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MICROBIOLOGY OF SEDIMENTS IN LAKES OF DIFFERING DEGREES
OF EUTROPHICATION

by

Mirna J.L. Godinho Orlandi

A thesis submitted for the degree of Doctor of Philosophy
in the University of Durham, England.

Department of Botany

April 1980



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Mirna J. Orlandi

Dedicated to Myrian, Miguel, Osmar
and Mara.

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Mirna J.L. Godinho Orlandi. Microbiology of sediments in lakes of differing degrees of eutrophication.

Abstract

A general survey was carried out on the sediments of seventeen lakes, ranging from oligotrophic to eutrophic, in the English Lake District. Several variables not directly concerned with the population of bacteria were measured to provide background information. Estimates of the total population of bacteria and of the population of filamentous bacteria were made using direct counts with acridine orange and fluorescein di-acetate, and counts by an MPN technique. The counts with acridine orange showed an upward trend with increasing degree of enrichment of the lakes, particularly at the eutrophic end of the spectrum. The distribution pattern obtained with the fluorochrome fluorescein di-acetate was different with an apparent upward trend in the intermediate lakes. The viable counts of the bacterial population in the sediments did not agree with the ranking of the lakes according to published information from the Freshwater Biological Association, although a slight upward trend was observed in the distribution of the viable filamentous bacteria.

The preliminary survey led to the selection of three lakes representing the oligotrophic, mesotrophic and eutrophic states in which a more detailed investigation on the population of filamentous bacteria was made. The profundal and littoral zones of the three lakes were investigated particularly in relation to the different groups of filamentous bacteria and their vertical distribution in the sediments. The groups of filamentous bacteria were described based on morphological and cytochemical tests. A tentative key for identification of filamentous bacteria was devised.

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Abbreviations

AO	acridine orange	rpm	revolutions per minute
°C	degree centigrade	s	second
cm	centimetre	SCY	sucrose-casitone-yeast extract
CPS	casein-peptone-starch	TSC	trypticase soy
E _h	electrode (redox) potential	v	volume
FDA	fluorescein di-acetate	v/w	volume/weight
Fig.	figure	wt	weight
G	group	μCi	microCurie
g	gramme	μg	microgramme
g	gravity, acceleration due to	μm	micron
h	hour		
kPa	kiloPascal		
l	litre		
lat.	latitude		
LMW	low molecular weight		
long.	longitude		
M	molar		
m	metre		
min	minute		
ml	millilitre		
mM	millimolar		
mm	millimetre		
MPN	most-probable-number		
mV	millivolt		
No	number		
nm	nanometre		
p	page		

1. INTRODUCTION

1.1 Bacteria in sediments

Bacterial population densities are far greater in sediments than in the water column (Henrici & McCoy, 1938). The realization of this fact brought about a fuller appreciation of the importance of the sediment in the life of a lake.

Although much more information was available on bacterial populations in the water column and despite the problems of sampling and enumeration, the early work on bacteria in the sediment (Henrici & McCoy, 1938; ZoBell, 1946) has thrown some light on this complex system. One of the major difficulties encountered by the earlier workers was the differentiation between inorganic particles and bacteria, since the only method available for direct count enumeration involved examination of smears by light microscopy. Even now, we face greater problems studying bacteria in sediments than in the water column. The presence of sharper chemical and physical gradients, the masking effect of detrital particles in direct counts and the presence of large numbers of spreading colonies in the standard spread plate technique are some of the difficulties encountered in sediment studies.

Henrici & McCoy (1938) observed the distribution of heterotrophic bacteria in sediments from lakes with a wide range of characteristics, using both plate counts and a direct count technique (a wet mount and a modification of the method used by Chododny in 1930). The plate counts showed that bacterial counts from the sediment were much higher than those from the water. It was found that counts from profundal stations were related to the productivity of the lakes being highest in the eutrophic lakes and lowest in the oligotrophic waters; dystrophic lakes gave high counts. The plate counts also showed a marked decrease



in bacterial number with depth in the sediment and this was, apparently, a logarithmic decline. Cooper et al. (1953) studied the horizontal distribution of bacteria in sediments of an oligotrophic lake using a standard plate count method. The study was made in two consecutive summers and sudden peaks in the number of bacteria were observed during both sampling periods. The peaks were not restricted to any particular area in the lake and were independent of the characteristics of the sediment. Each peak was formed apparently by a single organism, and each time a different one. Hayes & Anthony (1959) used plate counts to determine the relationship between bacterial numbers and other variables measured in several lakes of differing character. They observed that the number of bacteria was directly related to productivity, namely methyl orange alkalinity, conductivity and oxygen consumption over the mud, in all clear-water lakes. The brown-water lakes, low in alkalinity and conductivity, had high bacterial counts. A good correlation between bacterial count and water colour was detected. Vanderpost & Dutka (1971) studied the distribution of bacterial types and their densities and found that they varied greatly throughout the sampling area; bacterial densities were inversely related to sediment particle size and directly related to the amount of sediment organic matter. Aerobic bacteria were present in greater densities than anaerobes at both depths analysed (0 - 2 cm and 2 - 4 cm) but the two groups of bacteria were found to correlate with density variation in the two sediment layers. Vanderpost (1972) observed that bacterial counts, percentage organic carbon and water content decreased logarithmically with depth in the sediment. No seasonal variation of the population density of bacteria was detected and the effect of Eh was demonstrated by the increased ratio of anaerobic to aerobic bacteria with sediment depth. Bell & Dutka (1972) investigated the vertical distribution of bacteria, Eh and organic matter in

sediments. A sharp decrease was noted in the density of bacteria, paralleled by a decrease in the organic matter content and a change in Eh immediately below the 3 - 6 cm level. The samples were taken from different basins in the lake and the results obtained have shown that uniform conditions were present in the deep water sediments. In the above papers the techniques used to enumerate bacteria included membrane filtration plate count techniques, spread plates and most probable number counts (MPN). Strzelczyk (1976) used plate counts to study certain physiological groups of heterotrophic bacteria in water and in sediment. The lipolytic organisms proved to be the most numerous among the groups studied. The sediment also contained large populations of starch hydrolysing and denitrifying organisms, and also organisms more active in gelatin hydrolysis than the isolates from the water. No humic acid decomposers were observed. Collins (1977) provided information on the distribution of bacteria at the sediment-water interface, sediment surface and on the vertical distribution in different lakes, using either the pour plate method or MPN. The presence of viable bacteria in deep core sediment was also discussed. Higher counts of bacteria were observed in the more productive lakes and at the surface sediment. Studies of the vertical distribution of bacteria have shown that the more productive lakes have larger populations of heterotrophic and autotrophic bacteria.

The introduction of fluorochromes by Strugger (1948) in the study of bacteria in soil made it possible to apply this technique for other ecological studies of bacteria and therefore to examine new aspects and obtain more reliable estimates in sediments. Francisco et al. (1973) demonstrated the suitability of the epifluorescence count technique since it permitted the differentiation of bacteria from detritus and did not rely on cultural methods which estimate bacteria which can grow

on the specific media used.

Some of the work mentioned above was of comparative nature and concerned with lakes of differing trophic status. However it is not always easy to place a lake into a definite class (Cole, 1975) and as pointed out by Henrici & McCoy (1938) even though the number of bacteria tended to classify bottom deposits according to type, this classification is more clear at extremes than in the middle of a given range. Apart from the suggested classification of lakes based on the number of bacteria in sediments, several authors have tried to rank the lakes according to different criteria. Measurements of microbial biomass and activity (direct counts of bacteria, heterotrophic potential) (Gillespie, 1976), chlorophyll a and ATP concentration (Spencer, 1978), maturity indices based on other variables associated with the euphotic zone (Winner, 1972) and estimates of standing crop of surface water algae (Gorham, 1974) have all been used to classify lakes into series or groups (Jones et al., 1979).

Much of the recent research aimed at understanding the microbiology of sediments has been devoted to the measurements of processes, particularly those of geochemical significance. Reasonably reliable estimates have been obtained of rates of methanogenesis, sulphate reduction and denitrification (Rudd & Hamilton, 1978; Jørgensen, 1978; Sørensen, 1978) as has a better understanding of the interactions of the processes (Nedwell & Abram, 1979; Winfrey & Zeikus, 1979). Comparatively little has been done on the biology of the organisms and the consortia involved. This thesis is concerned with the filamentous bacteria, a group which is morphologically distinct but does not necessarily possess a unifying metabolic feature.

1.2 Description of the lakes

The English Lake District contains a group of lakes situated in a roughly circular area of about 50 km in diameter with its centre at lat. 54° 29' N long. 3° 03' W (Hutchinson, 1975). The geological formations of the Lake District consist of an elevated dome of Ordovician and Silurian rocks on which Carboniferous and later Triassic rocks were deposited unconformably. The doming of the region was supposed to have taken place in the mid-tertiary. Practically the whole Carboniferous and Triassic and all later deposits have now been eroded from the dome, and the present hydrography, due to uplift, was superimposed on a landscape of ancient rocks. These rock formations were later eroded into nine valleys due to widening and deepening by the glaciers of the ice-age. The valleys radiated from the dome and most of them contained a major lake depression. Moraine material was carried from the centre and deposited in the valleys mainly at the ends, where it sometimes formed a dam which raised the subsequent water level above the edge of the rock basin (Macan, 1970). The moraines and drifts which remained were those of the latest glaciation since in each successive glacial epoch, the deposits of previous glaciations were scoured away. Nearly all the existing lakes were the result of a combination of rock basin and a block or dam formed by moraines.

In its primitive state a glacial lake must have had an inorganic floor of glacial deposits, sand and gravel, silt and clay. At the end of the glaciation, no organic soil would remain on the ice-eroded surface of its drainage basin, and the absence of any microfossils from the basal lake sediments shows that the lakes were also quite barren (Pearsall & Pennington, 1973). With the spread of vegetation over the drainage basin, and accumulation of humus in stable soils, plant and animal life began in the waters of the lakes (Pearsall & Pennington, 1973).

Bearing this in mind it is clear that the diversity of the lakes partly reflects their basic geological history and partly the differentiation of the vegetation pattern.

As plants and animals grew in the lakes and contributed to the accumulating mud, changes took place both in the chemical composition of the lakes and in the plants and animals which flourished in them (Pearsall & Pennington, 1973). The freshwater sediment formed consisted of three primary components: organic matter in various stages of decomposition, particulate mineral matter and an inorganic component of biogenic origin. The organic material and mineral matter are of two types: autochthonous and allochthonous. The primary sedimented material was controlled by regional geology and climate and modified by biological processes in both the drainage basin of the lake and in the lake itself. The products of primary production within the lake are eventually mineralized by bacteria with attendant consumption of O_2 and evolution of CO_2 . The disappearance of O_2 in the hypolimnion, therefore, mirrors the basic production in the upper strata (Cole, 1975).

In the Lake District there are marked trophic differences between the lakes. Wastwater and Ennerdale at one end of the scale are the lakes which are more representative of those with rocky littoral zones; they are both very unproductive. At the other end of the scale are Esthwaite Water and Blelham Tarn in which the water is very much richer in all plant nutrients (Pearsall & Pennington, 1973). All lakes, productive and unproductive, silted or rocky, contain very similar thicknesses of sediment, which at the deepest point, consists of fine silts.

It was observed that the post-glacial sediment in Ennerdale was 6 m thick and in Esthwaite, 4.5 m thick. The reverse picture would be expected if organic matter production within the lake were the main source. Mackereth (1965) suggested that the degree to which lake

basins were filling with sediment must depend on balance between erosion of the drainage basins and the prevailing composition of hard ancient rocks. All Lake District rocks exposed today are hard, and yield little calcium to the water that flows over them. But based on the evidence of sediments, it is clear that lakes and tarns of all types were, in the early post-glacial period, both more productive and richer in calcium and other dissolved bases than they are today (Macan, 1970). The present productivity of each lake is positively correlated with the percentage of the drainage area which is cultivable and negatively correlated with the percentage of the lake bottom, to a water depth of 9m which is rocky, not silted (Pearsall, 1921). Even though many of the lakes in the Lake District are virtually untouched by cultural eutrophication, it is known that Windermere, Esthwaite Water, Grasmere and Blelham Tarn are becoming more productive. There is an increase in the concentration of certain nutrients and this is due to sewage effluent and agricultural fertilisers. Tables 1 and 2 summarize some of the chemical, physical and biological characteristics of the Cumbrian Lakes and Plate 1 is a Landsat satellite photograph of the same lakes.

The present work consisted initially of a general survey of the Cumbrian Lakes which led to the choice of three lakes for further studies. This choice was based in the case of Blelham Tarn on the high numbers of bacteria observed and the development of anoxic conditions in the hypolimnion during thermal stratification. The intermediate lake, Windermere South Basin, was chosen on the basis of its high input of nutrients and the absence of completely anoxic conditions but a substantial lowering of oxygen concentration in the hypolimnion. Wastwater was selected as the oligotrophic example since it is well known as one of the most nutrient poor of the English lakes with no biologically significant reduction in oxygen tension in the hypolimnetic water. Therefore the three selected lakes have characteristics that are entirely

Table 1. Some chemical and physical characteristics of the Cumbrian Lakes (Jones et al., 1979)

Lake	Maximum depth ¹ (m)	Volume ¹ (10 ⁶ m ³)	Human population ² per 10 ⁶ m ³ lake vol.	Depth of 1% light penetration ³ (m)	Total cations ⁴ (µeq l ⁻¹)	Phosphorus ⁵			pH ⁵	Sediment organic matter ⁴ (% loss on ignition)
						Total (µg l ⁻¹)	Soluble reactive (µg l ⁻¹)	Nitrate nitrogen ⁵ (µg l ⁻¹)		
1. Wastwater	76	115.6	1.32	38.4	329	2	0.5	256	6.85	14.4
2. Ennerdale	42	53.2	1.65	35.4	361	2	0.6	219	6.7	13.8
3. Buttermere	28.5	15.2	11.6	38.2	340	5	0.1	115	6.85	13.2
4. Crummock	44	66.4	4.8	24.2	340	3	0.4	154	6.7	12.0
6. Coniston	56	113.3	12.3 (18.5)	14.4	568	3	0.5	300	7.05	13.0
7. Thirlmere	46	52.5	3.1	14.4	354	3	0.6	179	6.6	19.5
8. Windermere (N)	64	201.8	21.95 (42.6)	14.4	500	13	0.7	309	7.4	19.0
9. Ullswater	62.5	223.0	6.1	13.0	507	7	0.2	146	7.15	10.2
10. Derwentwater	22	29.0	40.5	14.8	487	6	0.7	99	6.9	15.2
11. Bassenthwaite	19	27.9	273 (502)	6.1	609	21	0.9	194	7.6	11.9
12. Windermere (S)	42	112.7	77.4 (150)	13.0	545	20	1.5	294	7.9	17.4
13. Loweswater	16	5.4	12.96	8.5	509	7	1.3	404	7.05	11.7
14. Rydal Water	19	-	-	-	486	11	0.6	178	7.1	21.0
15. Grasmere	21.5	4.9	201 (612)	9.7	455	19	0.8	172 (14)	7.2	39.4 ⁶
16. Esthwaite Water	15.5	6.4	162 (212)	9.7	749	31	1.2	355	7.6	24.0
17. Blelham Tarn	14.5	0.69	141	8.7	755	29 (5.6)	1.8 (0.5)	342 (140)	7.4	26.5

Data in columns 6 to 9 are arithmetic means of data obtained from the epilimnion between April and September. Sources of data: ¹ from Ramsbottom (1976); ² from 1971 minor district census, () indicates increase due to tourist influx estimated from increased sewage effluent discharge; ³ from Talling (1971), values refer to penetration of green light; ⁴ largely from Gorham et al. (1974); ⁵ Freshwater Biological Association, unpublished data, () indicates standard deviation on seasonal mean; ⁶ Round (1957); Note Lake no. 5, Haweswater, is not included in the above table.

Table 2. Some biological features of the Cumbrian lakes (Jones et al., (1979)

Lake	Chlorophyll a^7 ($\mu\text{g l}^{-1}$)	Algal biomass ⁴ ($\mu\text{g dry wt l}^{-1}$)	Bacteria ml^{-1}		Phosphatase activity ⁷ ($\mu\text{mol PO}_4$ $\text{l}^{-1} \text{d}^{-1}$)
			10^{-3} x Plate counts ⁷	10^{-6} x Direct counts ¹⁰	
1. Wastwater	1.4	23	1.48	0.71	0.11
2. Ennerdale	1.0	93	0.82	2.04	0.36
3. Buttermere	1.6	70	1.28	1.25	0.38
4. Crummock	2.8	110	1.72	1.11	0.37
6. Coniston	4.2	790	1.54	2.43	0.49
7. Thirlmere	2.6	55	0.56	1.54	0.42
8. Windermere (N)	8.7	1400	1.49	1.84	0.64
9. Ullswater	4.7	3800	0.37	1.82	0.83
10. Derwentwater	3.1	1800	11.9	1.60	0.91
11. Bassenthwaite	11.1	4200	23.1	1.84	0.65
12. Windermere (S)	13.3	2800	1.23	2.28	0.97
13. Loweswater	6.8	2100	5.05	1.70	1.02
14. Rydal Water	9.6	1000*	6.45	2.52	1.41
15. Grasmere	15.6	910*	4.38(2.3) ⁸	1.87	1.01
16. Esthwaite Water	33.4	6800	3.61 ⁹	3.41 ⁹	2.45
17. Blelham Tarn	29.2(5.6)	3600	6.56(2.73)	4.32	1.83

Data in columns 1 and 3 to 5 are arithmetic means of data obtained from the epilimnion between April and September. Sources of data: ⁷ largely from Jones (1972) and unpublished data, () indicates standard deviation on seasonal mean; ⁸ from Hall et al. (1978) () indicates standard deviation on seasonal mean; ⁹ from Jones (1977); ¹⁰ from Jones et al. (1979). Note: Lake No. 5, Haweswater, is not included in the above table.

* Underestimates because no large algae were counted.

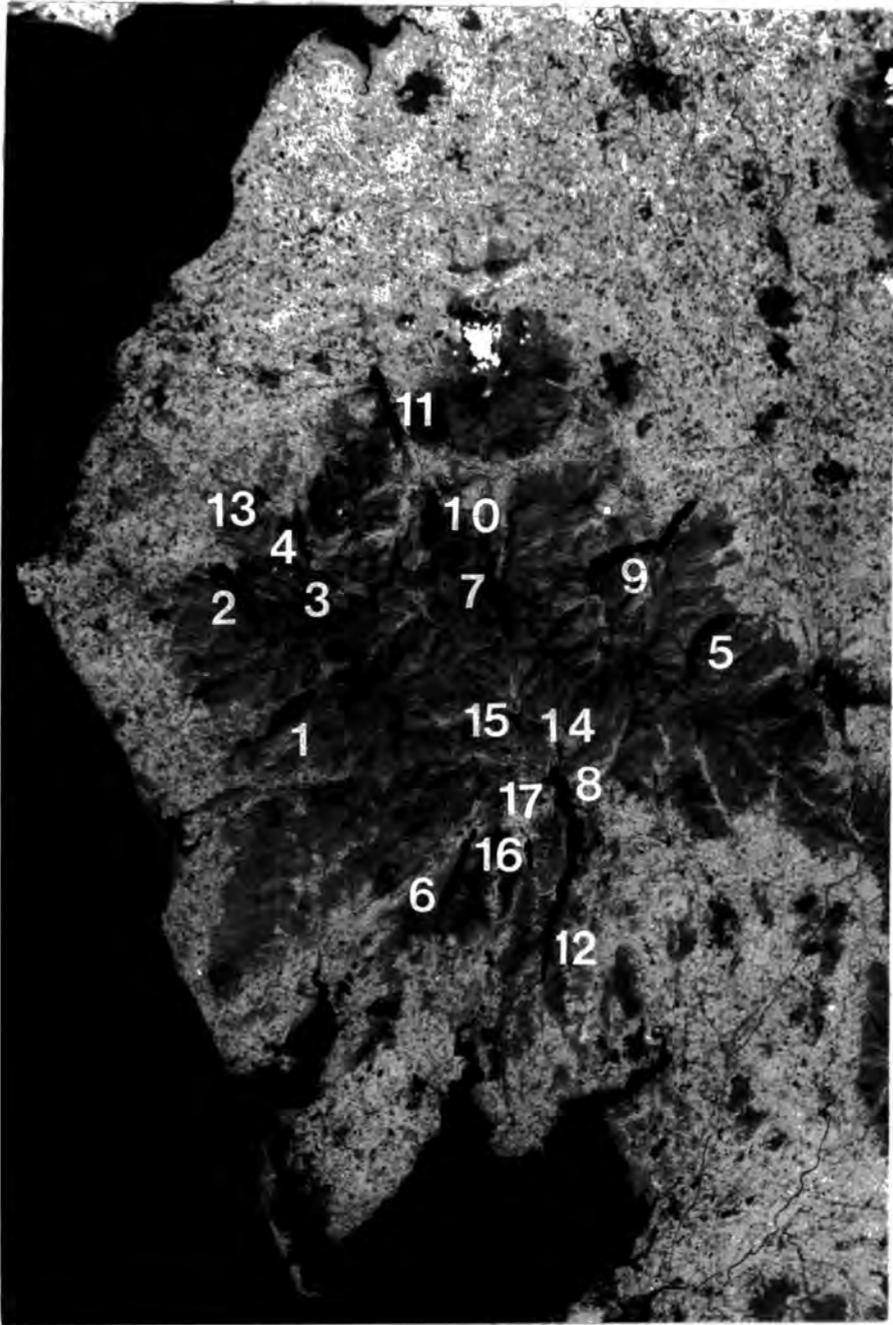


Plate 1. Landsat satellite photograph of the English Lake District

- | | |
|---------------------------|----------------------------|
| 1. Wastwater | 10. Derwentwater |
| 2. Ennerdale | 11. Bassenthwaite |
| 3. Buttermere | 12. Windermere South Basin |
| 4. Crummock Water | 13. Loweswater |
| 5. Haweswater | 14. Rydal Water |
| 6. Coniston Water | 15. Grasmere |
| 7. Thirlmere | 16. Esthwaite Water |
| 8. Windermere North Basin | 17. Blelham Tarn |
| 9. Ullswater | |

distinctive. Their general limnology may be summarized as follows, further details being obtainable in the references provided.

Wastwater, an oligotrophic lake, is the deepest lake in the English Lake District. Situated within the main mountain mass, this lake receives an inflow of water which has little nutrient content due to its bare rocky drainage area which has only a small proportion that is suitable for cultivation and human settlement (Macan, 1970). No deoxygenation of the hypolimnion takes place during thermal stratification and the oxidized zone is deeper compared with the other two lakes selected.

Windermere, a silted lake, is the largest in the district both in terms of length and volume. Biological studies have shown that the two basins may be considered as separate and distinct bodies of water (Macan, 1970). The lake is situated in more gentle terrain, where the rocks are softer and more weathered and where there is more intense cultivation (Macan, 1970). For this reason the water from its drainage basin is rich in dissolved substances. Also, the South Basin receives the sewage effluent of Windermere and Bowness and drainage from Esthwaite Water which receives the sewage effluent of Hawkshead into its main inflow. Some lowering of the oxygen concentration of the hypolimnion occurs during thermal stratification (the minimum saturation detected was 13% (K. Shepherd, personal communication)).

Blelham Tarn, is a slightly enriched lake (Lund, 1969) with a large percentage of its drainage area cultivable. In comparison with the other two lakes, it is very small and shallow. The hypolimnion becomes anoxic during stratification and the release of substances at the sediment-water interface follows the pattern described by Mortimer (1941-1942) for Esthwaite Water (Macan, 1970).

Whereas in Wastwater reed-beds are absent, and in Windermere they are confined to bays, they form a complete fringe around Blelham Tarn,

with the exception of a small strip of rocky shoreline (Macan, 1949).

The volume of the hypolimnion of Blelham Tarn is far smaller ($0.224 \times 10^6 \text{ m}^3$) than that of Wastwater ($68.9 \times 10^6 \text{ m}^3$) and of Windermere South Basin ($45.2 \times 10^6 \text{ m}^3$). Since one of the factors that controls the amount of oxygen in the hypolimnion is its size relative to that of the epilimnion (Lund, 1973) it is not surprising that the depletion of oxygen takes place rapidly in Blelham Tarn during thermal stratification. This does not occur in Windermere South Basin and Wastwater which always contain free oxygen.

Apart from the differences between the lakes summarized above, there are also differences within each lake. Comparisons were therefore made between the littoral and profundal sediments, not only because they differed so much in physico-chemical characteristics, but also to provide some information on the variability in the population of filamentous bacteria within the lake.

The profundal zone is the deep region of the lake where light penetration is considerably reduced or biologically insignificant, and temperature is relatively uniform throughout the year. The zone is characterized by processes of consumption and decomposition rather than production of organic matter (Cole, 1975). The sediment particles in this region are fine and largely made up of material produced within the lake (Cole, 1975).

The littoral zone is defined as the shallow region, usually within the euphotic zone (i.e. there is light penetration to the sediment), and subject to fluctuating temperature and erosion of shore materials. The result is a bottom region of relatively coarse sediment (Cole, 1975).

Some microbiological differences between the littoral and profundal zones have been observed in lakes in this district. Willoughby (1961) studied the population of lower fungi in marginal and submerged sediments

and found that the marginal zone provided excellent conditions for growth of saprophytic chytrids. Willoughby (1965) and Dick (1971) also reported that members of the Saprolegniales were more active in the marginal than in the deep water sediments. Willoughby (1969) found, however, that actinomycetes (bacteria) were more numerous in the profundal than in the littoral zone, and Jones (1979) observed differences in the microbial biomass and activity in sediments of the oxic littoral and anoxic profundal zones.

1.3 Filamentous bacteria

Although it is known that filamentous bacteria play an important part in the bulking of sewage sludge (Farquhar & Boyle, 1971, 1972; Sladká & Ottová, 1973; van Veen, 1973; Eikelboom, 1975) very little is known about their role in freshwater, with the possible exception of the genera Beggiatoa and Leptothrix.

The organisms of interest to the present investigation are listed below according to the classification given by Bergey's Manual (1974). The relevant literature is cited and details important to their taxonomy are discussed in that part of the main text concerned with production of a key for their identification.

Part 2: THE GLIDING BACTERIA

Order I Cytophagales

Family I Cytophagaceae

Genus I Flexibacter. Fundamental taxonomic work has been done on this genus (Lewin, 1969; Lewin & Lounsbery, 1969; Mandel & Lewin, 1969).

A review on Flexibacteria was made by Soriano (1973).

Genus II Herpetosiphon. This genus was first described by Holt & Lewin (1968). Lewin (1970) described three new species.

Genus III Flexithrix. The genus was first described by Lewin (1970).

Genus IV Saprospira. Fundamental taxonomic work has been done on this genus by Lewin (1962, 1963, 1965) and Lewin & Mandel (1970).

Family II Beggiatoaceae

Genus I Beggiatoa. Standard references to this genus include those of Faust & Wolfe (1961), Scotten & Stokes (1962), Pringsheim (1964), Burton & Lee (1978) and Strohl & Larkin (1978).

Genus II Vitreoscilla. The description of this genus was first given by Pringsheim (1951). Brzin (1966) described the effect of different incubation temperatures on cellular and colonial morphology of this organism. Gräf & Perschmann (1970) described a new species, Vitreoscilla proteolytica, with proteolytic properties and containing carotenoid pigments.

Genus III Thioploca. Maier & Murray (1965) pointed out the close resemblance between the trichomes of Thioploca and Beggiatoa and gave comparative details of the fine structure of both genera. Maier & Preissner (1979) provided some information about its occurrence.

Family III Leucotrichaceae

Genus I Thiothrix. Bland & Staley (1978) provided information on growth of this genus and also on the fine structure.

FAMILIES AND GENERA OF UNCERTAIN AFFILIATION

Genus Toxothrix. Details of the biology of this genus were given in a paper by Krul et al. (1970).

Family Pelonemataceae. Much of the information on this family was derived from the original descriptions.

Genus Pelonema. Skuja (1956)

Genus Achroonema Skuja (1948-1956).

Genus Peloploca Skuja (1956)

Genus Desmanthos Skuja (1958).

Part 3 THE SHEATHED BACTERIA

Genus Sphaerotilus and genus Leptothrix. The most recent detailed description of these genera was provided by van Veen et al. (1978).

Genus Streptothrix. Four strains morphologically similar to the description of this organism by Migula in 1895 were isolated by van Veen (Bergey's Manual, 1974). They have been assigned to the genus Haliscomenobacter.

Genus Lieskeella. This was originally described by Perfil'ev (1927) but has also been observed by Perfil'ev & Gabe (1969).

Genus Phragmidiothrix. Engler in 1883 provided the only description of this genus (Bergey's Manual, 1974).

Genus Crenothrix. Wolfe (1960) made some observations on this organism in activated sludge. Völker et al. (1977) studied its morphology and ultrastructure.

Genus Clonothrix. The original description was that of Roze in 1896 (Bergey's Manual, 1974). Kolk (1938) compared the filaments of Clonothrix fusca and Crenothrix polyspora and observed several differences between the two species.

The following organisms are not included in Bergey's Manual (1974):

Haliscomenobacter hydrossis. This nomenclature was proposed by van Veen et al. (1973) to replace the name Streptothrix hyalina, since it had been used before by Corda in 1839 to describe a fungus. Deinema et al. (1977) gave some structural and physiological characteristics of this organism.

Nostocoida limicola. This is a provisional name proposed by van Veen (1973) for non-motile, filamentous organism, forming chains of coccoid cells. They did not resemble any of those described in Bergey's Manual. They resemble cyanophyceae but photosynthetic pigments have never been detected. Two types of this organism

have been grown in pure cultures.

Microthrix parvicella. This nomenclature was proposed by van Veen (1973) based on morphological data only since the growth requirements and preservation of isolates have not yet been clarified. Both genera, Nostocoida and Microthrix are gram positive.

Chloronema. This nomenclature was proposed by Dubinina & Gorlenko (1975) for a straight or helical filamentous organism motile by gliding and resembling some types of cyanobacteria of the genus Lyngbya. The cells contain gas vacuoles, bacteriochlorophyll d and chlorobium vesicles. Two species, C. giganteum and C. spiroideum have been described.

Chloroflexus aurantiacus. This phototrophic organism of hot springs was described by Pierson & Castenholz (1974). It possesses gliding motility, chlorobium vesicles, bacteriochlorophyll a and c, a preference for photoorganoheterotrophic growth and ability to grow aerobically in the dark. Mesophilic species have been reported (Dubinina & Gorlenko, 1975).

Pseudanabaena. This organism was first described by Lauterborn (1914-1917). Rippka et al. (1979) characterized this genus as short filaments (usually 2 to 6 cells in length) containing polar gas vacuoles and exhibiting rapid gliding motility. Some strains of this genus were described previously by Stanier et al. (1971) as Synechococcus spp (Rippka et al., 1979).

Previous observations at this laboratory have shown that filamentous bacteria are scarce in the water column (Jones, J.G. personal communication). Henrici (1936) and Romanova (1958) studied the distribution of bacteria in the water column in the littoral zones and found that the bacteria were mostly non-filamentous types. ZoBell & Allen (1935) found that ovoid organisms, with a diameter of less than 1 μm were the most numerous

in sea water. The filamentous forms occasionally observed were reported as being actinomycetes and other branched filaments which they referred to as probably being Chlamydobacter. However, Henrici (1936) observed, using submerged slides in the open lake, an initial period of slow growth when haplobacteria (bacteria that occur singly or in irregular aggregates) were predominant followed by a period of more rapid development when the organisms were mainly of the filamentous type (trichobacteria). Leptothrix has also been observed in the water column (ZoBell & Allen, 1935; Jones, 1975) but the numbers were relatively small (10^2 ml^{-1}) compared with direct count of all bacteria (10^6 ml^{-1}) (Jones, 1978).

The present study was divided into three phases:

- 1) A general investigation to determine the microbiological and physico-chemical characteristics of the sediment of seventeen lakes in the English Lake District with a view to placing particular emphasis on the filamentous bacteria. It was hoped that, based on the data obtained, and also on the limnological studies previously done, one representative lake of each of the three main trophic states could be selected for the second phase of the investigation.
- 2) A more detailed study of selected lakes to determine whether differences in the populations of filamentous bacteria could be observed. This would comprise comparison between littoral and profundal zones of the sediment.
- 3) An attempt to identify, quantify and determine the function of the main groups of filamentous bacteria in three selected lakes. Although it is known that filamentous bacteria play an important part in the bulking of sewage sludge (Farquahr & Boyle, 1971, 1972; Sladká & Ottová, 1973; van Veen, 1973; Eikelboom, 1975) very little is known about them in freshwater with the possible exception of the genera Beggiatoa and Leptothrix. Preliminary observations at this laboratory had shown that the filamentous bacteria were largely confined to the benthic community and therefore this study concentrated on lake sediments.

2. MATERIAL AND METHODS

2.1 Pretreatment of the samples

The top 1 cm layer of the sediment was removed, with as little overlying water as possible, using a screw extruder of known pitch (0.2 cm revolution⁻¹). The sub sample was mixed and 1 ml dispensed into a test-tube. To this was added 1 ml of lake water which had been filtered through a 0.22 µm pore size membrane filter (Millipore Ltd). The mixture was homogenized for 1 min with an Ultra Turrax Homogenizer (Jenke & Kunkel KG) set at full power (20 000 rpm). 3 ml of filtered water was added to the homogenate, the contents of the tube were mixed and then diluted to a final concentration of 10⁻³. The diluents used in this procedure depended on the analysis to be performed and details are provided with the descriptions of each technique. Unless otherwise stated all samples were homogenized as described before dilution.

2.2 Microscopy

The microscope used was a Leitz Orthoplan equipped with a Ploem illuminator for epifluorescence studies. A 200 W mercury burner (HBO 200) served as a light source and the following filter combination was used: a Calflex B1/k2 interference heat filter and for excitation, a GG475, a 1.5 mm BG12, 2 x KP490 (= KP500) interference filters and the dichroic mirror/suppression filter combination Tk510/k515. The image was viewed through a k530 mm barrier filter at 1250 X magnification.

2.3 General survey of the Cumbrian lakes

2.31 Direct counts

2.311 Counts using acridine orange

The Jones & Simon (1975) procedure was used. Acridine orange fluorochrome C.I. 46005, 10 mg l⁻¹ final concentration was added to a 10⁻³ dilution of the homogenized sample and allowed to stand for 5 min.

To ensure even distribution of bacteria 4 ml of diluent (pre-filtered tap water) was dispensed onto the membrane before the 2 ml aliquot of sample. The stain solution and diluent (6 ml) were filtered through a black membrane filter (Sartorius) of 25 mm diameter and 0.22 μm pore size before use. After filtration the membrane was transferred, while moist, to a drop of immersion oil on a slide and covered with oil. The membrane was examined immediately. Fifty microscope fields were counted and the count was facilitated by the use of a graticule in the area range of $0.2 - 2.0 \times 10^3 \text{ mm}^2$.

2.312 Counts using fluorescein di-acetate (FDA).

The method used was based on that described by Paton & Jones (1975). The microscopy was that described in section 2.2 except that the exciter filter combination used consisted of a Calflex B1/k₂ interference heat filter and an S470 interference filter.

