5.76	14	33	37	7.7.76	11	31	45
16.5.76	14	33	37	8.7.76	11	31	45
17.5.76	13	32	40				
18.5.76	12	33	44				

Appendix 5.2 (contd)

BED 45

DATE	V_f	H	H/V_f X Vt	DATE	V_f	H	H/V_f X Vt
RUN 5				RUN 6 (Contd)			
15.7.76	4	7	28	27.10.76	16	42	42
16.7.76	4	7	29	28.10.76	16	43	43
17.7.76	9.5	10	18	29.10.76	15.5	43	45
18.7.76	12	15	20	30.10.76	15	45	45
19.7.76	15.5	18	19	31.10.76	14	45	51
20.7.76	15	19	20	1.11.76	12	45	61
21.7.76	15	19	20	2.11.76	12	46	61
22.7.76	15	21	22	3.11.76	11	51	74
23.7.76	15	21	22	4.11.76	12	50	67
24.7.76	15	22	23	5.11.76	11.5	50	69
25.7.76	14.5	22	24	6.11.76	12	51	69
26.7.76	14.5	23	25	7.11.76	12.5	51	66
27.7.76	14	23	26	8.11.76	10	51	82
28.7.76	14	24	27	9.11.76	9	52	93
29.7.76	14	26	30	10.11.76	9.5	54	91
30.7.76	14	27	31	11.11.76	9.5	53	90
31.7.76	14	28	32	12.11.76	10	56	90
1.8.76	13.5	30	36	13.11.76	10	50	80
2.8.76	13.5	30	36	14.11.76	9.5	57	96
3.8.76	13.5	31	37	15.11.76	9.5	58	101
4.8.76	13	31	38	16.11.76	9	59	106
5.8.76	13	32	39	17.11.76	8.5	60+	114+
6.8.76	13	32	39				
7.8.76	12	30	39	RUN 7			
8.8.76	12.5	33	42	15.1.77	5	35	112
9.8.76	12.5	33	42	16.1.77	9	35	62.4
10.8.76	12	33	44	17.1.77	13.5	36	43.2
11.8.76	12	33	44	18.1.77	16	42	41.6
12.8.76	12	33	44	19.1.77	16	40	40
13.8.76	12.5	33	42	20.1.77	15.5	36	36.8
14.8.76	12.5	38	49	21.1.77	16	37	36.8
RUN 6				22.1.77	16	36	36.8
				23.1.77	16	37	36.8
9.10.76	4	15	61	24.1.77	16	38	38.4
10.10.76	9.5	22	37	25.1.77	16	39	38.4
11.10.76	12	26	35	26.1.77	16	38	38.4
12.10.76	16.5	32	30	27.1.77	15	38	40
13.10.76	16	31	30	28.1.77	15	39	41.6
14.10.76	16	33	34	29.1.77	15	40	43.2
15.10.76	16	33	34	30.1.77	14	40	46.4
16.10.76	16	35	35	31.1.77	14	41	46.4
17.10.76	16	35	35	1.2.77	13	41	51.2
18.10.76	16	36	37	2.2.77	12.5	40	51.2
19.10.76	16	34	34	3.2.77	12.5	40.5	51.2
20.10.76	15	34	37	4.2.77	12.5	38	48
21.10.76	16	38	38	5.2.77	12	39	52.8
23.10.76	16	39	38	6.2.77	12	41.5	56
24.10.76	16	40	40	7.2.77	11	42	60.8
25.10.76	16	41	42	8.2.77	10.5	43	65.6
26.10.76	16	41	42	9.2.77	10.5	43	65.6

Appendix 5.2 (contd)

BED 45

DATE	V_f	H	H/V_f X Vt	DATE	V_f	H	H/V_f X Vt
RUN 7 (contd)				RUN 8 (Contd)			
10.2.77	11	43	62.4	6.4.77	15	47	49.6
11.2.77	10.5	43	65.6	7.4.77	14	45.5	52.8
12.2.77	10.5	43	65.6	8.4.77	14	43	49.6
13.2.77	10.5	43	65.6	9.4.77	13.5	41.5	49.6
15.2.77	10	43	68.8	10.4.77	13.5	41.5	49.6
16.2.77	12.5	44.5	57.6	11.4.77	13.5	41.5	49.6
17.2.77	14	45.5	52.8	12.4.77	13.5	43	51.2
18.2.77	14	47.5	54.4	14.4.77	13.5	45	52.8
19.2.77	14	47.5	54.4	15.4.77	13.5	44	52.8
20.2.77	14	47.5	54.4	16.4.77	13.5	45	52.8
21.2.77	13.5	50	59.2	17.4.77	12.5	44.5	57.6
22.2.77	14	50	57.6	18.4.77	13.5	45	52.8
23.2.77	13.5	50	59.2	19.4.77	13.5	47.5	56.0
24.2.77	14	51	57.6	20.4.77	13.5	52	62.4
25.2.77	12.5	50	64	22.4.77	13	47	57.6
26.2.77	13.5	54	64	23.4.77	12	51	68.8
27.2.77	12	49	65.6	24.4.77	11	51	73.6
28.2.77	12	54.5	72	25.4.77	10.5	52	80
1.3.77	11	51	73.6	26.4.77	10.5	51	78.4
2.3.77	11	50	72	27.4.77	11	52	80
3.3.77	11	49	72	28.4.77	10.5	53	76.8
4.3.77	10.5	49	75.2	29.4.77	10	53.5	81.6
5.3.77	10.5	51	78.4	30.4.77	10	53.5	86.4
7.3.77	10	54.4	88	1.5.77	10	59	94.4+
8.3.77	10	54	86.4	2.5.77	10	60	96+
9.3.77	10	55	88	3.5.77	9.5	60	100.8+
10.3.77	10	60+	96+	4.5.77	9	60+	107.2+
11.3.77	9.5	60+	100.8+	5.5.77	9	60+	107.2+
12.3.77	9.5	60+	100.8+	6.5.77	8.5	60+	113.6+
13.3.77	9.0	60	107.2	7.5.77	7.5	60+	128+
14.3.77	8.5	60+	113.6+	8.5.77	6.5	60+	147.2+
15.3.77	8.5	60+	113.6+	9.5.77	6	60+	160+
16.3.77	8.0	60+	120+	10.5.77	6	60+	160+
17.3.77	8.0	60+	120+	11.5.77	5.5	60+	174.4+
18.3.77	8.0	60+	120+	12.5.77	5	60+	192+
19.3.77	8.0	60	120	13.5.77	5	60+	192+
20.3.77	7.5	60	128	14.5.77	5	60+	192+
21.3.77	7.0	60+	137.6+				
22.3.77	7.0	60+	137.6+				
23.3.77	6.5	60+	147.2+				
24.3.77	5.5	60	174.4				
RUN 8							
29.3.77	5	31	99.2				
31.3.77	7.5	35.5	75.2				
1.4.77	11	36	52.8				
3.4.77	13	40	49.6				
4.4.77	12	39.5	52.8				
5.4.77	11	40	57.6				

Appendix 5.2 (contd)

BED 44

DATE	V_f	H	$H/V_f \times Vt$	DATE	V_f	H	$H/V_f \times Vt$
10.10.76	8.5	6	5.7	30.10.76	8	8	8
11.10.76	8	5	5	31.10.76	6.5	8	9.6
12.10.76	8	5	5	1.11.76	6	8	10.4
13.10.76	8	5	5	2.11.76	7	10	11.2
14.10.76	8	5	5	3.11.76	6	10	13.6
15.10.76	8	5	5	4.11.76	6	11	14.4
16.10.76	8	6	6	5.11.76	6	11	14.4
17.10.76	8	6	6	6.11.76	6	11	14.4
18.10.76	8	6	6	7.11.76	6	11	14.4
19.10.76	8	6	6	8.11.76	6	11	14.4
20.10.76	8	6	6	9.11.76	6	11	14.4
21.10.76	8	6	6	10.11.76	5.5	11	16
23.10.76	8	7	7	11.11.76	6.5	12	14.4
24.10.76	8	7	7	12.11.76	6	12	16
25.10.76	8	7	7	13.11.76	6	12	16
26.10.76	8	7	7	14.11.76	6	13	17.6
27.10.76	8	7	7	15.11.76	5.75	14	19.2
28.10.76	8	7	7	16.11.76	5.5	14	20
29.10.76	8	7	7	17.11.76	6.0	14	18.4

Appendix 5.3 REGRESSION TABLE : RATE OF CHANGE OF HEAD LOSS PER UNIT FLOW WITH TIME

RUN	TIME PERIOD (DAYS)	$\bar{X}$	$\ln\bar{Y}$	SLOPE	S.E. SLOPE	ln INTER-CEPT	VAR RATIO	D.F.	P(VAR. RATIO)
1	5 - 11	8.0	3.96	0.172	0.046	3.266	14.10	5	0.025
2	2 - 12	7.0	3.43	0.042	0.008	3.177	28.57	9	0.001
	12 - 15	13.5	4.12	0.402	0.041	3.115	96.86	2	0.025
3	4 - 13	8.5	3.32	0.010	0.004	3.266	7.81	8	0.025
	14 - 33	23.5	3.93	0.057	0.003	3.337	380.21	18	0.001
4	3 - 15	9	3.26	0.041	0.004	2.975	118.36	11	0.001
	16 - 27	21.5	3.72	0.020	0.003	3.589	51.55	10	0.001
5	2 - 16	9	3.16	0.040	0.002	2.837	390.42	13	0.001
	17 - 30	23.5	3.71	0.020	0.002	3.559	83.53	12	0.001
6	3 - 22	12.4	3.62	0.019	0.002	3.424	125.64	17	0.001
	23 - 40	31.5	4.37	0.038	0.004	4.009	103.69	16	0.001
7	6 - 32	18.5	3.90	0.029	0.009	3.508	543.31	24	0.001
	34 - 68	51.5	4.43	0.030	0.001	3.877	2315.10	33	0.001
8	4 - 14	7.23	3.94	0.0003	0.003	3.940	0.011	11	0.75
	14 - 47	31.2	4.45	0.038	0.002	3.760	386.49	30	0.001
BED 44									
	3 - 22	12.4	1.82	0.024	0.002	1.571	155.21	17	0.001
	26 - 40	33	2.76	0.025	0.004	2.559	50.20	13	0.001

APPENDIX 5.4 DEPTH DISTRIBUTION OF PARTICULATE ORGANIC CARBON CONTENT IN SAND CORES

(Units = μg C cm^{-3} sand)

RUN		3						4				5			
DEPTH (CM) / DAY	1	5	8	12	15	22	28	3	10	13	25	1	6	11	13	18
1		359	194	197	2671	2938	3300	93	113	632	2009	-	270	1061	615	1419
2		122	158	106	281	770	823	76	175	247	346	89	194	512	288	460
3		81	72	219	132	383	682	33	57	269	361	-	112	313	294	231
4		26	156	96	257	395	463	82	148	93	189	96	241	433	185	512
5		93	41	221	167	349	402	19	64	239	242	4	135	1084	292	96
6		27	145	145	235	331	302	35	173	95	40	207	244	329	172	216
7		69	145	178	201	355	265	-	135	261	181	126	127	175	221	49
8		135	115	100	285	303	187	86	176	29	36	177	145	317	101	158
9		-	116	192	180	224	-	97	73	225	130	89	90	168	196	219
10		119	114	-	322	254	206	59	241	81	141	182	172	227	162	116
11		59	41	140	209	198	-	-	119	169	115	-	97	186	231	48
12		-	127	73	308	255	156	68	149	39	7	189	191	249	147	64
13		4	100	93	208	177	177	-	56	145	130	88	29	125	278	46
14		92	114	70	253	238	171	103	129	40	-	295	213	195	13	148
15		12	42	121	121	204	141	44	-	296	70	45	99	120	228	157
16		173	96	22	192	262	66	57	126	91	16	152	209	219	93	-
17		51	57	151	110	176	283	-	-	176	89	14	88	117	191	86
18		-	142	104	161	216	186	101	166	84	95	230	101	285	78	123
19		-	94	159	170	167	205	307	88	204	146	48	64	275	155	299
20		-	85	52	313	217	103	153	161	51	27	155	164	253	46	53
21		-	2	207	142	-	185	-	256	154	137	27	46	123	129	200
22		32	185	135	192	135	138	218	173	48	198	239	104	175	180	145
23		-	47	175	134	241	244	-	95	55	126	86	86	112	163	308
24		-	61	74	121	111	162	145	274	86	52	182	214	265	89	96
25		48	-	198	138	199	277	-	501	148	305	111	142	91	161	246

Appendix 5.4 (Contd)