The sample treatment before the dilution was the same as that used for the direct count with acridine-orange except that the final sample dilution 10^{-3} was prepared with filtered phosphate buffered saline, pH 7.5 (subsequently referred to as buffer). The buffer preparation was based on that of Gomori (1955) except that NaCl 8 g l^{-1} was added. 2 ml of sample was dispensed onto the membrane and 0.2 ml of FDA (Koch Light) 100 mg l^{-1} solution freshly prepared with buffer (a stock solution of 5 g l^{-1} FDA in acetone was stored in the freezer compartment of a refrigerator) was added and allowed to stand for 30 s. 4 ml of buffer was then added and the mixture filtered through a black Millipore membrane filter (0.45 μm pore size and 25 mm diameter). The filter was placed on a drop of immersion oil on a slide and covered with oil and viewed, while still moist, under incident light illumination.

Another fluorescent stain (the magnesium salt of 1-anilino-8-naphthalene sulphonic acid, Mg-ANS, BDH) (Mayfield, 1977) was used with the

technique described above except that the final concentration was 3 mg ml⁻¹ and the time of contact with the sample was 1 min.

2.32 Viable counts

2.321 Agar plate

The sample was homogenized and diluted in sterile tap water. Two drops of sterile water were placed on each plate of solid medium and then 0.01 ml of the diluted sample was added. The mixture was spread with a sterile glass rod. Five replicates were used for each medium. The plates were incubated for 2 weeks at 15°C. The same procedure was used for exo-enzyme producers except that for medium with cellulose and Tween 80 a 0.1 ml inoculum was used. For anaerobe counts, the procedure was the same as for aerobes but an inoculum of 0.1 ml was used and the plates incubated for 10 days at 20°C in a Gas Pak 150 anaerobic system (BBL).

For the viable counts of aerobes the following media were tested:

- a) Basal medium (g l⁻¹): K₂HPO₄, 0.2; MgSO₄·7H₂O, 0.05; Fe Cl₃, trace (4 drops of a 0.01% solution); in distilled water.
- b) CPS (g l⁻¹) (Collins & Willoughby, 1962): Basal medium plus bacto-peptone (Difco), 0.5; soluble casein (BDH), 0.5; soluble starch, 0.5; glycerol, 1; agar (Difco), 15; in distilled water.
- c) LMW1 (g l⁻¹): Basal medium plus glucose, 0.05; sodium lactate, 0.05; sodium acetate, 0.05; sodium propionate, 0.05; sodium butyrate, 0.05; agar (Difco), 15; in distilled water.
- d) LMW2 As LMW1 but no sodium propionate was added.
- e) LMW3 As LMW1 but no sodium butyrate was added.

For exoenzyme producing bacteria the media were: Basal medium plus:

- a) Gluten and casein (5 g l⁻¹) for protease.
- b) Starch (1 g l⁻¹) for amylase.

c) Carboxymethylcellulose, medium and low viscosity, (Sigma) (5 g l^{-1}) and cellulose powder - Avicel RC 581 and Avicel RH - Honeywill & Stein Limited - 1 g l^{-1} and 3 g l^{-1} respectively.

d) Tributyrin (10 g l^{-1}) for lipase. A modification of the medium used by Sierra (1957) for lipase producers was also tested. It contained (g l^{-1} membrane filtered lake water); bactopectone (Difco), 5; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1; Tween 80, 2 ml; agar, 15. These media used for enumeration of exoenzyme producer organisms were tested with and without peptone (5 g l^{-1}). The zones of exoenzymes activity were developed (Hartman, 1968) using HgCl_2 (HgCl_2 , 15 g; conc. HCl, 20 ml; distilled water 1 l) for protease 50% lugol iodine for amylase and 1% aqueous hexadecyltrimethylammonium bromide for cellulose.

For anaerobes the media used were:

a) CPS A (g l^{-1}): Basal medium for anaerobes plus bactopectone, 0.5; soluble casein (BDH), 0.5; soluble starch, 0.5; glycerol, 1; agar (Difco) 15; in distilled water.

b) CPS B (g l^{-1}): basal medium for anaerobes plus glucose, 0.05; lactate, 0.05; acetate, 0.05; in distilled water.

c) CPS B plus propionate (0.05 g l^{-1}).

d) basal medium for anaerobes plus NaNO_3 (0.1 g l^{-1}), $\text{NaSO}_4 \cdot 10\text{H}_2\text{O}$ (0.1 g l^{-1}) and NaCO_3 (0.1 g l^{-1}).

The basal medium for anaerobes contained (g l^{-1}): $\text{k}_2 \text{HPO}_4$, 0.2; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05; Fe Cl_3 , trace (4 drops of a 0.01% solution); NaNO_3 , 0.1; $\text{NaSO}_4 \cdot 10\text{H}_2\text{O}$, 0.1; NaCO_3 , 0.1.

2.322 Drop plate method

In plates containing CPS medium (section 2.321) previously dried at 45°C , five circles were marked with a cork borer. To each one of them, 1 drop of sterile tap water was delivered aseptically from a Pasteur pipette; following that, 0.01 ml of the diluted sample was delivered with an Eppendorf pipette. The plates were shaken gently and

incubated at 10°C for 5 and 10 days.

2.323 Slide culture (Postgate et al., 1961). A 22 mm external diameter x 1.2 mm deep metal ring was laid in a clean microscope slide; both were stored in alcohol and flamed before use. Molten agar (0.25 ml) previously filtered through a 0.22 μ m Millipore membrane, was pipetted into the annulus, sealing it to the slide. When set, different volumes of the diluted sample (0.1 ml, 0.05 ml and 0.01 ml) were delivered over the agar and spread with a Platinum loop. When the sample had dried in, the agar was covered by a coverglass and sealed by allowing a drop of water to penetrate the interface between the coverglass and the annulus. The slides culture were incubated for 2, 4, 8 and 16 h in a moist chamber. Microscopic examination was done at 400 X magnification. The same technique was performed using a leucocyte migration plate (Sterilin Ltd) in which case, 0.42 ml of molten agar was delivered in each compartment.

2.324 Plate dilution frequency technique (Harris & Sommers, 1968).

Repli dishes (Sterilin Ltd) containing different solid media were used. Proceeding from the highest dilution (10^{-6}) of a 10 fold dilution series of tubes, an Eppendorf pipette was used to deliver 8 replicate 0.01 ml samples from each dilution to the centre of the compartments. The plates were incubated at 15°C for 2 weeks except for anaerobes which were incubated at 20°C for 10 days. After the incubation period the positive responses at each dilution were noted and the microbial enumeration achieved by consulting the appropriate table. The media used are those described in section 2.321. This technique was also applied using liquid media for enumeration of viable filamentous bacteria in which case a six four fold serial dilution with eight replicates at each dilution was used. Detailed description in the use for filamentous bacteria is given in section 2.523.

2.33 Chlorophyll a and chloroplast counts

To 18 ml of 90% (V/v) aqueous acetone (Analar) 2 ml of neat sediment was added, mixed thoroughly to aid efficient extraction, and then left to stand at 4°C for 12 h. The sample was then agitated vigorously and the sediment allowed to settle. The extract was centrifuged at $2-3 \times 10^3$ rpm and aliquots of the supernatant were used for spectrophotometric analysis carried out with a Unicam SP 600 spectrophotometer. The absorbance of the supernatant was read in 4 cm cells at 665 nm and 750 nm before and after acidification. The reading was made against 90% acetone blank for the untreated sample and 90% acidified acetone for the treated sample. To acidify the sample 0.12 ml 1 N HCl was added to 12 ml of extract and this was allowed to stand for 1 minute. The readings were again made at the required wavelengths. The data obtained were substituted in the general equation (Vollenweider, 1974):

$$\text{Chl. } \underline{a} = (D_b - D_a) [R/R-1] (\bar{v}/l) (10^3 / \alpha c)$$

Chl a = concentration of chlorophyll a in $\mu\text{g}/\text{sample}$

D_a = optical density of extract after acidification

D_b = optical density of extract before acidification

where D_a and D_b are the differences between the 665 and 750 nm readings.

αc = specific absorption coefficient for chlorophyll a (units of $\text{g}/\text{l cm}$)

v = volume of solvent used to extract the sample in ml

l = path length of spectrophotometer cell in cm.

$R = D_b/D_a$ for pure chlorophyll a (neglecting the small change in molecular weight when chlorophyll is changed to phaeophytin, it also equals the ratio of the specific absorption coefficients of chlorophyll and phaeophytin. For the present work the values of 2.43 for $R/R-1$ and 84 for αc (Vollenweider, 1974) were substituted in the general equation which gave:

$$\mu\text{g Chl } \underline{a} = 11.9 \{2.43 (D_b - D_a)\} \cdot v/l$$

For the chloroplast counts the microscopy used was that described in section 2.2. The following exciter filter combinations were tested: BG 12 (3.0 mm), BG (1.5 mm), heat filter; BG 12 (1.5 mm), GG 475, heat filter and BG 12 (1.5 mm), $\lambda > 420$, heat filter. The sediment was homogenized and diluted in tap water. 5 ml of the diluted sample was filtered through a black membrane (Sartorius, 0.45 μm pore size and 25 mm diameter). The membrane was transferred to a slide using the same procedure described for counting bacteria in section 2.311 except that no acridine orange was added.

2.34 Determination of carbon (C) and nitrogen (N)

Samples were dried to constant weight at 80°C to prevent the loss of volatile substances (Vollenweider, 1974) and their C and N content determined on a Hewlett-Packard F & M 185 CHN analyser.

2.35 Loss on ignition

Sediment samples of known volume and wet weight were dried at 80°C overnight and reweighed. The oven dried sediment samples were heated for 20 h in a muffle furnace at 550°C, allowed to cool in a desiccator and reweighed.

2.36 Determination of NO_3^- -N, NH_4^- -N and phosphorus

The bottom water samples were taken at 0.5 m above the sediment-water interface using a Friedinger bottle. The top samples were taken using a 5 m tube (Lund et al., 1958).

Nitrate was determined by reduction to nitrite in an alkaline buffered solution using spongy cadmium. The nitrite was determined spectrophotometrically after diazotisation reaction (Elliot & Porter, 1971).

Ammonia was determined by the indophenol blue method (Chaney & Marbach, 1962; Lubochinsky & Zalta, 1954).

Phosphate was determined as the molybdate complex using method of Stephens (1963).

Full details of the analytical procedures and their performance characteristics are given by Mackereth et al. (1978).

2.37 Electrode potential measurements

The measurements were taken as soon as the samples arrived in the laboratory (usually within 0.5 - 2 h of collection). The core was placed in the screw extruder so that the sediment was within reach of the tip of platinum electrode (Radiometer P 101). The platinum electrode was clamped vertically and the sediment was raised with minimum disturbance to the sediment upper layer. The reference calomel electrode (two types of calomel electrodes were used, a Radiometer calomel electrode and a fast flow calomel electrode with glass sleeve - EIL) was placed so that the tip was immersed in the water. The electrode potential readings were taken at 2 mm depth intervals with a PHM 62 pH meter set in a mV scale (Radiometer) immediately and after 60 s. The electrode was cathodically and anodically cleaned but the final method of cleaning chosen involved polishing with aluminium oxide powder (BDH, particle size about 0.05 μm). The electrode was cleaned immediately before use and then checked against ZoBell's solution (1/300 M $\text{K}_3\text{Fe}(\text{CN})_6$ and 1/300 M $\text{K}_4\text{Fe}(\text{CN})_6$ in 0.1 M KCl (Hayes et al. 1958)). This should give a reading of + 192 mV. The values of electrode potentials were not corrected for pH.

2.38 Oxygen, temperature and pH determinations

Dissolved oxygen and temperature were measured in the field, 0.5 m above the sediment-water interface, with a combination thermistor/oxygen electrode (Mackereth, 1964). The pH readings were taken with a PHM 62 standard pH meter (Radiometer) at the sediment-water interface. No attempt was made to take a pH profile due to inadequacy of the electrode

available for taking pH measurements in sedimentary systems.

2.39 Analysis of data

The relationship between variance (S^2) and mean (\bar{x}) of the series of counts was used as a guide for the necessary transformations as described by Taylor's Power Law (Elliott, 1971). After the transformations were applied to data, the adequacy was tested by Bartlett's test and checked regularly by calculations of the ratio of maximum to minimum variance of the transformed data. The differences between and within cores, i.e. measure of variability, as well as differences between lakes were analysed. Samples for direct counts were always sufficiently large ($n = 50$) for central limit to apply (Elliott, 1971) so that 95% confidence limit could be calculated without transformation.

2.4 Detailed survey of three selected lakes

2.41 Direct count of filamentous bacteria with marked slides

(Troll denier, 1973)

To 10 ml of sediment (10^{-1} dilution), 1 ml of a freshly prepared 1% agar solution, cooled down to 50°C , was added to give a uniform distribution of the particles in the dried film. The suspension was then hand shaken vigorously for 30 s. 10 μl of the suspension was transferred with an Eppendorf pipette on to a special slide on which two concentric circles of 7.72 mm and 11.3 mm in diameter had been engraved. These circles had an area of 1 cm^2 . The suspension was then evenly distributed within the circular area and allowed to dry at room temperature. Following that, the smear was covered with acridine orange (20 mg l^{-1}) for 10 min, washed with tap water and covered with a coverslip. Fifty fields were counted, while still wet, using a Calflex B1/k2 interference heat filter, an S 470 interference filter and the barrier filter 530. The counts were made inside the inner circle, i.e. 1.6 - 1.7 mm from the border where the film had an average thickness, at a magnification of 500 x.

The vertical distribution of filamentous bacteria was observed.

The Eh results were used to help the choice of sampling depth i.e., the counts were made to the depth where the Eh became constant.

2.42 Viable count of filamentous bacteria with agar coated slides

Clean glass slides, ignited at 500 °C for 2 hours, were coated thinly with molten 2% agar, prepared with filtered lake water, cooled to about 60 °C. After draining off the excess agar, the slides were dried in a dust free environment. Gelatin (0.5%), and Ullrich's adhesive prepared by diluting 1 ml of 40% sodium silicate and 1 ml of concentrated NH_4OH in 98 ml of distilled water (Brock & Brock, 1968) were also used for coating the slides. The dry slides were inserted vertically into the core of sediment, leaving ca 2 cm above the sediment-water interface. The core were incubated at 8 °C for 7 and 14 days. After the incubation the slides were removed, wiped clean on one side, covered with a coverslip and examined under phase-contrast illumination at a magnification of 400 x. Plate 2 shows slides incubated in the sediment core.

2.43 Counts of filamentous bacteria in wet mount preparation

Fairly accurate counts can be obtained by the method described by Edmondson (1974). A 10^{-1} dilution of sediment was prepared and shaken by hand vigorously for 30 s. Acridine orange was added to the diluted sediment to a final concentration of 10 mg l^{-1} and the sample was allowed to stand for five minutes and then re-shaken. 40 μl of the sample was then delivered onto a clean glass slide, covered with a coverslip (22 x 50 mm) and examined at a final magnification of 500 x.

2.44 Determination of carbon and nitrogen

These were made as described in section 2.34.

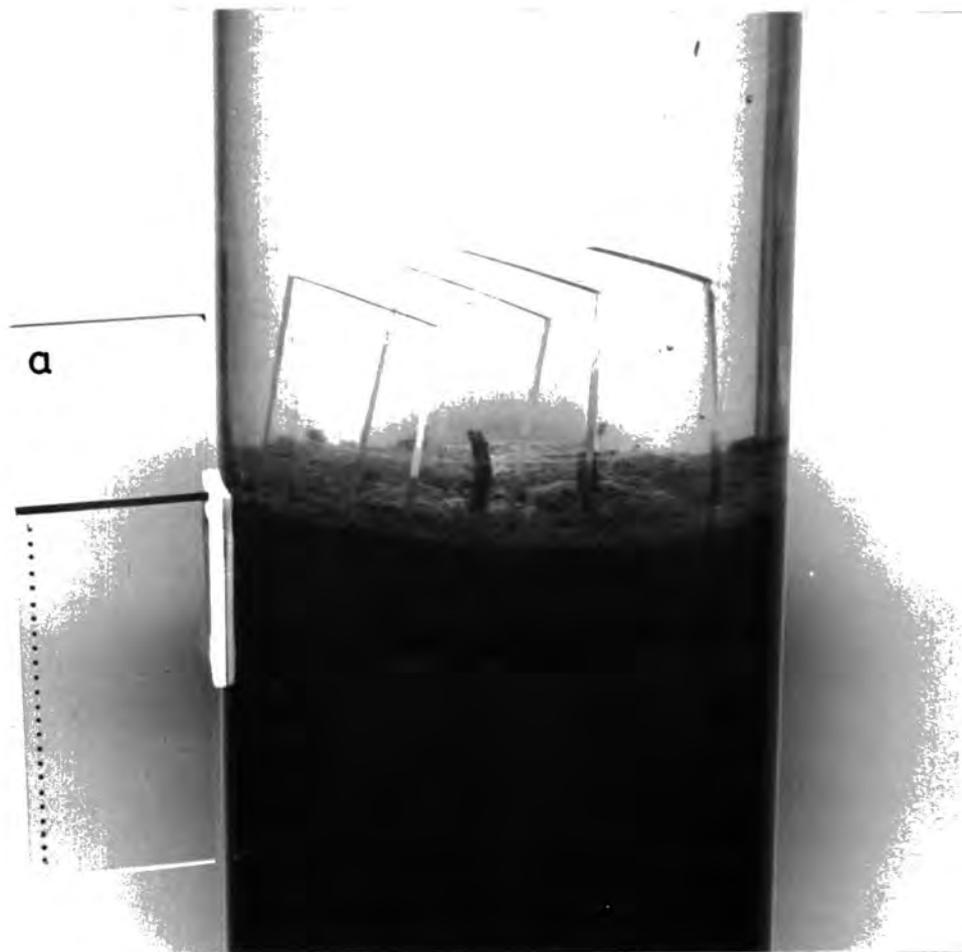


Plate 2. Slides incubated in the sediment core. a. Slide demonstrating the marks used in the counts of vertical distribution of filamentous bacteria.

2.45 Electrode potential measurements

These were made as described in section 2.37.

2.5 Biology of filamentous bacteria

2.51 Direct observation of filamentous bacteria

The microscopic examination of sediment was made using both wet mounts and fixed preparations. The wet mount technique consisted of mixing a drop of sediment with a drop of water on a clean glass slide covered with a coverslip. For the fixed preparation a 10^{-3} dilution of sediment was made using membrane filtered lake water. To 4 ml of diluent (membrane filtered lake water) 2 ml of diluted sample was added and then filtered through white Millipore membrane, pore sizes of 0.22 μm and 0.45 μm . The membrane was laid on a clean glass slide and dried at 60 °C for 20 min. Drops of cedarwood oil were then placed over the membrane which became transparent. A coverslip was placed over the membrane and observations were made under bright field and phase-contrast microscopy at 500 x magnification.

2.52 Methods for isolation of filamentous bacteria

2.521 Culture media

The culture media used are listed below.

- 1) I Medium (van Veen, 1973) contained (g l⁻¹ distilled H₂O): glucose, 0.15; (NH₄)₂SO₄, 0.5; Ca(NO₃)₂, 0.01; k₂HPO₄, 0.05; MgSO₄·7H₂O, 0.05; kCl, 0.05; CaCO₃, 0.1; vitamin B₁₂, 1 x 10⁻⁵; thiamine 4 x 10⁻⁴; Agar (Difco), 10.
- 2) CPS. This was prepared as described in section 2.321; for liquid CPS the agar was omitted.
- 3) Sediment extract medium. Equal amounts of sediment and overlaying water were mixed together and brought to the boil. The mixture was allowed to settle and the supernatant filtered through a filter paper

and centrifuged at 4×10^3 rpm for 20 min. For diluted sediment extract 1:2 dilution was prepared using membrane filtered overlaying water. For sediment extract agar, 15 g l^{-1} of agar (Difco) was added.

4) Lake water agar. 20.0 g l^{-1} of agar (Difco) was added to lake water and dissolved by boiling.

5. Medium A (Pringsheim, 1951) contained (g l^{-1} distilled H_2O): yeast extract, 3; bacto peptone, 3; agar (Difco), 15. pH 6.8.

6) Heterotrophic medium contained (g l^{-1} distilled H_2O): K_2HPO_4 , 0.2; MgSO_4 , 0.05; Glucose, 0.01; peptone, 0.01; yeast extract, 0.01; Fe Cl_3 trace (4 drops of a 0.01% solution).

7) Autotrophic medium contained (g l^{-1} distilled H_2O): K_2HPO_4 , 0.2; MgSO_4 , 0.05; NaHCO_3 , 0.1; Fe Cl_3 , trace (4 drops of a 0.01% solution).

8) SCY medium (van Veen, 1973) contained (g l^{-1} distilled H_2O): sucrose, 1; casitone (Difco), 0.75; yeast extract (Difco), 0.25; trypticase soy broth without dextrose (BBL), 0.25; thiamine, 4×10^{-4} ; vitamin B_{12} , 1×10^{-5} ; agar (Difco), 10.

9) TSC medium (Farquhar & Boyle, 1971) contained (g l^{-1} distilled H_2O): Trypticase soy, 1; agar (Difco), 15.

10) ASM-1 medium (Gorham et al. 1964) was prepared as follows: Stock A: NaNO_3 , 1.7; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.49; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.41; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.29. These amounts were all added to 2 l distilled water. Stock B: K_2HPO_4 , 0.87; Na_2HPO_4 , 0.705. These are both added to 1 litre distilled water. Stock C: $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 1.08; H_3BO_3 , 2.48; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.39; ZnCl_2 , 0.335; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.019; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.0014. These were all added to 1 l distilled water. Stock D: $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 1.86. This was made up to 1 litre. The four stocks were prepared and mixed as follows to 1 l of distilled water: stock A, 200 ml; stock B, 10 ml; stock C, 1 ml; stock D, 4 ml.

11) Chitin agar medium (Willoughby, 1968) contained (g l^{-1} distilled H_2O): purified chitin, 2.5; CaCO_3 , 0.02; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; KCl , 1.71; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 4.11; Agar (Difco), 18.

After sterilization it was added cycloheximide at a final concentration of 0.05 g l^{-1} .

12) Medium for Beggiatoa. It was used a modification of the medium described by Strohl & Larkin (1978): $\text{CH}_3\text{COONa}_3\text{H}_2\text{O}$, 0.5 g l^{-1} ; catalase, 22 units ml^{-1} ; sediment extract, 1 l.

2.522 Isolation on solid media

a) The sample was treated as described in section 2.1. The homogenized sample was used to inoculate plates containing solid medium. The homogenized samples were also used in two other ways. Firstly the sediment particles were allowed to settle and the supernatant was used as inoculum. Secondly the sample was centrifuged at 5000 g for 10 min. and the supernatant discarded. The pellet was suspended in 4 ml of membrane filtered tap water, shaken by hand and then used as inoculum. $50 \mu\text{l}$ of the different inocula was delivered in the centre of the agar plates. Streak plate method was also used. In all cases I medium (section 2.521) was used and the plates incubated for 2 weeks at 15°C .

b) Scored surface agar plates

Glass slides coated with 2% agar, 0.5% gelatin and Ullrich's adhesive (Brock & Brock, 1968) were incubated in the sediment for 1 week at 8°C . After this period of incubation the slides were removed, examined for the presence of filaments and put on the surface of agar plates which had previously been scored with a dry calcium alginate swab (Burton & Lee, 1978). The side of the coated slide containing filaments was placed in contact with the agar surface for 5 minutes or during the whole period of incubation. The slides were placed at right angles to the score marks providing therefore direction for the motility of the filamentous bacteria from the contaminated areas. All plates were incubated for a week at 8°C . The media used were: CPS, CPS at 10% strength and lake water agar (section 2.521). In this case both

CPS media were prepared with lake water.

c) Membrane technique

Sterile Millipore membranes of 25 mm and 47 mm with pore size of 0.45 μm and 0.70 μm and also Nuclepore membranes (47 mm, 1 μm pore size) were placed on the surface of agar plates. Then 50 μl and 200 μl of sediment and sediment extract (section 2.521) respectively were dispensed onto the centre of the membranes which would allow the filamentous bacteria to migrate onto the agar plates (Canale-Parola *et al.* 1966). The plates were incubated at 15 °C for 12 h, after which the membranes were removed and the incubation of the plates was continued for 3, 7 and 9 days. A modification of the above method was also used.

a) A 1:2 dilution of sediment was filtered through a Whatman filter paper (no. 40) and 17 ml of the filtrate was filtered through a Millipore membrane of 25 mm diameter and 0.7 μm pore size. It was used on Millipore stirred cell for the second filtration step and in that way removal of some unicellular bacteria occurred. The membrane was placed on the surface of agar plate. The plates were incubated as above.

b) 200 μl of a suspension of a scrape from the agar coated slides incubated into the sediment plus 4 ml of sterile core water was filtered through membranes of 0.45 μm and 1 μm pore size using a Millipore stirred cell. The membranes were transferred, face downwards, onto agar plates for 12 h at 15 °C and then removed. Incubation of the plates continued at the same temperature for 3, 7 and 9 days. In all cases the culture media used were CPS, 10% CPS, lake water agar, sediment extract agar and medium A (section 2.521).

2.523 Isolation in liquid media

The culture media used were heterotrophic medium, autotrophic medium, sediment extract medium, SCY, CPS, TSC and ASM-1 (section 2.521)

a) A drop each of sediment and medium were delivered onto a cavity slide, previously dipped into alcohol and passed through a flame. The cavity

was covered with a coverslip and sealed with petroleum jelly (A. Gallenkamp & Co. Ltd). The slides were incubated in the dark (heterotrophic medium) and in the light, ca 3000 lux (autotrophic medium) for 4, 17, 25, 48 h and 8 days.

b) 3 ml aliquots of the different media mentioned above were distributed into Repli dishes (Sterilin Ltd) and inoculated with:

- 1) 1 ml of sediment without treatment
- 2) 0.5 ml " " "
- 3) 0.25 ml " " "
- 4) 1 ml of diluted sediment (treated as described in section 2.1)
- 5) 1 ml of supernatant from sample treated as described in section 2.1
- 6) 1 ml of diluted sediment (10^{-3} dilution homogenized for 1 min with Rotamixer).

The autotrophic medium plus inoculum was incubated in the light (ca 3000 lux) at 18 °C. The dark incubation (heterotrophic medium and sediment extract) was at 15 °C. The samples were examined after 2 weeks.

c) 3 ml of autotrophic medium, heterotrophic medium, SCY, CPS and TSC media (section 2.521) were dispensed into Repli dishes (Sterilin, Ltd). Inocula of 1 ml, 0.5 ml, 0.25 ml of sediment and 1 ml of a 10^{-1} dilution of sediment were delivered into the various compartments. A small quantity of cellulose powder (Avicel-PH, Honeywill & Stein Ltd), carboxymethylcellulose (medium viscosity, Sigma), small sterile pieces of filter paper, small sterile pieces of cellophane PT 400 (BCL) and of ordinary cellophane were added separately to each medium. The samples were incubated under dark and light conditions for one week at 15 °C and 18 °C respectively.

d) Plate dilution technique (MPN)

A 3 ml aliquot of heterotrophic medium (section 2.521) was dispensed into each compartment of a Repli dish (Sterilin Ltd). 1 ml of sediment was pipetted into each of the first eight compartments. Serial dilutions

were then performed by using sterile 1 ml pipette tips. The pipette tips were used to mix the inoculum and medium together. Six fourfold serial dilutions with eight replicates of each dilution were used. Finally to each compartment a piece of sterile cellophane (PT 400) was added. The plate was then agitated gently, covered, and sealed with Sello-tape. The samples were incubated at 15 °C for 10 days.

e) Enrichment for filamentous bacteria

A four fold serial dilution was prepared as described in item d) of this section except that the sediment used was diluted (10^{-1}) before being dispensed into the 8 first compartments.

The following media and conditions were employed:

1) ASM-1 (section 2.521) incubated under dark and light conditions.

2) Basal medium contained (g l^{-1}): K_2HPO_4 ; MgSO_4 , 0.05; peptone, 0.01; yeast extract, 0.01; in membrane filtered lake water plus:

a) the monomers (g l^{-1}) glucose, 0.01; CH_3COONa , 0.01; glutamic acid, 0.01. b) small quantities of gluten and starch. c) $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ 0.2 g l^{-1}

d) ferric ammonium citrate, 0.2 g l^{-1} e) $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ plus ferric ammonium citrate 0.2 g l^{-1}

f) $\text{Mn}(\text{C}_2\text{H}_3\text{O}_2)_2$, 0.2 g l^{-1} g) MnCO_3 , 0.2 g l^{-1} h) $\text{Mn}(\text{C}_2\text{H}_3\text{O}_2)_2$ plus MnCO_3 , 0.2 g l^{-1}

i) $\text{Na}_2\text{S}_9\text{H}_2\text{O}$. An approximately equal amount of granules were dispensed

into five empty compartments of each plate. In all cases a piece of sterile cellophane (PT 400) and one sterile coverslip were added to each compartment containing medium. The sealed Repli dishes were incubated for two weeks at 15 °C. The sediment used was from the profundal and littoral zones, at depths where maximum and minimum numbers of filamentous bacteria were observed (based on the direct count made previously).

f) The use of agar coated slides inoculum

The agar coat of slides, incubated in the sediment for 7 days was scraped into a petri dish containing 0.22 μm membrane filtered core

water. 50 μ l of this suspension was used to inoculate different culture media dispensed into Repli dishes. The media used were: liquid CPS, liquid 10% CPS, TSC, heterotrophic medium sediment extract (section 2.521) and membrane filtered core water. The plates were prepared in duplicate and streptomycin (25 mg l^{-1}) was added to one. The plates were incubated under aerobic and anaerobic conditions for 7 days at 15°C .

2.524 The use of agar coated slides

The slides were prepared as described in section 2.42 and were incubated at 8°C for 1 week. They were then removed, covered with a coverslip and examined under phase contrast-microscopy at 400 x magnification. Apart from the standard 2% agar coating, other media were also tested for coating the slides. They were as follows: CPS, chitin medium, medium for Beggiatoa (section 2.521) and agar containing 0.25% of Cerophyl (Sigma). Inhibitory substances and substrates for enrichment were added to the agar as follows:

a) Substrates for enrichment g l^{-1} : Na_2SO_4 , 0.2; glucose, 1; glutamic acid, 1; starch, 1; Na_2HCO_3 , 0.5. b) Inhibitory substances. 25 mg l^{-1} ; cycloheximide (Upjohn), streptomycin (Sigma), penicillin (Glaxo), neomycin (Sigma), chloramphenicol (Sigma). Each substrate was tested with five different antibiotics. The coated slides were incubated in sediment cores as described above. The slides containing Na_2HCO_3 were incubated in the light and in the dark. The incorporation of 3mM of 2,3,5-triphenyltetrazolium-chloride in the agar for coating the slides was also tested. The agar was prepared by adding 500 ml of phosphate buffer (pH 7), 0.5 ml of 4 m sodium hydrogen malate and 10 g of agar. The buffer-malate mixture was autoclaved and tetrazolium added after autoclaving. The incubation was at 15°C for 7 days.

Slides coated with agar only were also incubated in sediment and placed in the buffer-malate solution plus tetrazolium (Patriquin & Döbereiner, 1978) for 24 h after incubation. They were examined microscopically. The use of 2-(p-iodophenyl)-2(p-nitrophenyl)-5-pehnyl tetrazolium chloride (INT) (3 mM) and sodium bicarbonate buffer (4M) was also tested. Thick agar coated slides were also prepared. The slides were incubated in the sediment for 7 days at 15 °C and then removed and examined microscopically. The area of the agar coating where the filaments were more numerous was excised and placed in a petri-dish with sterile lake water. The filaments were brushed off and the suspension of filaments dispensed into small vials and allowed to settle for 1, 2, 3 and 4 h. The supernatant was then discarded and the filaments washed twice allowing them to settle each time. The pellet was then removed and examined. If filamentous forms were present they were inoculated onto CPS, 10% CPS, lake water agar and sediment extract agar (section 2.521). The filaments growing on the thick agar coated slides were also used to inoculate sloppy agar tubes containing amorphous ferrous sulphide to a final concentration of 0.02 g l⁻¹ (Brock & O'Dea, 1977). The tubes were incubated for 7 and 14 days at 15 °C.

2.525 Investigation of sheath development

Agar coated slides were incubated in the sediment for 2 days at 8 °C and then removed, covered with a coverslip and examined. The position of the filamentous bacteria in the field were recorded by a rough sketch map and noting the readings on the stage verniers. The coverslip was then sealed with petroleum jelly and the slides incubated inside a high humidity chamber at 8 °C. Microscopic observations were made after 12 h, 24 h, 7 days and 12 days.

2.526 The use of microcapillaries

Flat glass capillaries (Perfil'ev & Gabe, 1969) of 0.05, 0.2

and 0.4 mm viewing path length (Camlab, Cambridge) were mounted on a glass slide and autoclaved. The slides were then incubated in the sediment. Round glass capillaries were attached to a support through a rubber band. These capillaries were incubated in the sediment, empty, filled with lake water and with lake water plus streptomycin (25 mg l^{-1}). Different diameters were used. Plastic capillaries were also tested. In all cases capillaries were incubated for 7 days at 8°C and examined afterwards for the presence of filamentous bacteria.

2.527 The agar sausage technique

The agar sausages were prepared by boiling membrane filtered lake water or sediment extract with different concentrations of agar. The agar solution was dispensed into sterile test tubes and allowed to solidify. The agar sausages were removed by heating the closed end of the test tubes. The following concentrations of agar were used: 2%, 1%, 0.5%, 0.33% and 0.25%. The agar sausages were inserted vertically into the sediment and also placed horizontally at the sediment-water interface. They were incubated for 7 days at 15°C .

2.528 Enrichment for filamentous bacteria with the use of membrane holders.

The 25 mm and 47 mm membrane holders (Swin Lock, Nuclepore [®]), were used in an attempt to select some filamentous bacteria. The filter holders contained Millipore membranes of pore sizes $0.45 \mu\text{m}$ and $0.7 \mu\text{m}$ and a small rubber bung was placed on the cylindrical end. After sterilization, liquid medium was dispensed into them aseptically. The culture media used were: heterotrophic medium and sediment extract medium (section 2.521). The device was then incubated vertically and horizontally at the sediment-water interface and incubated for 7 days at 15°C . Examination of the liquid media and membrane was made after the incubation period.

2.529 Micromanipulation and reinoculation of isolated organism into the sediment.

Micropasteur pipettes and micromanipulator needles (Scott, 78) were used to remove material from agar coated slides and to inoculate defined areas of agar plates. The media used were lake water agar, sediment extract agar and CPS (section 2.521). The plates were incubated at 15 °C and 8 °C and examined periodically.

The reinoculation of isolated organisms into the sediment was carried out as follows: sediment and overlaying water were dispensed into 50 ml beakers and autoclaved. Agar coated slides were dipped into each beaker and Micromonospora was inoculated. The incubation was at 8 °C for 7 days under aerobic and anaerobic conditions. The sediment to be incubated anaerobically was saturated with nitrogen. After the incubation period the slides were removed and examined.