RUN	DAY / DEPTH (CM)	6				7				8				BED 44			
		5	12	18	26	40	3	10	19	32	45	1	9	17	28	3	10	24	33
1		167	105	649	464	1354	56	124	2723	-	1155	129	198	1077	1467	131	150	395	388
2		148	107	221	523	398	21	-	967	161	237	317	133	189	334	110	409	283	222
3		146	400	216	316	319	28	-	91	174	184	95	117	210	231	77	39	135	214
4		166	186	234	357	412	38	40	103	114	189	123	108	148	168	393	37	157	231
5		-	161	165	320	289	-	52	Contaminated	45	189	67	110	245	216	158	-	173	224
6		164	94	223	219	261	-	-		-	-	-	-	-	-	221	151	218	248
7		-	202	170	299	265	56	8		76	183	124	101	136	128	90	-	155	262
8		76	95	116	210	180	-	-		-	-	-	-	-	-	226	91	217	221
9		100	30	103	196	239	28	-		67	116	143	131	392	240	429	58	213	198
10		38	253	140	129	197	-	-		-	-	-	-	-	-	1057	101	193	203
11		138	127	184	112	252	50	23		87	153	101	98	136	168	288	41	162	137
12		162	150	253	152	241	-	-		-	-	-	-	-	-	77	105	227	124
13		67	131	245	139	199	49	58		117	172	92	74	187	141	165	34	93	146
14		153	120	248	287	251	-	-		-	-	-	-	-	-	50	120	161	94
15		148	110	205	188	262	32	198		117	91	124	153	130	183	240	-	116	137
16		46	198	207	163	145	-	-		-	-	-	-	-	-	100	174	138	162
17		246	118	143	129	137	37	13		135	150	98	119	135	146	135	174	173	226
18		217	131	237	151	148	-	-		-	-	-	-	-	-	213	208	206	154
19		138	204	187	124	89	58	52		137	199	121	146	124	146	316	844	166	159
20		49	272	205	158	94	-	-		-	-	-	-	-	-	178	16	270	102
21		140	150	144	282	71	31	15		178	168	311	162	163	136	194	190	144	109
22		177	166	254	144	111	-	-		-	-	-	-	-	-	199	163	160	108
23		116	150	230	200	63	100	10		161	28	144	97	150	126	223	88	139	127
24		91	13	222	172	88	-	-		-	-	-	-	-	-	153	112	150	62
25		152	166	153	270	112	-	3		180	6	110	154	142	130	232	121	85	105

Appendix 5.5 PARTICULATE ORGANIC CARBON PRESENT IN THE TOP WATER AND FILTRATE WATER OF BED 45 DURING THE STUDY PERIOD

(Data obtained from the Thames Water Authority)

Carbon recorded in $\mu g\ l^{-1}$ water

TOP = Top Water
FIL = Filtrate

DATE	TOP	FIL	DATE	TOP	FIL
3.3.76	176	46	11.10.76	496	132
10.3.76	144	38	18.10.76	293	52
30.3.76	517	48	25.10.76	335	120
13.4.76	650	154	1.11.76	113	52
20.4.76	474	93	8.11.76	142	39
			29.11.76	127	37
4.5.76	195	68			
11.5.76	271	112	6.12.76	172	33
18.5.76	370	47	13.12.76	102	68
25.5.76	518	41	20.12.76	176	60
			29.12.76	169	59
14.6.76	221	120			
21.6.76	223	114	4.1.77	110	75
28.6.76	463	62	17.1.77	120	41
			24.1.77	134	71
20.7.76	438	49			
27.7.76	9.2	16.4	3.2.77	163	73
			7.2.77	160	82
3.8.76	429	29	17.2.77	122	85
10.8.76	717	43	21.2.77	229	46
24.8.76	350	62			
			1.3.77	261	38
6.9.76	406	54			
13.9.76	174	26	18.4.77	201	38
22.9.76	544	182	25.4.77	308	47
27.9.76	429	118			
			2.5.77	223	23
			9.5.77	203	42

Appendix 5.6 REGRESSION TABLE : CHANGE IN PARTICULATE ORGANIC CARBON CONTENT OF SAND WITH DEPTH

RUN	DAY	SLOPE	S.E. SLOPE	INTERCEPT	VAR RATIO	dF	p
3	5	-0.051	0.037	4.591	1.871	15	0.25
	8	-0.049	0.026	5.015	3.610	22	0.10
	12	-0.014	0.015	4.967	0.810	21	0.50
	15	-0.043	0.015	5.911	7.884	23	0.01
	22	-0.065	0.013	6.427	26.064	22	0.001
	28	-0.069	0.017	6.436	16.039	21	0.001
4	3	0.058	0.019	3.710	9.770	16	0.01
	10	0.030	0.014	4.554	4.650	21	0.05
	13	-0.042	0.020	5.333	4.398	23	0.05
	25	-0.049	0.031	5.342	2.514	21	0.25
5	1	0.015	0.033	4.320	0.211	19	0.75
	6	-0.026	0.014	5.176	3.205	23	0.10
	11	-0.058	0.013	6.215	18.970	23	0.001
	13	-0.045	0.019	5.625	5.901	23	0.025
	18	-0.032	0.023	5.450	1.907	22	0.25
6	5	-0.004	0.015	4.843	0.083	22	>0.75
	12	-0.012	0.019	5.032	0.408	23	0.75
	18	-0.008	0.010	5.406	0.723	23	0.5
	26	-0.028	0.020	5.732	7.348	23	0.025
	40	-0.080	0.009	6.290	77.011	23	0.001
7	3	0.023	0.015	3.468	2.237	10	0.25
	10	-0.080	0.041	4.382	3.833	8	0.10
	32	0.024	0.014	4.454	3.073	12	0.25
	45	-0.102	0.027	6.079	14.057	13	0.005
8	1	0.004	0.014	4.802	0.077	13	>0.75
	9	0.001	0.009	4.797	0.026	13	>0.75
	17	-0.037	0.017	5.682	4.946	13	0.05
	28	-0.049	0.017	5.854	8.650	13	0.025
BED 44	3	0.008	0.018	5.104	0.178	23	0.75
	10	0.015	0.026	4.460	0.351	20	0.75
	24	-0.021	0.008	5.420	6.428	23	0.025
	33	-0.045	0.007	5.673	45.679	23	0.001

Appendix 5.7 PARTICULATE ORGANIC CARBON DENSITIES ($mg\ Ccm^{-2}$) OF SAND CORES $1cm^2$ IN SURFACE AREA AND 25 cm DEEP, OBTAINED BY SUMMATION OR INTEGRATION OF VALUES IN APPENDIX 5.4

RUN	DAY	P.O.C. ($mg\ Ccm^{-2}$)	RUN	DAY	P.O.C. ($mg\ Ccm^{-2}$)
3	5	1.25	7	3	0.66
	8	2.75		10	0.59
	12	3.36		*19	3.88
	15	7.50		32	2.75
	22	7.45		45	3.90
	28	7.42			
			8	1	3.33
4	3	2.26		9	2.92
	10	3.64		17	4.62
	13	3.95		28	4.64
	25	4.23			
			BED		
5	1	3.21	44	3	5.56
	6	3.58		10	3.75
	11	7.41		24	4.53
	13	4.71		33	4.36
	18	4.67			
6	5	3.16			
	12	3.84			
	18	5.35			
	26	5.70			
	40	6.18			

* Only the top 4 cm are included as the remaining samples became contaminated.

Appendix 5.8 REGRESSION TABLE : CHANGE IN CARBON DENSITY WITH TIME DURING THE FILTRATION RUNS

RUN	DEPTH (cm)	PERIOD (days)	SLOPE	S.E. SLOPE	ln INTERCEPT	$\bar{X}$	$\ln\bar{Y}$	VAR. RATIO	dF	p (Var. ratio)	S/NS*
3	2 - 10	5 - 28	0.07	0.01	4.11	15.0	5.19	76.28	49	0.001	S
	11 - 25	5 - 15	0.15	0.03	2.90	10.71	4.50	23.22	50	0.001	S
		15 - 28	-0.003	0.009	5.25	21.51	5.19	0.09	41	>0.75	NS
4	2 - 10	3 - 13	0.099	0.03	3.72	8.88	4.60	11.05	24	0.005	S
		13 - 25	0.003	0.03	4.89	19.00	4.95	0.01	16	>0.75	NS
	11 - 25	3 - 25	-0.02	0.01	4.99	13.76	4.65	3.17	49	0.1	NS
5	2 - 10	1 - 11	0.14	0.04	4.27	6.19	5.12	12.88	24	0.005	S
		11 - 18	-0.07	0.04	6.48	14.00	5.44	3.69	25	0.1	NS
	11 - 25	1 - 11	0.06	0.02	4.46	6.11	4.81	5.76	42	0.025	S
		11 - 18	-0.04	0.03	5.53	13.91	4.91	1.83	42	0.25	NS
6	2 - 10	5 - 40	0.03	0.01	4.65	20.55	5.21	20.21	42	0.001	S
	11 - 25	5 - 40	0.003	0.005	4.94	20.20	5.01	0.46	73	0.5	NS
7	2 - 10	3 - 45	0.04	0.01	3.42	23.59	4.38	13.70	20	0.001	S
	11 - 25	3 - 45	0.03	0.01	3.43	23.13	4.08	6.69	29	0.025	S
8	2 - 10	1 - 28	0.023	0.008	4.76	13.75	5.07	8.56	22	0.01	S
	11 - 25	1 - 28	0.007	0.005	4.81	13.75	4.90	2.15	30	0.25	NS
BED 44	2 - 10	3 - 33	0.0095	0.0096	5.00	17.94	5.17	0.98	32	0.5	NS
	11 - 25	3 - 33	-0.004	0.006	5.00	17.63	4.93	0.41	57	0.75	NS

* S = significant change in carbon density over the time period.
NS = insignificant change in carbon density over the time period.

Appendix 5.9 WEIGHT COMPOSITION OF THE SEVEN GRADES OF SAND DETERMINED IN THREE REPLICATE CORES

Core A

Depth (cm) \ Pore diameter of sieve		4.0 mm	2.0 mm	850μm	500μm	250μm	125μm	63μm	TOTAL
1	Weight (g)	1.61	2.97	8.39	15.02	25.03	2.03	-	55.05
	% composition	2.93	5.39	15.24	27.29	45.47	3.68	-	100
2	Weight (g)	1.55	3.05	7.94	15.00	29.15	2.60	0.04	59.33
	% composition	2.61	5.15	13.38	25.28	49.13	4.39	0.07	100.01
5	Weight (g)	0.87	1.33	4.93	13.29	30.68	3.32	-	54.42
	% composition	1.59	2.44	9.07	24.43	56.37	6.10	-	100
9	Weight (g)	1.20	1.35	4.52	11.49	28.30	3.25	-	50.11
	% composition	2.39	2.70	9.03	22.93	56.48	6.48	-	100.01
13	Weight (g)	1.49	3.08	8.67	16.40	35.00	3.36	0.02	68.02
	% composition	2.19	4.53	12.74	24.12	51.46	4.94	0.02	100
19	Weight (g)	0.98	3.68	8.02	15.59	34.69	3.22	0.02	66.2
	% composition	1.49	5.56	12.11	23.55	52.40	4.87	0.03	100.01
25	Weight (g)	1.02	1.96	4.69	12.34	32.49	3.82	0.01	56.33
	% composition	1.81	3.48	8.33	21.91	57.68	6.77	0.02	100

Appendix 5.9 (contd)

Core B

Depth (cm) / Pore diameter of sieve		4.0 mm	2.0 mm	850μm	500μm	250μm	125μm	63μm	TOTAL
1	Weight (g)	1.33	3.55	9.04	16.09	32.17	3.25	0.01	65.45
	% composition	2.04	5.43	13.82	24.58	49.16	4.96	0.01	100
2	Weight (g)	0.39	2.12	8.07	15.58	35.58	3.43	0.01	65.18
	% composition	0.60	3.26	12.38	23.90	54.59	5.26	0.02	100.01
5	Weight (g)	0.95	1.71	6.69	15.09	34.80	3.45	0.01	62.70
	% composition	1.51	2.74	10.67	24.07	55.50	5.51	0.01	100.01
9	Weight (g)	0.97	1.25	5.10	12.39	33.46	3.82	0.01	57.00
	% composition	1.69	2.20	8.94	21.74	58.70	6.71	0.01	99.99
13	Weight (g)	1.07	2.04	5.86	13.16	27.29	2.24	0.01	51.67
	% composition	2.06	3.95	11.34	25.47	52.83	4.33	0.01	99.99
19	Weight (g)	0.53	1.87	6.60	16.12	32.40	2.85	0.01	60.38
	% composition	0.88	3.09	10.93	26.70	53.66	4.72	0.01	99.99
25	Weight (g)	1.59	2.79	9.35	19.65	36.38	3.17	0.01	72.94
	% composition	2.19	3.82	12.82	26.94	49.87	4.35	0.01	100

Core C

Depth (cm) / Pore diameter of sieve		4.0 mm	2.0 mm	850μm	500μm	250μm	125μm	63μm	TOTAL
1	Weight (g)	2.58	3.66	10.42	20.70	30.62	2.00	0.01	69.99
	% composition	3.69	5.23	14.88	29.57	43.76	2.86	0.01	100
2	Weight (g)	1.54	2.77	9.53	19.10	28.46	2.22	0.01	63.62
	% composition	2.42	4.35	15.0	30.03	44.73	3.48	0.01	100.02
5	Weight (g)	1.33	3.03	5.78	15.52	34.30	3.19	0.01	63.16
	% composition	2.10	4.79	9.16	24.57	54.31	5.06	0.01	100
9	Weight (g)	-	1.58	4.60	14.99	32.16	2.57	0.01	55.91
	% composition	-	2.83	8.23	26.81	57.52	4.60	0.01	100
13	Weight (g)	0.58	1.10	6.19	13.08	30.17	2.43	0.01	53.57
	% composition	1.09	2.06	11.56	24.43	56.32	4.53	0.01	100
19	Weight (g)	0.20	0.98	3.87	13.23	31.12	2.49	0.01	51.90
	% composition	0.38	1.90	7.46	25.49	59.97	4.79	0.01	100
25	Weight (g)	1.32	1.71	4.88	14.84	31.58	2.42	0.01	56.75
	% composition	2.32	3.02	8.59	26.15	55.64	4.27	0.01	100

Appendix 7.1 VARIATION OF SPECIES DIVERSITY WITH DEPTH

RUN 3 (28.4.76 - 7.6.76)

Depth (cm)	No. of species present per cm^3						
	Day 1	Day 5	Day 8	Day 12	Day 15	Day 22	Day 28
1	8	6	11	10	8	11	7
2	2	8	7	12	14	13	8
4	3	6	5	7	9	11	12
7	4	8	7	9	8	11	12
10	6	6	6	9	9	12	8
15				9	8	10	8
20				8	7	-	8
25				9	7	9	-

RUN 4 (11.6.76 - 11.7.76)

Depth (cm)	No. of species present per cm^3			
	Day 3	Day 10	Day 13	Day 25
1	9	11	13	11
2	6	8	8	10
4	7	10	9	16
7	6	11	0	12
10	5	6	0	13
15	5	10	1	8
20	3	7	0	4
25	3	11	8	4

Appendix 7.1 (contd)

RUN 5 (14.7.76 - 15.8.76)

Depth (cm)	No. of species present per cm^3				
	Day 1	Day 6	Day 11	Day 13	Day 18
1	8	12	11	10	14
2	7	9	14	11	9
4	2	9	19	5	13
7	4	10	12	12	12
10	1	8	11	6	8
15	2	6	13	6	6
20	3	4	4	8	6
25	2	6	8	7	4

RUN 6 (8.10.76 - 19.11.76)

Depth (cm)	No. of species present per cm^3				
	Day 5	Day 12	Day 18	Day 26	Day 40
1	7	3	10	9	9
2	6	6	16	17	12
4	5	10	14	17	14
7	5	7	14	13	12
10	3	7	11	10	12
15	4	7	13	10	10
20	1	7	11	10	9
25	4	5	5	10	8

Appendix 7.1 (contd)

RUN 7 (14.1.77 - 25.3.77)

Depth (cm)	No. of species present per cm^3				
	Day 3	Day 10	Day 19	Day 32	Day 45
1	2	6	6	8	13
2	2	4	8	12	16
4	7	3	6	7	10
7	2	6	4	9	8
10	1	2	4	10	7
15	0	1	5	8	5
20	0	1	3	6	6
25	1	2	2	6	8

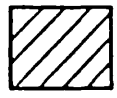
RUN 8 (28.3.77 - 16.5.77)

Depth (cm)	No. of species present per cm^3			
	Day 1	Day 9	Day 17	Day 28
1	4	13	15	12
2	4	6	12	9
4	4	5	10	14
7	5	3	10	10
10	7	7	13	7
15	2	8	9	8
20	5	6	12	10
25	5	5	7	8

BED 44 (11.10.76 - 10.11.77)

Depth (cm)	No. of species present per cm^3			
	Day 3	Day 10	Day 24	Day 33
1	8	8	14	9
2	5	4	16	14
4	3	8	15	13
7	2	2	10	12
10	1	4	12	9
15	5	4	14	9
20	0	4	10	11
25	3	6	10	2

Appendix 7.2 Depth distribution of the common ciliate species and the flagellates



Depths not analysed.

Glaucoma sp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	25	13	62	12	68	110	97	148	10	40	20	40	30
2	0	0	20		52	0	18	10	68	48	158	14	4	0
3	0	1	7	70										
4	0	0	4		20	8	2	3	36	28	12	70	2	0
5	0	11	40	14										
6			13		4	4	153	42	54	94				
7				19	9	4	188	76	18	60	24	64	10	1
10	0	0	0	18	6	4	56	16	24	102	52	126	2	7
12								6						
13											26	70		
15													16	4
16											8	66		
20											20	54		6
25											56	28	16	

Glaucoma sp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	41	10	90	20	0	1	0	0	0	0	0	0	150	20
2	25	20	180	20	0	1	0	0	0	0	0	0	74	22
4	8	12	90	13	0	8	1	0	0	0	0	0	28	14
7	2	16	0	0	2	5	0	0	0	0	0	0	10	0
10	3	0	0	1	0	0	0	0	0	0	0	0	6	0
15	7	20	0	0	0	0	0	0	0	0	0	16	22	0
20	8	2	0	0	0	0	0	0	0	0	0	22	22	0
25	3	0	52	0	1	3	0	0	0	0	0	0	54	0

Glaucoma sp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	12	0	240	20	9	22	4	6	0	0	0	0
2	0	6	36	72	6	2	29	14	4	0	0	0	0
4	7	1	10	24	4	0	5	6	0	0	0	20	0
7	6	3	9	2	2	1	0	2	0	0	0	36	7
10	2	1	9	32	0	2	10	4	4	0	0	16	0
15	0	0	11	2	0	0	13	2	0	0	0	10	4
20	0	3	3	0	4	0	10	0	1	0	0	12	0
25	0	0	4	2	0	0	7	2	0	0	0	8	0

Cyclidium heptatrichum

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	0	311	6	38	90	0	12	30	190	120	0	20
2	0	0	0		29	0	200	0	12	30	192	2	0	0
3	0	1	0	516										
4	0	0	0		20	0	0	2	56	32	110	14	0	12
5	0	1	0	84										
6			0		6	0	9	0	30	26				
7				29	3	0	6	0	38	12	132	18	40	4
10	0	0	0	22	4	0	7	2	32	46	34	66	18	30
12								0						
13											28	84		
15													18	3
16											22	28		
20											26	46		4
25											24	60	18	

Cyclidium heptatrichum

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	1	0	30	0	0	9	70	60	90	0	0	0	10	0
2	1	0	80	0	3	4	30	70	130	31	1	44	64	0
4	1	36	10	3	11	0	1	10	44	3	106	322	740	22
7	1	1	0	0	8	5	16	5	23	0	50	172	24	374
10	0	2	0	2	0	3	1	1	14	0	18	74	52	25
15	1	2	0	1	0	0	2	0	10	0	12	40	14	19
20	0	0	0	0	0	0	0	0	1	0	5	20	8	26
25	0	2	0	0	0	0	2	3	2	3	7	8	88	21

Cyclidium heptatrichum

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	0	0	0	0	0	0	0	2	2	11	120	180
2	0	0	2	2	2	1	1	0	0	0	14	132	101
4	15	1	0	2	12	0	4	6	4	1	14	186	52
7	2	3	0	12	26	0	8	12	0	0	1	46	51
10	0	0	0	24	20	1	2	37	0	0	4	52	26
15	0	0	0	14	0	0	2	24	1	0	6	26	8
20	0	0	0	2	6	1	1	36	1	0	1	28	16
25	0	0	0	2	8	2	0	18	9	0	2	26	0

Cinetochilum margaritaceum

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	0	0	0	0	0	0	0	0	30	0	0	0
2	0	0	0		3	0	0	0	0	0	2	0	0	0
3	0	0	0	0										
4	0	0	0		0	0	0	0	0	0	4	0	0	15
5	0	0	0	0										
6			0		0	1	0	0	0	0				
7				0	1	0	0	2	2	2	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12								0						
13											0	0		
15													0	0
16											2	0		
22											0	0		0
25											0	0	0	

Cinetochilum margaritaceum

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	5	200	650	0	1	6	260	300	0	6	7	80	100	180
2	0	20	340	0	1	25	230	920	0	1	8	42	62	40
4	0	56	40	5	0	50	100	590	7	0	44	44	4	36
7	3	5	0	7	0	30	34	19	0	2	16	66	0	16
10	1	78	0	2	0	0	34	10	0	1	5	18	4	19
15	0	176	4	5	0	0	37	4	0	0	15	124	10	9
20	0	37	0	2	0	0	8	9	0	0	5	40	24	7
25	0	8	34	1	0	0	1	6	0	0	3	36	18	5

Cinetochilum margaritaceum

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	10	0	0	0	0	1	7	157	8	0	2	30	230
2	2	1	0	0	4	0	2	55	54	0	0	36	5
4	1	0	0	0	2	1	0	44	4	0	9	44	2
7	0	1	0	18	12	0	4	35	20	0	0	38	59
10	0	0	0	14	22	1	4	23	16	0	4	8	15
15	0	1	0	2	22	0	4	18	10	1	2	24	14
20	0	0	0	26	24	2	1	12	5	0	1	34	21
25	0	0	0	12	12	2	1	10	10	3	3	52	0

Chilodonella sp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	0	0	0	0	0	0	0	60	0	0	60	10
2	0	0	0		0	0	0	0	0	0	10	12	6	10
3	0	0	0	0										
4	0	0	0		1	0	1	0	2	0	0	8	6	0
5	0	0	0	0										
6			0		0	0	1	0	0	0				
7				0	0	0	0	0	14	16	0	0	42	5
10	0	0	0	0	0	0	0	0	4	4	2	0	12	6
12								0						
13											2	2		
15													2	0
16											0	2		
20											0	0		2
25											0	0	2	

Chilodonella sp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	13	11	40	0	5	32	0	30	110	3	0	20	10	50
2	3	0	0	0	1	6	0	0	50	0	0	12	18	4
4	1	0	0	0	3	8	1	0	1	0	0	10	10	26
7	2	0	0	0	8	10	0	1	0	0	0	4	8	2
10	1	0	0	0	3	8	0	0	0	0	0	4	2	6
15	4	0	0	0	1	1	0	0	0	0	1	6	4	4
20	0	0	0	0	0	0	0	0	0	0	1	8	0	1
25	0	0	0	0	1	5	0	0	0	0	0	0	0	3

Chilodonella sp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	0	10	0	0	0	0	14	26	0	0	0	20
2	0	0	0	6	0	0	0	4	10	0	0	4	2
4	0	0	0	0	0	0	0	2	6	0	11	4	4
7	0	0	0	0	0	0	0	2	2	0	0	14	7
10	0	0	0	0	0	0	0	1	6	0	0	10	5
15	0	0	0	0	0	0	2	3	6	0	0	16	4
20	0	0	0	2	0	0	1	2	2	0	0	14	14
25	0	0	0	0	6	2	2	0	6	0	0	4	2

Lacrymaria olor

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	0	0	3	0	0	0	0	0	0	11	0	0
2	0	0	0		0	0	1	0	0	4	0	8	18	0
3	0	0	0	0										
4	0	0	1		0	0	0	0	0	0	0	0	4	0
5	0	0	0	0										
6			0		0	0	0	0	0	0				
7				0	0	0	0	0	2	0	0	0	4	0
10	0	0	0	0	0	0	0	0	0	0	2	0	4	0
12								0						
13											0	0		
15													2	0
16											0	0		
20											0	0		0
25											1	0	3	

Lacrymaria olor

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	0	0	0	0	0	0	0	0	0	0	0	10	0	10
2	0	0	0	0	0	0	0	20	10	0	0	8	2	6
4	0	0	0	0	0	0	2	0	1	0	2	6	2	16
7	0	1	0	0	0	0	0	1	1	0	0	2	2	2
10	0	0	0	0	0	0	0	0	0	0	0	6	0	0
15	0	0	0	0	0	0	0	0	0	0	0	2	2	0
20	0	0	0	0	0	0	0	0	0	0	0	0	2	0
25	1	0	2	0	0	0	0	0	1	0	0	0	0	0

Lacrymaria olor

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	0	0	0	10	0	2	10	0	1	0	0	0
2	0	0	0	2	0	0	0	4	0	0	0	2	0
4	0	0	0	2	0	0	0	0	1	0	0	2	2
7	0	0	0	0	0	0	0	0	2	0	0	0	0
10	0	0	0	2	0	0	0	1	0	0	0	0	0
15	0	0	0	0	2	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	2	2	0	0	0	1
25	0	0	0	0	0	0	0	0	2	0	0	0	0

Litonotus spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	0	0	0	0	0	2	0	11	10	0	2	0
2	0	0	0		0	0	1	0	2	0	24	3	8	10
3	0	0	0	0										
4	0	0	0		0	0	0	0	0	0	2	34	0	1
5	0	0	0	0										
6			0		0	0	0	0	2	0				
7				0	0	0	0	0	0	0	0	20	0	3
10	0	0	0	0	0	0	0	0	0	0	0	13	2	30
12								0						
13											0	2		
15													0	1
16											10	10		
20											0	2		6
25											4	2	0	

Litonotus spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	0	20	20	30	0	4	0	10	130	4	0	70	50	30
2	0	70	0	0	0	0	0	20	80	1	0	50	82	4
4	0	124	20	8	0	0	2	0	19	0	2	84	8	10
7	1	8	0	4	0	0	4	6	8	0	0	20	6	0
10	0	3	0	0	0	0	1	0	1	0	1	0	1	3
15	0	24	0	2	0	0	4	1	4	0	1	14	0	5
20	0	5	0	3	0	0	0	0	2	0	0	2	1	1
25	0	2	4	13	0	0	1	0	2	0	2	2	10	4

Litonotus spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	0	0	0	10	0	2	2	0	0	7	41	10
2	0	0	0	0	24	0	1	8	6	0	0	27	37
4	0	0	0	0	2	1	0	6	2	0	2	32	2
7	0	0	0	0	0	1	0	5	2	0	0	4	10
10	0	0	0	4	0	2	0	2	0	0	0	6	5
15	0	0	0	0	0	0	0	3	3	1	0	2	0
20	0	0	0	0	0	1	0	4	1	0	0	2	2
25	0	0	0	0	2	0	0	5	1	0	2	0	0