2.53 Autoradiography

The isotopes used were D-[U-¹⁴C] glucose and D-[3-³H] glucose and they were obtained from Radiochemical Centre, Amersham, Buckinghamshire. The final concentration used was 10 µCi ml⁻¹. 200 µl of a 10⁻¹ dilution of sediment was delivered into small vials and 200 µl of ¹⁴C glucose was added. One vial was previously fixed with 40 µl of 38% neutral formalin and served as a control. The vials were incubated for 1 h and 2 h in the dark at 15 °C. After the incubation time the reaction was stopped by adding 40 µl of 38% neutral formalin. 10 µl of the sample was then transferred to the centre of a circle engraved in a glass slide (section 2.41). The slides were previously coated with Ullrich's adhesive (Brock & Brock, 1968). The sample was spread on the circle with a disposable plastic needle and left to dry overnight in a desiccator under vacuum. The formalin and unincorporated radioactivity were removed by passing the slides through five changes of

distilled water. After drying the slides at room temperature they were stained. Two stains were tested: 5% erythrosine in 5% phenol for 5 min and acridine orange (20 mg l^{-1}) for 10 min. Ilford liquid nuclear emulsion, L4, was used to prepare the autoradiograms. The method of diluting was as follows. 10 ml aliquots of distilled water were dispensed into clean 30 ml containers with a tight fitting cap. These containers and the emulsion were put in a water bath at 43°C for 2 h. The emulsion was then stirred with a clean glass rod and 10 ml was dispensed into each container using a 5 ml piston pipette. The slides were dipped singly into the diluted emulsion and the excess wiped off the back. They were placed on a cooled metallic plate until the emulsion had gelled (Rogers, 1973). The handling of the emulsion was done in total darkness. The slides were stored for 3 and 5 days at 4°C inside a container with drying agent. After the incubation the slides were transferred to a glass slide staining rack for development. The developer (Ilford ID-2, diluted with an equal amount of water) was added and left in contact with the slides for 4 min. The reaction was stopped with 1% acetic acid for 10 s. The fixative (Kodak unifix diluted with an equal amount of water) was used for 10 min. The development was done in total darkness. The slides were then rinsed through 5 changes of tap water over 10 min (Ramsay, 1974). After the slides had been dried at room temperature they were examined under oil ($1250 \times$ magnification) by means of bright field optics and under fluorescence microscopy. The staining procedure was tested by running a similar experiment using bacteria grown in lake water enriched with glucose and peptone and incubated for 12 h with continuous stirring. Slides were coated with 0.5% gelatin and Ullrich's adhesive. The stains and times used were as follows: aqueous safranin, 0.05% (30 s); erythrosine 5% in 5% phenol (30 s); methylene blue, 0.3% (30 s). The

staining was done before the coating of slides with the emulsion and also at the end of the autoradiographic process.

Autoradiography using coverslips. Coverslips (22 x 50 mm) coated with 0.5% gelatin, 2% agar and Ullrich's adhesive were incubated in sediment for 7 days at 8 °C, and then examined microscopically. Coverslip strips were cut where most filamentous bacteria were present. The strips were placed in a Leucocyte migration plate (Sterilin Ltd) and covered with 50 µl of D-[U-¹⁴C] glucose. Formalin was added to one strip to serve as a control. A coverslip was placed over each chamber of the plate and sealed with petroleum jelly. The samples were then incubated in the dark at room temperature. The reaction was stopped with formalin and the strips were removed and left to dry. They were then fixed to a glass slide by gluing the corners with an epoxy resin (Araldite, Ciba Geigy).

The washing, emulsion coating and development were as described previously. The samples were stained with phenolic erythrosine for 30 s.

Autoradiography in slides. Slides coated with 2% agar or 0.5% gelatin or Ullrich's adhesive were incubated in the sediment at 8 °C for 7 days. After microscopic examination the slides were cleaned leaving only the area with the highest number of filamentous bacteria. This area was then ringed with petroleum jelly and 4% formalin was added to the control slides which were left to stand for half an hour. The formalin was then removed using a piston pipette. The area was then covered with 10 µCi ml⁻¹ of D-[U-¹⁴C] glucose or D-[6-³H] glucose and incubated in a humidity chamber for 5 h at 15 °C. The reaction was stopped with 4% formalin. The liquid was then removed using a piston pipette and the slides left to dry. Formalin and unincorporated

radioactivity was removed by washing gently under running water. The slides were left to dry, coated with emulsion and exposed for 5 days at 4 °C. The development was as described above except that the developer used was Ilford Phen-X. The slides were finally stained with 5% phenolic erythrosine for 30 s.

2.54 Microscopic observations of filamentous bacteria

The initial observations of filamentous bacteria were made using wet mount preparations with the top centimetre of sediment. Following that, extensive observations were made using agar coated slides incubated into the sediment core. During those observations the filamentous bacteria were sketched, and the following were noted: trichome description (presence or absence of sheath; diameter and length; branched or unbranched; motility; segmented or not), cell description (diameter, length and shape) and presence of intracellular deposits. Some other particulars such as flexibility of the filament, colour under phase-contrast microscopy and breakage during microscopic observations were also noted.

The use of uncoated glass slides, incubated in the sediment for 7 days at 8 °C were used for different tests to be applied. Whenever necessary the slides were incubated for shorter periods of time, varying from 7 hours to 7 days. The slides were removed from the sediment, wiped clean on the underside and examined by phase-contrast microscopy at a magnification of 400 x. The presence of filamentous forms were recorded by a rough sketch map and the readings on the microscope stage verniers. Both wet mount and dried slides were used, in which case the drying process was always followed by microscopic observation. The dried slides were gently fixed by heat. The techniques used to examine various morphological and cytochemical features are given below.

a) Lipid inclusions (Burdon, 1946).

The reagents were: sudan black, 0.3 g in 100 ml 70% (V/v) ethanol;

0.5% (w/v) aqueous safranin.

The heat fixed slide was flooded with sudan black and allowed to stand at room temperature for 15 min. The excess stain was drained off without washing and the slide blotted dry. The slide was then cleaned with xylol by dipping it in and out of the solvent and blotted dry. After counterstaining with safranin for 5 to 10 s it was washed in water and blotted dry. Lipids (poly β -hydroxybutyrate mainly) inclusions were blue-black in colour and contrasted with the pale red cells.

b) Volutin granules (Norris & Ribbons, 1971).

The reagents were as follows. Solution 1: Toluidine blue, 0.15 g; malachite green, 0.2 g; glacial acetic acid, 1 ml; 95% (v/v) ethanol, 2 ml; distilled water, 100 ml. Solutions: Iodine, 2 g; KI, 3 g; distilled water 300 ml. The heat fixed slide was stained in solution 1 for 5 min and the excess stain was drained off without washing. Solution 2 was then added for 1 min. The slide was then washed briefly with water, blotted dry and examined. Volutin (polymetaphosphate) granules stained black in contrast to the light green of the cells.

c) Sulphur inclusions

The wet slide was covered with a cover slip and examined by phase-contrast microscopy at 400 x magnification inside a fume cupboard. While observing the organisms, a drop of pyridine was added to one edge of the coverslip and drawn under with blotting paper. Pyridine dissolved the sulphur inclusions. The addition of ethanol was also tested.

d) Iron deposition

A heat-fixed slide was covered with 2% (w/v) $K_4Fe(CN)_6 \cdot 3H_2O$ and left to stand for 15 min. The excess solution was drained off and the slide dried in air. The slide was then covered with 0.2% (v/v) HCl for 10 s, rinsed with tap water and dried at room temperature. Sheaths containing iron were stained blue.

e) Sheath stain

A modification of the technique described by Farquhar & Boyle (1971) was tested. The area of the heat fixed slide containing filamentous bacteria was ringed with petroleum jelly. 400 μ l of lysozyme and 400 μ l of EDTA were then added to give a final concentration of 125 μ g ml⁻¹ and 500 μ g ml⁻¹ respectively. The slide was incubated in a high humidity chamber at 37 °C for 45 min. After this time 800 μ l of sodium lauryl sulphate was added to a final concentration of 0.01 M and the slide incubated for 30 min at 27 °C. The ring of grease was removed, the slide allowed to dry and stained with Hucker's crystal violet (2 g of crystal violet dissolved in 20 ml of 95% (V/v) ethanol. The 20 ml was added to 80 ml 1% (W/v) aqueous ammonium oxalate) for 1 min. The empty sheath stained pale purple. Use of nigrosin was also tested. The reagents were as follows: Nigrosin, 1 g; HCl, 2 ml; distilled water to 100 ml. A drop of the stain was added to the slide with the filamentous bacteria and covered with a coverslip. The excess liquid was removed with absorbent paper and examined. The use of 3% NaCl (the cells could possibly shrink and make sheath evident) followed by staining with Tyler's crystal violet (crystal violet, 0.1g; glacial acetic acid, 0.25 ml; distilled water, 100 ml) (Manual of Microbiological Methods, 1957) was also tested.

f) Cell wall stain (Norris & Ribbons, 1971)

The reagents were 10% (W/v) aqueous solution of tannic acid and 0.02% (W/v) crystal violet.

The heat fixed slide was flooded with tannic acid heated for 5 min, washed and stained with crystal violet for 1 min. The bacterial cytoplasm was pale purple and contrasted with the darker cell wall.

g) Spore stain

The reagents were 5% (W/v) aqueous malachite green and 0.5% (W/v) safranin.

A heat fixed slide was flooded with malachite green and heated for 4 min ensuring that the stain did not boil or dry. The slide was washed thoroughly with cold water, covered with safranin for 30 s, washed again and blotted dry. The spores stained green against pink cytoplasm.

h) Gas vacuoles

A coverslip was placed on the wet preparation and this was examined microscopically for the presence of gas vacuolated bacteria. The slide was transferred in a horizontal position to a pressure cell similar to that described by Walsby (1974) and exposed to a pressure of 965.27 KPa. The pressure was raised quite rapidly, within 5 to 10 s and immediately released at about the same speed. The slide was replaced on the microscope stage and the bright refractile granules which disappeared after this treatment were considered to be gas vacuoles.

i) Flagella stain (Mayfield & Inniss, 1977)

Stock solutions of saturated (w/v) $KAl(SO_4)_2$, 20% tannic acid, saturated (w/v) $HgCl_2$ and saturated alcoholic (w/v) basic fuchsin were prepared and mixed in the proportions of 5:2:2:0.8 on the day of use. The prepared stain was placed in a 10 ml syringe fitted with a syringe filter holder containing a 13 mm diameter, 0.22 μm Millipore membrane filter, thus allowing for filtration during application. The wet slide was covered with a coverslip so that a thin layer of liquid was present. After about 10 min, 2 drops of the staining solution were placed on the edge of the coverslip so that capillary action would draw the solution underneath. The slide was observed using phase-contrast and bright field optics for the presence of flagellated bacteria.

j) Gram stain

The reagents were as follows. Hucker's crystal violet (see e) in this section); iodine solution (KI, 2 g and I_2 , 1 g in 100 ml distilled water); absolute ethanol and 0.5% (w/v) aqueous safranin. The dried and heat fixed slides were flooded in crystal violet for 30 s, washed with water for 2 s, flooded with iodine solution for 30 s and washed

in water for a further 2 s. The slide was decolorized in absolute ethanol for 5 s, washed in water for 2 s and then counterstained with safranin for 10 s. After washing in water for 1 s it was blotted dry and examined under bright field illumination.

k) Chlorophyll fluorescence

The microscope used was described in section 2.2. The excitation was through a Calflex B₁/K₂ interference filter, a BG38 4mm, a Panchrom (Grün) and a dichroic mirror / suppression filter combination TK 580/580. The dichroic mirror / suppression filter combination TK 455/K460 and TK 510/K 515 was also used for excitation. The wet slide was covered with a coverslip and examined under phase-contrast microscopy. The different filamentous forms observed were tested for the presence of chlorophyll under fluorescence.

3. RESULTS

3.1 Sampling sites

Sediment samples were taken at a fixed station near the deepest point in each lake except for Blelham Tarn which was sampled at 13.5 m depth in its northern basin.

The more detailed sampling programme in the three selected lakes included sampling the profundal and littoral zones. The profundal samples were collected at points as described above. The littoral samples were taken at the nearest point to the shore with soft sediment which corresponded a depth of ca 15% of that in the deepest point i.e. 10 m in Wastwater, 6 m in Windermere South Basin and 2 m in Blelham Tarn. The sampling sites are shown in Figs 1, 2 and 3. Table 3 summarizes some morphometric and chemical characteristics of three lakes in which a more detailed study was carried out.

3.2 Development of methods

3.21 Direct count using AO The problems involved in the use of this technique have been discussed by Jones & Simon (1975) and therefore its application to sediments gave due consideration to these results. During the preliminary tests the direct count was performed using distilled water for dilution. A very red background was observed probably due to the pH of the water, but this was reduced by the use of tap water. The effect of different homogenization times using different volumes of water was investigated and the results are shown in Table 4. That procedure which gave the highest mean count (1 min Ultra-Tunax homogenization) was chosen for the survey.

3.22 Direct count using FDA. The storage of the solution of FDA (5 g l^{-1} in Analar acetone) was checked at -15°C and 5°C ; freshly made solution was also tested. Storage at -15°C gave a slightly brighter fluorescence. The reagent solution (100 mg l^{-1}) was tested using

DEPTH CONTOURS IN METRES

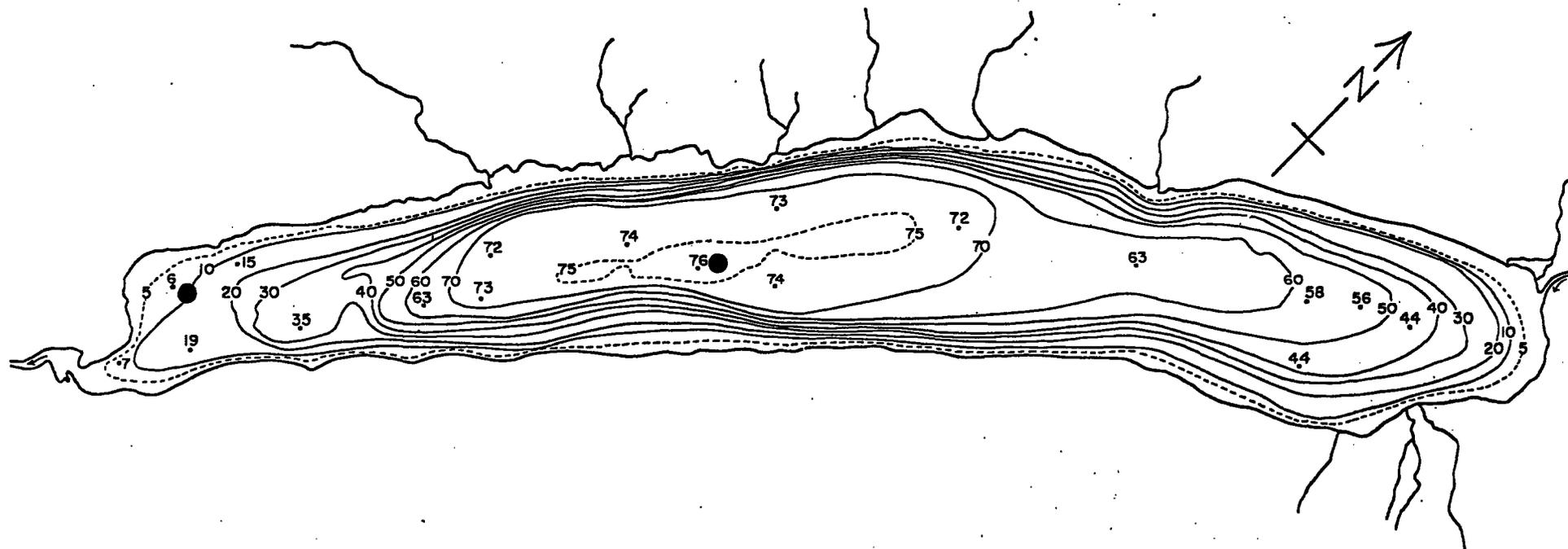
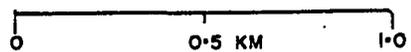


Fig. 1. Bathymetric map of Wastwater (Ramsbottom, 1976). ● Sampling locations.

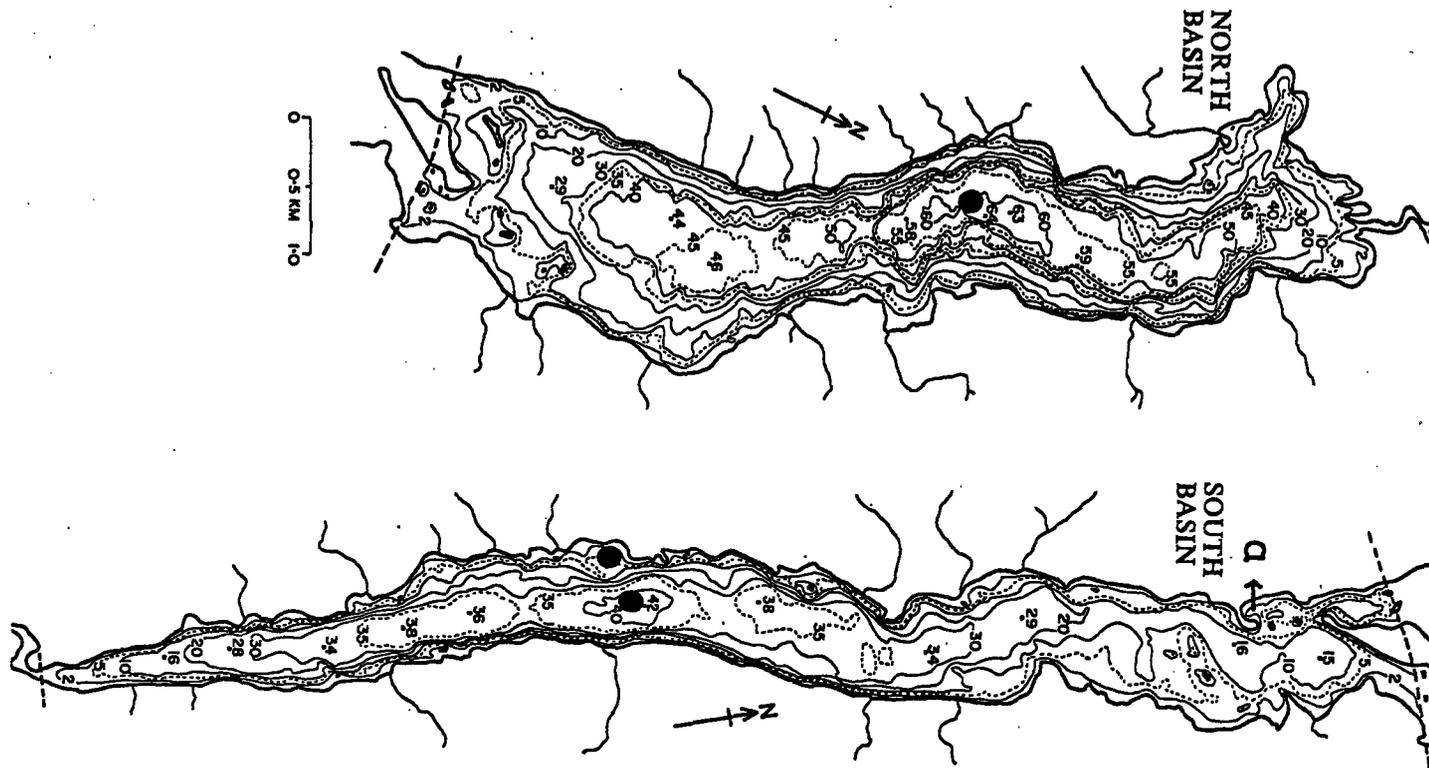


Fig. 2. Bathymetric map of Windermere (Ramsbottom, 1976). ● Sampling locations.

(a) Mitchell Wyke.

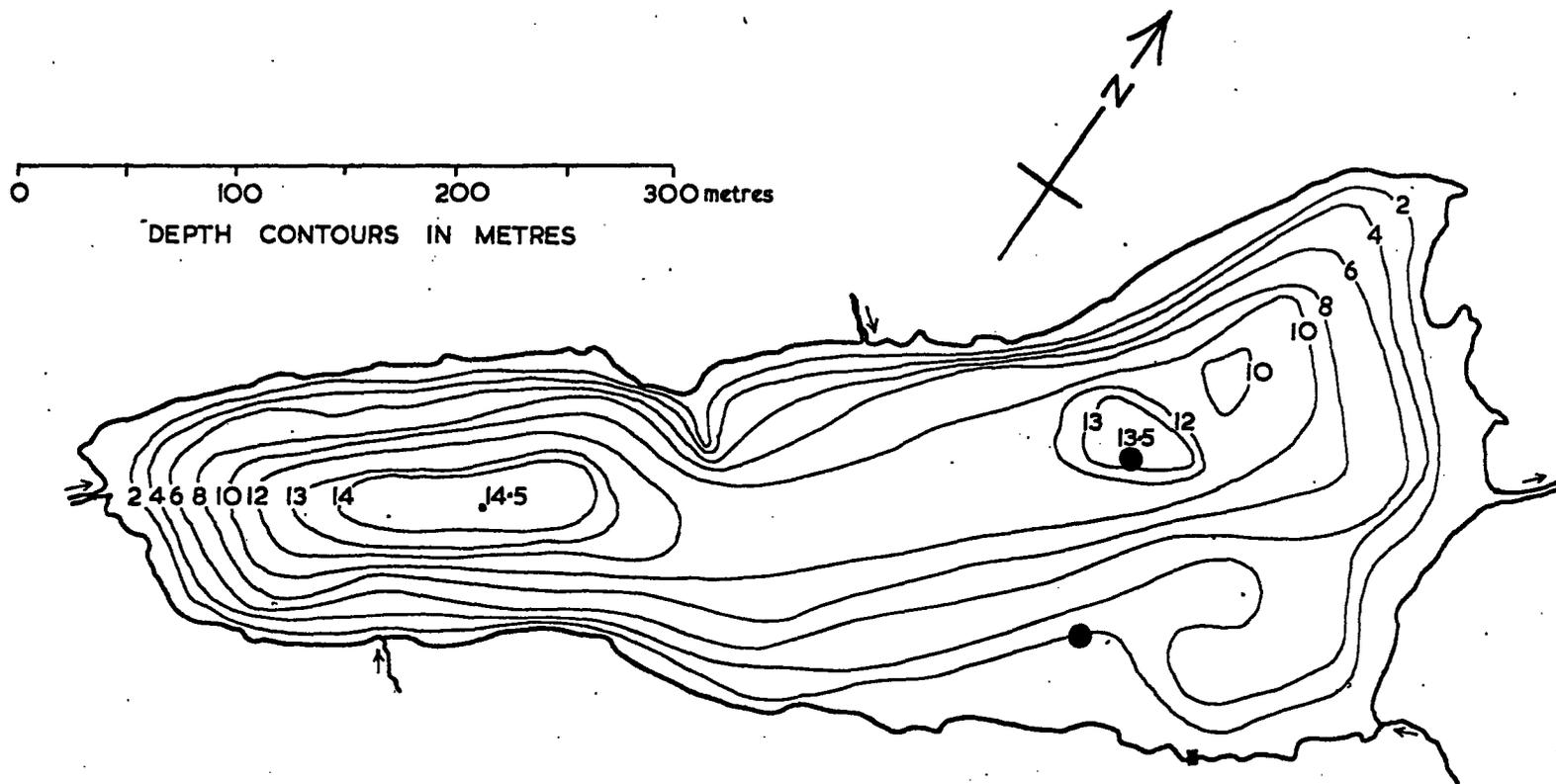


Fig. 3. Bathymetric map of Blelham Tarn (Ramsbottom, 1976). ● Sampling locations.

Table 3. Morphometric and chemical characteristics of three Cumbrian lakes.

	Wastwater	Windermere South Basin	Blelham Tarn
Morphometric characteristics			
altitude ¹ (m)	61.0	39.0	45.0
area ¹ (km ²)	2.91	6.72	0.102
area of drainage basin ² (km ²)	48.5	-	4.3
length ² (km)	4.8	-	0.67
maximum breadth ³ (m)	805.0	-	317.0
maximum depth ² (m)	76.0	42.0	14.5
volume ² (10 ⁶ m ³)	115.6	112.7	0.69
drainage area ⁴ / volume ratio	0.45	-	4.31
Chemical characteristics			
Ca ¹	109	309	438
Mg ¹	56	57	131
Na ¹	156	165	165
K ¹	8	14	21
total cations ¹	321	545	755
total anion ¹ $\mu\text{eq l}^{-1}$	324	551	755
HCO ₃ ¹	51	184	325
Cl ¹	164	190	190
SO ₄ ¹	99	159	222
Total phosphorus ⁵ ($\mu\text{g l}^{-1}$)	2.0	20.0	29.0 (5.6)
Soluble reactive phosphorus ⁵ ($\mu\text{g l}^{-1}$)	0.5	1.5	1.8 (0.5)
NO ₃ -N ⁵ ($\mu\text{g l}^{-1}$)	256.0	294.0	342.0 (140)
NH ₄ -N ⁵ ($\mu\text{g l}^{-1}$)	8.8*	12.11	24.93
SiO ₂ ⁵ ($\mu\text{g l}^{-1}$)	2430.0*	511.36	607.50

Sources of data: ¹ Gorham et al (1974); ² Ramsbottom (1976)

³ Macan (1970); ⁴ Jones (1972); ⁵ Freshwater Biological Association, unpublished data (the values are arithmetic mean of data obtained from the epilimnion between April and September). * result of one measurement.

() indicates standard deviation on seasonal mean.

Table 4. The effect of homogenization procedure and sample dilution on direct counts of benthic bacteria

Homogenizer	Homogenization time	Dilution	Number of bacteria/ graticule area	
			\bar{x}	s^2
Ultra-Turrax	1 min	1 ml sediment + 3 ml water	4.28	5.84
Hand-grinder	20 times	1 ml sediment + 3 ml water	3.66	10.31
Ultra-Turrax	30 s	1 ml sediment + 1 ml water	3.38	7.87
Ultra-Turrax	1 min	1 ml sediment + 1 ml water	5.10	10.24

distilled water, membrane filtered lake water (0.22 μm membrane) and buffer (pH 7.5). The last procedure proved to be the best and was used in a variety of counting techniques including:

a) Wet mount. A drop of the diluted sample was put onto a slide and a drop of FDA solution was added to it. A coverslip was placed over the sample which was then examined immediately.

b) Use of membrane filters. 2 ml of diluted sample plus 4 ml of diluent (tap water and buffer were tested) were filtered through a black membrane. The membrane was then transferred to a slide and a drop of FDA solution was added. After covering with a coverslip, it was examined. A modification of this procedure was also tried where, before adding the FDA solution, the membrane was laid on a filter paper for a few seconds, transferred to a glass slide and then the FDA was added. After that, the membrane was again laid in a filter paper and then transferred to a glass slide, covered with oil and examined. When the membrane was placed on a drop of FDA, and left for 30 s, 20 min and 2 h, instead of using the above procedures, no fluorescing bacteria were observed except for a very few after the longer incubation periods.

c) Staining before filtration. 2 ml of the diluted sample, 4 ml of buffer and 0.2 ml of FDA were filtered through a black membrane. The membrane was laid in a glass slide, covered with oil and examined. This was repeated at a higher FDA concentration (50 mg l^{-1}) but in both cases very few bacteria displayed fluorescence. If, however, buffer was used for the dilution, and the sample plus 0.2 ml of FDA was left in the filter funnel for 30 s before addition of a further 4 ml of buffer and filtration, a satisfactory fluorescence image was obtained when the membrane was examined in the wet state under a coverslip. Contact of the sample with FDA must be for a very short time otherwise crystals, due to precipitation of FDA in aqueous solution, interfere with the counts. The magnesium salt of 1-anilino-8-naphthalene sulphonic acid was also tested but found

to be unsatisfactory.

Once the optimum experimental conditions had been selected the counts were then made using different membranes. Comparisons of FDA and AO counts were made and many combinations of exciter filters were tested (Table 5). It was found that the Millipore membranes gave a red and more "cloudy" background when compared with Sartorius membranes. Although the intensity of fluorescence with the use of FDA did decrease after some time it was still possible to count the bacteria. Nuclepore membranes were tested but gave very poor results, uneven distribution and very low contrast. Sartorius, black, 0.22 μm pore size membranes were eventually chosen.

3.23 Viable counts. The agar slide technique and drop plate technique failed to give good results. The spread plate procedure was initially used for counting aerobes and anaerobes. However the presence of spreading bacterial colonies caused problems during counting and made it impossible to determine the exact number of zones of exo-enzymes activity. Therefore the spread plate method was not adopted as a standard method and a most probable number technique was chosen. The effect of homogenization of the sample was tested in the same way as for the direct counts (section 3.21). Higher counts were obtained using 1 ml of sediment and 1 ml of sterile tap water during the homogenization. The effect of temperatures on incubation was tested and 15 °C proved to be the most satisfactory. For the counts of aerobic bacteria CPS and LMW3 proved to be the best media; for the exo-enzyme tests, starch (2 g l^{-1}), casein (10 g l^{-1}), carboxymethylcellulose, medium viscosity, (2 g l^{-1}) and tributyrin (10 g l^{-1}) were chosen. Peptone enhanced the number of colonies in all cases except for the medium counting casein. For counts of anaerobes CPSA and CPSB were the media chosen.

3.24 Chloroplast counts. Homogenization of the sample for chloroplast counts was tested using the Ultra-Turrax homogenizer and a Rotamixer (Hook

Table 5. The effect of membrane and staining on the apparent uptake of fluorochrome by bacteria

Fluorochrome	Membranes	10^8 x Bacteria ml ⁻¹	Filter combinations
1. FDA	Sartorius, black (0.45 μ m)	6.2	BG12(1.5mm), BG12(3.0mm), heat filter
2. FDA (control)	Sartorius, black (0.45 μ m)	6.1	as in 1
3. AO	Sartorius, black (0.45 μ m)	30.4	BG12(1.5mm), GG475, heat filter
4. AO (control)	Sartorius, black (0.45 μ m)	19.2	as in 2
5. FDA	Millipore, GB (0.45 μ m)	10.2	S470, heat filter
6. FDA	Millipore* (0.22 μ m)	6.8	as in 5
7. FDA	Sartorius, black (0.45 μ m)	7.5	as in 1

* stained with Dylon (Jones & Simon, 1975)

& Tucker Ltd.) both at full strength. The results are presented in Table 6 and show that a higher mean count with lower relative standard deviation was obtained with the Ultra-Tunax homogenizer. Several optical filter combinations were tested for the fluorescence microscopy and a combination of a BG12 (1.5 mm) with a BG12 (3 mm) proved to be the most suitable.

3.25 Statistical analysis and procedures for obtaining reliable quantitative estimates.

Before reliable confidence limits and parametric statistical tests can be applied to population estimates it is necessary to confirm that the data conform to the assumptions of these calculations i.e., that they are normally distributed. Two preconditions which must be met are assurance of homogeneity of the variances and independence of the mean and the variance. One of the simplest methods of testing for these conditions is to plot the variance of the data against the mean on a double log scale, the slope of the regression line describing the relationship may then be used to transform the data (Taylor, 1961). The adequacy of the transformation should then be tested by replotting the transformed data and testing for homogeneity of the variances. In this study the slopes of the regression lines, b , for counts on membranes using acridine orange fluorochrome (Fig. 4), agar coated slides (Fig. 5), wet mount (Fig. 6) and marked slide technique (Fig. 7) were 1.33, 1.33, 1.07 and 0.93 respectively indicating the need for a square root transformation. A logarithmic transformation was also tested. When these transformations were applied, however, it was discovered that the transformed data still did not exhibit independence of the mean and variance nor homogeneity of variances. It was therefore necessary to use non-parametric statistics on small samples, or use sufficiently large samples that the normal approximation could be assumed (Central Limit Theorem,

Table 6. The effect of homogenization procedure on direct counts of chloroplasts

Core No	Ultra - Turrax		Rotamixer	
	\bar{x}	s^2	\bar{x}	s^2
1	1.5	5.8	1.2	5.5
2	1.2	2.7	0.6	0.9
3	1.4	2.4	1.3	5.7
4	0.8	1.1	0.8	3.2
5	1.3	1.5	1.6	9.2
	Overall mean 1.24	Overall coefficient of variation 21.77%	Overall mean 1.10	Overall coefficient of variation 36.00%

Fig. 4. Regression line obtained with data from counts of bacteria on membrane using acridine orange

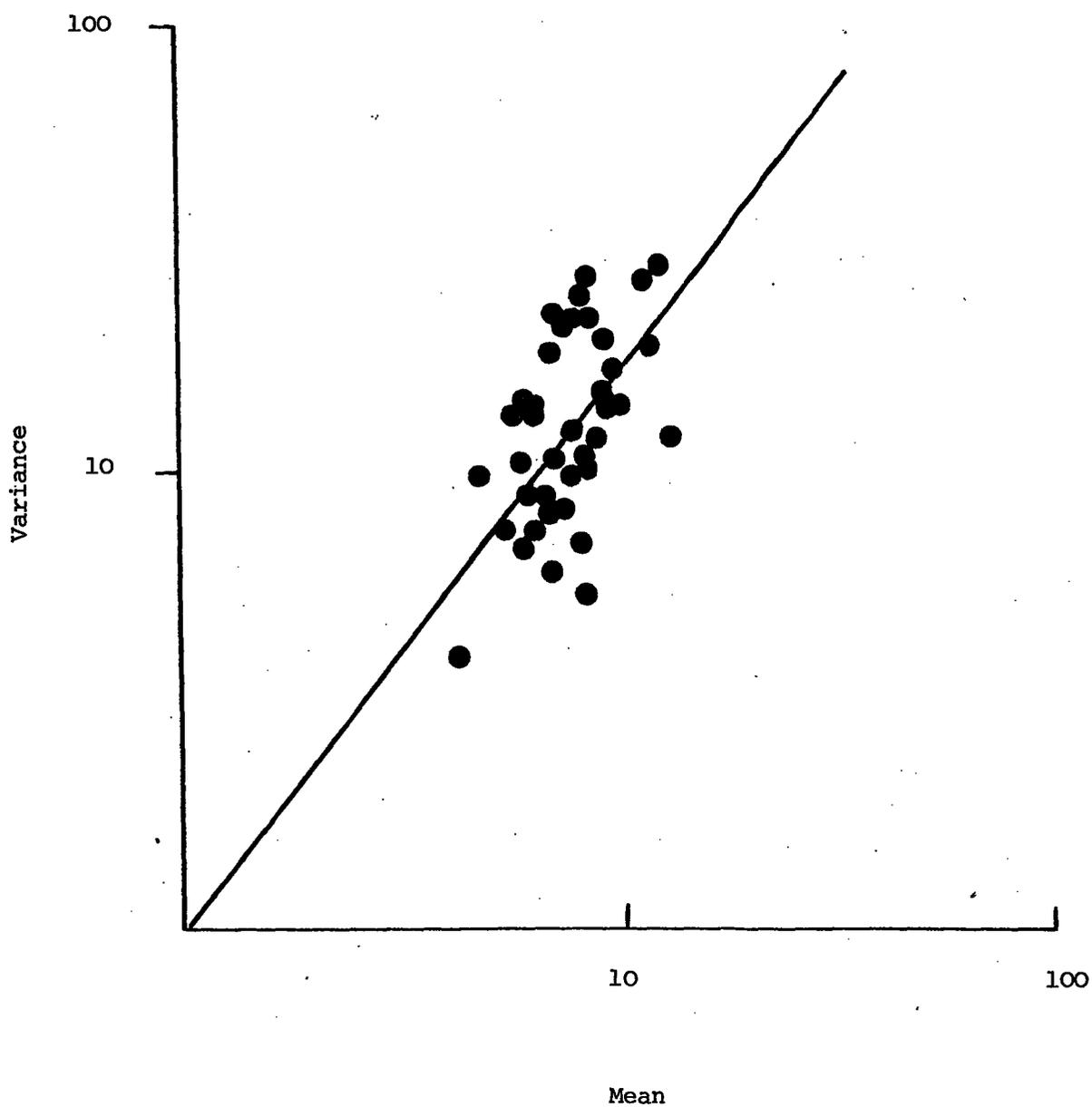


Fig. 5. Regression line obtained with data from counts of filamentous bacteria on agar coated slides.