Tachysoma pellionella

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	139	454	36	38	1020	7	16	520	984	64	71	10
2	0	0	4		68	43	188	0	51	31	232	37	30	0
3	0	0	1	108										
4	0	0	7		32	22	62	2	61	16	27	66	44	6
5	0	1	0	21										
6			1		12	13	69	6	92	22				
7				10	22	7	86	8	86	6	34	79	18	2
10	0	0	0	44	0	8	51	2	84	52	38	68	10	0
12								20						
13											24	80		
15													8	1
16											18	24		
20											10	24		0
25											47	11	10	

Tachysoma pellionella

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	42	80	220	70	3	37	190	80	110	6	4	60	70	20
2	8	110	0	30	1	20	50	20	0	1	136	60	48	16
4	8	92	0	16	0	2	42	30	5	2	71	120	22	12
7	0	24	0	17	0	6	74	15	2	5	63	58	12	18
10	1	35	0	4	0	13	61	1	15	0	64	84	14	5
15	3	25	0	9	0	4	60	9	6	1	38	124	2	5
20	1	42	0	3	0	1	36	10	1	0	22	80	18	3
25	0	243	6	1	0	2	120	13	0	14	18	6	18	0

Tachysoma pellionella

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	1	10	220	300	200	3	24	557	31	3	2	125	0
2	0	6	197	42	59	1	11	40	48	0	3	48	5
4	1	4	87	28	10	2	0	24	9	1	1	76	4
7	0	2	67	34	10	2	0	27	5	1	0	14	1
10	0	1	38	52	8	1	1	25	2	0	0	8	2
15	0	0	60	44	12	0	0	2	1	1	0	2	2
20	0	0	24	50	22	0	0	14	5	0	0	0	2
25	0	4	18	24	2	0	0	14	3	1	1	2	0

Aspidisca costata

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	0	2	0	12	2840	8	80	380	70	90	70	0
2	0	0	0		2	2	70	8	86	22	36	18	4	0
3	0	0	0	12										
4	0	0	0		0	2	12	0	36	0	4	10	10	23
5	0	0	0	6										
6			0		0	2	131	8	264	30				
7				30	8	0	50	50	210	42	6	6	2	4
10	0	0	0	6	2	16	39	6	260	16	0	12	4	3
12								86						
13											2	12		
15													4	3
16											0	8		
20											2	8		8
25											4	2	2	

Aspidisca costata

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	1	110	117	230	0	5	220	260	110	5	0	0	20	72
2	2	20	60	10	1	1	0	60	0	11	2	8	12	26
4	2	16	0	20	0	2	60	10	19	6	25	54	6	144
7	0	1	0	13	0	6	46	20	18	2	39	50	28	10
10	0	6	0	3	0	6	44	5	38	1	65	60	10	49
15	0	8	0	11	0	2	34	31	44	2	45	126	12	47
20	1	0	0	12	1	1	13	23	0	4	7	124	50	40
25	2	0	14	7	0	4	23	0	0	7	12	34	56	25

Aspidisca costata

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	1	0	100	250	4	2	45	34	1	2	80	30
2	1	0	14	10	92	2	0	18	30	1	8	37	6
4	1	0	1	6	28	3	1	13	17	2	20	36	11
7	0	0	2	4	70	3	0	37	16	2	6	16	20
10	0	0	0	28	64	1	4	42	14	1	6	18	3
15	0	0	0	34	94	0	1	25	0	8	3	40	1
20	0	0	0	4	66	1	1	30	1	0	4	8	10
25	0	1	0	18	24	2	1	24	2	2	3	6	4

Oxytricha spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	3	29	0	6	0	70	1	16	0	50	13	0	0
2	0	2	5		1	5	6	0	6	8	2	45	0	0
3	0	1	0	2										
4	0	0	0		0	0	3	0	2	2	2	9	0	0
5	0	0	9	6										
6			3		0	3	3	0	2	6				
7				1	0	0	2	0	4	2	2	6	0	0
10	0	0	0	1	0	0	4	0	0	4	10	10	0	0
12								0						
13											0	10		
15													0	0
16											2	2		
20											4	0		0
25											6	2	0	

Oxytricha spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	6	0	30	20	3	18	50	0	0	0	0	30	0	0
2	2	0	40	20	1	0	0	0	0	0	9	38	30	2
4	0	12	30	5	0	1	4	0	2	1	2	32	31	2
7	1	2	0	9	0	5	2	4	0	1	1	10	10	26
10	1	0	0	3	0	1	1	0	1	0	0	24	8	7
15	1	4	0	1	1	0	3	6	0	0	0	2	2	7
20	0	1	0	0	0	0	5	6	2	0	2	0	2	4
25	0	12	2	0	0	0	0	3	1	2	0	0	10	2

Oxytricha spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	4	90	0	30	0	9	12	19	2	0	10	20
2	0	3	16	8	46	0	3	6	34	1	0	4	2
4	2	0	11	4	62	0	1	2	10	0	1	7	7
7	0	1	7	0	18	0	0	0	6	0	0	2	3
10	0	0	0	2	4	1	2	0	10	0	1	4	2
15	0	0	3	2	38	2	0	2	1	0	0	4	4
20	0	0	0	2	26	1	0	1	4	0	0	4	2
25	0	0	0	8	16	0	0	1	3	0	0	4	0

Stentor polymorphus

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE														
	RUN 1				RUN 2			RUN 3							
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	0	0	1	0	0	0	0	3	0	0	0	0
2	0	0	0		0	0	0	0	0	0	0	0	2	1
3	0	0	0	0										
4	0	0	0		0	0	4	0	0	0	0	0	0	6
5	0	0	0	0										
6			0		0	0	1	0	0	2				
7				0	0	0	6	0	0	0	0	0	0	5
10	0	0	0	0	0	0	0	0	0	0	0	4	0	1
12								0						
13											0	0		
15													0	0
16											0	0		
20											0	0		0
25											0	0	0	

Stentor polymorphus

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	0	0	0	0	0	0	11	0	10	0	0	0	0	0
2	0	0	0	10	0	0	10	0	0	0	0	2	0	0
4	0	0	0	29	0	0	1	0	0	0	0	2	2	0
7	0	0	0	4	0	0	0	0	1	0	0	0	0	0
10	0	0	0	2	0	0	0	0	0	0	0	0	0	0
15	0	0	0	1	0	0	0	0	0	0	0	0	0	1
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Stentor polymorphus

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	1	0	0	0	0	1	4	8	0	0	1	1
2	0	0	0	0	7	0	0	0	25	1	0	5	6
4	0	0	0	0	1	0	0	0	6	0	0	4	4
7	0	0	0	0	0	0	0	0	2	0	0	0	1
10	0	0	0	0	0	0	0	0	0	0	0	4	0
15	0	0	0	0	0	0	0	0	1	0	0	0	0
20	0	0	0	0	0	0	0	1	0	0	0	8	0
25	0	0	0	0	0	0	0	0	0	0	0	1	0

Vorticella spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	1	2	7	34	10	5	27	1	1	7	6	2	32	33
2	0	0	0		1	2	35	0	1	0	7	14	345	100
3	0	1	1	23										
4	0	0	1		1	0	5	0	0	0	0	14	98	147
5	0	0	0	21										
6			0		0	1	17	0	0	0				
7				10	4	0	2	0	0	0	3	6	52	48
10	0	0	1	7	2	0	1	0	0	0	1	8	7	38
12								0						
13											0	0		
15													5	14
16											3	0		
20											1	2		4
25											1	0	5	

Vorticella spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	0	67	212	103	0	6	233	98	494	0	2	70	2	21
2	0	12	73	121	0	1	135	24	74	0	1	22	26	291
4	0	9	10	50	0	4	26	5	21	0	7	0	31	80
7	0	2	0	30	0	2	12	1	7	0	0	2	20	49
10	0	1	0	20	0	0	8	0	2	1	2	2	4	5
15	0	4	0	1	0	0	5	0	0	1	0	3	4	2
20	0	1	0	0	0	1	0	0	0	0	0	2	3	1
25	0	7	0	0	0	1	5	1	0	0	0	0	5	1

Vorticella spp.

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	0	5	24	29	148	0	4	20	13	3	0	38	35
2	0	0	12	49	143	0	0	13	4	0	0	16	24
4	0	0	1	0	23	0	0	6	11	0	0	13	15
7	0	1	0	8	26	0	0	3	0	0	0	10	4
10	0	0	2	3	14	0	1	2	0	0	0	5	2
15	0	0	2	2	0	0	0	0	0	0	0	4	1
20	0	0	1	0	0	0	1	4	0	0	0	2	1
25	0	0	0	0	1	0	1	0	0	0	0	2	0

Flagellates

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	0	0	7	20	52	294	200	73	280	1020	1310	320	120	210
2	0	0	38		1000	50	1200	136	306	440	402	122	46	2540
3	0	0	7	1000										
4	0	0	69		450	300	287	40	120	156	82	302	30	59
5	0	0	48	213										
6			20		260	146	131	84	168	228				
7				90	430	188	82	86	124	1240	150	246	114	138
10	0	0	0	412	185	74	46	95	74	180	0	478	62	103
12								124						
13											0	378		
15													14	19
16											82	154		
20											20	86		41
25											74	62	26	

Flagellates

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	48	890	1250	110	56	223	2880	2470	510	27	29	410	250	310
2	4	180	7400	60	194	197	1780	1720	230	56	75	226	290	136
4	14	496	180	108	246	402	213	2250	9060	69	201	530	768	394
7	9	20	8	71	72	594	154	114	624	104	222	402	916	176
10	19	53	40	47	20	30	93	118	389	55	168	68	216	160
15	12	178	14	26	38	43	96	171	77	43	203	326	90	408
20	7	17	4	49	134	13	46	83	0	39	74	218	206	84
25	1	10	98	41	152	57	72	123	0	62	79	120	282	76

Flagellates

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	45	80	1522	3860	820	9	23	120	62	130	81	236	450
2	23	49	60	74	318	22	7	58	96	13	44	432	177
4	425	71	76	30	42	38	5	94	114	21	308	302	108
7	242	251	80	34	50	13	14	79	44	32	54	200	112
10	158	28	58	32	26	23	16	77	174	8	53	142	65
15	134	174	117	28	58	8	9	52	107	14	75	234	61
20	72	82	89	130	76	21	24	45	132	39	69	134	132
25	50	50	134	104	80	14	26	25	171	14	58	98	72

Total Ciliates

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 1				RUN 2			RUN 3						
	1	4	7	11	3	5	12	1	5	8	12	15	22	28
1	1	31	206	879	76	165	4222	119	273	1043	1402	387	394	217
2	0	2	33		159	52	530	18	250	146	666	178	479	141
3	0	4	14	732										
4	0	2	4		74	32	91	7	193	82	161	231	215	254
5	0	14	49	153										
6			19		24	25	388	56	454	185				
7				100	48	11	340	146	315	140	215	207	202	82
10	0	0	2	98	14	28	162	30	260	224	152	313	96	118
12								116						
13											86	267		
15													68	37
16											73	150		
20											67	138		34
25											145	110	74	

Total Ciliates

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE													
	RUN 4				RUN 5					RUN 6				
	3	10	13	25	1	6	11	13	18	5	12	18	26	40
1	115	541	1425	566	28	130	1183	871	1353	45	14	460	491	310
2	42	255	781	321	11	60	530	1355	544	94	159	296	446	425
4	22	367	205	220	14	79	262	645	169	14	264	696	943	370
7	10	64	0	112	20	71	213	83	72	12	175	393	146	504
10	7	125	0	54	3	34	174	29	77	4	157	286	118	124
15	16	265	4	36	2	23	153	53	70	6	113	460	74	101
20	11	91	0	26	3	4	62	60	9	4	45	305	136	84
25	6	287	116	27	2	22	160	29	7	27	42	88	270	64

Total Ciliates

DEPTH (CM)	NUMBER PER CUBIC CENTIMETRE												
	RUN 7					RUN 8				BED 44			
	3	10	19	32	45	1	9	17	28	3	10	24	33
1	12	34	394	988	784	17	79	846	162	35	29	496	558
2	3	16	281	346	425	7	47	176	227	8	26	331	206
4	28	6	116	70	151	8	12	115	80	4	59	441	117
7	8	11	85	86	168	8	14	125	60	3	7	183	176
10	2	2	49	167	133	9	24	143	54	1	15	139	63
15	0	1	78	102	168	4	25	82	25	12	13	135	39
20	0	3	28	86	148	6	15	109	24	0	8	115	72
25	2	5	22	66	71	10	12	74	37	6	13	109	6

Appendix 7.3 CALCULATION OF THE DOWNWARD RATE OF FLOW OF INTERSTITIAL WATER

Porosity of sand = 38.81% (See p.120)

$\therefore$ rate at which water passes through the sand = 100/38.81 = 2.58 times as fast as in the surface water.

Depth of top water through sand = 16" hr^{-1}

= 40.64 cm hr^{-1}

$\therefore$ velocity of water passing through the sand

$$= \frac{40.64 \times 2.58}{60} \text{ cm min}^{-1}$$

$$= 1.75 \text{ cm min}^{-1}$$

VOLUME (μm^3) = L X W X $\frac{\pi}{4}$ X C where C = factor for depth of animal (taken as $\frac{1}{2}$ or $\frac{1}{3}$ of W depending on species)

(See p.167 for corrected equation)

Glaucoma sp. (C = $\frac{1}{3}$)

DEPTH (CM)	DAY 1		DAY 9		DAY 17		DAY 28	
	IND	TOTAL	IND	TOTAL	IND	TOTAL	IND	TOTAL
1	1.77×10^3	1.59×10^4	7.96×10^2	1.75×10^4	4.95×10^2	1.98×10^3	4.95×10^2	2.97×10^4
2	1.77×10^3	3.54×10^3	7.96×10^2	2.31×10^4	7.96×10^2	1.11×10^4	4.13×10^2	1.65×10^3
4	-	-	7.96×10^2	3.98×10^3	4.95×10^2	2.97×10^3	-	-
7	1.77×10^3	1.77×10^3	-	-	1.06×10^2	2.12×10^3	-	-
10	1.77×10^3	3.54×10^3	7.96×10^2	7.96×10^3	4.95×10^2	1.98×10^2	4.95×10^2	1.98×10^3
15	-	-	7.96×10^2	1.03×10^4	4.95×10^2	9.90×10^2	-	-
20	-	-	7.96×10^2	7.96×10^3	-	-	4.95×10^2	4.95×10^2
25	-	-	4.13×10^2	2.89×10^3	4.13×10^2	8.26×10^2	-	-
X	1.77×10^3		7.41×10^2		6.07×10^2		4.95×10^2	

Cyclidium sp. (C = $\frac{1}{3}$)

DEPTH (CM)	DAY 1		DAY 9		DAY 17		DAY 28	
	IND	TOTAL	IND	TOTAL	IND	TOTAL	IND	TOTAL
1	-	-	-	-	-	-	5.78×10^2	1.16×10^4
2	5.78×10^2	5.78×10^2	5.78×10^2	5.78×10^3	-	-	-	-
4	-	-	6.60×10^2	2.64×10^3	6.60×10^2	3.96×10^3	5.78×10^2	2.31×10^3
7	-	-	6.60×10^2	5.28×10^3	7.43×10^2	8.92×10^3	-	-
10	5.78×10^2	5.78×10^2	1.72×10^2	3.44×10^3	5.78×10^2	2.14×10^4	-	-
15	-	-	6.60×10^2	1.32×10^3	5.78×10^2	1.39×10^4	5.78×10^2	5.78×10^2
20	9.91×10^2	9.91×10^2	1.33×10^3	1.33×10^3	5.78×10^2	2.08×10^4	8.26×10^2	8.26×10^2
25	5.78×10^2	1.16×10^3	-	-	6.60×10^2	1.19×10^4	7.43×10^2	6.69×10^3
X	6.81×10^2		9.35×10^2		6.33×10^2		6.61×10^2	

Cinetochilum sp. (C = $\frac{1}{3}$)

DEPTH (CM)	DAY 1		DAY 9		DAY 17		DAY 28	
	IND	TOTAL	IND	TOTAL	IND	TOTAL	IND	TOTAL
1	3.44×10^3	3.44×10^3	2.38×10^3	1.66×10^4	2.38×10^3	3.74×10^5	1.56×10^3	1.25×10^5
2	-	-	2.64×10^3	5.28×10^3	1.36×10^3	7.48×10^4	1.56×10^3	8.42×10^4
4	9.29×10^2	9.29×10^2	-	-	1.56×10^3	6.86×10^5	2.38×10^3	9.52×10^4
7	-	-	2.38×10^3	9.52×10^3	3.44×10^3	1.20×10^5	1.75×10^3	3.50×10^4
10	1.19×10^3	1.19×10^3	2.38×10^3	9.52×10^3	1.56×10^3	3.59×10^4	2.38×10^2	3.81×10^3
15	-	-	2.38×10^3	9.52×10^3	1.56×10^2	2.81×10^4	9.29×10^3	9.29×10^4
20	1.56×10^3	3.11×10^3	2.38×10^2	2.38×10^2	9.29×10^2	1.11×10^3	2.38×10^2	8.0×10^3
25	9.29×10^2	1.86×10^3	9.29×10^2	9.29×10^2	9.29×10^2	9.29×10^3	9.29×10^2	9.29×10^3
$\bar{X}$	1.61×10^3		2.21×10^3		1.71×10^3		1.73×10^3	

Tachysoma sp. (C = $\frac{1}{3}$)

DEPTH (CM)	DAY 1		DAY 9		DAY 17		DAY 28	
	IND	TOTAL	IND	TOTAL	IND	TOTAL	IND	TOTAL
1	1.09×10^4	3.27×10^4	8.95×10^3	2.15×10^5	1.34×10^4	7.47×10^6	1.05×10^4	3.26×10^6
2	5.81×10^3	5.81×10^3	7.92×10^3	8.71×10^4	4.28×10^3	1.71×10^5	4.09×10^3	1.96×10^5
4	8.26×10^3	1.65×10^4	-	-	7.92×10^3	1.90×10^5	6.20×10^3	5.58×10^4
7	7.58×10^3	1.52×10^4	-	-	1.13×10^4	3.06×10^5	5.55×10^3	2.77×10^4
10	7.58×10^3	7.58×10^3	-	-	7.92×10^3	1.98×10^4	6.60×10^4	1.32×10^4
15	-	-	-	-	1.05×10^4	2.09×10^4	1.34×10^4	1.34×10^4
20	-	-	-	-	6.33×10^4	8.87×10^5	7.58×10^3	3.79×10^4
25	-	-	-	-	1.18×10^3	1.65×10^5	7.58×10^3	2.27×10^4
$\bar{X}$	8.03×10^3		8.43×10^3		9.18×10^3		7.69×10^3	

Appendix 7.4 (contd)

Aspidisca sp. ($C = \frac{1}{2}$)

DEPTH (CM)	DAY 1		DAY 9		DAY 17		DAY 28	
	IND	TOTAL	IND	TOTAL	IND	TOTAL	IND	TOTAL
1	9.69×10^3	2.91×10^4	7.20×10^3	1.44×10^4	5.17×10^3	2.33×10^5	3.56×10^3	1.21×10^6
2	9.69×10^3	1.94×10^4	-	-	9.69×10^3	1.74×10^4	2.18×10^3	6.54×10^4
4	9.69×10^3	2.91×10^4	2.33×10^3	2.33×10^3	3.56×10^3	4.63×10^5	2.33×10^3	3.96×10^4
7	8.89×10^3	2.67×10^4	-	-	6.54×10^3	2.42×10^5	2.33×10^3	3.73×10^4
10	5.17×10^3	5.17×10^3	9.69×10^3	3.88×10^4	7.20×10^3	3.02×10^5	2.33×10^3	3.26×10^4
15	-	-	5.17×10^3	5.17×10^3	7.20×10^3	1.80×10^5	-	-
20	7.85×10^3	7.85×10^4	5.17×10^3	5.17×10^3	7.85×10^3	2.36×10^5	5.17×10^3	5.17×10^3
25	9.69×10^3	1.94×10^4	9.69×10^3	9.69×10^3	7.20×10^3	1.73×10^5	2.33×10^3	4.66×10^3
$\bar{X}$	8.67×10^3		6.54×10^3		6.80×10^3		2.89×10^3	

Appendix 7.5 REGRESSION TABLE FOR ANALYSES PERFORMED ON THE INTER-DEPENDENCE OF CILIATE FREQUENCY AND CARBON DENSITY WITH DEPTH

RUN	DAY	$\ln\bar{x}$	$\ln(\bar{Y}+1)$	ln INTER-CEPT	SLOPE	S.E. SLOPE	VAR. RATIO	N	p
3	5	4.376	5.641	5.848	-0.047	0.139	0.116	6	0.75
	8	5.012	5.325	-6.742	2.408	2.205	1.193	6	0.50
	12	4.611	5.246	1.410	0.832	0.436	3.648	9	0.10
	15	5.714	5.326	3.483	0.323	0.130	6.151	9	0.05
	22	6.090	5.138	0.964	0.685	0.199	11.829	7	0.025
	28	5.921	4.610	1.523	0.522	0.181	8.262	7	0.05
4	3	4.449	2.924	4.220	-0.291	0.916	0.101	8	0.75
	10	5.178	5.316	5.443	-0.025	0.622	0.002	8	0.75
	13	5.134	3.203	-6.657	1.921	1.343	2.047	8	0.25
	25	5.163	4.561	1.131	0.664	0.254	6.860	8	0.05
5	1	4.665	1.887	1.165	0.155	0.811	0.036	7	0.75
	6	5.124	3.640	-3.874	1.467	1.112	1.740	8	0.25
	11	5.587	5.464	0.915	0.814	0.297	7.531	8	0.05
	13	5.266	4.973	-1.447	1.219	0.738	2.732	8	0.25
	18	5.351	4.493	-1.266	1.076	0.434	6.155	8	0.05
6	5	4.682	2.769	-3.269	1.290	0.541	5.693	8	0.10
	12	5.102	4.490	1.411	0.604	0.991	0.371	8	0.75
	18	5.385	5.794	2.800	0.556	0.467	1.417	8	0.50
	26	5.592	5.470	-1.971	1.331	0.436	9.302	8	0.025
	40	5.614	5.251	1.464	0.675	0.287	5.539	8	0.10
7	3	3.649	1.516	-3.984	1.507	1.413	1.137	7	0.5
	10	3.260	1.851	1.831	0.006	0.288	0.001	7	0.75
	32	4.822	4.715	4.474	0.050	0.758	0.004	7	0.75
	45	4.929	5.273	3.190	0.423	0.114	13.725	8	0.01
8	1	5.016	2.203	3.452	-0.249	0.312	0.638	8	0.50
	9	4.902	3.168	-3.002	1.259	0.936	1.809	8	0.25
	17	5.245	4.989	-0.351	1.018	0.132	59.958	8	0.001
	28	5.399	4.144	0.510	0.673	0.289	5.432	8	0.10
BED									
44	3	5.390	1.661	5.718	-0.753	0.550	1.875	8	0.25
	10	4.575	2.890	2.510	0.083	0.278	0.090	8	0.75
	24	5.223	5.329	1.770	0.681	0.412	2.731	8	0.25
	33	5.236	4.461	-7.862	2.354	0.614	14.713	8	0.01

lnx = ln carbon density

ln (Y + 1) = ln (ciliate frequency + 1)

Appendix 8.1 INTEGRALS OF TOTAL CILIATE POPULATION DENSITY ESTIMATES IN CORES OF 1 cm^2 CROSS SECTION AND ASSOCIATED DENSITY PER CUBIC CENTIMETRE

RUN 1

	DAY 1		DAY 4		DAY 7		DAY 11	
MAX. DEPTH EXAMINED (CM)	6		10		10		10	
SPECIES	INTEGRAL	$NQCM^{-3}$	INTEGRAL	$NQCM^{-3}$	INTEGRAL	$NQCM^{-3}$	INTEGRAL	$NQCM^{-3}$
Glaucoma	-	-	8.90	0.89	43.30	4.33	316.10	31.61
Tachysoma	-	-	6.82	0.68	75.99	7.60	723.12	72.31
Vorticella	-	-	1.90	0.19	6.76	0.68	152.46	15.25
Total Ciliates	-	-	112.85	11.28	174.47	17.45	3076.50	307.65
Total Protozoa	-	-	119.67	11.97	369.28	36.93	6570.42	657.04
Flagellates	-	-	-	-	181.97	18.20	3491.63	349.16

Appendix 8.1 (contd)

RUN 2

	DAY 3		DAY 5		DAY 12	
MAX. DEPTH EXAMINED (CM)	10		10		10	
SPECIES	INTEGRAL	NO.CM^{-3}	INTEGRAL	NO.CM^{-3}	INTEGRAL	NO.CM^{-3}
Glaucoma	165.90	16.59	53.20	5.32	825.54	82.55
Cyclidium	113.89	11.39	9.31	0.93	398.85	39.89
Tachysoma	264.09	26.41	175.35	17.54	1066.53	106.65
Aspidisca	30.19	3.02	32.29	3.23	1167.29	116.73
Vorticella	16.90	1.69	6.54	0.65	68.39	6.84
Total Ciliates	609.24	60.92	290.39	29.04	3768.25	376.83
Flagellates	4271.17	427.12	1546.01	154.60	3166.98	316.70
Total Protozoa	4889.21	488.92	1857.09	185.71	6993.25	699.33

RUN 3	DAY 1		DAY 5		DAY 8		DAY 12	
MAXIMUM DEPTH (CM)	12		10		10		25	
SPECIES	INT	NO.CM^{-3}	INT	NO.CM^{-3}	INT	NO.CM^{-3}	INT	NO.CM^{-3}
Glaucoma	318.78	26.57	389.98	39.00	538.83	53.88	827.67	33.11
Cyclidium	8.65	0.72	308.80	30.88	238.23	23.82	1482.31	59.29
Tachysoma	57.60	4.80	655.14	65.51	310.18	31.02	1224.98	49.00
Aspidisca	209.72	17.48	1427.63	142.76	295.97	29.60	129.30	5.17
Vorticella	0.13	0.01	2.19	0.22	0.50	0.05	16.24	0.65
Total Ciliates	627.85	52.32	2818.91	281.89	1523.07	152.31	4107.83	164.31
Flagellates	980.17	81.68	1500.43	150.04	4714.12	471.41	2429.22	97.17
Total Protozoa	1647.83	137.32	4447.88	444.79	6287.72	628.77	6717.76	268.71