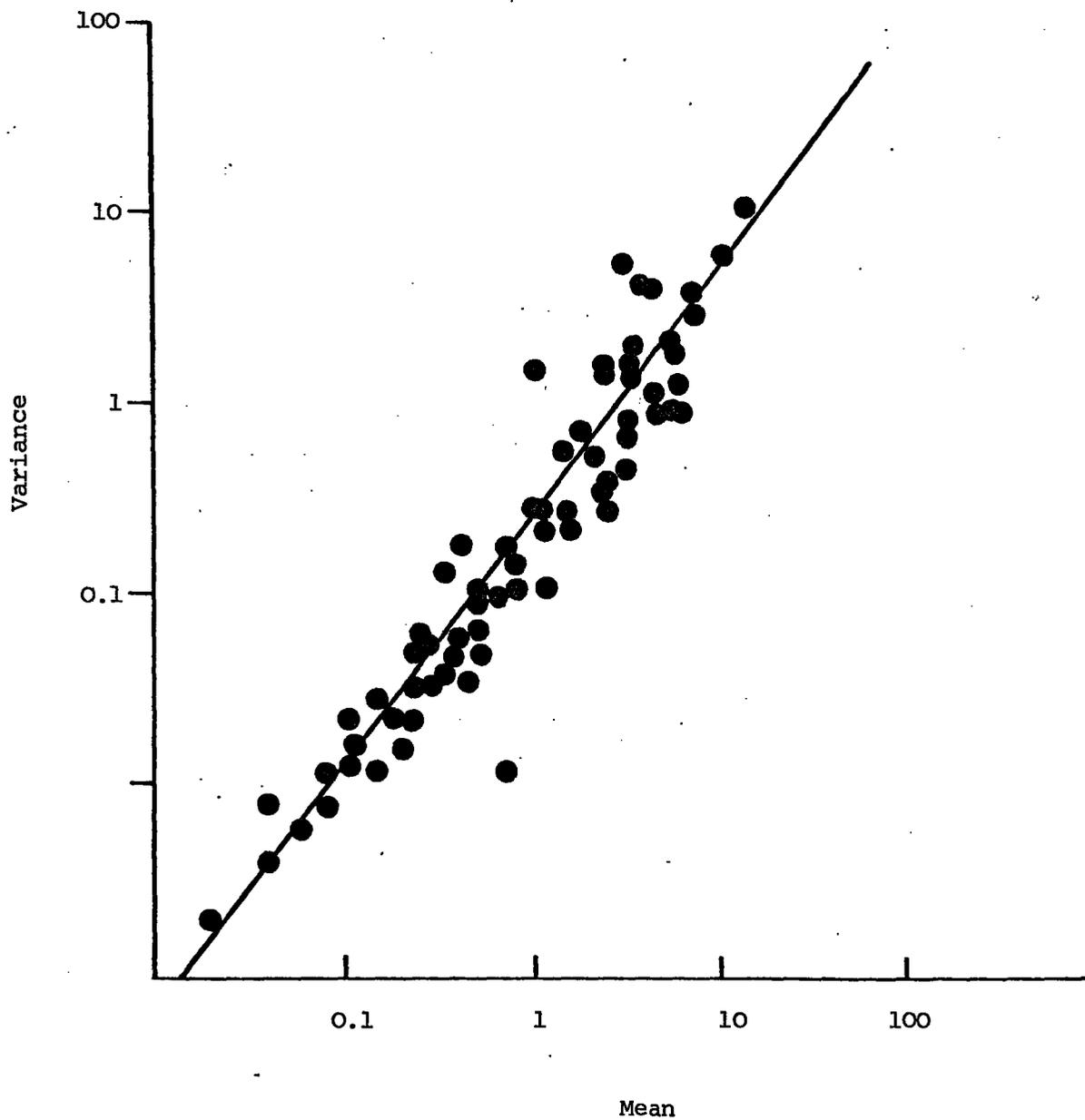


Fig. 6. Regression line obtained with data from counts of filamentous bacteria on wet mount preparations using acridine orange.

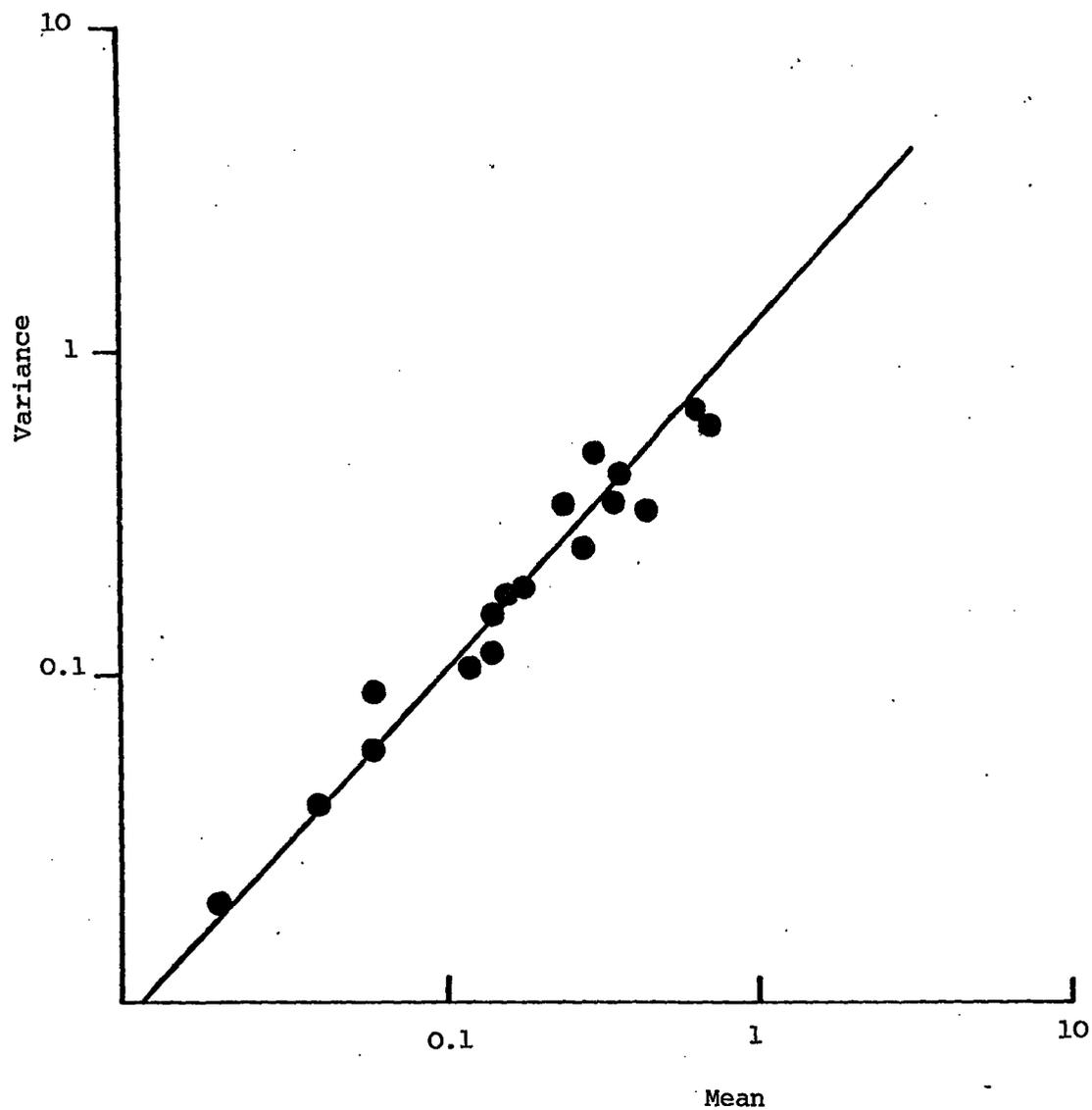
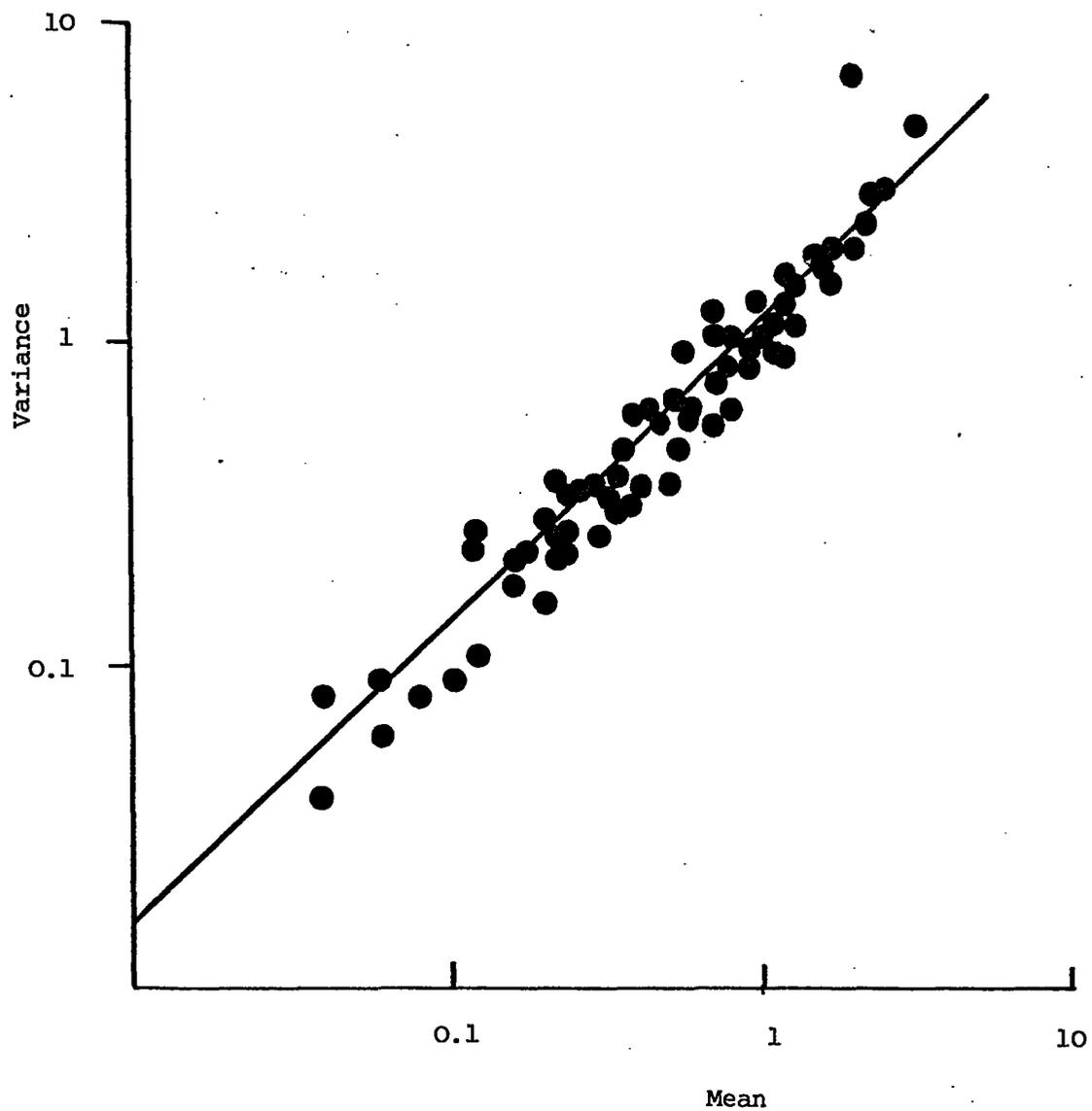


Fig. 7. Regression line obtained with data from counts of filamentous bacteria on marked slides.



Elliot, 1971). Under the circumstances of extremely low and variable counts it was decided that the use of large samples was the more desirable for all the field data collected.

Comparison of variability between and within sediment cores were made to determine the number of sampling units required for estimation of the population with a given degree of precision. Table 7 summarises the data obtained.

As might be expected within-sample variability was considerably less than that between cores. The most reproducible results were obtained with total counts (AO) and estimates of chloroplasts. It was interesting to observe that, although generally speaking, the counts of filaments were more variable than those of the total bacterial population, this was certainly not true of the between core variability of the FDA stained filaments.

3.3 Field data

3.3.1 Direct counts

A general survey was required to provide basic information on the lakes sediments before choosing of sites for more detailed studies of filamentous bacteria. The acridine orange fluorochrome which stains the DNA, was chosen to estimate the total bacterial population. The direct count of the population of bacteria stained with AO (total counts - AO) and of the filamentous bacteria also stained with AO (filamentous bacteria - AO) are summarized in Figs. 8 and 9. There was an upward trend in the counts with increasing degree of enrichment of the lakes particularly at the eutrophic end of the spectrum, and the ranges observed were wider in the nutrient rich lakes than in the nutrient poor ones. The total counts ranged between $1.5 \times 10^{10} \text{ g}^{-1}$ dry weight (Haweswater) and $15.7 \times 10^{10} \text{ g}^{-1}$ (Blelham Tarn) whereas those of filamentous bacteria ranged between $0.03 \times 10^8 \text{ g}^{-1}$ in Bassenthwaite and $12.03 \times 10^8 \text{ g}^{-1}$ in Blelham Tarn.

Table 7. Comparisons of bacterial counts and number of chloroplasts between and within sediment cores.

	Total bacteria / graticule area				Filamentous bacteria / microscopic field area				Chloroplasts / microscopic field area	
	AO		FDA		AO		FDA		Between	Within
	Between	Within	Between	Within	Between	Within	Between	Within		
\bar{x}	5.73	8.96	1.54	7.81	2.37	2.27	0.78	0.73	1.24	18.36
s	1.07	0.53	0.76	0.37	0.52	0.41	0.20	0.21	0.27	0.99
s ²	1.15	0.28	0.58	0.14	0.27	0.17	0.04	0.04	0.07	0.99
n	5	5	5	5	5	5	5	5	5	5
C _v	18.67%	5.92%	49.35%	4.74%	21.94%(22)	18.06%	25.64%	28.77%	21.77%	5.39%
sampling units required	*3.50(4)	0.35(1)	24.20(24)	0.23(1)	4.82(5)	3.40(3)	6.67(7)	8.00	3.50(4)	0.29(1)
	**9.67(10)	0.97(1)	67.20(67)	0.64(1)	13.38(13)	9.44(9)	18.51(19)	22.21(22)	9.70(10)	0.81(1)

* to obtain a standard error of 10% of the mean

** to obtain 95% confidence limit of 10% of the mean

() rounded figures expressing the sampling units required.

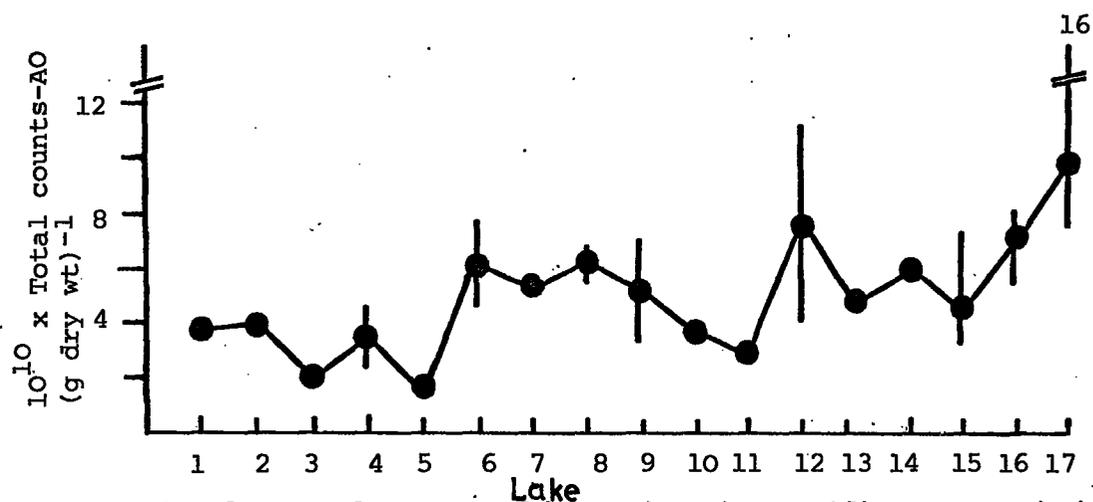


Fig. 8. Total counts of bacteria using acridine orange (AO) from the surface sediment of 17 Cumbrian lakes. The range and arithmetic means in lakes 1 to 14 are the result of two values and in lakes 15, 16 and 17 the results of four values. The lakes are numbered as in Plate 1.

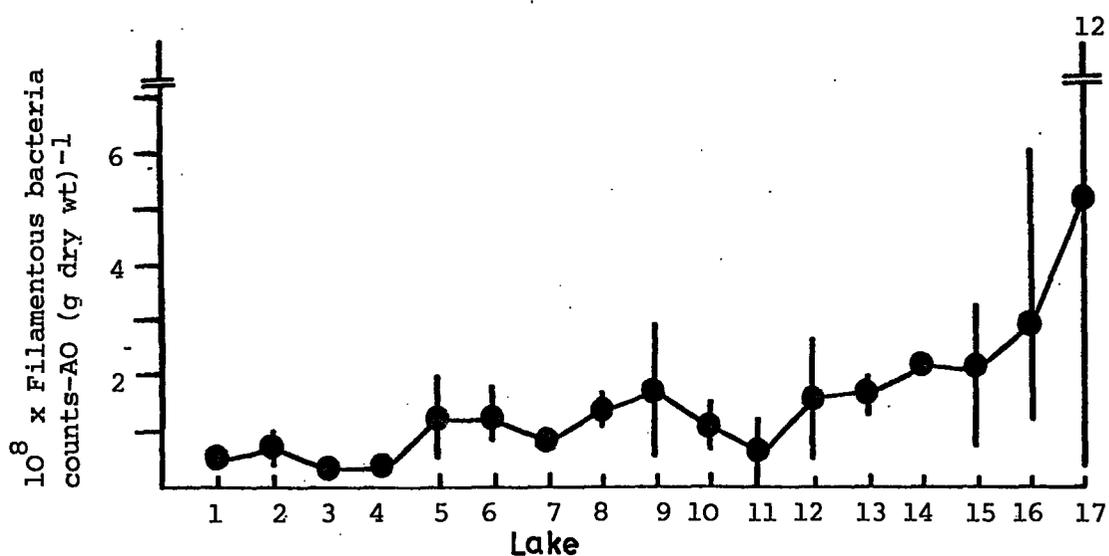


Fig. 9. Filamentous bacteria counts-AO from the surface sediment of the 17 lakes sampled. Conditions as for Fig. 8

To estimate the viable population of bacteria fluorescein diacetate staining (FDA) was chosen. The FDA, an esterified dye, is considered to give an indication of the integrity of the membrane and therefore of the viability of the cell. The distribution pattern of bacteria stained with FDA was rather different with an apparent upward trend in the intermediate lakes (Fig. 10 and 11), this being more marked in the counts of the total bacterial population stained with FDA (total counts - FDA) than those of the filamentous bacteria (filamentous bacteria - FDA). Fig. 12 expresses the total bacterial counts - FDA expressed as a percentage of the total bacterial count - AO and the highest values were once again observed in the sediment of the intermediate lakes. When the counts of filamentous bacteria were expressed as a percentage of the total, the FDA counts gave higher values than those obtained with AO in all lakes except Haweswater and Ullswater (Fig. 13). The total and filamentous counts - FDA showed greater variability between the lakes than the counts obtained using AO. The minimum and maximum values of the total counts from the lakes sampled, using either AO or FDA, differed by an order of magnitude and the total counts - AO were 1.3 to 15.8 times greater than those obtained with total counts - FDA. A considerable range in the counts of filamentous bacteria was obtained throughout the seventeen lakes; this was greater with AO where the maximum value was 400 times the minimum value. A smaller range was observed with FDA the maximum value being 16 times the minimum.

3.32 Viable counts

Since little was known about the sediments in the lakes sampled, viable counts were performed so that if any significant trend was to be present, this should not be missed. Estimates of the viable heterotrophic bacteria are presented in Figs. 14 to 19. The viable counts did not agree with the ranking of the lakes and a wide scatter of points

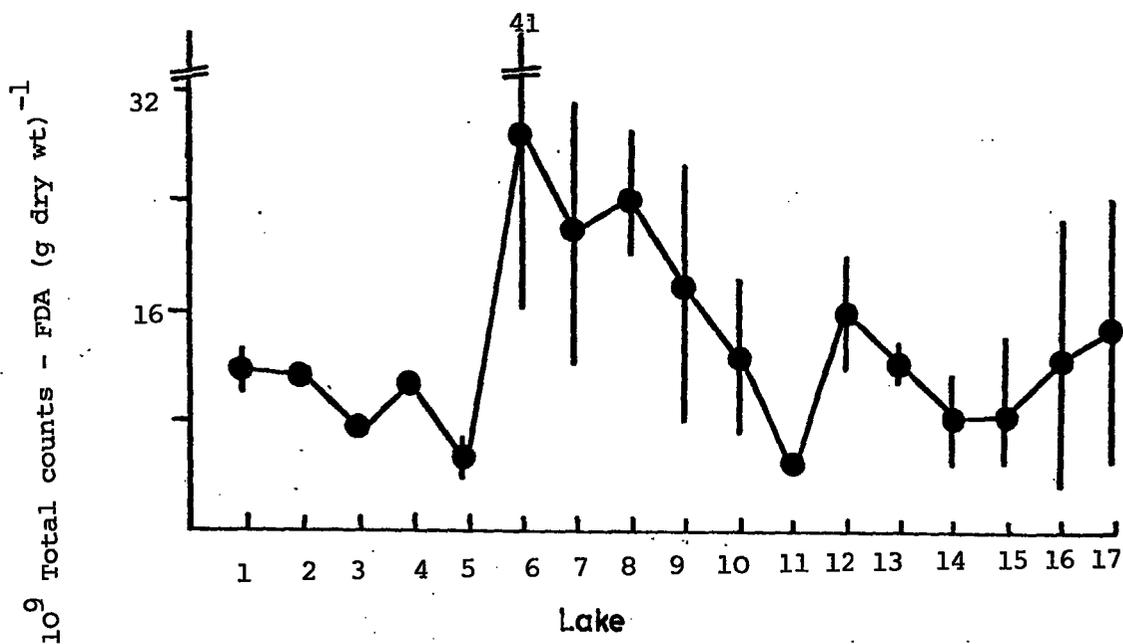


Fig. 10. Total fluorescein di-acetate (FDA) counts of bacteria from the surface sediment of the 17 lakes sampled. Conditions as for Fig. 8.

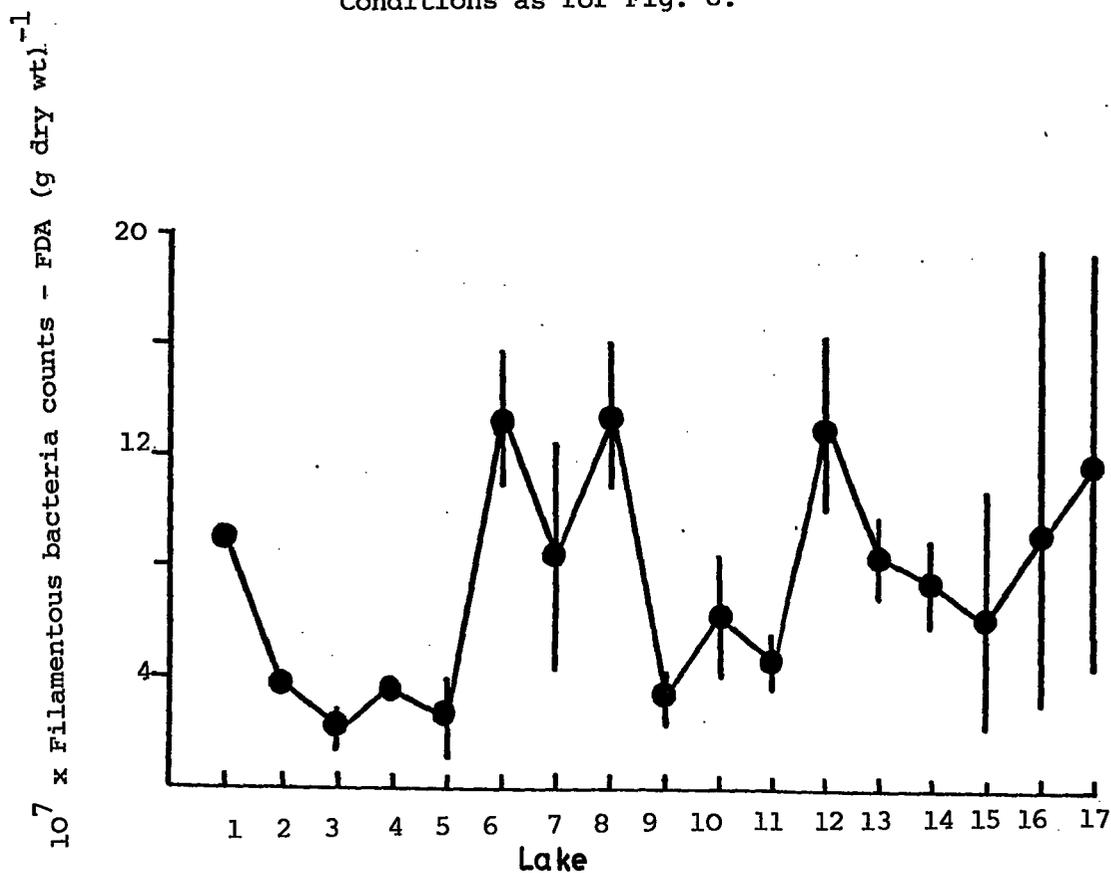


Fig. 11. Filamentous bacteria counts-FDA from the surface sediment of the 17 lakes sampled. Conditions as for Fig. 8.

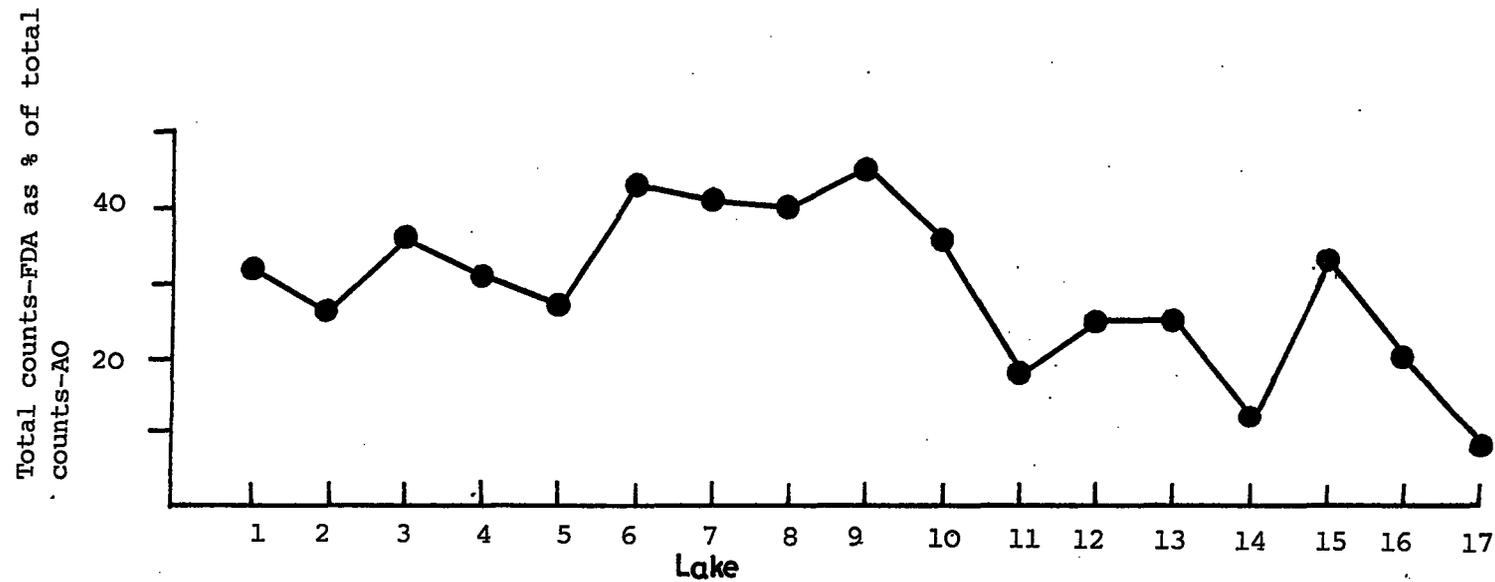


Fig. 12. Total bacteria counts - FDA as a percentage of total bacteria counts - AO in the 17 lakes sampled. The lakes are numbered as in Plate 1.

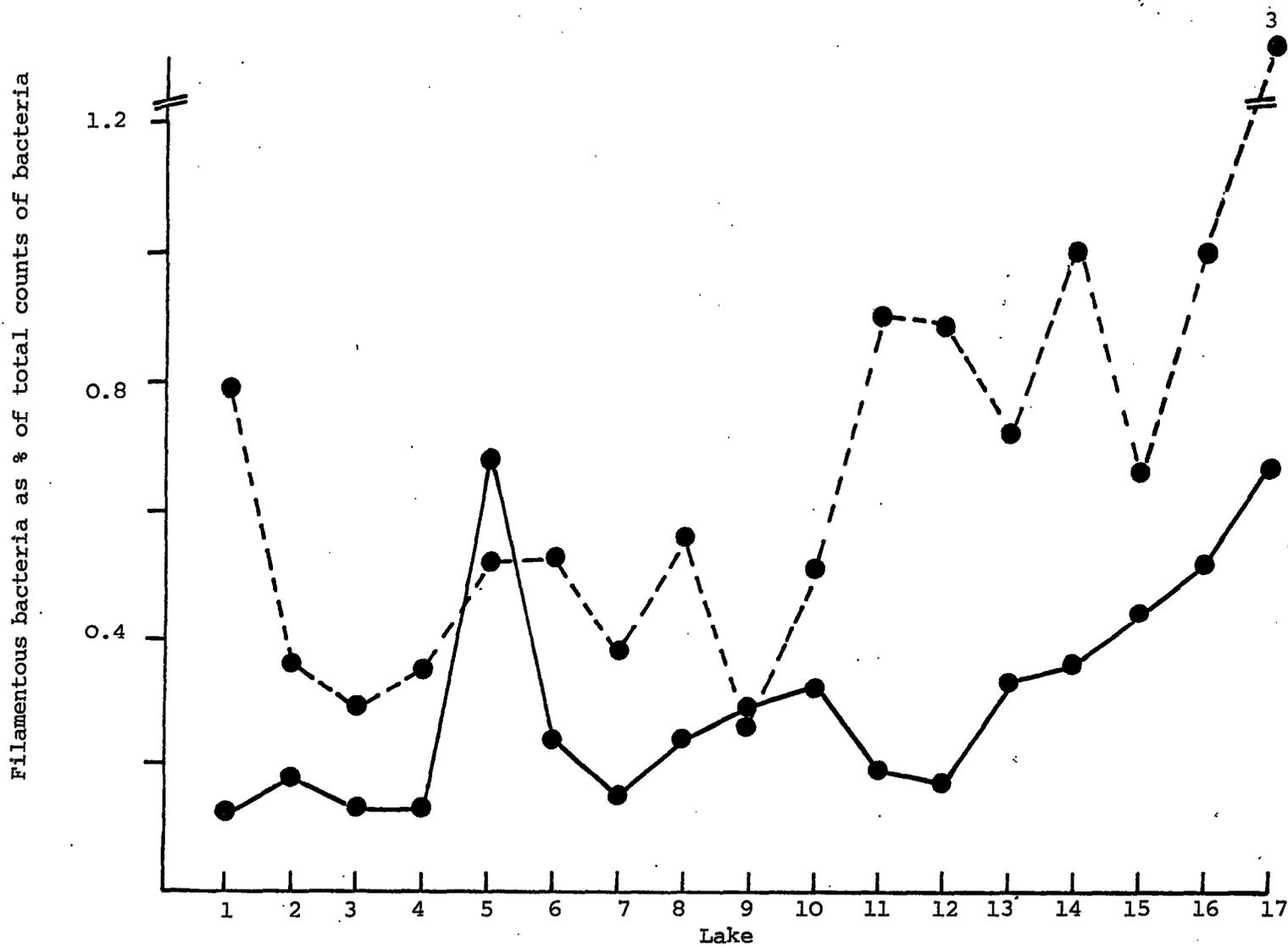


Fig. 13. Filamentous bacteria counts (AO and FDA) expressed as a percentage of total bacterial counts (AO and FDA) in the 17 lakes sampled. The lakes are numbered as in Table 1. (●—●) AO; (●---●) FDA.

was observed. In 71% of the lakes sampled the CPS medium gave counts higher or equal to those on LMW3 medium (Fig. 14). The values varied from $1.9 \times 10^6 \text{ g}^{-1}$ in Rydal Water to $176 \times 10^6 \text{ g}^{-1}$ in Blelham Tarn. In Blelham Tarn a high value was observed with LMW3 ($170\,000 \times 10^6 \text{ g}^{-1}$). Fig. 15 shows that the trends obtained with viable counts were similar on a dry weight as well as on volume basis. The scatter observed was of a similar order of magnitude. Fig. 16 shows the distribution of viable filamentous bacteria in the seventeen lakes. The values as well as the confidence limits were smaller than those obtained for viable counts. A slight upward trend was observed in the richer lakes, bearing in mind that the results are presented in log scale. The estimates of exo-enzyme producers are summarized in Fig. 17. The highest values of amylase producers were obtained in Haweswater, Bassenthwaite and Blelham Tarn. In the majority of the lakes sampled the numbers of amylase producers were higher than those of protease and lipase producers. The lowest individual counts for amylase, protease and lipase were obtained in Blelham Tarn, Esthwaite and Windermere North Basin respectively. Under the dilutions used, no cellulolytic isolates were obtained. When the counts of exo-enzyme producers were expressed as percentage of the estimated aerobic viable population on CPS medium (Fig. 18) no real trend was observed, although at the eutrophic end of the series, higher values for all three types were observed in samples from Rydal Water. Values over 100% were observed for amylase producers in three of the oligotrophic lakes probably reflecting the inadequacy of the medium used to estimate the viable population of bacteria in these lakes.

The estimates of anaerobic viable bacteria presented in Fig. 19 showed that in 65% of the lakes the counts on CPSB medium (basal medium plus glucose, lactate and acetate) were higher or equal to those on CPSA (basal medium plus peptone, casein, starch and glycerol).

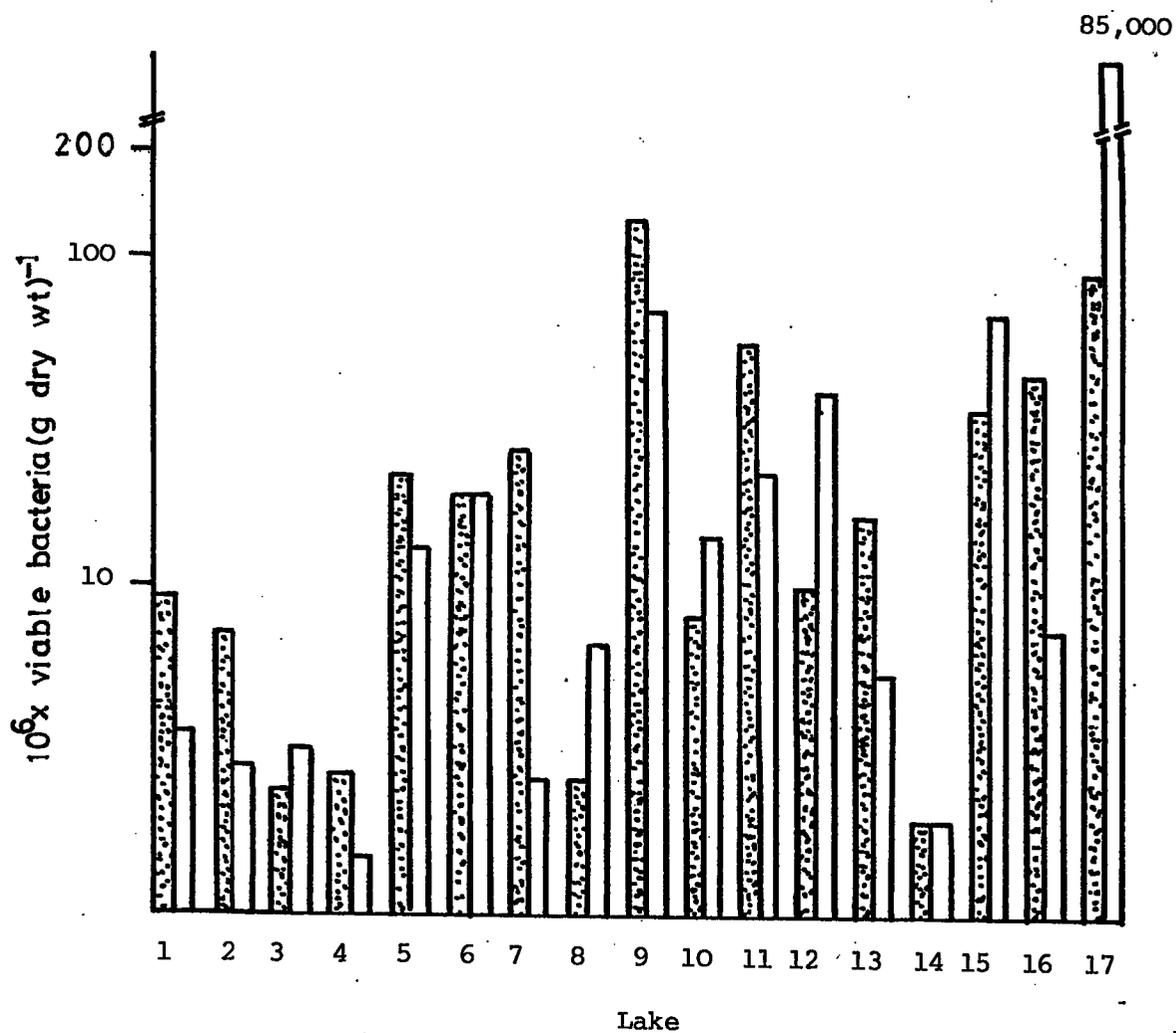


Fig. 14. Plate counts of aerobic bacteria on CPS and LMW3 media incubated for 2 weeks at 15 °C from surface sediment of the 17 lakes sampled. The lakes are numbered as in Plate 1. Each sample count is represented by a column except for lakes 15, 16 and 17 where the columns represent the arithmetic mean of two sample counts (▨) CPS; (□) LMW3.

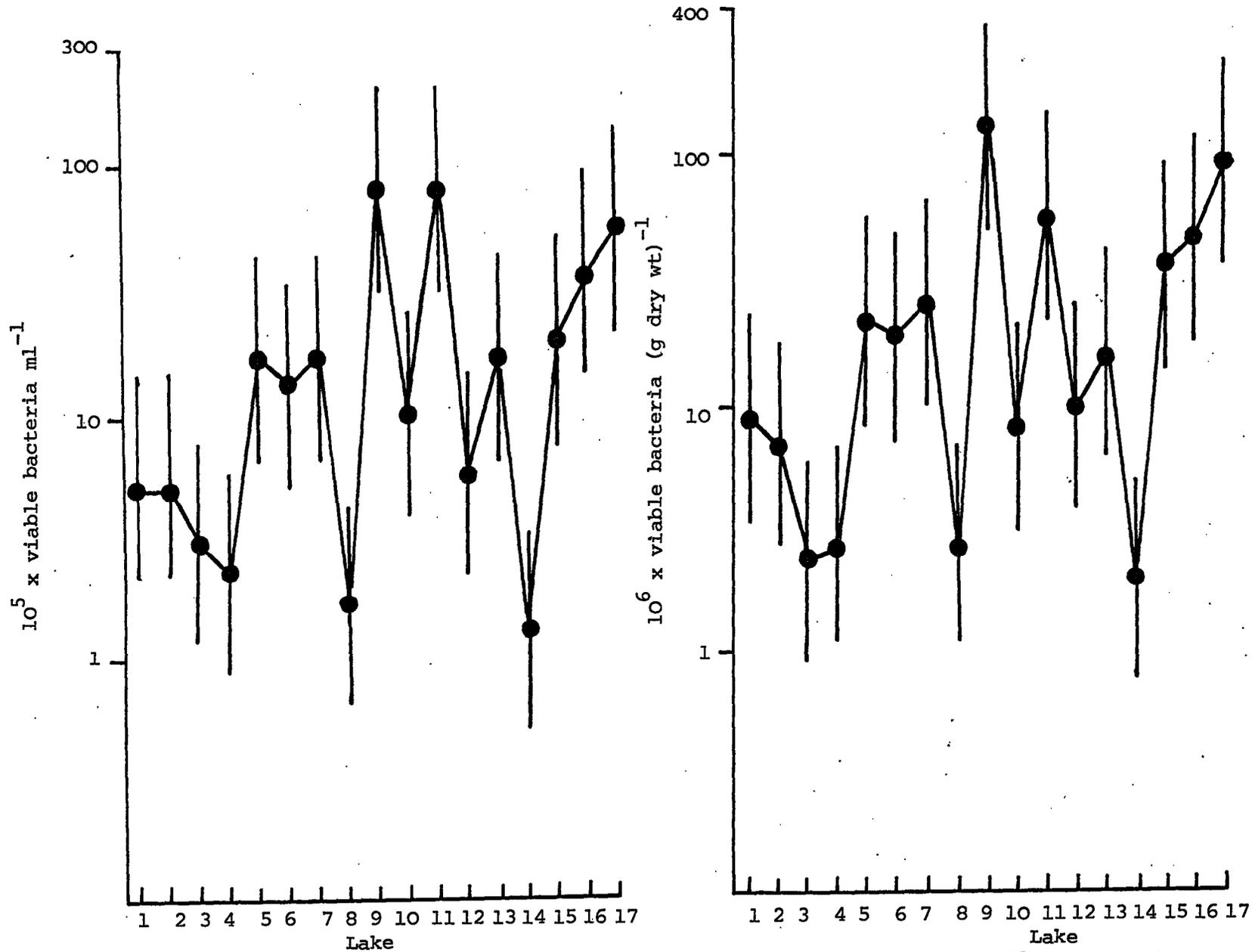


Fig. 15. Plate counts of aerobic bacteria on CPS medium incubated for two weeks at 15°C expressed on a volume (a) and dry weight basis (b). The values are results of one sample count except for lakes 15, 16 and 17 where the arithmetic mean of 2 values is represented. The 95% confidence limits are shown. The lakes are numbered as in Plate 1.

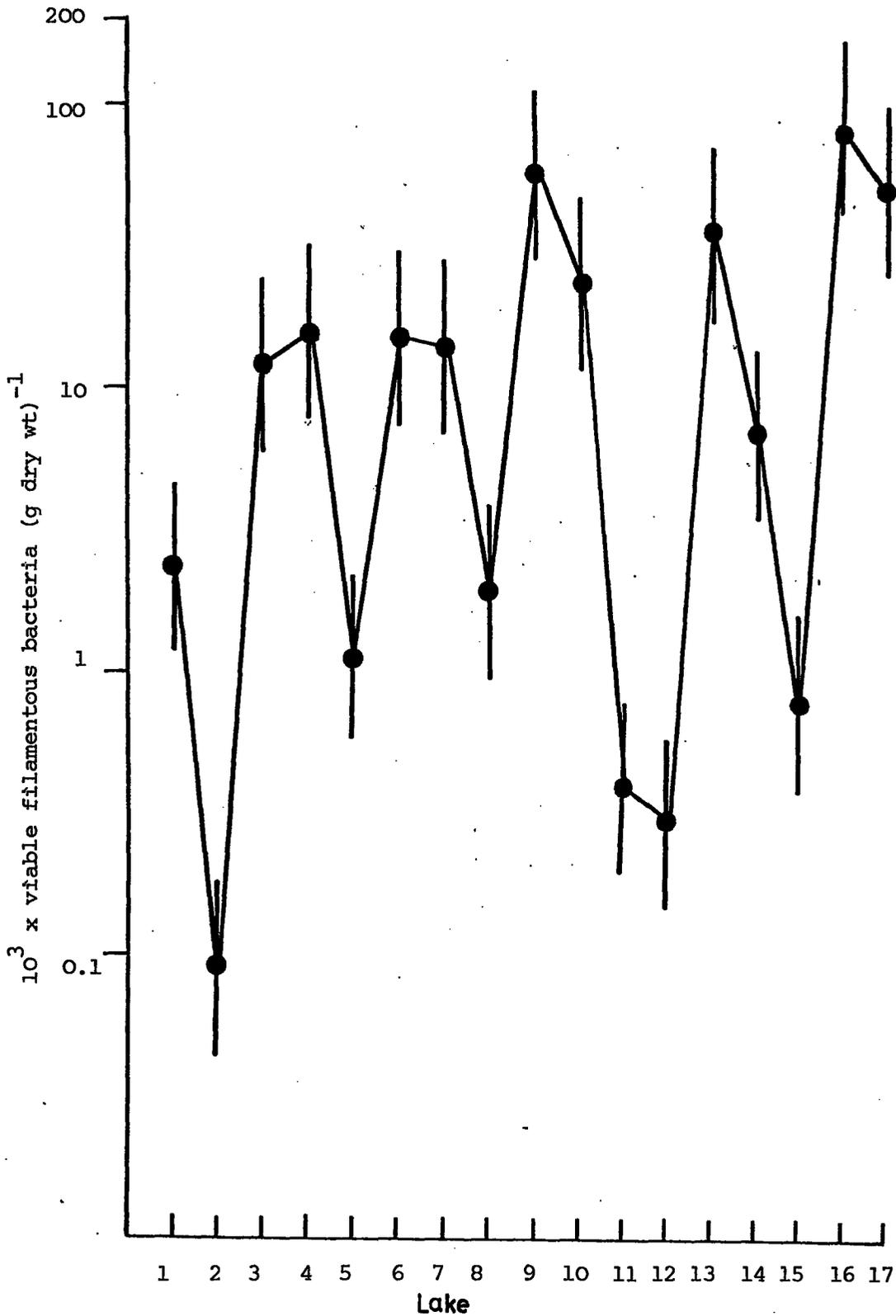


Fig. 16. Viable counts of filamentous bacteria estimated by MPN using liquid heterotrophic medium plus pieces of cellophane. Incubation was at 15°C for 10 days. Samples from the surface sediment of the 17 lakes considered. The 95% confidence limits are shown. The lakes are numbered as in Plate 1.

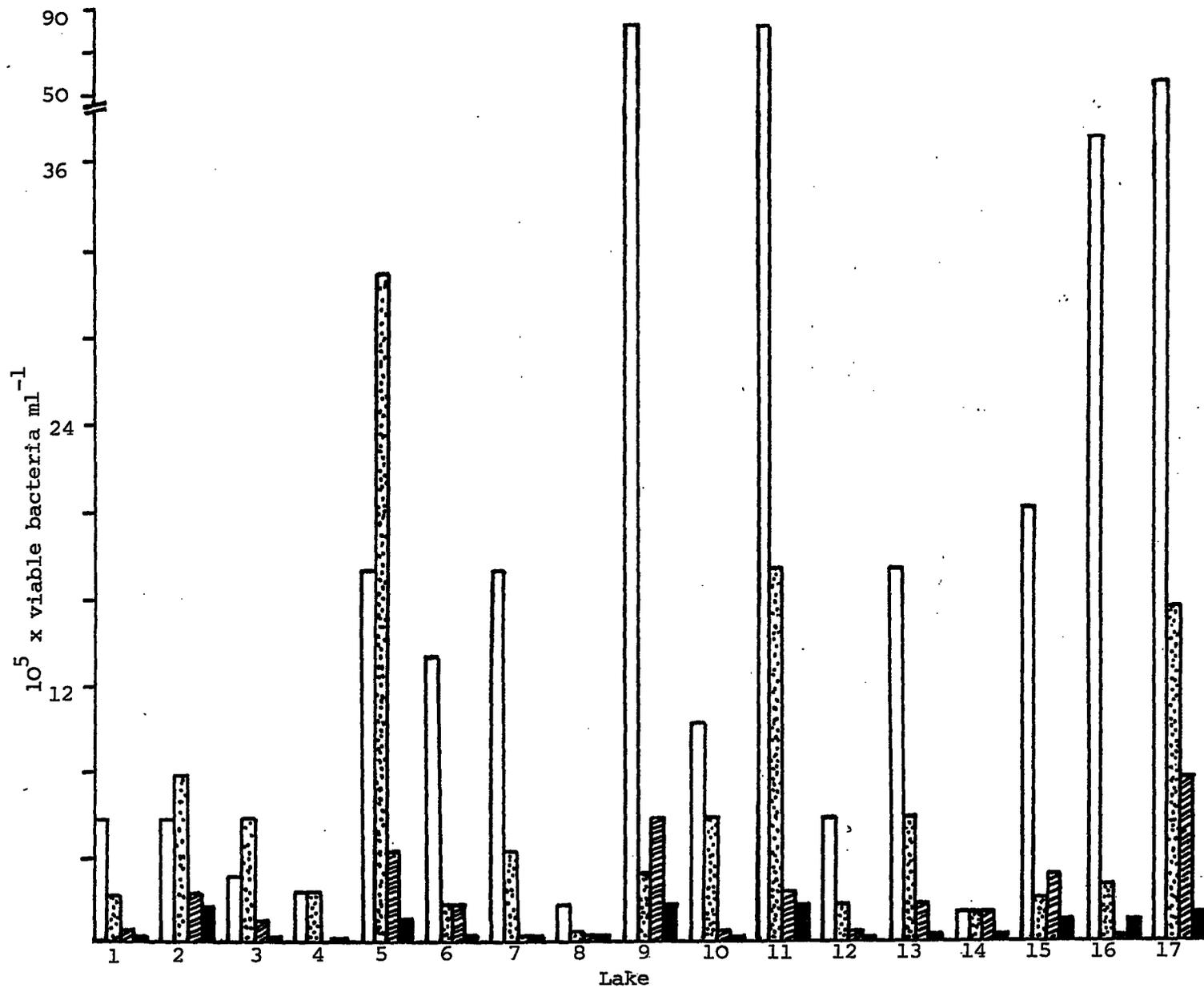


Fig. 17. Plate counts of exo-enzyme producing Bacteria compared with the plate counts of aerobic bacteria in CPS medium⁰⁶ in the 17 lakes sampled. Conditions as for Fig. 14. (□) viable bacteria (CPS); (▨) amylase producers; (▩) protease producers; (■) lipase producers.

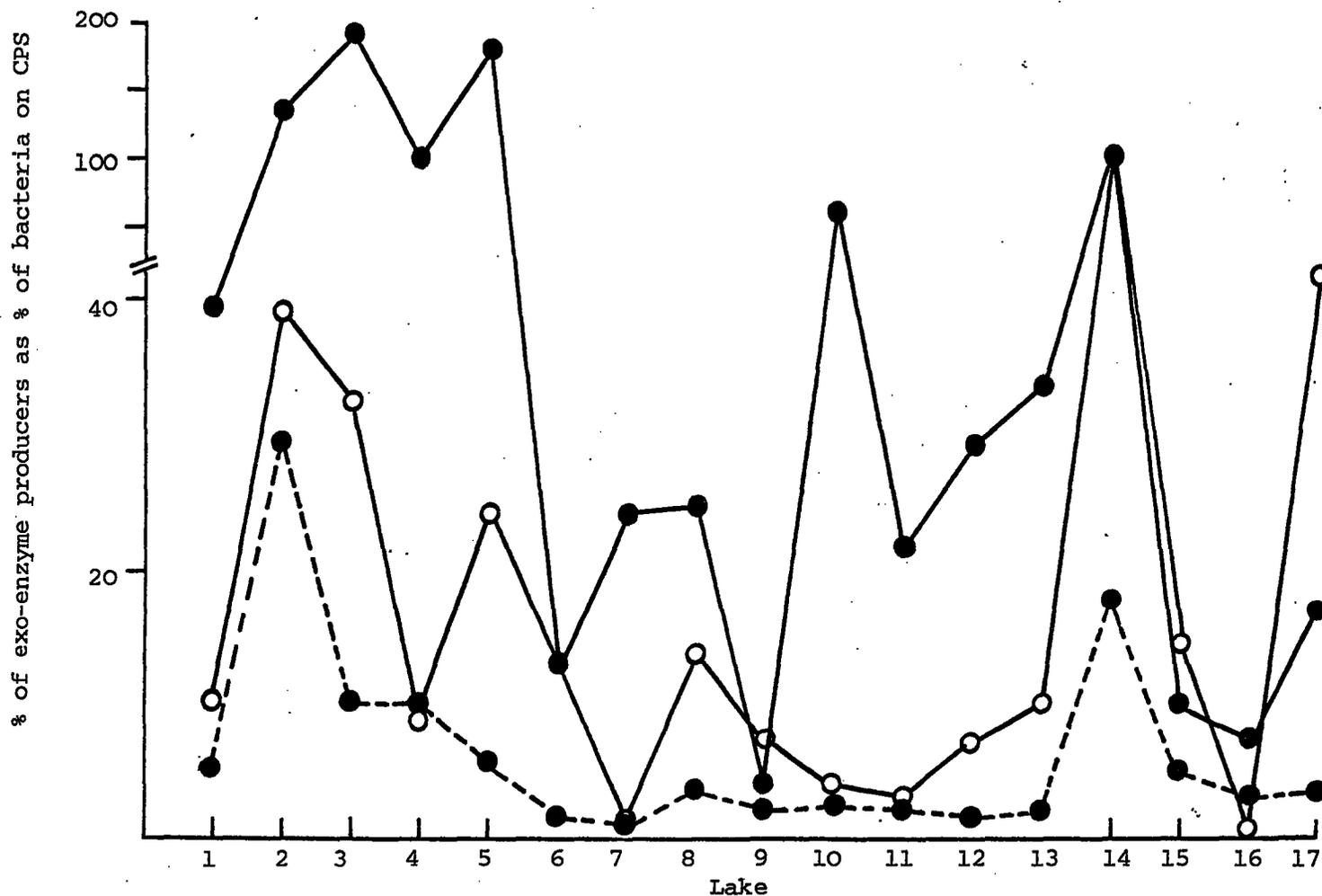


Fig. 18. Variations in number of exo-enzyme producers expressed as a percentage of the plate counts of aerobic bacteria on CPS medium. The lakes are numbered as in Plate 1. (●—●) amylase producers, (○) protease producers; (●---●) lipase producers. Note break and change in scale of ordinate.

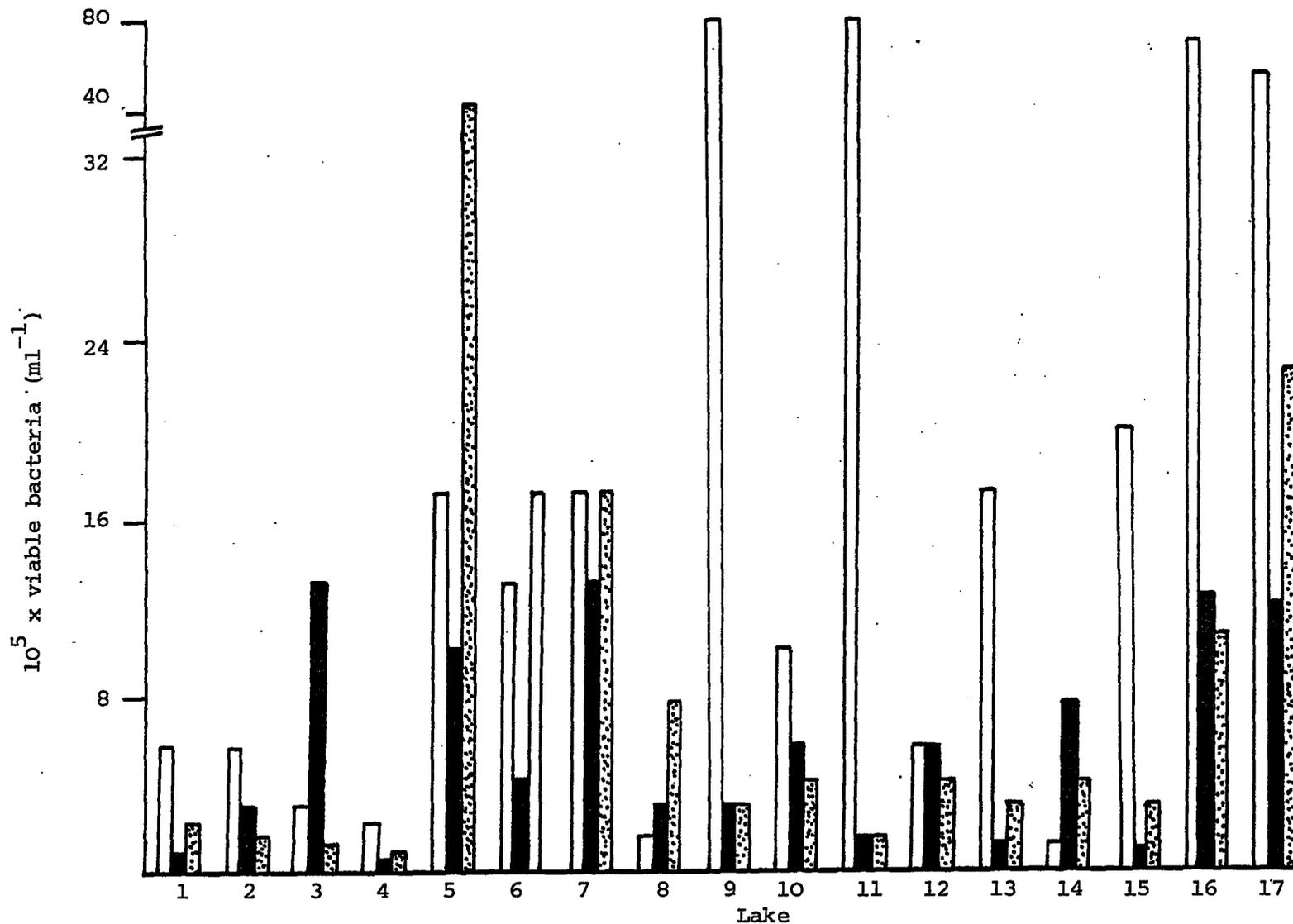


Fig. 19. Plate counts of anaerobic bacteria on CPSA and CPSB incubated for 10 days at 20°C compared with the plate counts of aerobic bacteria on CPS medium. Conditions are as for Fig. 14 (□) CPS, aerobes; (■) CPS A anaerobes; (▨) CPS B anaerobes. Note break and change in scale of ordinate.

The total counts - AO were 500 to 31000 times (i.e. 2 to 4 orders of magnitude) higher than the viable counts (CPS). The total counts - FDA were 60 to 10700 (1 to 4 orders of magnitude) higher than the viable counts. If the counts of aerobic and anaerobic were considered together (total viable counts) the total counts - AO were still 500 to 7300 times greater and the total counts - FDA were 48 to 1900 times greater. Fig. 20 shows the total viable counts expressed as a percentage of the total counts - AO and Fig. 21 gives the viable filamentous bacteria as percentage of the total viable counts. Expressed as a percentage of the viable counts the filamentous bacteria were proportionally higher than their direct counts when compared with the counts of the total bacteria - FDA and much higher than their AO counts when compared with the counts of the total bacteria - AO. The ratio of viable anaerobes (CPSB) to aerobes (CPS) was extremely variable (Fig. 22) with high values in Haweswater, Windermere North Basin and Rydal Water.

3.33 Chloroplast counts and chlorophyll a content of the sediments

The chloroplasts and chlorophyll a were estimated to provide basic information about their content in the sediments. The counts of chloroplasts or filaments containing chlorophyll under fluorescence microscopy was made also to give some indication of the proportion of thin filamentous forms showing fluorescence. The results obtained are presented in Figs. 23 and 24. There was an increase in chloroplast numbers with increasing degree of enrichment of the lakes. The values ranged from $0.07 \times 10^8 \text{ g}^{-1}$ in Ennerdale to $21.6 \times 10^8 \text{ g}^{-1}$ in Blelham Tarn. The same trend was observed for the chlorophyll a the maximum value being observed in Blelham Tarn ($532 \mu\text{g (g dry weight)}^{-1}$) and the minimum in Coniston ($2.1 \mu\text{g (g dry weight)}^{-1}$). Based on chloroplast and chlorophyll a values it would appear that Bassenthwaite and Ullswater are incorrectly placed in the ranking of the lakes studied.

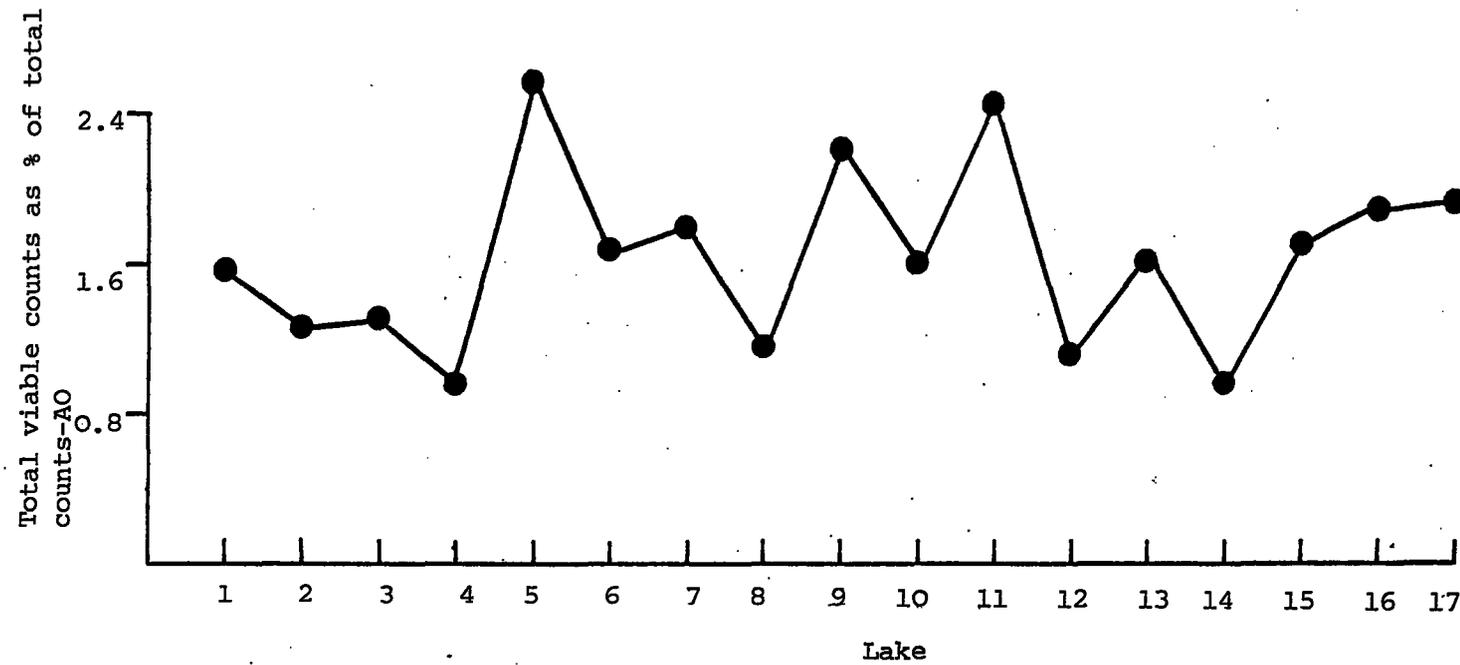


Fig. 20. Total viable counts (CPS aerobes + CPSB anaerobes) expressed as a percentage of total bacterial counts-AO in the surface sediment of the seventeen lakes sampled. The lakes are numbered as in Plate 1.

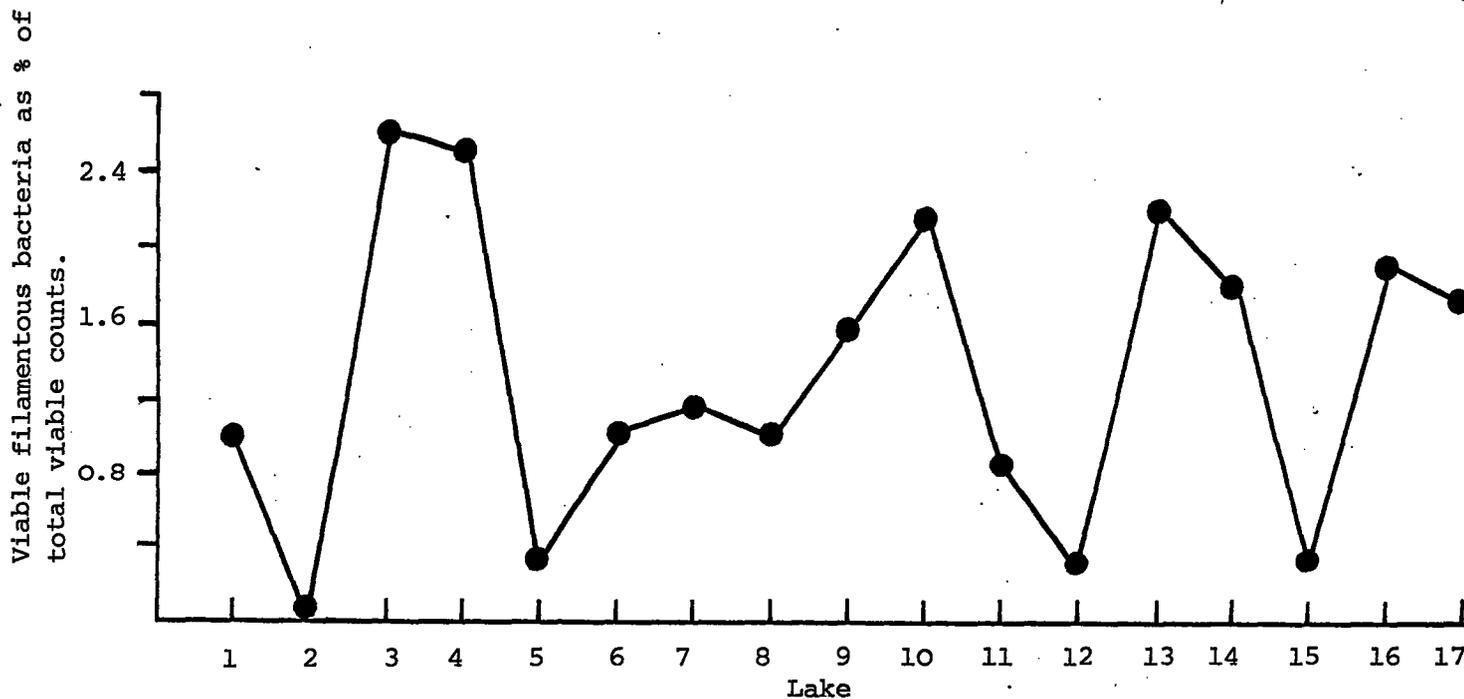


Fig. 21. Viable filamentous bacteria counts expressed as a percentage of total viable counts (CPS aerobes + CPSB anaerobes) from the surface sediment of the 17 lakes sampled. The lakes are numbered as in Plate 1.

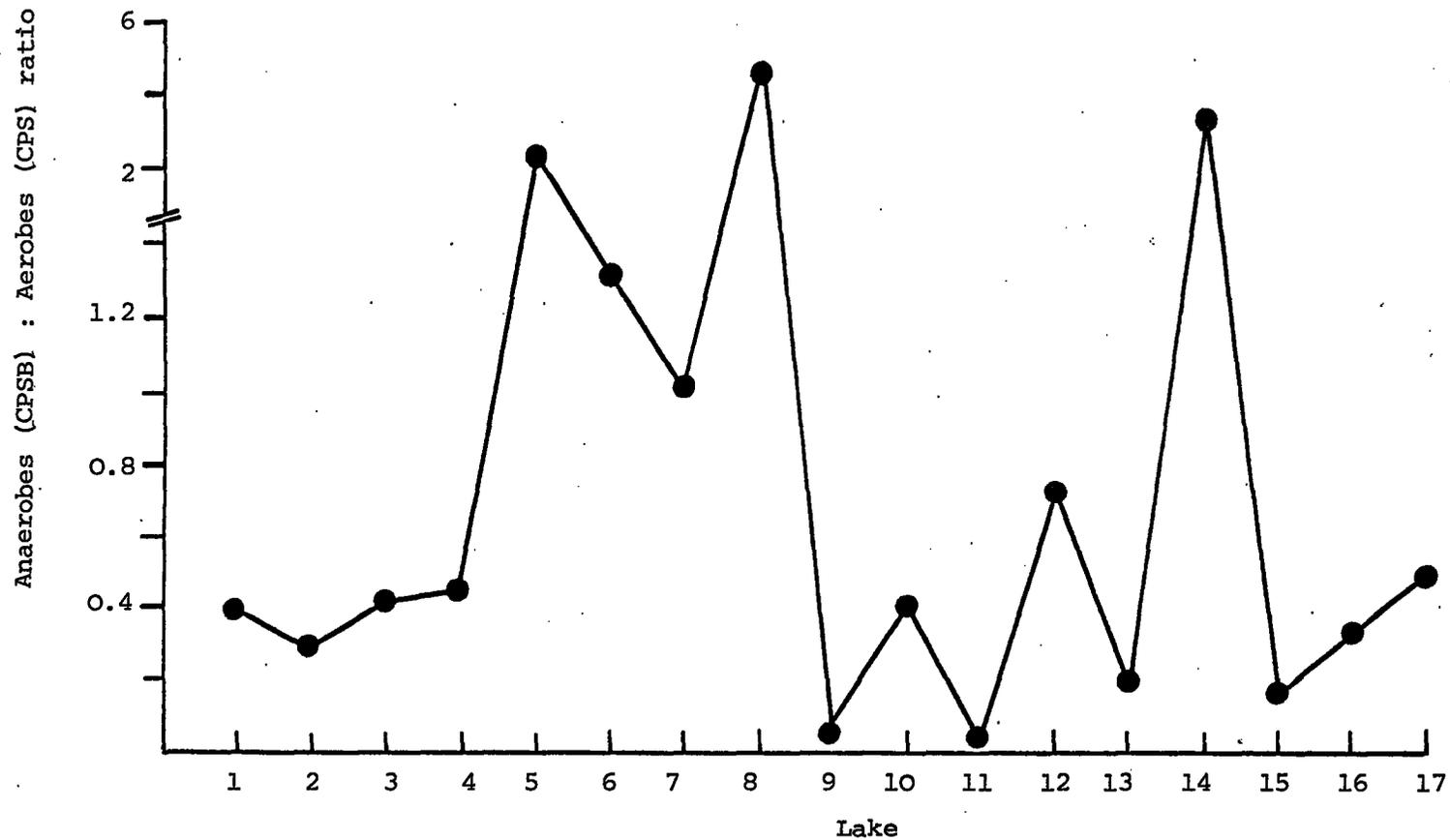


Fig. 22. Ratio of anaerobic bacteria estimated in CPS B medium and aerobic bacteria estimated in CPS medium in the 17 lakes sampled. The lakes are numbered as in Plate 1. Note break and change in scale of ordinate.