Appendix 8.1 (contd)

RUN 3	DAY 15		DAY 22		DAY 28	
MAXIMUM DEPTH (CM)	25		25		20	
SPECIES	INT	NOCM^{-3}	INT	NOCM^{-3}	INT	NOCM^{-3}
Glaucoma	1547.12	61.88	297.23	11.89	75.96	3.80
Cyclidium	1039.07	41.56	441.93	17.68	195.80	9.79
Tachysoma	1161.68	46.47	380.15	15.21	31.35	1.57
Aspidisca	244.35	9.77	112.81	4.51	119.59	5.98
Vorticella	7.54	0.30	465.56	18.62	128.13	6.41
Total Ciliates	4781.40	191.26	3314.25	132.57	1948.49	97.42
Flagellates	5463.74	218.55	956.43	38.26	4759.56	237.98
Total Protozoa	10522.32	420.89	4491.70	179.67	6823.56	341.18

RUN 4

	DAY 3		DAY 10		DAY 13		DAY 25	
MAX. DEPTH EXAMINED (CM)	25		25		25		25	
SPECIES	INTEGRAL	$NOCM^{-3}$	INTEGRAL	$NOCM^{-3}$	INTEGRAL	$NOCM^{-3}$	INTEGRAL	$NOCM^{-3}$
Glaucoma	175.30	7.01	217.27	8.69	636.20	25.45	77.10	3.08
Cyclidium	13.07	0.52	126.24	5.05	140.45	5.62	22.11	0.88
Cinetochilum	13.64	0.55	1702.12	68.08	898.13	3.57	78.91	3.16
Tachysoma	73.16	2.93	1492.21	59.69	89.31	3.57	235.96	9.44
Aspidisca	18.32	0.73	183.60	7.34	151.77	6.07	333.74	13.35
Vorticella	-	-	21.52	0.86	58.04	2.32	295.30	11.81
Total Ciliates	369.68	14.79	4674.05	186.96	2406.17	96.25	2161.57	84.46
Flagellates	267.44	10.70	3236.24	129.45	11647.07	465.88	1296.72	51.87
Total Protozoa	745.81	29.83	8042.72	321.71	14151.37	566.05	3552.87	142.11

RUN 5

	DAY 1		DAY 6		DAY 11		DAY 13		DAY 18	
MAX. DEPTH EXAMINED (CM)	25		25		25		25		25	
SPECIES	INTEGRAL	$NOCM^{-3}$	INTEGRAL	$NOCM^{-3}$	INTEGRAL	$NOCM^{-3}$	INTEGRAL	$NOCM^{-3}$	INTEGRAL	$NOCM^{-3}$
Glaucoma	7.89	0.32	43.14	1.73	3.17	0.13	-	-	-	-
Cyclidium	60.15	2.41	35.09	1.40	128.62	5.14	175.89	7.04	518.99	20.76
Cinetochilum	2.19	0.09	270.40	10.82	1155.11	46.20	3217.25	128.69	70.12	2.80
Tachysoma	2.44	0.10	151.43	6.06	1445.54	57.82	335.37	13.41	160.68	6.43
Aspidisca	7.01	0.28	83.03	3.32	841.45	33.66	554.42	22.18	547.59	21.90
Vorticella	-	-	12.70	0.51	218.02	8.72	32.55	1.30	154.39	6.18
Total Ciliates	166.60	6.66	918.28	36.73	4608.74	184.35	5034.39	201.38	2655.00	106.20
Flagellates	2904.44	116.18	3776.56	151.06	5748.09	229.92	12180.38	487.22	29646.06	1185.84
Total Protozoa	3076.31	123.05	4732.18	189.29	10941.42	437.66	17693.50	707.74	32834.63	1313.39

RUN 6

	DAY 5		DAY 12		DAY 18		DAY 26		DAY 40	
MAX. DEPTH EXAMINED (CM)	25		25		25		25		25	
SPECIES	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}
Glaucoma	-	-	-	-	209.00	8.36	625.57	25.02	79.11	3.16
Cyclidium	57.19	2.29	607.77	24.31	2056.11	82.24	2745.17	109.81	1418.20	56.73
Cinetochilum	13.11	0.52	308.87	12.35	1371.98	54.88	418.11	16.72	1418.20	56.73
Tachysoma	54.77	2.19	1174.50	46.98	2030.43	81.22	390.66	15.63	172.75	6.91
Aspidisca	89.61	3.58	739.16	29.57	1941.75	77.67	607.94	24.32	1209.98	48.40
Vorticella	10.06	0.40	32.81	1.31	28.40	1.14	218.60	8.74	554.33	22.17
Total Ciliates	339.11	13.56	2990.73	119.63	8950.97	358.04	5990.95	239.64	4676.90	187.08
Flagellates	1342.11	53.68	3556.53	142.26	6368.48	254.74	8113.97	324.56	5199.75	207.99
Total Protozoa	1681.57	647.26	6637.40	265.50	16115.47	644.62	14462.44	578.50	10767.53	430.70

RUN 7

	DAY 3		DAY 10		DAY 19		DAY 32		DAY 45	
MAX. DEPTH EXAMINED (CM)	25		25		25		25		25	
SPECIES	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}
Glaucoma	44.65	1.79	44.27	1.77	219.23	8.77	407.20	16.29	54.28	2.17
Cyclidium	46.78	1.87	13.04	0.52	4.12	0.16	226.99	9.08	239.03	9.56
Cinetochilum	9.69	0.39	9.96	0.40	-	-	286.62	11.46	404.69	16.19
Tachysoma	3.58	0.14	40.43	1.62	1072.64	42.91	1100.80	44.03	427.72	17.11
Aspidisca	4.37	0.17	2.95	0.12	30.81	1.23	423.30	16.93	1636.06	65.44
Vorticella	-	-	0.13	0.005	13.54	0.54	53.12	2.12	185.94	7.44
Total Ciliates	120.34	4.81	119.80	4.79	1872.50	74.9	3081.81	123.27	4102.10	164.08
Flagellates	3716.92	148.68	2493.70	99.75	2636.46	105.46	2847.27	113.89	1961.85	78.47
Total Protozoa	3858.56	154.34	2617.35	104.69	4515.24	180.61	6284.83	251.39	6984.44	279.38

RUN 8

	DAY 1		DAY 9		DAY 17		DAY 28	
MAX. DEPTH EXAMINED (CM)	25		25		25		25	
SPECIES	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}
Glaucoma	18.44	0.74	239.46	9.58	73.81	2.95	30.35	1.21
Cyclidium	15.24	0.61	57.53	2.30	563.87	22.55	40.36	1.61
Cinetochilum	22.65	0.91	60.22	2.41	621.01	24.84	306.66	12.27
Tachysoma	17.86	0.71	28.58	1.14	612.14	24.49	163.11	6.52
Aspidisca	34.25	1.37	33.19	1.33	693.58	27.74	212.12	8.48
Vorticella	-	-	12.23	0.49	39.59	1.58	19.96	0.80
Total Ciliates	170.84	6.83	498.01	19.92	2967.93	118.72	1306.45	52.26
Flagellates	458.13	18.33	367.89	14.72	1482.33	59.29	2946.63	117.87
Total Protozoa	652.48	26.10	910.43	36.42	4543.06	181.72	4393.87	175.75

BED 44

	DAY 3		DAY 10		DAY 24		DAY 33	
MAX. DEPTH EXAMINED .(CM)	25		25		25		25	
SPECIES	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}	INTEGRAL	NOCM^{-3}
Glaucoma	-	-	-	-	331.85	13.27	44.87	1.79
Cyclidium	3.99	0.16	121.71	4.87	1427.49	57.12	722.44	28.90
Cinetochilum	11.50	0.46	64.61	2.58	730.60	29.22	498.68	19.95
Tachysoma	13.42	0.54	8.85	0.35	408.36	16.33	50.51	2.02
Aspidisca	107.98	4.32	152.57	6.10	554.98	22.20	183.70	7.35
Vorticella	-	-	-	-	154.47	6.18	113.90	4.56
Total Ciliates	118.30	4.73	429.70	17.19	4475.61	179.02	2147.35	85.89
Flagellates	556.98	22.28	2198.28	87.93	4768.07	190.72	2464.28	98.57
Total Protozoa								

Appendix 8.2 REGRESSION TABLE : INSTANTANEOUS RATES OF CHANGE IN POPULATION DENSITY

SPECIES	TIME PERIOD (DAYS)	SLOPE	S.E. SLOPE	INTER-CEPT	VAR. RATIO	dF	p (VAR. RATIO)
RUN 1							
Glaucoma	4 - 11	0.213	0.097	1.025	4.846	12	0.05
Tachysoma	4 - 11	0.576	0.211	-2.361	7.443	9	0.025
Vorticella	4 - 11	0.332	0.078	-1.160	18.174	10	0.005
Total Ciliates	1 - 11	0.541	0.095	-0.531	32.759	16	0.001
Flagellates	7 - 11	0.513	0.188	-0.486	7.489	10	0.025
RUN 2							
Glaucoma	3 - 12	0.166	0.082	1.691	4.127	15	0.1
Cyclidium	3 - 12	0.108	0.085	1.902	1.610	10	0.25
Tachysoma	3 - 12	0.199	0.059	2.329	11.202	15	0.005
Aspidisca	3 - 12	0.400	0.096	-0.322	17.473	12	0.005
Vorticella	3 - 12	0.090	0.071	0.515	1.610	12	0.25
Total Ciliates	3 - 12	0.263	0.066	2.739	15.770	16	0.005
Flagellates	3 - 12	-0.040	0.056	5.634	0.497	16	0.5
RUN 3							
Glaucoma	1 - 8	0.129	0.080	2.909	2.574	17	0.25
	8 - 28	-0.119	0.025	5.016	21.966	34	0.001
Cyclidium	1 - 12	0.218	0.049	1.578	19.933	21	0.001
	12 - 28	-0.110	0.030	5.272	13.374	26	0.005
Cinetochilum	1 - 28	0.073	0.033	0.516	4.830	7	0.10
Tachysoma	1 - 5	0.584	0.113	1.116	26.870	10	0.001
	5 - 28	-0.097	0.025	4.802	14.847	39	0.001
Aspidisca	1 - 5	0.521	0.143	2.212	13.339	10	0.005
	5 - 28	-0.123	0.027	4.507	20.255	37	0.001
Vorticella	1 - 22	0.132	0.039	-0.757	11.235	15	0.005
	22 - 28	-0.045	0.119	3.403	0.145	10	0.75
Total Ciliates	1 - 5	0.453	0.239	3.373	14.326	11	0.005
	5 - 28	-0.036	0.015	5.755	6.269	42	0.025
Flagellates	1 - 8	0.212	0.050	4.176	18.309	17	0.001
	8 - 28	-0.062	0.028	5.979	4.975	35	0.05
RUN 4							
Glaucoma	3 - 13	0.190	0.061	1.245	9.881	16	0.01
	13 - 25	-0.200	0.064	7.133	9.847	6	0.025
Cyclidium	3 - 13	0.281	0.076	-1.034	13.677	11	0.005
	13 - 25	-0.230	0.057	6.357	16.487	4	0.25
Cinetochilum	3 - 13	0.335	0.109	0.074	9.510	14	0.01
	13 - 25	-0.258	0.074	7.529	12.176	9	0.01
Tachysoma	3 - 10	0.371	0.086	0.385	18.589	12	0.001
	10 - 25	-0.123	0.040	5.301	9.302	16	0.01
Aspidisca	3 - 13	0.321	0.076	-0.618	17.883	12	0.005
	13 - 25	-0.098	0.069	5.108	2.039	9	0.25

Appendix 8.2 (contd)

SPECIES	TIME PERIOD (DAYS)	SLOPE	S.E. SLOPE	INTER-CEPT	VAR. RATIO	dF	p (VAR. RATIO)
RUN 4 (Contd)							
Vorticella	10 - 25	0.121	0.064	-0.078	3.543	11	0.1
Total Ciliates	3 - 10	0.352	0.062	1.793	31.863	14	0.001
	10 - 25	-0.050	0.043	5.779	1.318	20	0.5
Flagellates	3 - 13	0.244	0.091	1.579	7.129	22	0.025
	13 - 25	-0.033	0.077	4.879	0.181	14	0.75
RUN 5							
Glaucoma	1 - 11	-0.019	0.089	0.822	0.043	7	0.75
Cyclidium	1 - 18	0.068	0.052	1.309	1.719	26	0.25
Cinetochium	1 - 13	0.242	0.104	0.792	5.414	20	0.05
	13 - 18	-0.474	0.333	9.858	2.027	8	0.25
Tachysoma	1 - 11	0.412	0.069	-0.457	35.161	16	0.001
	11 - 18	-0.263	0.096	6.524	7.505	22	0.025
Aspidisca	1 - 11	0.431	0.063	-1.206	46.253	15	0.001
	11 - 18	-0.009	0.084	3.608	0.011	18	0.75
Vorticella	6 - 11	0.463	0.166	-2.374	7.793	8	0.025
	11 - 18	0.059	0.136	1.886	0.189	11	0.75
Total Ciliates	1 - 11	0.366	0.055	1.302	44.425	24	0.001
	11 - 18	-0.144	0.097	6.854	2.179	24	0.25
Flagellates	1 - 18	0.108	0.035	4.194	9.795	40	0.005
RUN 6							
Glaucoma	18 - 26	0.054	0.098	1.959	0.307	8	0.75
	26 - 40	-0.033	0.045	4.210	0.519	9	0.50
Cyclidium	5 - 18	0.172	0.071	0.712	5.904	15	0.05
	18 - 40	-0.017	0.032	4.199	0.264	19	0.75
Cinetochilum	5 - 18	0.254	0.034	-0.745	55.335	18	0.001
	18 - 40	-0.035	0.024	4.246	2.189	13	0.25
Tachysoma	5 - 18	0.223	0.046	0.345	23.622	20	0.001
	18 - 40	-0.081	0.022	5.290	12.974	21	0.005
Aspidisca	5 - 18	0.199	0.038	0.359	26.890	20	0.001
	18 - 40	0.0003	0.021	3.489	0.0002	21	0.75
Vorticella	5 - 40	0.062	0.023	-0.060	7.446	24	0.025
Total Ciliates	5 - 18	0.241	0.035	1.501	46.024	22	0.001
	18 - 40	-0.024	0.017	6.167	1.963	22	0.25
Flagellates	5 - 26	0.085	0.015	3.661	31.766	30	0.001
	26 - 40	-0.034	0.025	6.583	1.897	14	0.25
RUN 7							
Glaucoma	3 - 32	0.054	0.026	0.928	4.408	21	0.05
	32 - 45	-0.016	0.073	5.076	1.099	10	0.5
Cyclidium	3 - 45	0.027	0.017	0.830	2.588	17	0.25
Cinetochilum	3 - 45	0.047	0.015	0.389	10.760	16	0.005