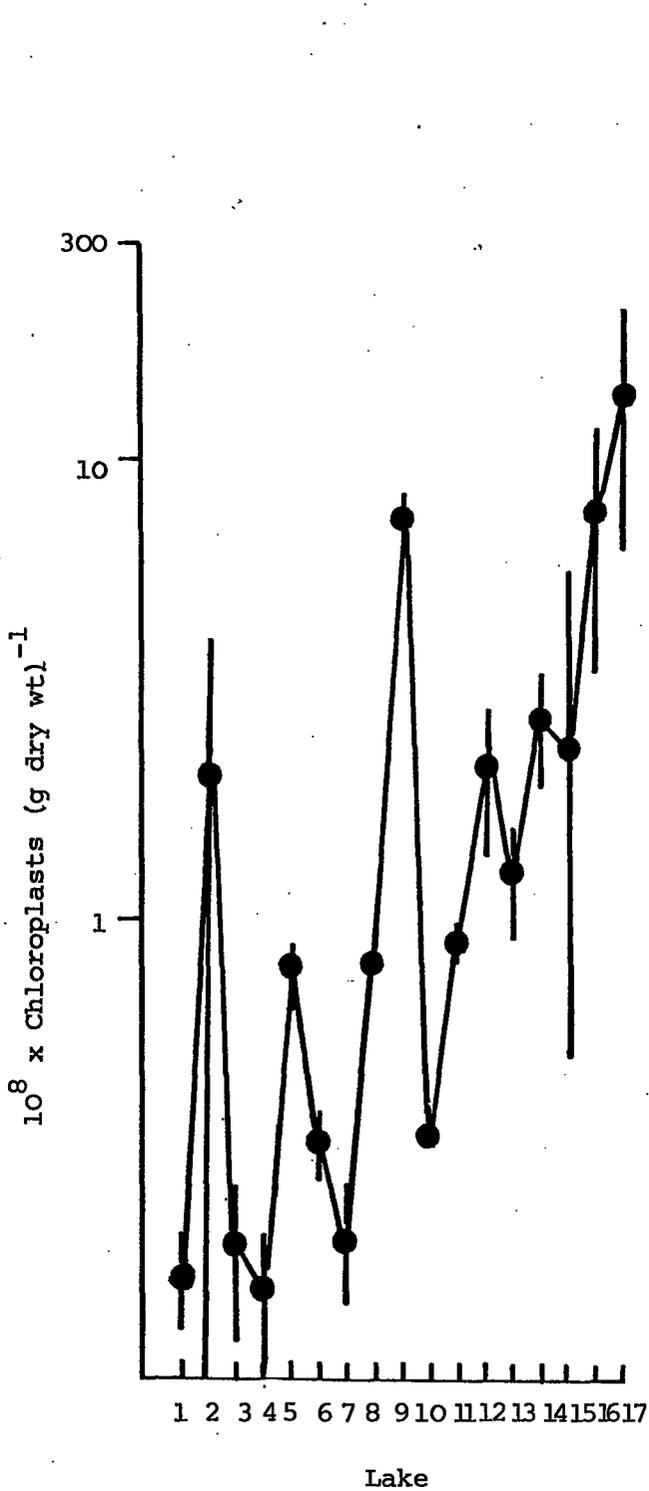


Fig. 23. Direct counts of chloroplasts in surface sediment of the 17 lakes sampled. The arithmetic mean and range of values are shown. Conditions are as for Fig. 8.

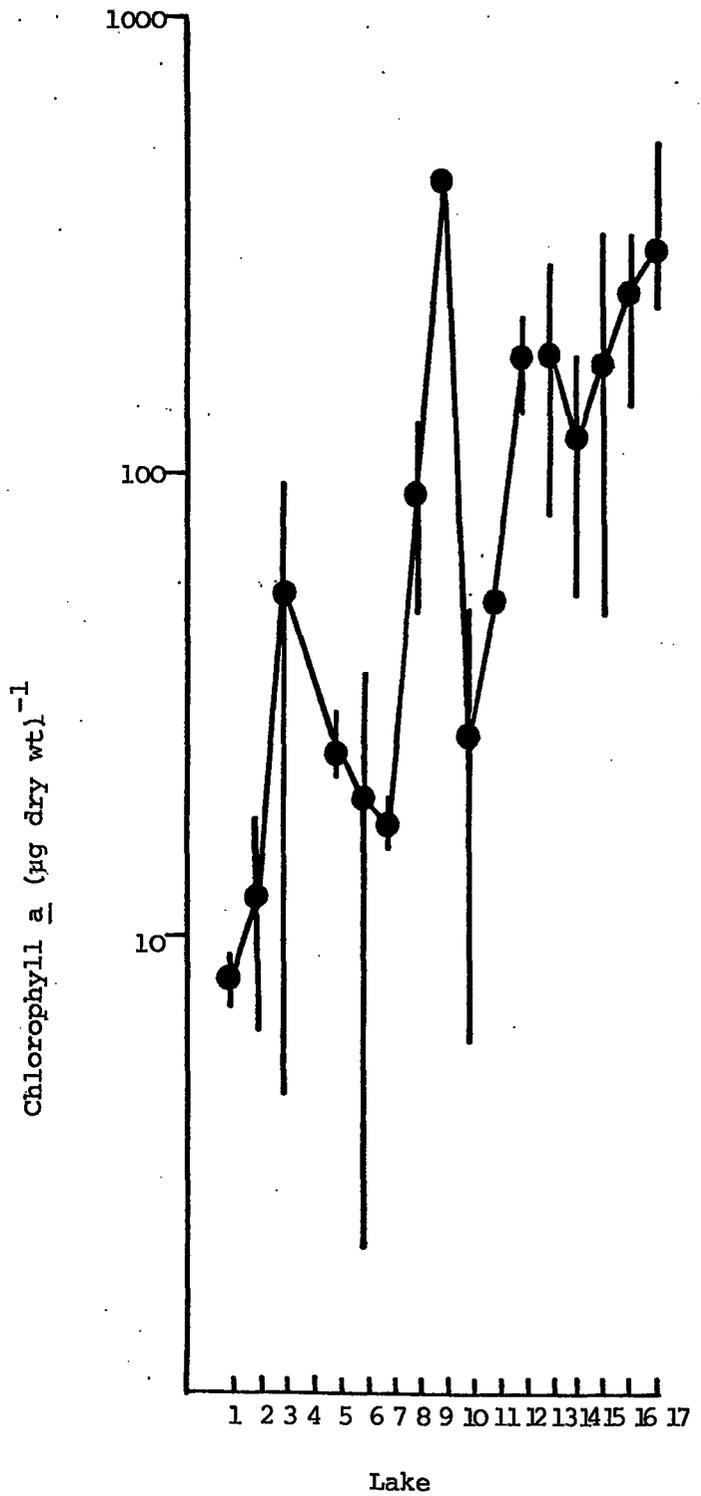


Fig. 24. Estimates of chlorophyll a in surface sediment of the 17 lakes sampled. The arithmetic mean and range of values are shown. Conditions are as for Fig. 8.

3.34 Analysis of the carbon (C), nitrogen (N), moisture and organic matter content of the sediments.

The analyses were made to obtain some estimate of potential carbon substrate concentration available for the bacteria. The C and N contents of the sediment are shown in Figs. 25 and 26 respectively. Higher values were observed particularly in the four lakes at the eutrophic end of the range. The values fluctuated within narrow ranges in all the seventeen lakes sampled. Fig. 27 shows the C/N ratios and no trend was observed.

The results obtained for moisture and organic matter content are summarized in Figs. 28 and 29. The values for moisture content did not show any trend but low values were observed in Buttermere and Bassenthwaite. The values for loss on ignition varied between 10% and 40% with no consistent upward trend with increasing lake enrichment. In Derwentwater an unaccountably high value (73%) was obtained.

3.35 Chemical analysis of the water overlying the sediments and of the surface 5 m

The results are presented in Table 8. The data were obtained from samples taken towards the end of the stratification period. With the exception of nitrate concentration in Esthwaite Water and Blelham Tarn the values obtained were in most cases higher 0.5 m above the sediment than in the 5 m surface waters tube samples and also higher in the more enriched lakes than in the nutrient poor ones. These values are included not as a comprehensive survey of the chemistry of the lake, but more to provide some general idea of their water chemistry and the degree to which enrichment (e.g. in phosphorus) occurs in the anoxic hypolimnia of the eutrophic lakes.

3.36 The electrode potential of sediments

The individual E_h profiles from each lake are presented in Figs. 30 to 46 and represent an E_h range from - 240 mV to + 595 mV. The

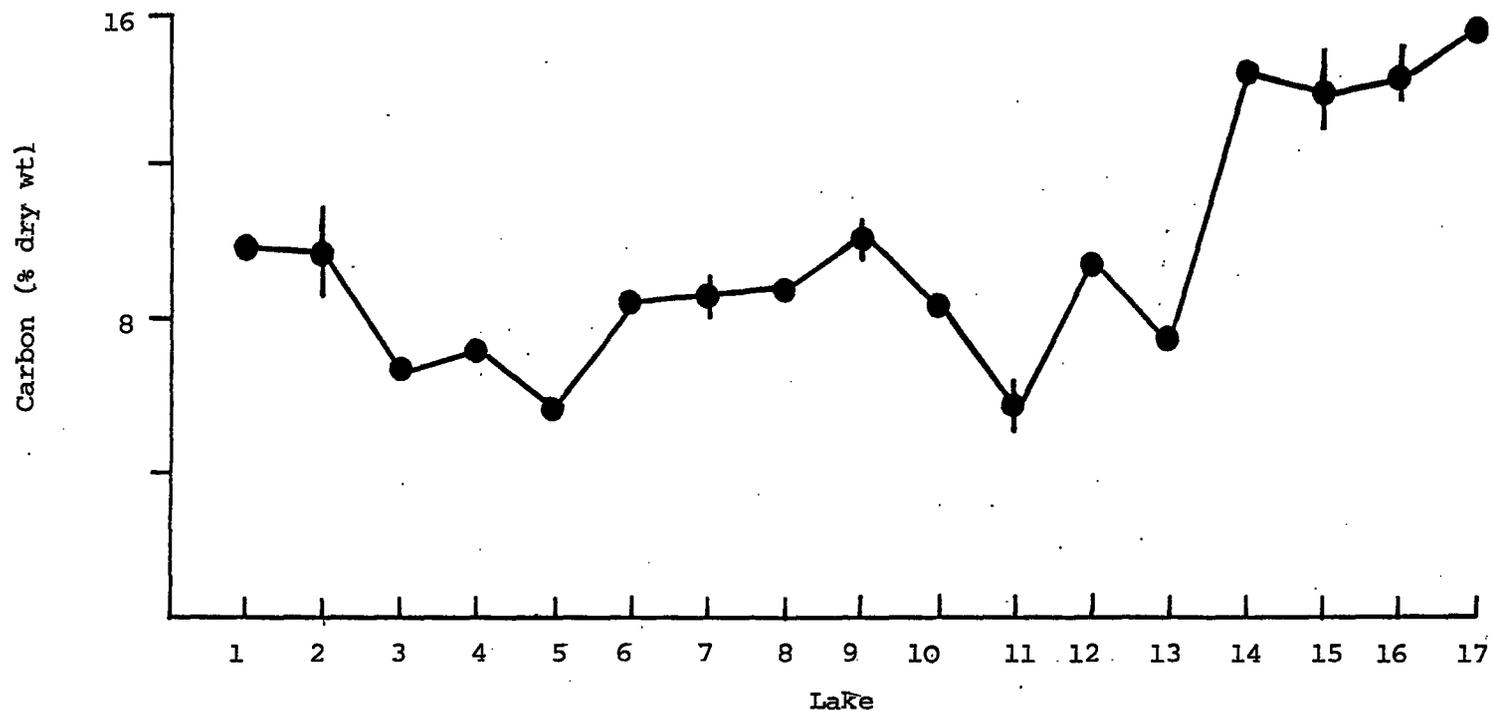


Fig. 25. Carbon content of surface sediment in the 17 lakes sampled. The range and arithmetic mean obtained are shown. Conditions are as for Fig. 8.

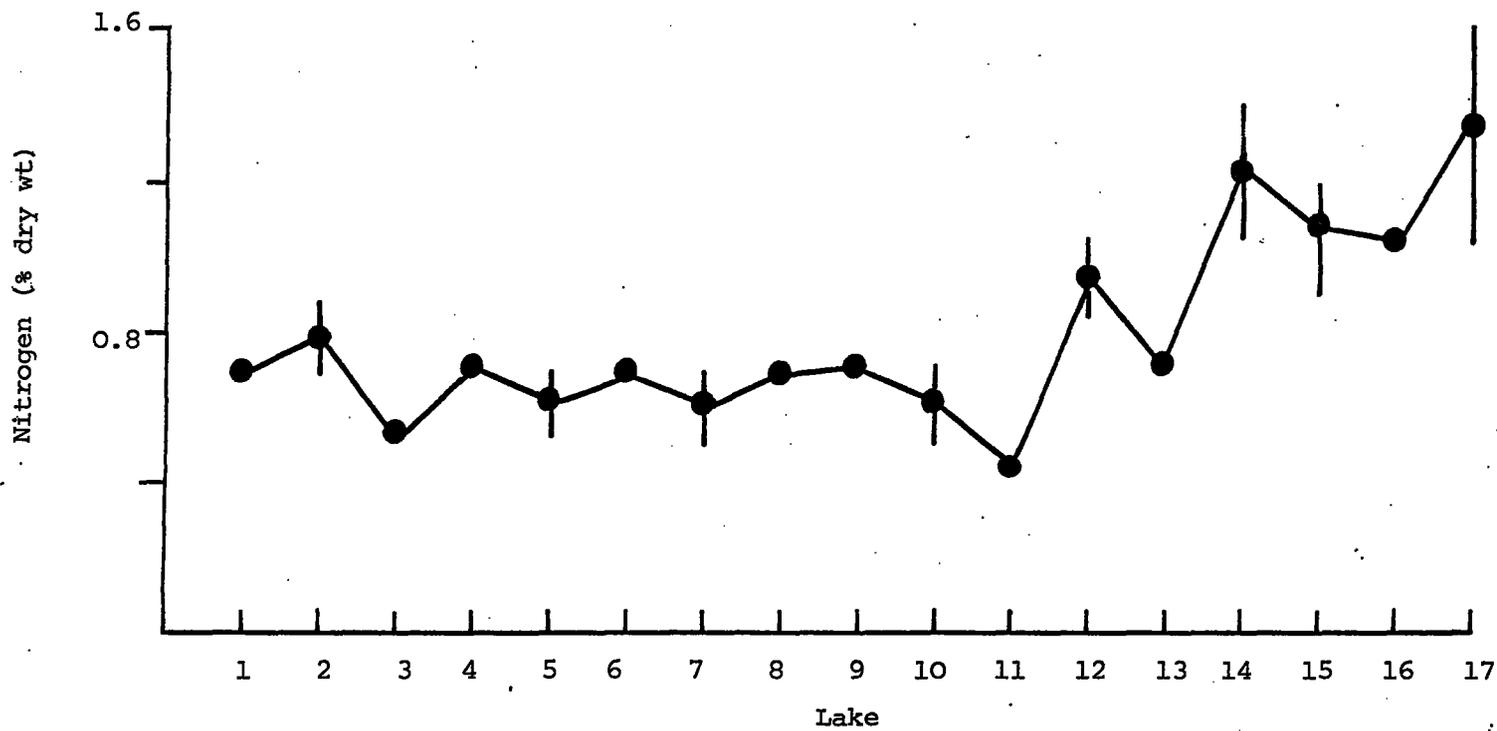


Fig. 26. Nitrogen content of surface sediment in the 17 lakes sampled. The range and arithmetic mean obtained are shown. Conditions are as for Fig. 8.

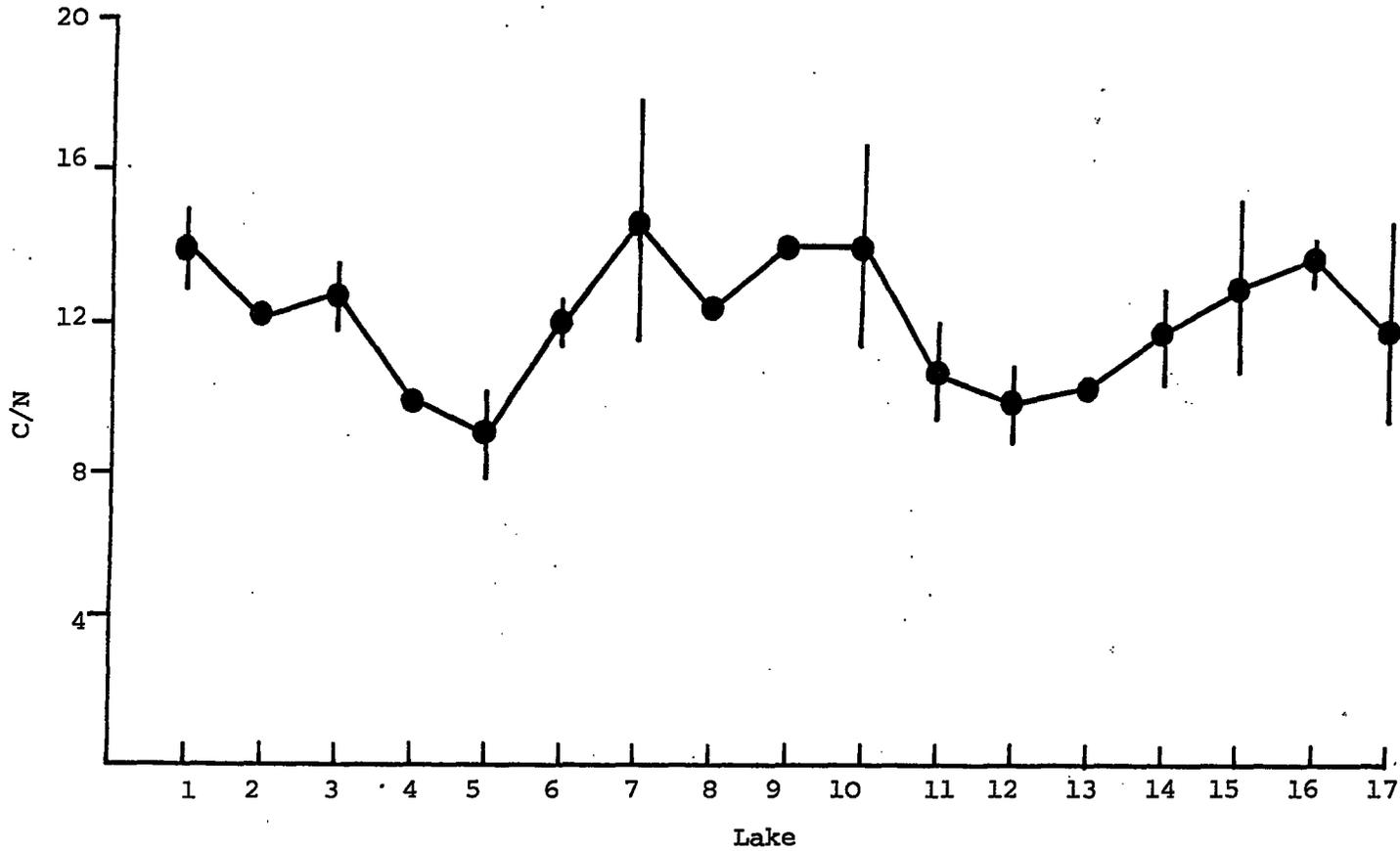
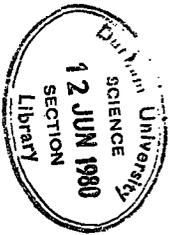


Fig. 27. C/N ratio from surface sediment in the 17 lakes sampled. The range and arithmetic mean obtained are shown. Conditions are as for Fig. 8.

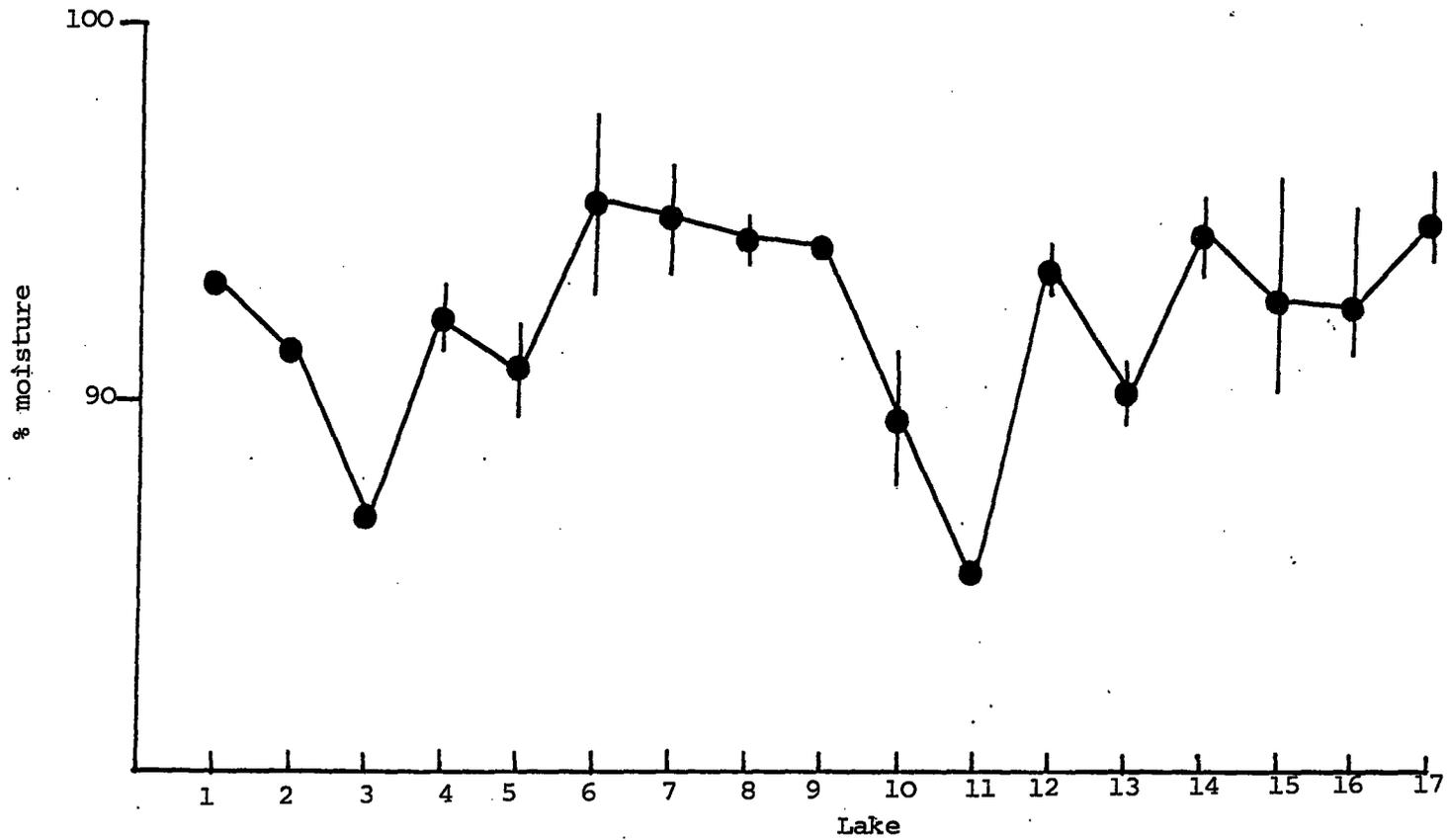


Fig. 28. Water content in the sediment surface expressed as percentage of moisture in the 17 lakes sampled. Conditions are as for Fig. 8.

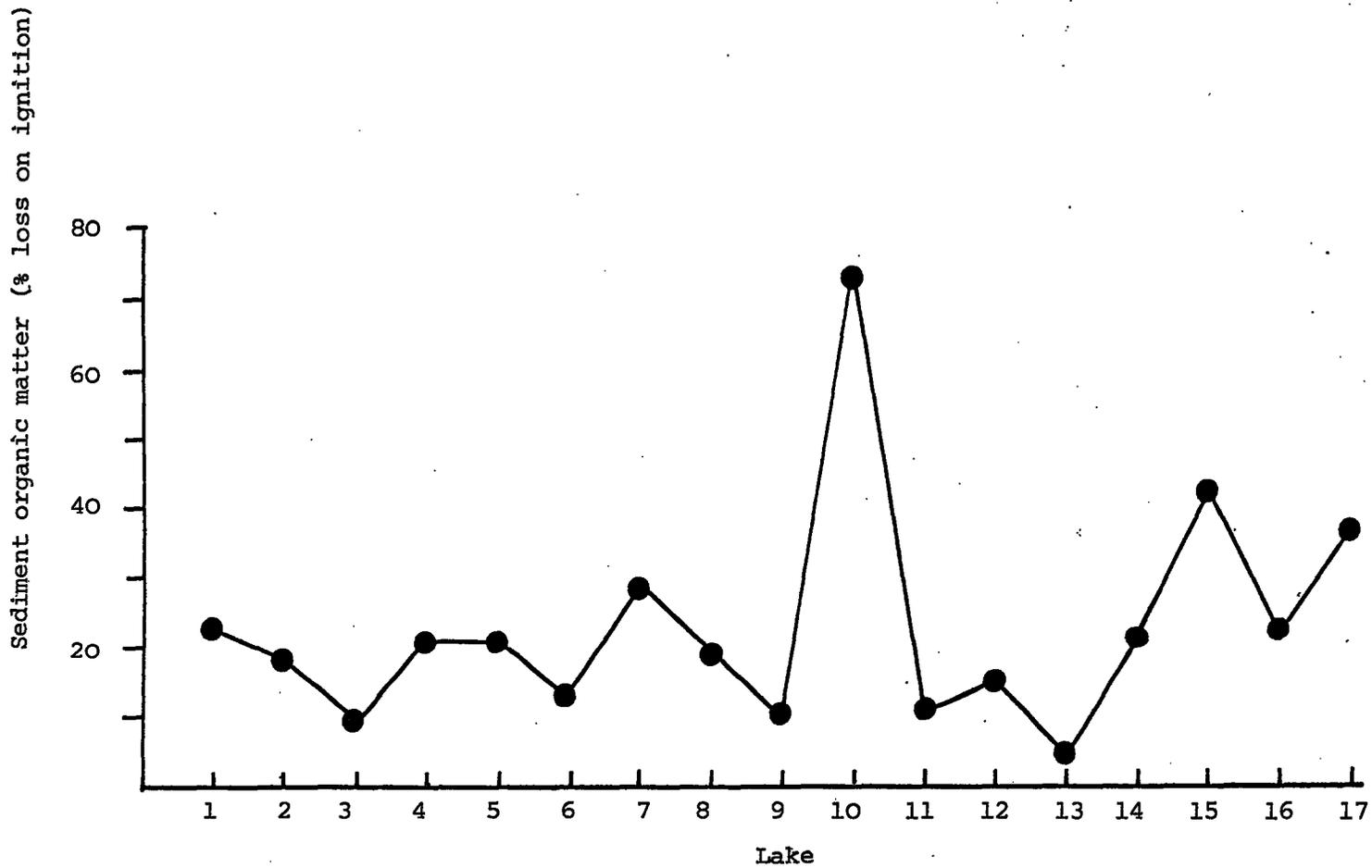


Fig. 29. Sediment organic matter expressed as percentage of loss on ignition in the 17 lakes sampled. The lakes are numbered as in Plate 1.

Table 8 : Chemical analyses of water 0.5 m above the sediment (bottom)
and from 5 m surface water (top) in the 17 lakes sampled.

Lakes	phosphorus (μgl^{-1})		$\text{NO}_3\text{-N}$ (μgl^{-1})		$\text{NH}_4\text{-N}$ (μgl^{-1})	
	top	bottom	top	bottom	top	bottom
1	< 0.6	< 0.6	297	344	9	12
2	< 0.6	< 0.6	222	305	8	15
3	< 0.6	< 0.6	133	130	< 5	14
4	1.0	4.0	194	228	-	-
5	< 0.6	< 0.6	261	264	9	13
6	0.6	< 0.6	422	527	36	41
7	0.6	< 0.6	216	311	40	58
8	1.7	7.4	280	588	19	< 5
9	6.5	1.6	261	258	34	51
10	< 0.6	< 0.6	150	122	21	20
11	3.3	1.0	261	228	-	-
12	5.4	44.7	341	594	13	7
13	< 0.6	< 0.6	633	633	27	41
14	< 0.6	< 0.6	164	133	9	329
15	1.5	25.0	119	474	62	157
16	2.0	188.0	20	15	30	1320
17	2.1	350.0*	48	31*	52	1540*

* 1.5 m above sediment

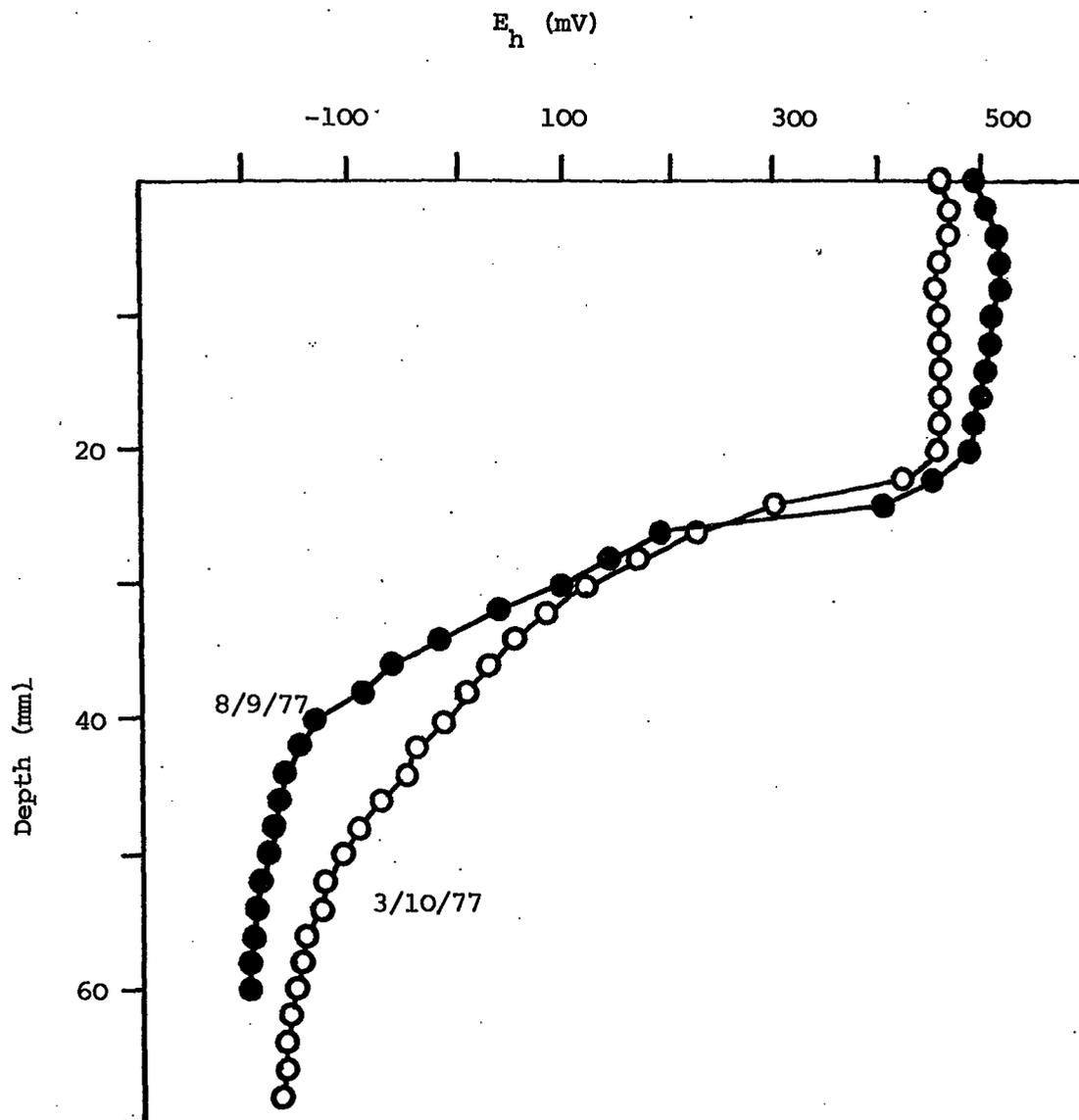


Fig. 30. Wastewater

Figs. 30 - 46. Individual readings of electrode potential of the 17 lakes sampled.