Appendix 8.2 (contd)

SPECIES	TIME PERIOD (DAYS)	SLOPE	S.E. SLOPE	INTER-CEPT	VAR. RATIO	dF	p (VAR RATIO)
RUN 7 (Contd)							
Tachysoma	3 - 19	0.281	0.036	-1.290	59.895	14	0.001
	19 - 45	-0.052	0.020	5.273	6.575	22	0.025
Aspidisca	3 - 45	0.110	0.014	-0.803	64.483	21	0.001
Vorticella	10 - 45	0.073	0.031	-0.632	5.503	12	0.05
Total Ciliates	3 - 19	0.183	0.040	0.633	20.759	20	0.001
	19 - 45	0.032	0.017	3.871	3.472	22	0.1
Flagellates	3 - 45	0.000	0.012	4.554	0.001	38	0.75
RUN 8							
Glaucoma	1 - 9	0.195	0.058	0.701	11.485	9	0.01
	9 - 28	-0.072	0.024	2.901	8.848	16	0.01
Cyclidium	1 - 17	0.179	0.030	-0.347	34.506	14	0.001
	17 - 28	-0.188	0.045	6.119	17.438	9	0.005
Cinetochilum	1 - 17	0.204	0.030	-0.315	46.355	18	0.001
	17 - 28	-0.088	0.039	4.905	5.071	14	0.05
Tachysoma	1 - 17	0.169	0.046	0.329	13.722	14	0.005
	17 - 28	-0.127	0.065	5.366	3.752	14	0.1
Aspidisca	1 - 17	0.166	0.030	0.037	30.305	19	0.001
	17 - 28	-0.094	0.046	4.900	4.160	13	0.01
Vorticella	9 - 28	0.080	0.023	-0.569	12.160	10	0.01
Total Ciliates	1 - 17	0.182	0.021	1.758	77.980	22	0.001
	17 - 28	-0.078	0.036	6.312	4.776	14	0.05
Flagellates	1 - 28	0.079	0.011	2.455	49.839	30	0.001
BED 44							
Glaucoma	24 - 33	-0.115	0.047	5.469	5.978	6	0.05
Cyclidium	3 - 24	0.183	0.027	-0.333	45.189	16	0.001
	24 - 33	-0.042	0.054	5.078	0.619	13	0.5
Cinetochilum	3 - 24	0.152	0.021	-0.309	54.657	14	0.001
	24 - 33	-0.056	0.065	4.741	0.737	13	0.5
Tachysoma	3 - 24	0.127	0.032	-0.390	15.470	14	0.005
	24 - 33	-0.210	0.081	7.768	6.788	11	0.025
Aspidisca	3 - 24	0.117	0.019	0.332	40.024	21	0.001
	24 - 33	-0.131	0.055	6.242	5.642	14	0.05
Vorticella	3 - 33	0.007	0.042	1.578	0.029	14	0.75
Total Ciliates	3 - 24	0.171	0.020	1.195	77.143	21	0.001
	24 - 33	-0.099	0.058	7.697	2.888	14	0.05
Flagellates	3 - 24	0.099	0.016	3.020	37.385	22	0.001
	24 - 33	-0.060	0.032	6.739	3.512	14	0.1

Appendix 8.3 TOTAL CILIATE BIOVOLUME ($\mu m^3 cm^{-3}$)

CALCULATED FOR EACH SAMPLE CORE

RUN 4	3	10	13	25	
BIO-VOLUME	7.47×10^4	1.00×10^6	7.99×10^5	1.61×10^6	
RUN 5	1	6	11	13	18
BIO-VOLUME	5.31×10^4	2.25×10^5	3.68×10^6	1.39×10^6	2.25×10^6
RUN 6	5	12	18	26	40
BIO-VOLUME	8.21×10^4	5.94×10^5	1.84×10^6	6.91×10^5	2.35×10^6
RUN 7	3	10	19	32	45
BIO-VOLUME	8.58×10^3	4.71×10^4	8.39×10^5	1.63×10^6	2.84×10^6
RUN 8	1	9	17	28	
BIO-VOLUME	3.64×10^4	8.28×10^4	1.12×10^6	1.22×10^6	
BED 44	3	10	24	33	
BIO-VOLUME	3.05×10^4	4.37×10^4	8.02×10^5	1.96×10^5	

Appendix 8.4 OXYGEN BALANCE IN BED 45 DURING RUNS 5 (JULY/AUGUST) AND 8 (MARCH/MAY)

T = top water dissolved oxygen

F = filtrate water dissolved oxygen

RUN 5 (July/August, 1976)

DAY	T - F $mgO_2cm^{-2}d^{-1}$	DAY	T - F $mgO_2cm^{-2}d^{-1}$	DAY	T - F $mgO_2cm^{-2}d^{-1}$
1	3.38	10	-0.82	19	2.15
2	2.46	11	0	20	2.66
3	1.43	12	0.82	21	2.87
4	0.72	13	1.23	22	3.79
5	-1.03	14	1.23	23	3.59
6	-0.92	15	1.84	24	5.33
7	-4.30	16	1.64	25	4.61
8	-2.87	17	1.84	26	6.45
9	-1.74	18	1.84	27	4.61

RUN 8 (March/May, 1977)

DAY	T - F $mgO_2cm^{-2}d^{-1}$	DAY	T - F $mgO_2cm^{-2}d^{-1}$	DAY	T - F $mgO_2cm^{-2}d^{-1}$
1	1.74	18	0.31	29	2.87
2	0.51	19	0	30	2.46
3	0.41	20	0.72	31	3.38
4	1.33	21	0.82	32	1.64
5	1.54	22	1.43	33	2.26
6	0.61	23	-0.11	34	2.87
7	0.61	24	1.12	35	2.15
8	0.92	25	1.33	36	0.41
9	0.11	26	1.12	37	1.74
15	-4.30	27	0.82	38	2.76
16	-0.20	28	1.54	39	1.54
17	0.72				

Appendix 9.1 Depth distributions of bacteria, ciliates and particulate organic carbon in sand cores from Bed 44, February 1978

(a) Bacteria removed from sand by shaking with sterile water

DEPTH (CM)	Density (Nos. X 10^6 cm^{-3}), mean of two counts						
	DAY 0	DAY 1	DAY 2	DAY 4	DAY 7	DAY 8	DAY 10
1	4.54	1.20	8.14	6.82	10.10	14.44	14.65
5	↓	0.87	4.68	6.78	12.15	10.90	8.48
10		1.78	13.62	11.74	7.70	8.97	4.41
20		1.33	6.38	7.70	8.44	12.25	4.17
30		0.76	2.08	10.16	3.53	7.82	3.58

(b) Ciliates

DEPTH (CM)	Density (Nos. cm^{-3})				
	DAY 1	DAY 2	DAY 7	DAY 8	DAY 10
1	34	12	65	123	72
5	4	8	34	38	NO DATA
10	20	2	12	12	NO DATA

(c) Particulate Organic Carbon

DEPTH (CM)	Concentration (mg. cm^{-3}), mean of two results						
	DAY 0	DAY 1	DAY 2	DAY 4	DAY 7	DAY 8	DAY 10
1	1.152	1.32	0.90	1.02	2.06	2.02	2.39
5		0.66	0.67	1.21	1.34	1.14	1.97
10		0.71	0.99	0.71	1.59	1.55	3.06
20		0.44	0.74	1.29	1.61	1.66	2.10
30		1.42	1.25	1.33	1.30	1.01	2.55

Appendix 9.2 Integral densities of bacteria and ciliates and concentrations of particulate organic carbon from cores in Bed 44 analysed in February 1978

(a) Bacteria removed from sand by shaking with sterile water to core depth of 30 cm.

DAY	TOTAL DENSITY NOS. cm^{-2}	DENSITY NOS. cm^{-3}
1	3.79×10^{7}	1.26×10^{6}
2	2.21×10^{8}	7.37×10^{6}
4	2.59×10^{8}	8.63×10^{6}
7	2.37×10^{8}	7.90×10^{6}
8	3.11×10^{8}	1.04×10^{7}
10	1.55×10^{8}	5.17×10^{6}

(b) Ciliates to core depth of 10 cm

DAY	TOTAL DENSITY NOS. cm^{-2}	DENSITY NOS. cm^{-3}
1	108	11
2	66	7
7	304	30
8	405	41
10	* 319	32

* Estimated value as no data were recorded for depths 5 and 10 cm.

Appendix 9.2 (contd)

(c) Particulate Organic Carbon to core depth of 30 cm

DAY	TOTAL CONCENTRATION mg cm^{-2}	CONCENTRATION mg cm^{-3}
1	19.3	0.7
2	23.6	0.8
4	29.2	1.0
7	39.5	1.5
8	43.3	1.4
10	63.9	2.4

Appendix 9.3 Carbon concentrations and ciliate densities recorded after draining the model filter column

(a) Column drained after 8 days (5.1.78, 8.5°C)

DEPTH (CM)	SAND WET WT (G)	CARBON CONC (μg)	*CARBON (μg cm^{-3})	MEAN CONC (μg cm^{-3})
1	0.928	125.26	215.70	
1	0.835	162.86	311.68	264.21
1	0.945	156.86	265.25	
5	0.858	480.74	895.36	968.36
5	0.970	632.12	1041.37	
10	0.730	280.18	613.33	
10	1.014	374.92	590.85	614.87
10	1.092	437.65	640.44	
20	1.285	371.69	462.23	
20	0.968	341.01	562.95	483.35
20	0.932	247.79	424.86	
30	0.997	354.04	567.46	
30	0.818	222.53	434.72	487.36
30	0.899	258.73	459.90	

DEPTH (CM)	SAND WET WT (G)	CILIATE NOS.	*CILIATES (NO. CM^{-3})
1	0.933	18	31
5	0.677	87	205
10	0.749	80	171

(b) Column drained after 37 days (30.11.77, 9.5°C)

DEPTH (CM)	SAND WET WT. (G)	CARBON CONC (μg)	*CARBON μg cm^{-3}
1	0.789	803.94	1628.26
5	0.354	255.93	1155.30
10	0.356	259.69	1165.69

DEPTH (CM)	SAND WET WT (G)	CILIATE NOS.	*CILIATES (NO. CM^{-3})
1	0.478	35	117

* Conversion factor 1 cm^3 = 1.598 g sand wet weight.

Appendix 9.4 Development of Interstitial Bacteria in Sand obtained from the Hampton Washing Bays during a model filter column run. (12.1.78 - 23.1.78, 7°C)

AGE OF RUN DAYS	SAND DEPTH (CM)	COUNT (NOS. ML^{-1})	MEAN COUNT (NOS. ML^{-1})
1	1	1.58×10^4	
1	1	1.62×10^4	1.60×10^4
1	5	2.04×10^4	
1	5	2.82×10^4	2.43×10^4
1	10	2.83×10^4	
1	10	8.78×10^3	1.85×10^4
1	20	3.02×10^4	
1	20	3.23×10^4	3.13×10^4
1	30	1.43×10^4	
1	30	1.58×10^4	1.51×10^4
5	1	2.62×10^4	
5	1	8.99×10^4	5.81×10^4
5	5	1.42×10^5	
5	5	7.27×10^4	1.07×10^5
5	10	9.55×10^4	
5	10	4.33×10^4	6.94×10^4
5	20	7.00×10^4	
5	20	7.03×10^4	7.02×10^4
5	30	5.81×10^4	
5	30	5.09×10^4	5.45×10^4
7	1	1.92×10^4	
7	1	4.67×10^4	3.30×10^4
7	5	9.44×10^4	
7	5	2.59×10^4	6.02×10^4
7	10	4.59×10^4	
7	10	2.17×10^4	3.38×10^4
7	20	1.27×10^4	
7	20	3.08×10^4	2.18×10^4
7	30	7.61×10^4	
7	30	4.02×10^4	5.82×10^4
9	1	3.41×10^4	
9	1	3.27×10^4	3.34×10^4
9	5	2.34×10^4	
9	5	3.37×10^4	2.86×10^4
9	10	6.19×10^3	
9	10	1.17×10^4	8.95×10^3
9	20	2.74×10^4	
9	20	2.38×10^4	2.56×10^4
9	30	1.76×10^4	1.76×10^4
11	1	6.87×10^4	
11	1	1.00×10^5	8.44×10^4
11	5	4.00×10^4	
11	5	3.55×10^4	3.78×10^4
11	10	3.98×10^4	
11	10	1.76×10^4	2.87×10^4
11	20	3.31×10^4	
11	20	4.11×10^4	3.71×10^4
11	30	1.76×10^4	
11	30	2.81×10^4	2.29×10^4

Appendix 9.5 Development of interstitial bacteria in filter column sand used in a previous filtration run with the top cm. replaced with washed sand, during a further model filter column run. (29.12.77 - 4.1.78, 9°C)

AGE OF RUN (DAYS)	DEPTH OF SAND (CM)	COUNT (NOS. ML^{-1})	MEAN COUNT (NOS. ML^{-1})
1	1	7.16×10^4	
1	1	8.64×10^4	7.90×10^4
1	5	2.74×10^5	
1	5	2.48×10^5	2.61×10^5
1	10	2.93×10^5	2.93×10^5
1	20	1.31×10^5	
1	20	2.64×10^5	1.98×10^5
1	30	1.83×10^5	
1	30	1.99×10^5	1.91×10^5
2	1	7.82×10^4	
2	1	6.31×10^4	7.07×10^4
2	5	9.87×10^4	
2	5	3.74×10^5	6.81×10^4
2	10	1.79×10^5	
2	10	7.38×10^4	1.26×10^5
2	20	5.89×10^4	
2	20	5.70×10^4	5.80×10^4
2	30	1.03×10^5	
2	30	5.07×10^4	7.69×10^4
3	1	1.04×10^5	
3	1	1.89×10^5	1.47×10^5
3	5	1.47×10^5	
3	5	1.48×10^5	1.48×10^5
3	10	7.84×10^4	
3	10	8.16×10^4	8.00×10^4
3	20	1.16×10^5	
3	20	7.57×10^4	9.59×10^4
3	30	4.52×10^4	
3	30	6.64×10^4	5.58×10^4
4	1	1.33×10^5	
4	1	5.97×10^4	9.64×10^4
4	5	1.22×10^5	
4	5	6.75×10^4	9.48×10^4
4	10	7.27×10^4	
4	10	7.42×10^4	7.35×10^4
4	20	2.83×10^4	
4	20	3.37×10^4	3.10×10^4
4	30	2.56×10^4	
4	30	4.24×10^4	3.40×10^4

Appendix 9.5 (Contd)

AGE OF RUN (DAYS)	DEPTH OF SAND (CM)	COUNT (NOS. ML^{-1})	MEAN COUNT (NOS. ML^{-1})
5	1	4.49×10^4	
5	1	1.34×10^5	8.95×10^4
5	5	4.43×10^4	
5	5	2.59×10^4	3.51×10^4
5	10	3.78×10^4	
5	10	2.98×10^5	3.38×10^4
5	20	1.27×10^5	
5	20	7.44×10^4	1.01×10^5
5	30	4.99×10^4	
5	30	2.37×10^4	3.68×10^4
6	1	6.09×10^4	
6	1	3.28×10^4	4.69×10^4
6	5	4.40×10^4	
6	5	3.50×10^4	3.95×10^4
6	10	3.31×10^4	
6	10	1.70×10^4	2.51×10^4
6	20	1.29×10^4	
6	20	1.54×10^4	1.42×10^4
6	30	1.12×10^4	
6	30	1.33×10^4	1.23×10^4
7	1	2.84×10^4	
7	1	3.78×10^4	3.31×10^4
7	5	3.68×10^4	
7	5	4.00×10^4	3.84×10^4
7	10	1.95×10^4	
7	10	3.98×10^4	2.97×10^4
7	20	2.29×10^4	
7	20	1.27×10^3	1.78×10^4
7	30	5.73×10^4	
7	30	6.26×10^4	3.42×10^4

Appendix 9.6 Redox potentials recorded during two model filter column runs

(a) Column filled with clean sand obtained from the washing bays at Hampton (12.1.78 - 23.1.78, 7°C)

Depth (cm)	Day 1	Day 5	Day 7	Day 9	Day 11
1	395.8	372.8	398.3	415.6	411.5
2.5	531.9	516.8	533.3	525.3	554.0
5	442.8	415.7	458.6	465.5	404.6
7.5	459.3	459.1	475.7	487.8	477.9
10	524.5	520.7	479.5	515.3	513.7
20	529	490.7	505.6	513.6	497.7
30	550.8	541.5	543.8	546.7	544.0

(b) Column filled with sand used in a previous filtration run, with the top cm replaced with washed sand (29.12.77 - 4.1.78, 9°C)

Depth (cm)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	421.6	389.6	399.6	432.4	431.2	436.7	419.5
2.5	444.3	454.4	464.5	500.4	539.0	563.5	572.6
5	440.7	449.4	428.3	447.8	444.6	434.6	442.6
7.5	467.9	459.0	433.0	462.2	462.2	459.1	463.1
10	531.5	523.2	515.2	487.5	517.4	514.1	513.0
20	513.5	502.9	498.0	509.6	496.6	502.5	478.4
30	553.0	532.9	543.1	548.6	551.3	548.4	548.8

Appendix 9.7 Diurnal variation in the depth distribution of interstitial bacteria in the model filter column

Run 1 (26.1.78, 7.25°C)

TIME (HOURS)	DEPTH (CM)	COUNT ML^{-1}	MEAN COUNT ML^{-1}
04.00	SURFACE WATER	4.82×10^4	3.53×10^4
	SURFACE WATER	2.24×10^4	
	1	4.08×10^4	4.56×10^4
	1	5.03×10^4	
	5	6.40×10^4	5.45×10^4
	5	4.49×10^4	
	10	3.31×10^4	3.13×10^4
	10	2.95×10^4	
	20	3.45×10^4	2.42×10^4
	20	1.38×10^4	
	30	2.41×10^4	2.46×10^4
	30	2.51×10^4	
08.00	SURFACE WATER	1.03×10^4	1.04×10^4
	SURFACE WATER	1.05×10^4	
	1	1.78×10^4	3.65×10^4
	1	5.51×10^4	
	5	4.54×10^4	3.83×10^4
	5	3.11×10^4	
	10	2.10×10^4	2.59×10^4
	10	3.08×10^4	
	20	2.16×10^4	1.97×10^4
	20	1.78×10^4	
	30	3.11×10^4	3.22×10^4
	30	3.32×10^4	
12.00	SURFACE WATER	1.49×10^4	1.30×10^4
	SURFACE WATER	1.11×10^4	
	1	7.73×10^4	7.73×10^4
	5	5.41×10^4	5.07×10^4
	5	4.73×10^4	
	10	2.30×10^4	1.61×10^4
	10	9.26×10^3	
	20	1.31×10^4	1.26×10^4
	20	1.20×10^4	
	30	1.16×10^4	1.14×10^4
	30	1.12×10^4	

Appendix 9.7 (contd)

Run 1 (contd)

TIME (HOURS)	DEPTH (CM)	COUNT ML^{-1}	MEAN COUNT ML^{-1}
16.00	SURFACE WATER	1.09×10^4	1.10×10^4
	SURFACE WATER	1.11×10^4	
	1	2.23×10^4	2.66×10^4
	1	3.09×10^4	
	5	2.56×10^4	2.85×10^4
	5	3.14×10^4	
	10	1.57×10^4	1.87×10^4
	10	2.17×10^4	
	20	1.14×10^4	1.91×10^4
	20	2.68×10^4	
	30	2.05×10^4	1.65×10^4
	30	1.25×10^4	
20.00	SURFACE WATER	6.36×10^3	6.45×10^3
	SURFACE WATER	6.54×10^3	
	1	2.42×10^4	2.53×10^4
	1	2.63×10^4	
	5	2.50×10^4	1.88×10^4
	5	1.25×10^4	
	10	3.12×10^4	2.08×10^4
	10	1.03×10^4	
	20	2.30×10^4	1.77×10^4
	20	1.24×10^4	
	30	1.84×10^4	1.77×10^4
	30	1.70×10^4	
24.00	SURFACE WATER	7.91×10^3	1.13×10^4
	SURFACE WATER	1.47×10^4	
	1	1.96×10^4	3.06×10^4
	1	4.16×10^4	
	5	3.06×10^4	5.57×10^4
	5	8.08×10^4	
	10	3.52×10^4	2.81×10^4
	10	2.10×10^4	
	20	1.75×10^4	3.02×10^4
	20	4.28×10^4	
	30	1.51×10^4	2.08×10^4
	30	2.66×10^4	

Appendix 9.7 (contd)

Run 2 (11.2.78, 4.5°C)

TIME (HOURS)	DEPTH (CM)	COUNT ML^{-1}	MEAN COUNT ML^{-1}
04.00	SURFACE WATER	2.80×10^4	3.39×10^4
	SURFACE WATER	3.97×10^4	
	1	4.25×10^4	3.81×10^4
	1	3.36×10^4	
	5	6.63×10^4	7.03×10^4
	5	7.43×10^4	
	10	5.44×10^4	4.95×10^4
	10	4.46×10^4	
	20	9.22×10^4	7.54×10^4
	20	5.86×10^4	
	30	4.85×10^4	4.46×10^4
	30	4.07×10^4	
08.00	SURFACE WATER	1.33×10^4	1.01×10^4
	SURFACE WATER	6.96×10^3	
	1	2.68×10^4	4.12×10^4
	1	5.56×10^4	
	5	4.85×10^4	4.41×10^4
	5	3.96×10^4	
	10	3.27×10^4	3.02×10^4
	10	2.77×10^4	
	20	5.86×10^4	6.20×10^4
	20	6.54×10^4	
	30	6.93×10^4	4.46×10^4
	30	1.99×10^4	
12.00	SURFACE WATER	1.53×10^4	1.12×10^4
	SURFACE WATER	7.14×10^3	
	1	4.67×10^4	4.02×10^4
	1	3.36×10^4	
	5	5.65×10^4	4.91×10^4
	5	4.16×10^4	
	10	5.26×10^4	5.75×10^4
	10	6.24×10^4	
	20	5.06×10^4	5.70×10^4
	20	6.33×10^4	
	30	4.16×10^4	4.27×10^4
	30	4.37×10^4	
16.00	SURFACE WATER	1.61×10^4	1.49×10^4
	SURFACE WATER	1.37×10^4	
	1	1.99×10^4	2.48×10^4
	1	2.97×10^4	
	5	8.33×10^4	8.58×10^4
	5	8.83×10^4	
	10	2.17×10^4	1.79×10^4
	10	1.40×10^4	

Appendix 9.7 (contd)

Run 2 (contd)

TIME (HOURS)	DEPTH (CM)	COUNT ML^{-1}	MEAN COUNT ML^{-1}
16.00 (contd)	20	2.47×10^4	2.98×10^4
	20	3.48×10^4	
	30	1.12×10^4	3.58×10^4
	30	6.04×10^4	
20.00	SURFACE WATER	1.39×10^4	1.14×10^4
	SURFACE WATER	8.92×10^3	
	1	3.48×10^4	4.08×10^4
	1	4.67×10^4	
	5	4.25×10^4	6.19×10^4
	5	8.12×10^4	
	10	2.77×10^4	2.77×10^4
	10	2.77×10^4	
	20	3.48×10^4	2.38×10^4
	20	1.28×10^4	
	30	4.97×10^4	4.97×10^4
24.00	SURFACE WATER	1.19×10^4	1.30×10^4
	SURFACE WATER	1.41×10^4	
	1	6.93×10^4	5.10×10^4
	1	3.27×10^4	
	5	9.01×10^4	7.33×10^4
	5	5.65×10^4	
	10	7.43×10^4	4.95×10^4
	10	2.47×10^4	
	20	1.99×10^4	1.64×10^4
	20	1.28×10^4	
	30	3.36×10^4	3.62×10^4
	30	3.87×10^4	

Appendix 9.8 Diurnal variation in the depth profile of redox potential in the model filter column

Run 1 (26.1.78, 7.25°C)

DEPTH (CM)	TIME (HOURS)					
	04.00	08.00	12.00	16.00	20.00	24.00
1	404.3	406.9	413.8	413.8	413.0	-
2.5	473.3	469.1	474.5	478.4	481.9	484.4
5	502.4	489.7	507.5	508.9	511.1	519.4
10	510.6	506.2	505.8	506.8	509.9	510.1
20	525.6	526.9	527.4	522.6	527.6	529.2
30	529.9	-	524.9	526.3	541.8	540.6

Run 2 (11.2.78, 4.5°C)

DEPTH (CM)	TIME (HOURS)					
	04.00	08.00	12.00	16.00	20.00	24.00
1	-	525.9	560.8	574.1	577.7	-
2.5	-	482.5	445.6	455.7	439.4	-
5	-	554.8	547.2	539.6	549.5	-
7.5	-	540.6	537.1	541.4	524.6	-
10	-	512.3	513.0	510.5	511.0	-
20	-	690	568.1	554.8	552.4	-
30	-	534.2	555.8	539.5	539.5	-