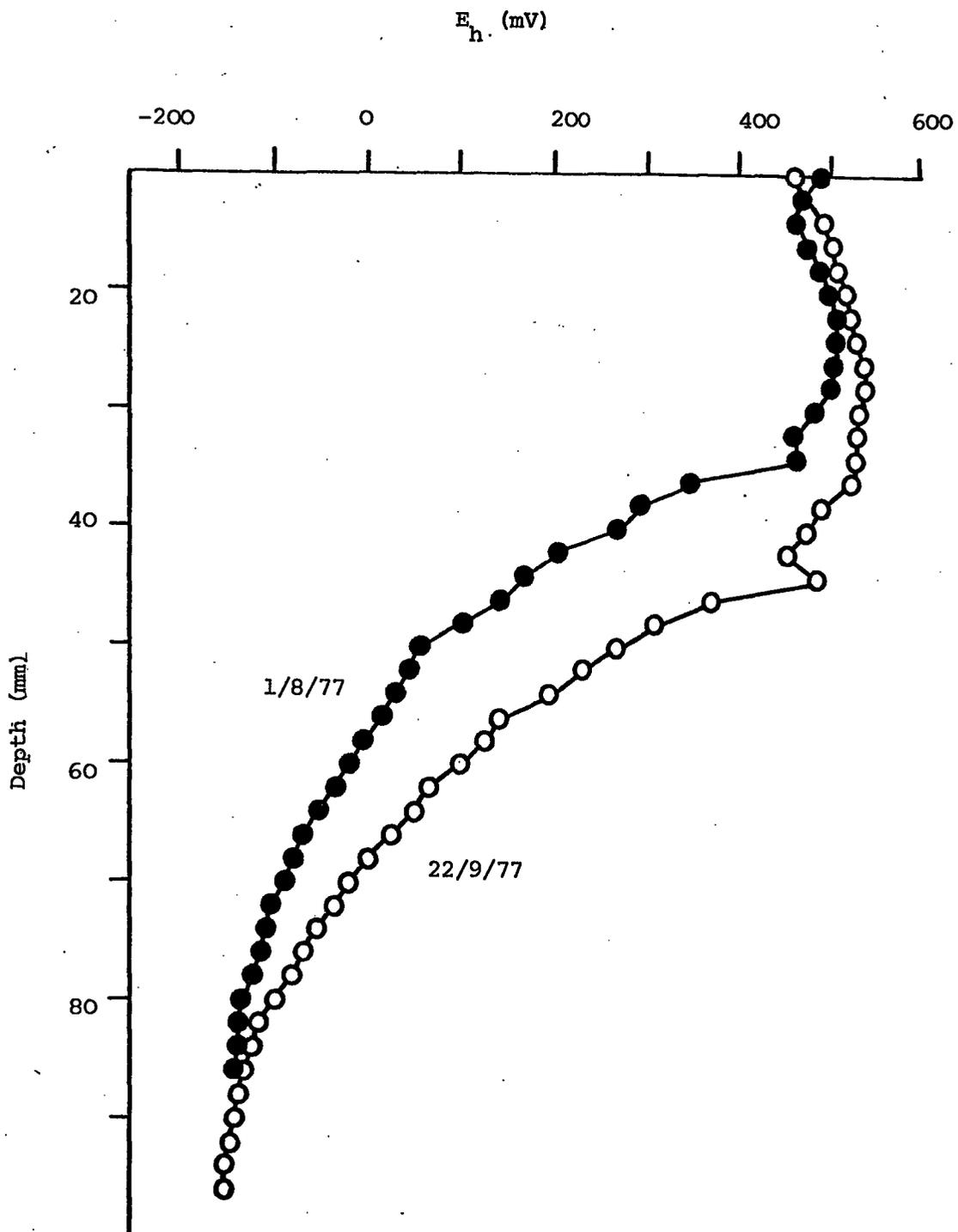


Fig. 31 Ennerdale

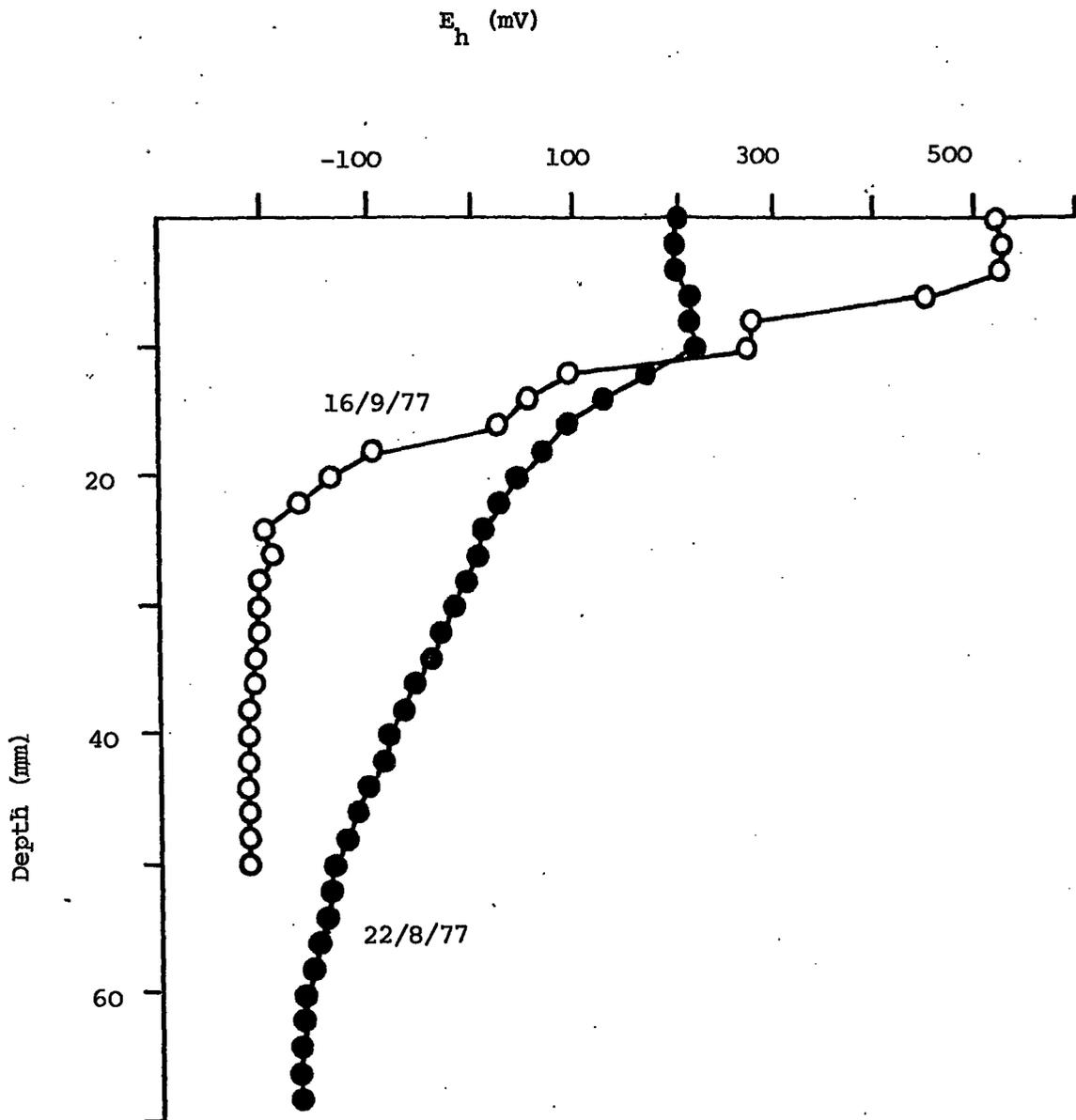


Fig. 32. Buttermere

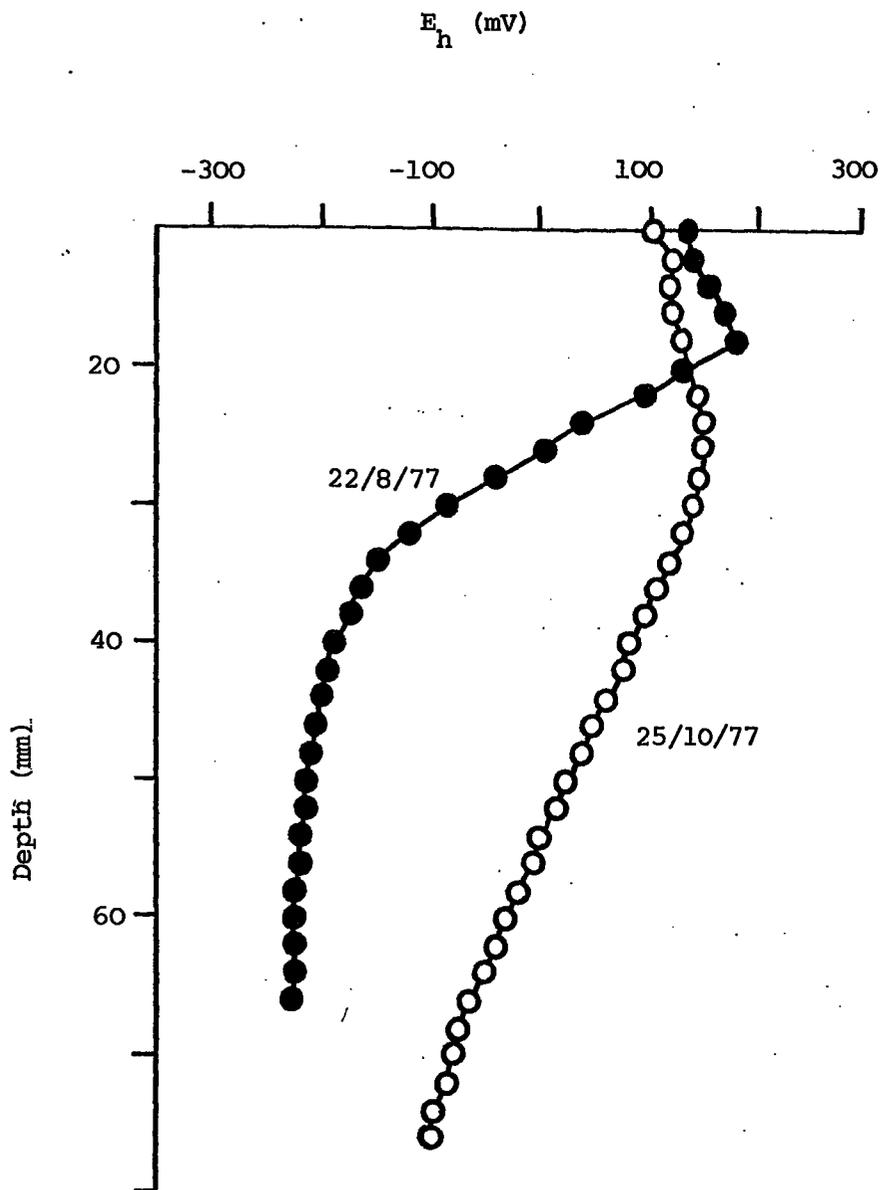


Fig. 33. Crummock Water.

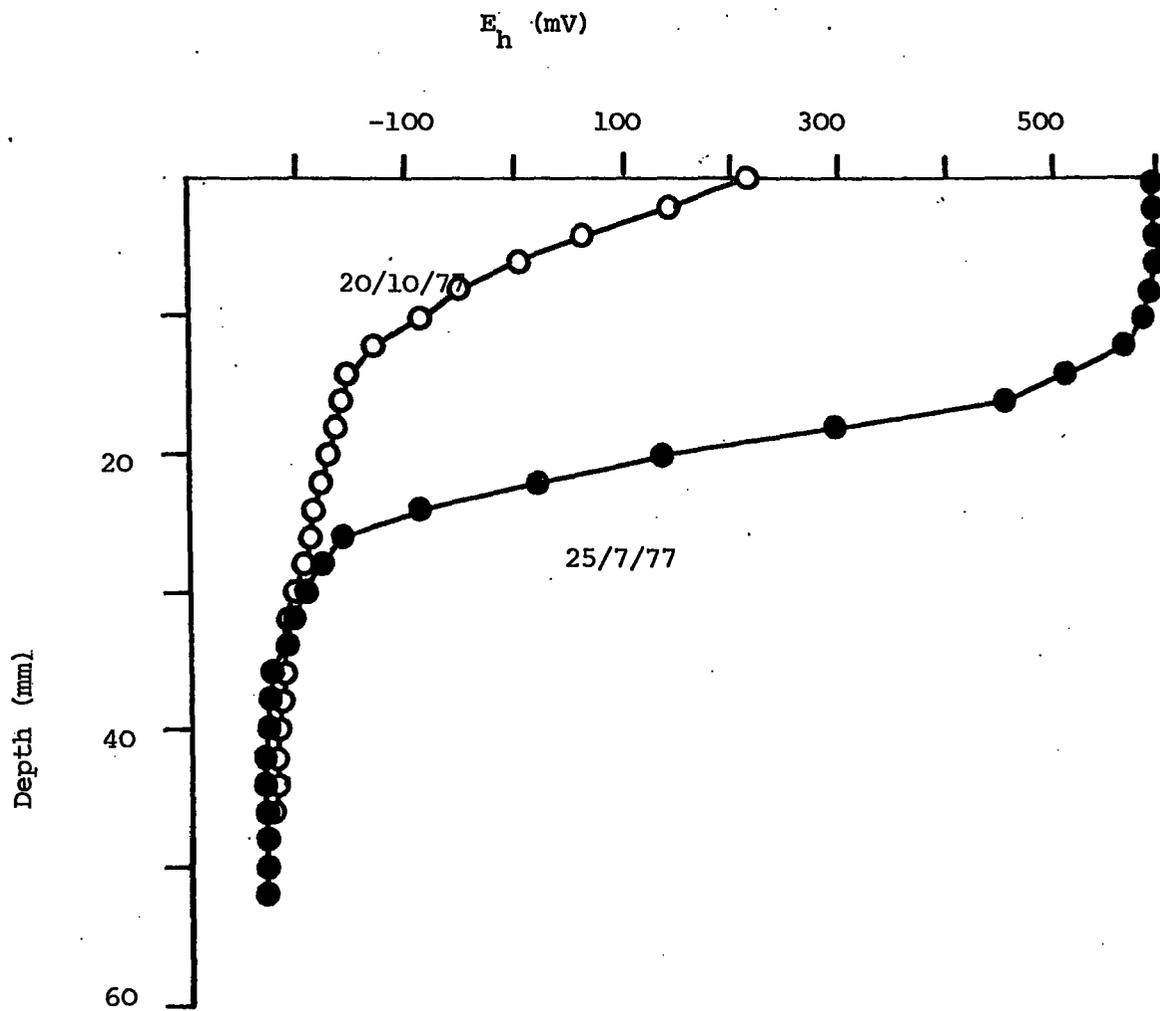


Fig. 34. Haweswater

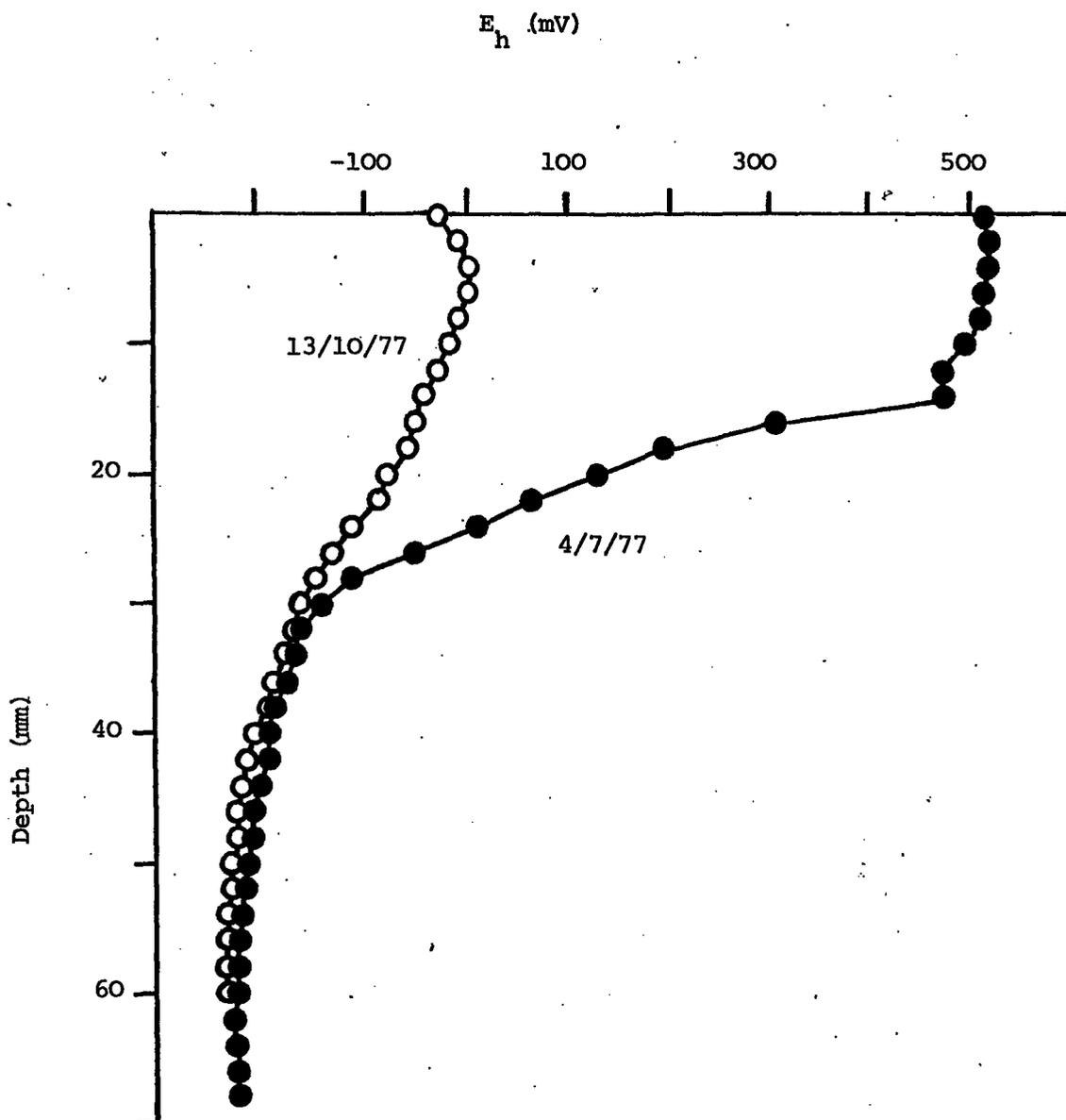


Fig. 35. Coniston Water

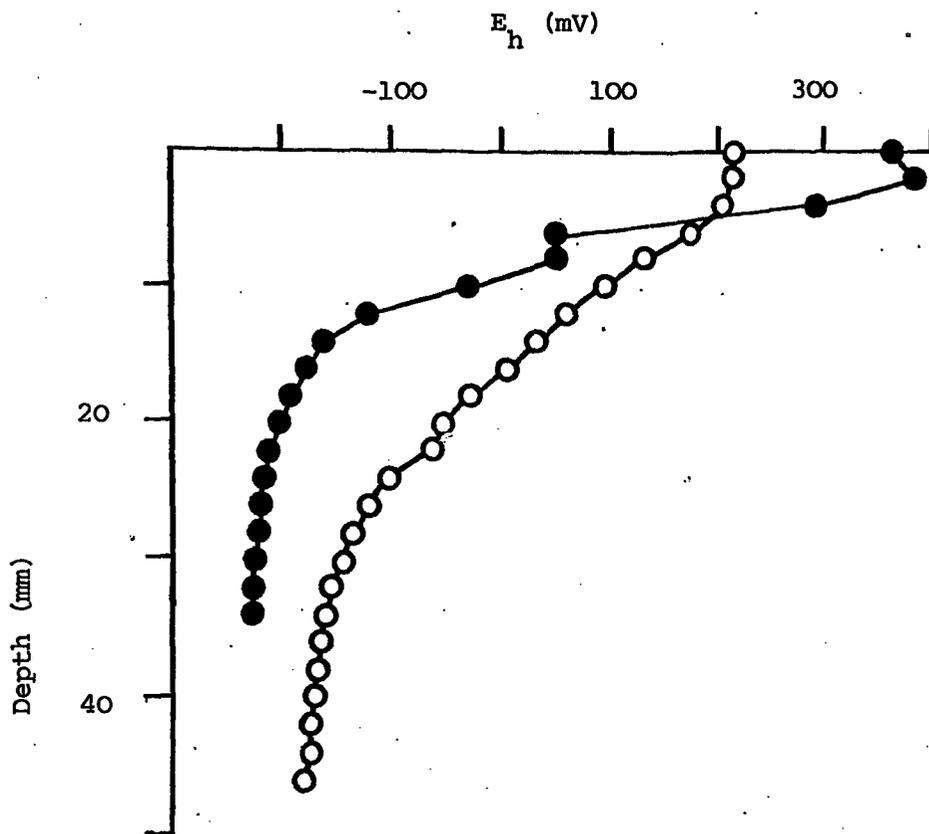


Fig. 36. Thirlmere

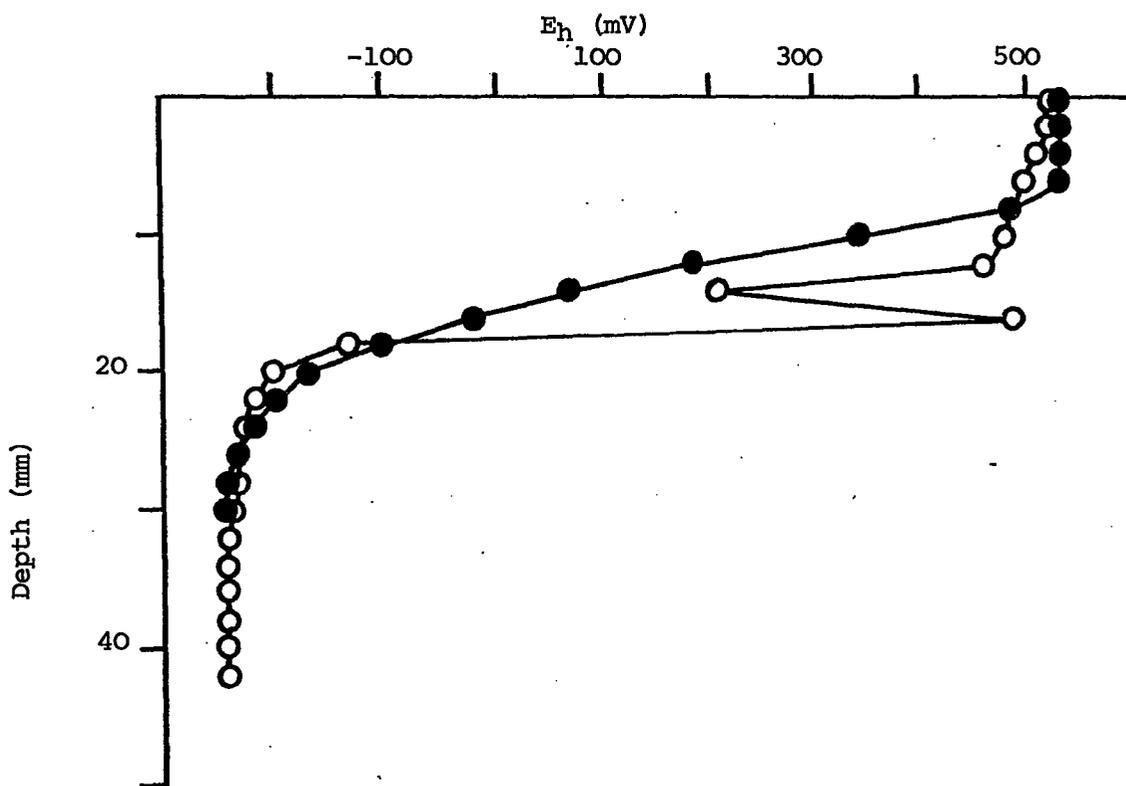


Fig. 37. Windermere North Basin

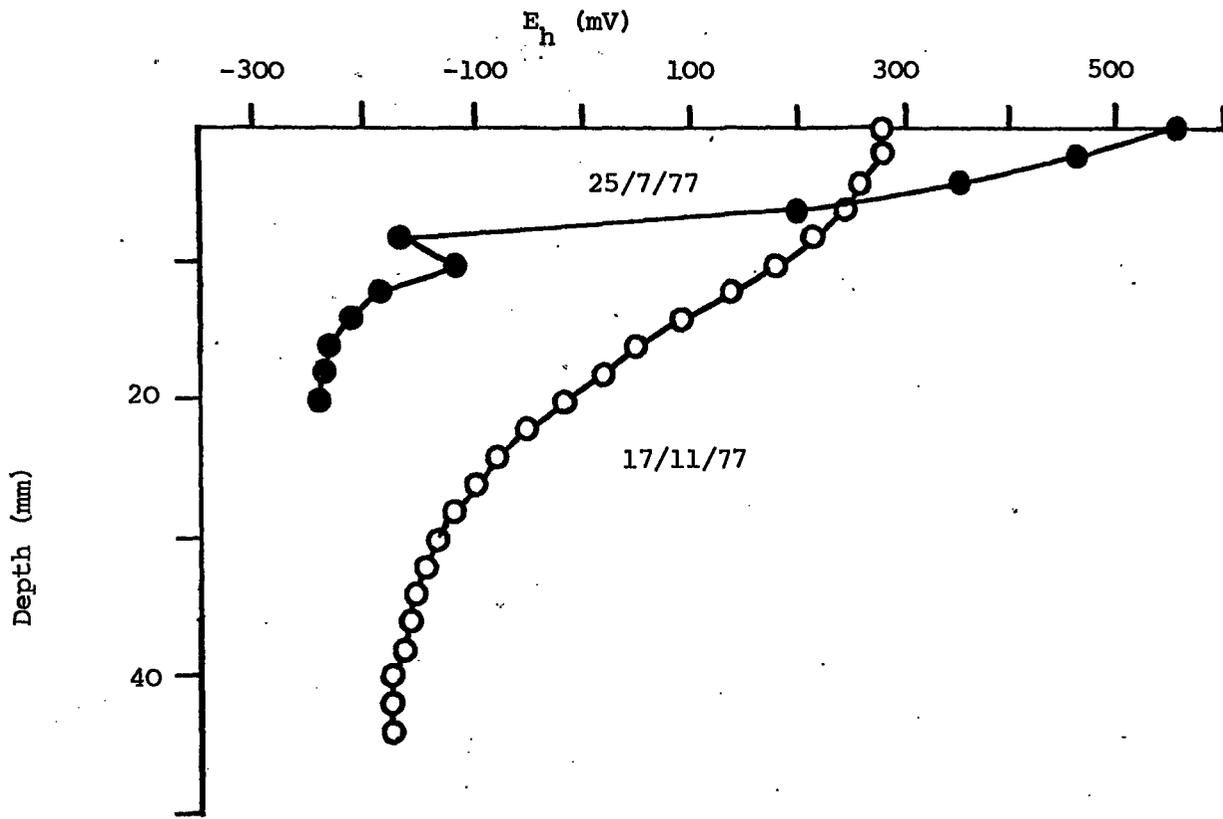


Fig. 38. Ullswater

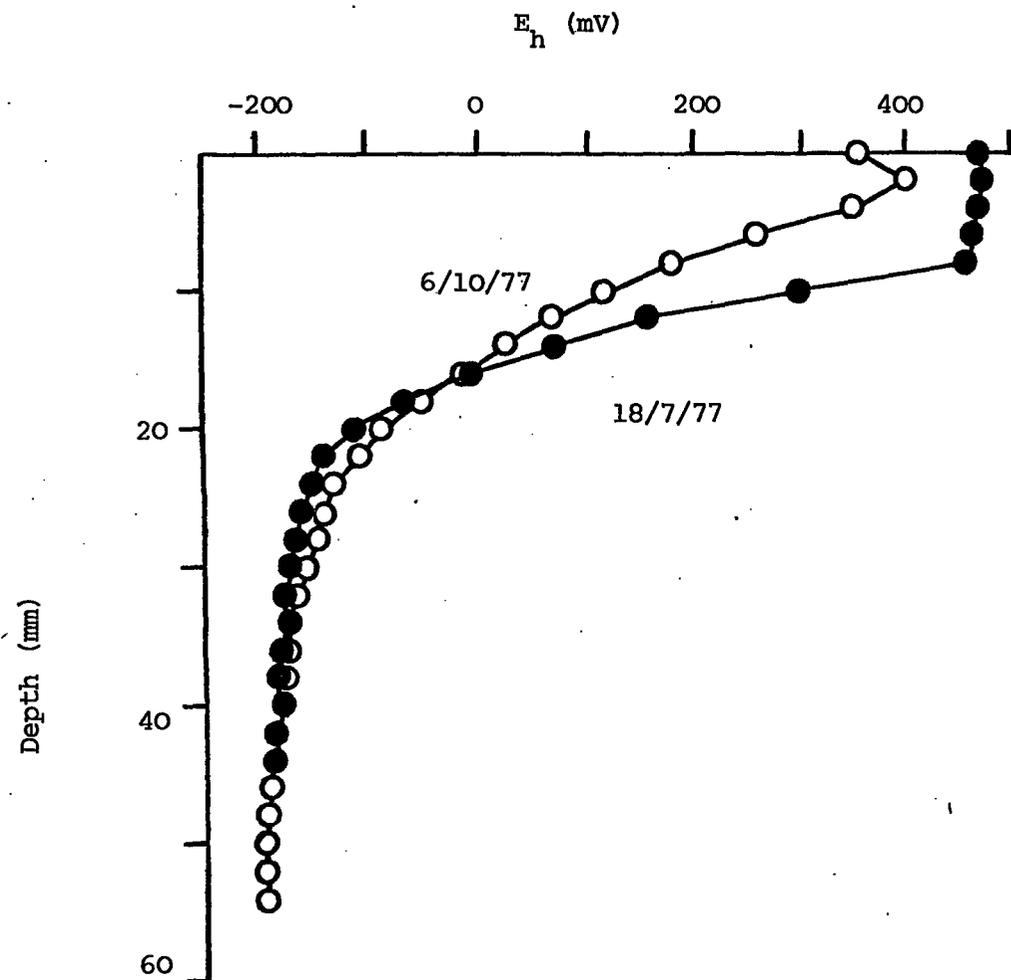


Fig. 39. Derwentwater

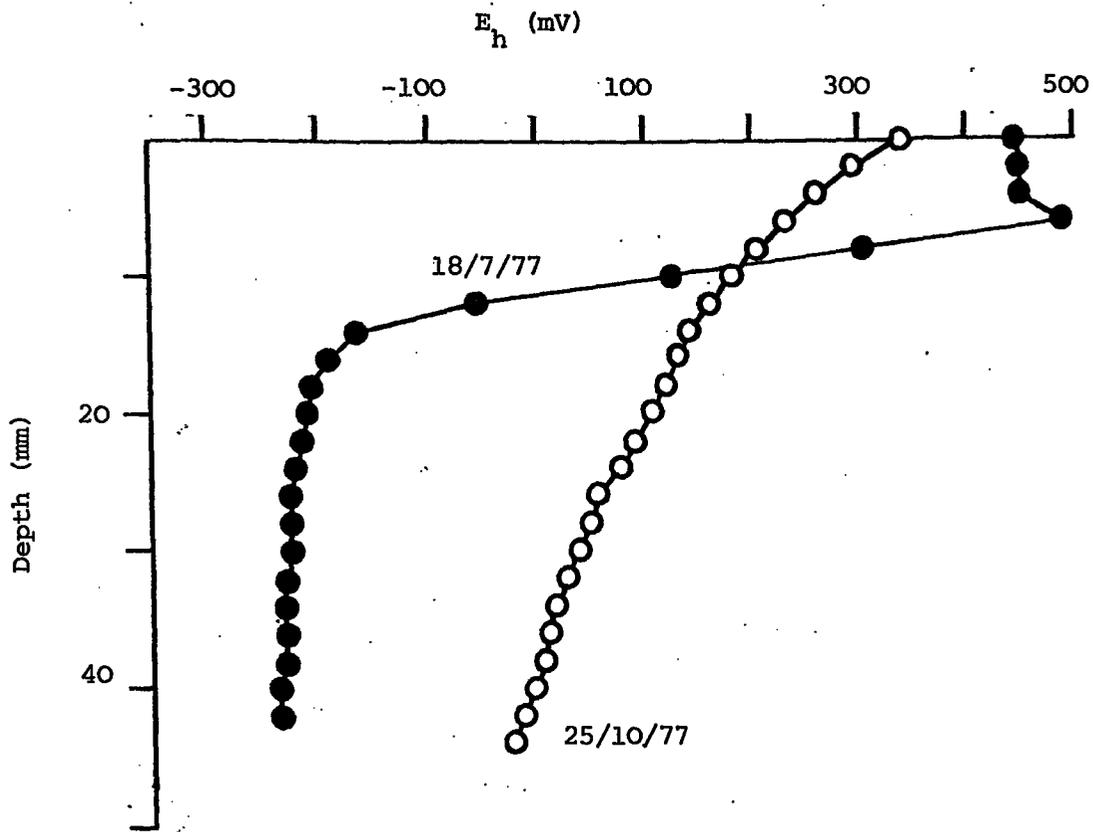


Fig. 40. Bassenthwaite

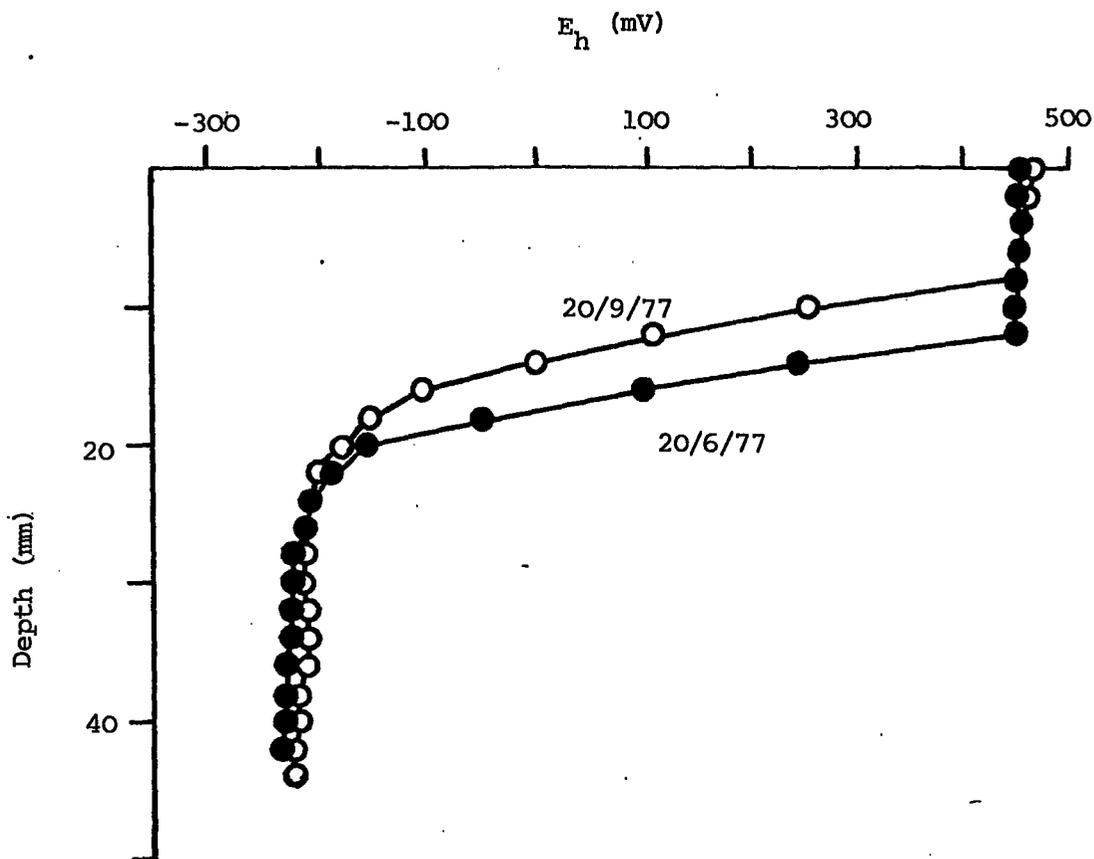


Fig. 41 Windermere South Basin

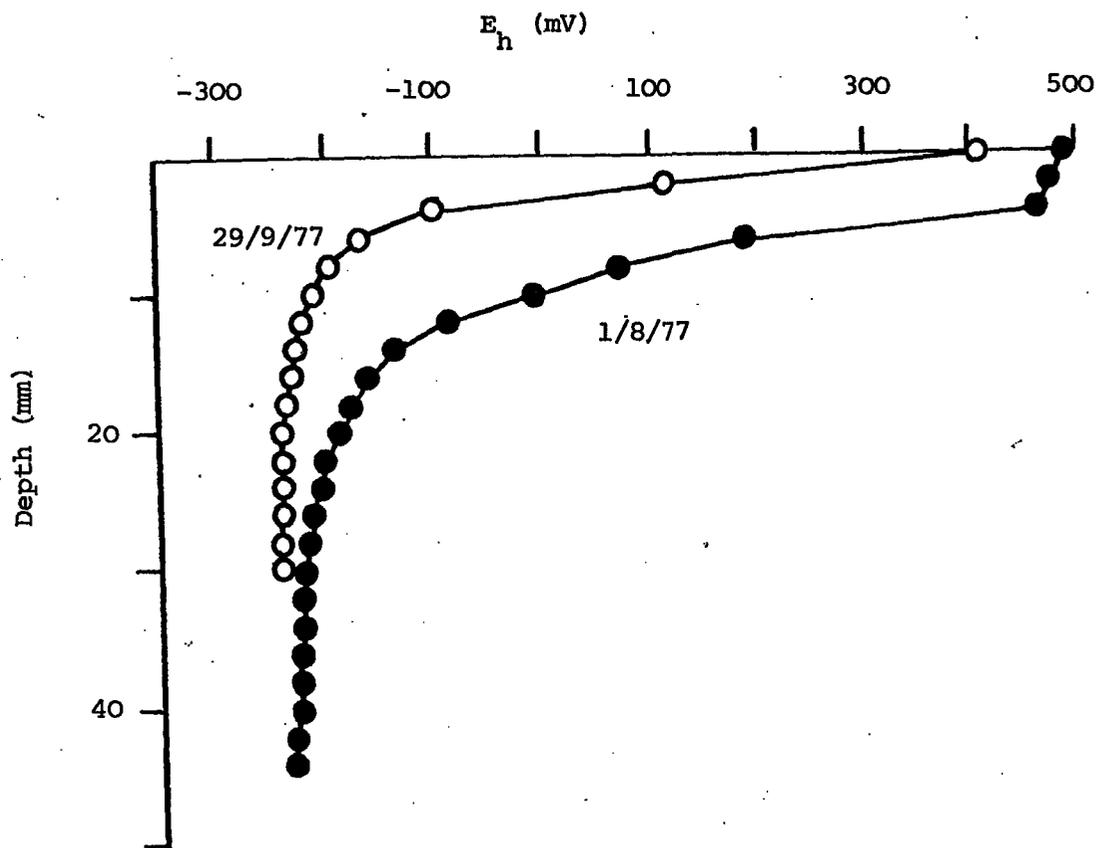


Fig. 42. Loweswater

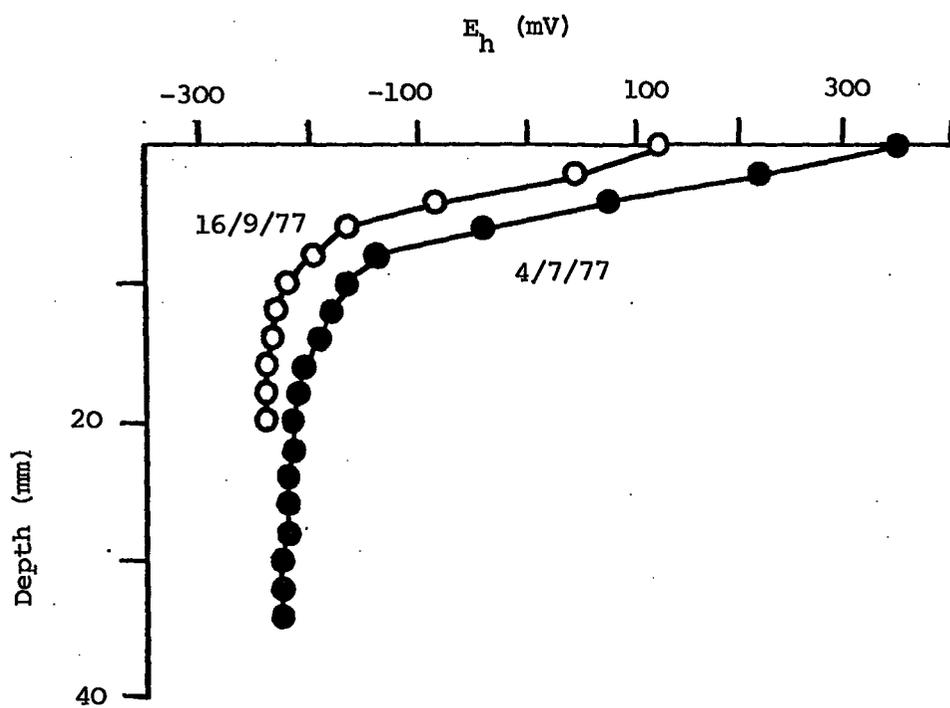


Fig. 43. Rydal Water

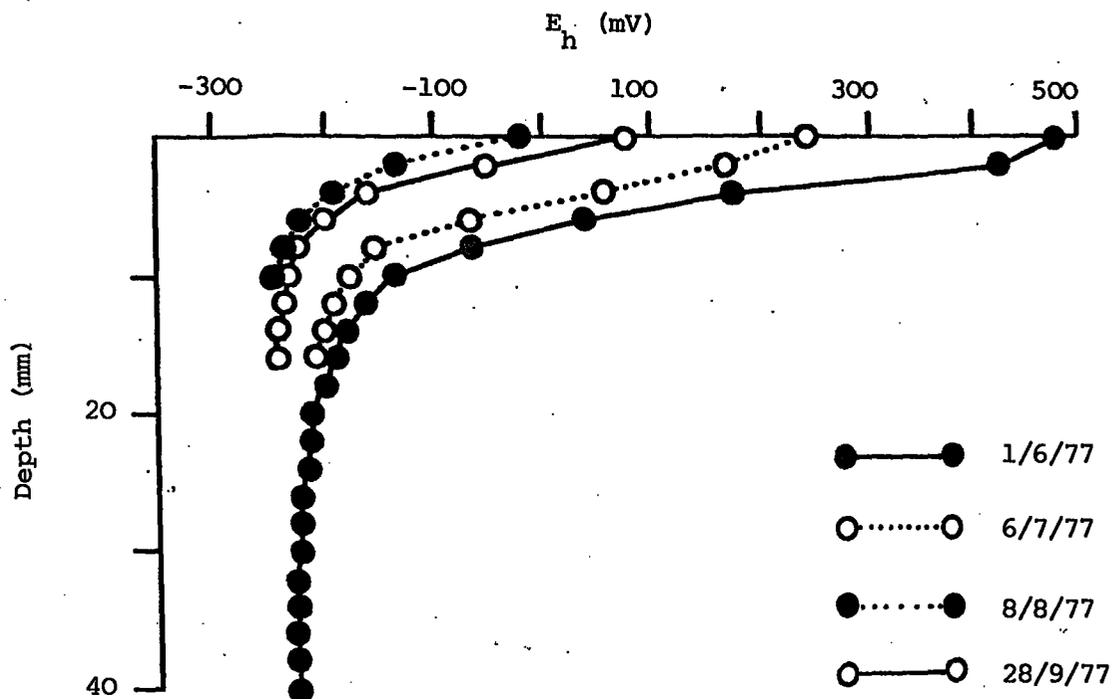
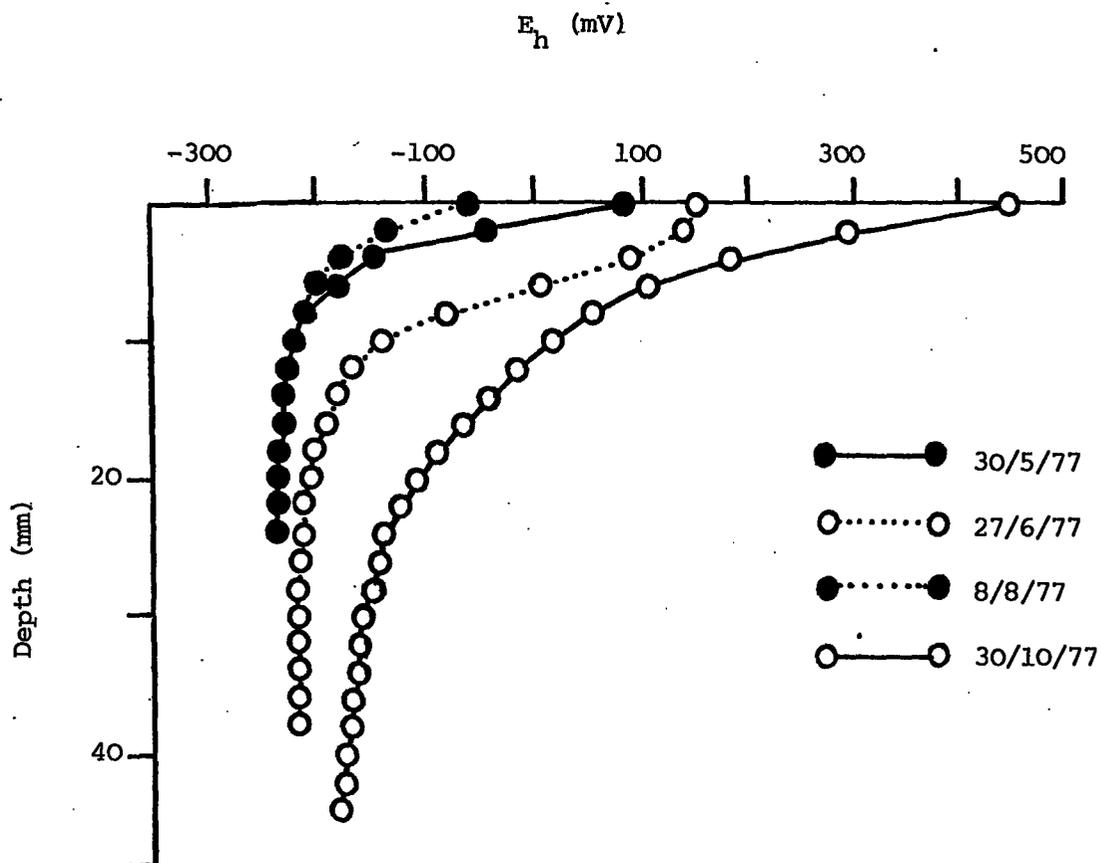


Fig. 44. Grasmere



Fit. 45. Esthwaite Water

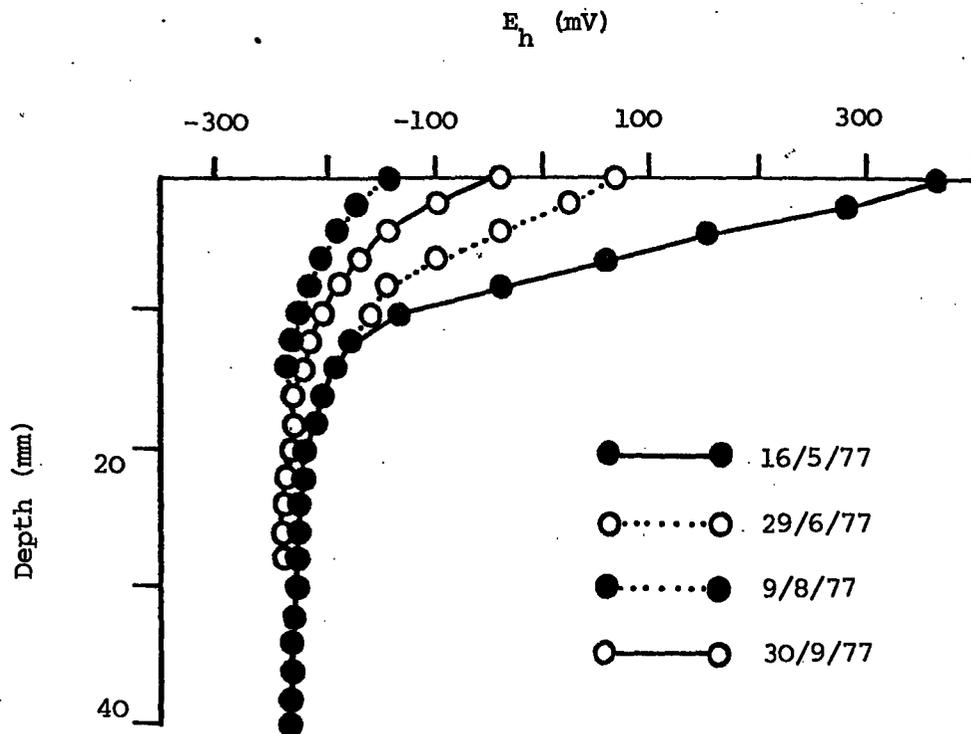
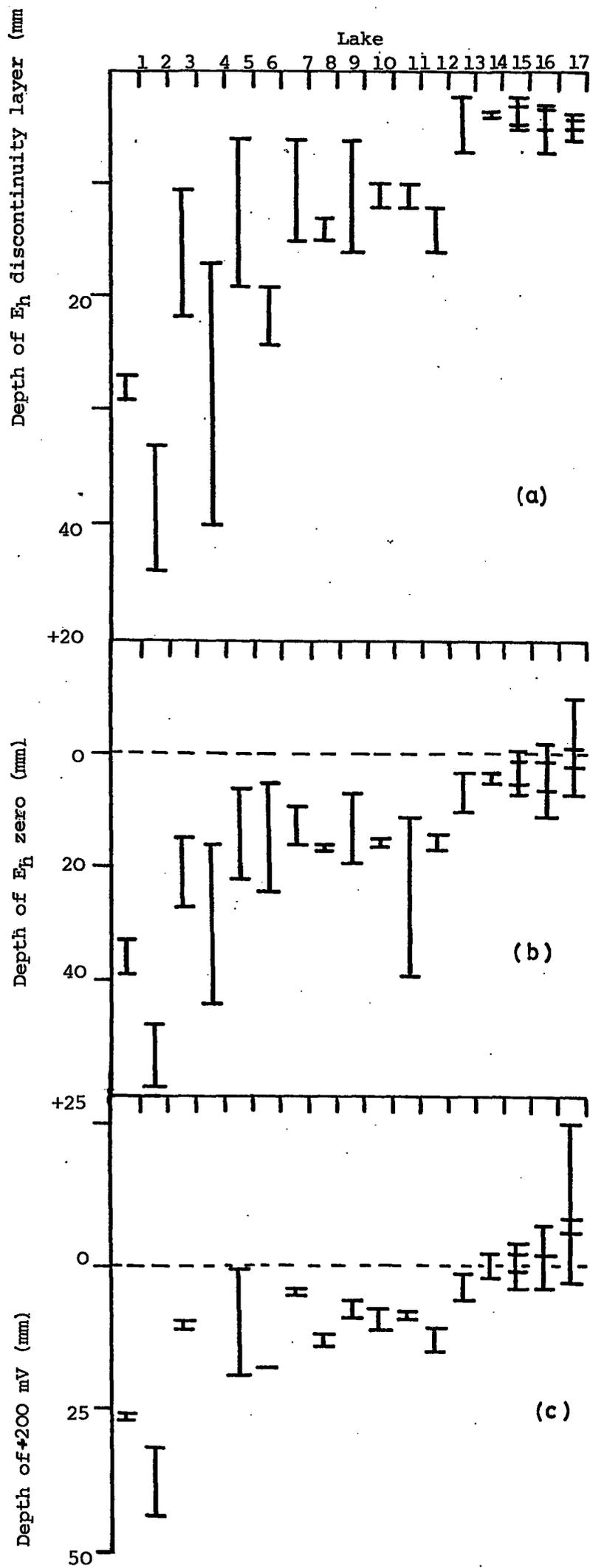


Fig. 46 Blelham Tarn

E_h was most positive at the sediment-water interface in the lakes with high nutrient contents. In the nutrient poor and intermediate lakes the highest value was found between 2 mm and 8 mm depth. The values decreased to zero between 1.2 mm (Esthwaite Water and Grasmere) and 58.4 mm (Ennerdale). The lowest values were found between 10 mm (Grasmere) and 84 mm (Ennerdale). The position of the discontinuity layer between oxidizing and reducing conditions varied between the lakes. It was very deep in the oligotrophic lakes such as Ennerdale (between 24 - 40 mm) and Wastwater (between 20 - 40 mm). In the lakes with high nutrient content (Esthwaite Water and Blelham Tarn) the discontinuity layer was between 0 - 20 mm and 0 - 12 mm respectively. The range of variation in E_h was very wide in the enriched lakes due to the deoxygenation of hypolimnion and the associated upward movement of the discontinuity layer. Fig. 47 summarizes the E_h results by showing the depth of the E_h discontinuity layer (a), the depth of E_h zero (b) and depth of the +200 mV (c). These figures show the more reduced sediment present in the more eutrophic lakes and also that the + 200 mV values migrated into the overlaying water in Rydal Water, Grasmere, Esthwaite Water and Blelham Tarn. The depth of the discontinuity layer in Grasmere, Esthwaite Water and Blelham Tarn gradually decreased with the continual decrease in oxygen content. In Loweswater and Rydal Water the sediment was more reduced in September than in July and August even though at that time the oxygen content was higher. This is probably because it took some time after the overturn before the oxidized layer developed at the mud surface. In the nutrient poor lakes the oxidized layer did not appear to be destroyed during the period of stratification. In some lakes (Windermere South Basin, Derwentwater and Ullswater) the oxidized layer became reduced in thickness, although the de-oxygenation of the hypolimnion did not take place. Haweswater and Coniston Water

Fig. 47. Analysis of E_h profiles in the sediments from 17 Cumbrian lakes. The ranges of readings obtained during the period of thermal stratification are shown (thermal stratification is defined, for this and subsequent figures, as the period between the onset of a 1°C temperature difference between surface and bottom water, and the autumn overturn when isothermal conditions are re-established). The result of each profile is represented by a horizontal bar. (a) Depth of the E_h discontinuity layer (b) Depth of E_h zero (c) Depth of the +200 mV.



had a more reduced sediment in October than in July. Although the hypolimnia in both lakes are normally well oxygenated throughout the year the reduction in the sediment could have been due to continual decomposition during the summer. In Crummock Water no clear discontinuity layer was observed in October. Windermere North Basin showed a discontinuity in the E_h values between 12 mm and 16 mm on 29th September 1977.

3.37 Oxygen, temperature and pH determinations

The results are summarized in Figs 48, 49 and 50. There was a significant lowering of oxygen concentration in Blelham Tarn, Esthwaite, Grasmere and Rydal Water, some in Derwentwater, Bassenthwaite and Windermere South Basin and very little in the other lakes samples. These trends are typical of stratified temperate lakes with deoxygenation of the more eutrophic water bodies. The temperature ranges varied considerably and reflected not only the depth and degree of exposure of individual lakes but the temperature of the water at the onset of thermal stratification. The highest temperature detected was 14.1 °C (Bassenthwaite) and the lowest 6.5 °C (Wastwater). A great variation in pH was not observed between the lakes but the values were slightly higher in the richer lakes. The highest value observed during the sampling period was 7.61 in Rydal Water.

3.4 Development of methods for estimation of the population and activity of benthic filamentous bacteria

The preliminary survey of lakes of differing degrees of eutrophication led to the selection of three lakes: Wastwater, Windermere South Basin and Blelham Tarn as representatives of the oligotrophic, mesotrophic and eutrophic states respectively. The number of bacteria present and the extent to which anoxic conditions were developed in the hypolimnion were the two main factors considered in making this choice. Comparisons between the littoral and profundal sediments were carried out in the

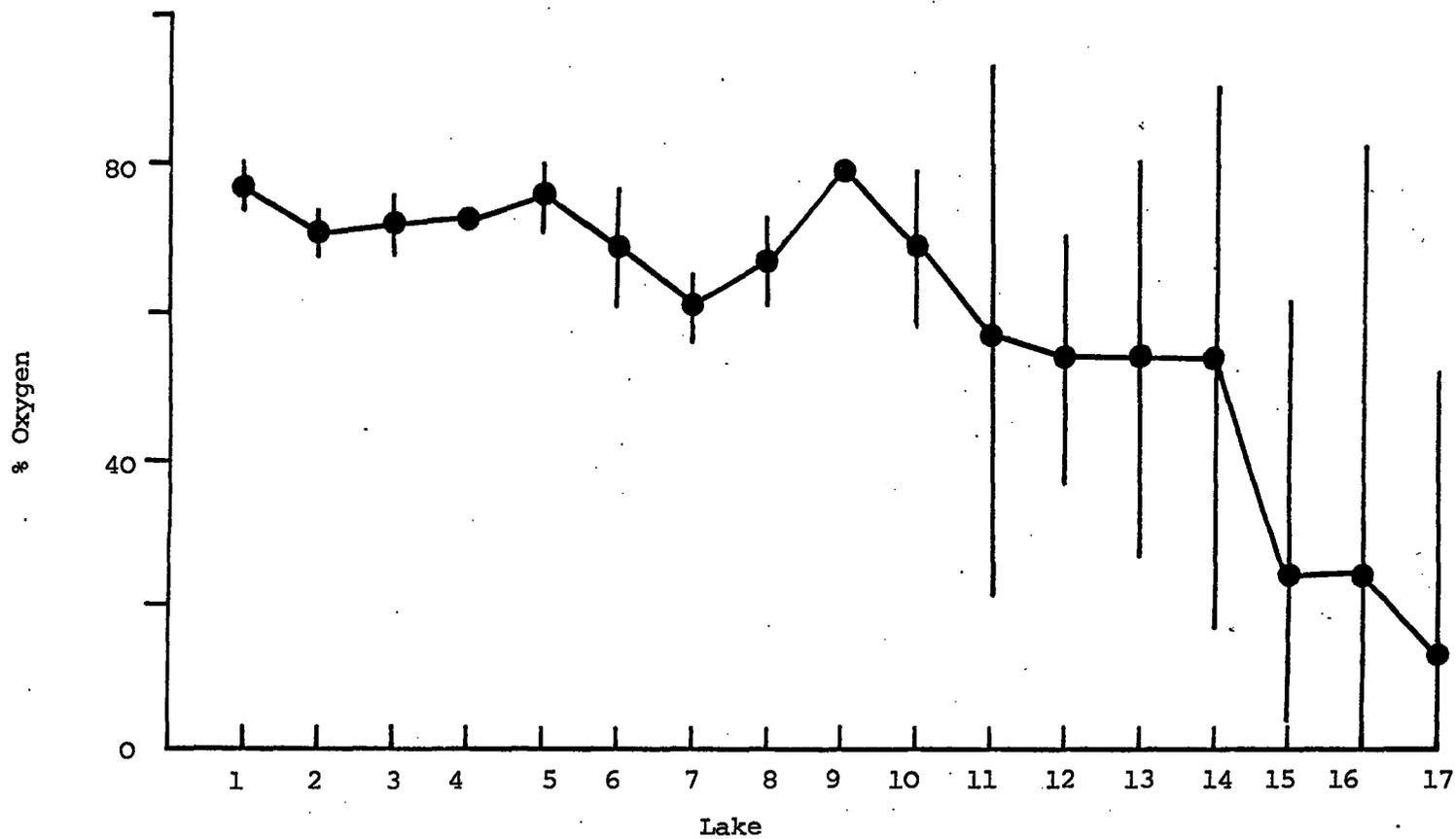


Fig. 48. Concentration of oxygen 0.5 m above the sediment-water interface in the hypolimnia of 17 Cumbrian lakes. The range and arithmetic mean obtained are shown. Where the ranges are not visible they are within the size of the point. The arithmetic means in lakes 1 to 14 are results of two readings and in lakes 15, 16 and 17 results of four readings. The lakes are numbered as in Plate 1.

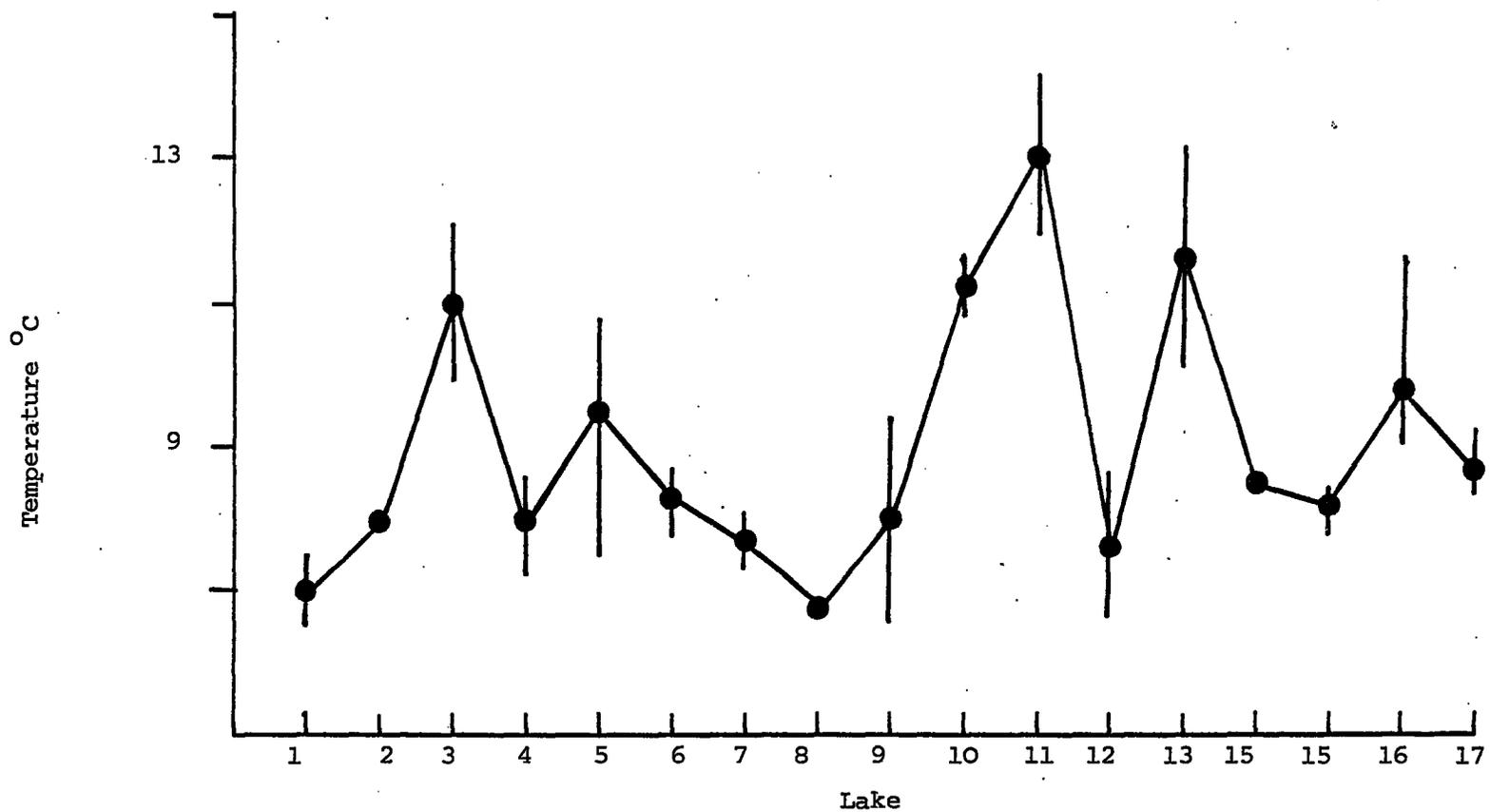


Fig. 49. Temperature at 0.5 m above the sediment-water interface in the hypolimnia of 17 Cumbrian lakes. Conditions are as for Fig. 48.

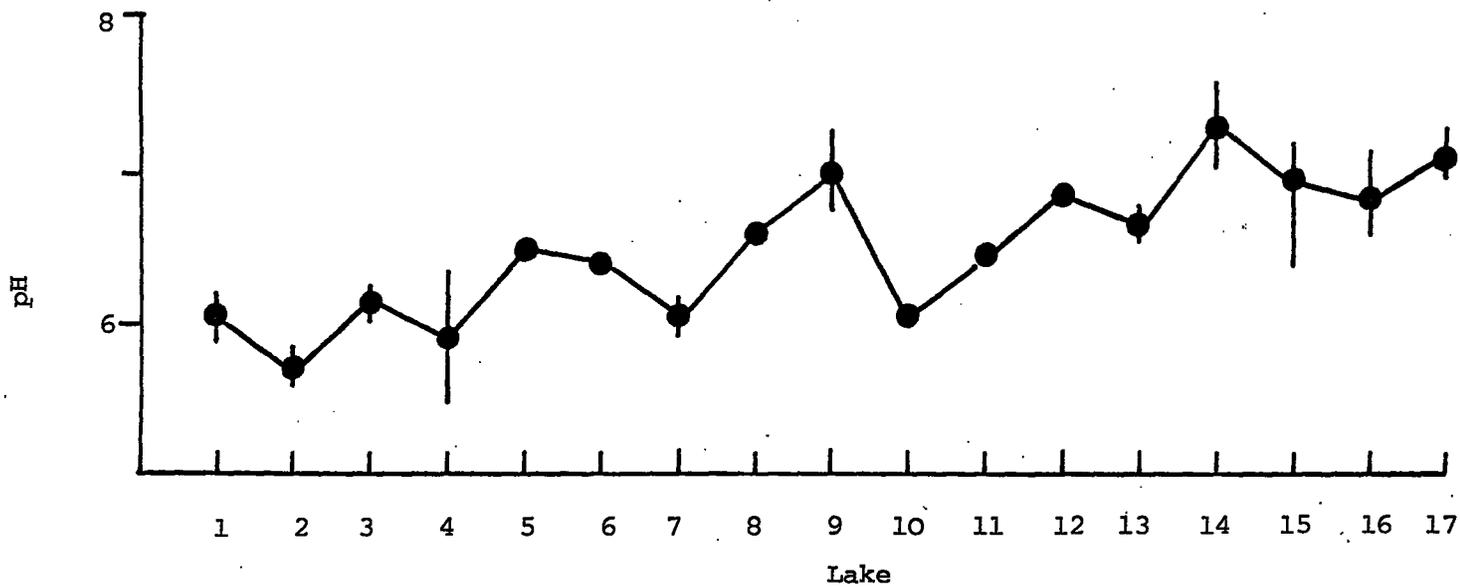


Fig. 50. Readings of pH at the sediment-water interface in the hypolimnia of 17 Cumbrian lakes. Conditions as for Fig. 48.

three lakes not only because they differed so much in physico-chemical characteristics, but also to provide some information on the variability in population of filamentous bacteria within the lakes.

3.41 Selection of a method for enumerating filamentous bacteria

Results from the preliminary survey of the lakes had shown that although it was possible to obtain relative estimates of the populations of the filamentous bacteria, the handling procedures, particularly homogenization, may have been destructive. Therefore an investigation of counting methods was necessary before detailed examination of the sediment population could start. Two techniques involving the use of acridine orange fluorescence were tested. The first was enumeration in known volumes of a wet mount of a given dilution of the sediment and the second (the marked slide technique) incorporation of the sediment suspension in an agar solution which was then spread over a known area and counted in the dry state (Trolldenier, 1973). Difficulties were encountered with the wet mounts due to the masking effect of particulate matter in the sediment samples. The marked slide technique proved to be more satisfactory resulting in a clearer preparation which was easier to count. Table 9 provides an example of the results obtained when the two techniques were used to enumerate bacteria in the littoral and profundal sediments of the oligotrophic Wastwater and the more eutrophic Blelham Tarn. The marked slide technique gave higher counts in both lakes the difference between the two techniques being more marked in Blelham Tarn. Higher values were also observed in the profundal zone when the marked slide technique was used. The wet mount counts in Blelham Tarn were higher in the littoral zone; this was possibly due to fluorescence quenching under the anoxic conditions of the sediment. In Wastwater the wet mount values were higher at the profundal zone except for the two lower depths examined (1.5 - 2.0 cm and 2.0 - 2.5 cm). When

Table 9 Comparison between AO direct counts of filamentous bacteria
in the littoral and profundal sediments of two lakes.

10^7 x Number of filamentous bacteria (g dry weight)⁻¹

Site	Depth (cm)	Marked slides		Wet mount	
		Blelham Tarn	Wastwater	Blelham Tarn	Wastwater
Profundal	0.0-0.5	4.50 (1.05)	0.48 (0.27)	0.23 (0.35)	0.45 (0.22)
	0.5-1.0	2.85 (0.67)	0.39 (0.23)	0.10 (0.16)	0.19 (0.14)
	1.0-1.5	1.36 (0.62)	1.46 (0.35)	0.14 (0.21)	0.06 (0.09)
	1.5-2.0	1.18 (0.42)	0.39 (0.18)	0	0.04 (0.06)
	2.0-2.5	2.19 (0.72)	0.31 (0.13)	0.07 (0.13)	0
Littoral	0.0-0.5	1.68 (0.32)	0.21 (0.13)	0.62 (0.22)	0.19 (0.12)
	0.5-1.0	1.68 (0.34)	0.23 (0.14)	0.85 (0.26)	0.17 (0.11)
	1.0-1.5	1.13 (0.27)	0.25 (0.18)	0.25 (0.14)	0.03 (0.09)
	1.5-2.0	0.59 (0.21)	0.10 (0.12)	0.32 (0.16)	0.06 (0.07)
	2.0-2.5	0.77 (0.21)	0.21 (0.16)	0.04 (0.05)	0.03 (0.05)

() 95% confidence limits

the wet mount counts were expressed as percentage of the marked slide counts the values ranged from 3% (Blelham Tarn, profundal zone) to 94% (Wastwater, profundal zone).

3.42 Analysis of lake sediments

3.421 The vertical distribution of filamentous bacteria using marked slides.

The depth distribution of filamentous bacteria at the three study sites was examined at the onset of thermal stratification and in late summer after it was well established and deoxygenation of the hypolimnion of Blelham Tarn had occurred. The counts in the profundal sediments were higher than those in the littoral during May and June (Fig. 51) as well as in August and September (Figs 52 and 53). The only exceptions were noted at two depths (0.0 - 0.5 cm and 4.0 - 4.5 cm) in Windermere South Basin where littoral sediments gave higher counts. The filamentous bacteria in Blelham Tarn were concentrated in the top centimetre and numbers decreased with depth in both profundal and littoral sediments; this pattern was not observed in Windermere South Basin and in Wastwater. The highest numbers, in both littoral and profundal samples, were observed in Blelham Tarn, the lowest in Wastwater. The range of value was from 0.11×10^7 to 8×10^7 filamentous bacteria (g dry wt)⁻¹ in the profundal sediments; in the littoral sediments the values ranged from 0.01×10^7 to 2.1×10^7 filamentous bacteria (g dry wt)⁻¹. The counts of filamentous bacteria obtained with the marked slides expressed as a percentage of total counts - AO are shown in Table 10. They tended to be higher in the profundal than the littoral sites and higher in Blelham Tarn than in the other two lakes.

3.422 Viable counts of filamentous bacteria with coated slides.

A coated slide technique was chosen to provide further information on the distribution of filamentous bacteria in the sediments. Since

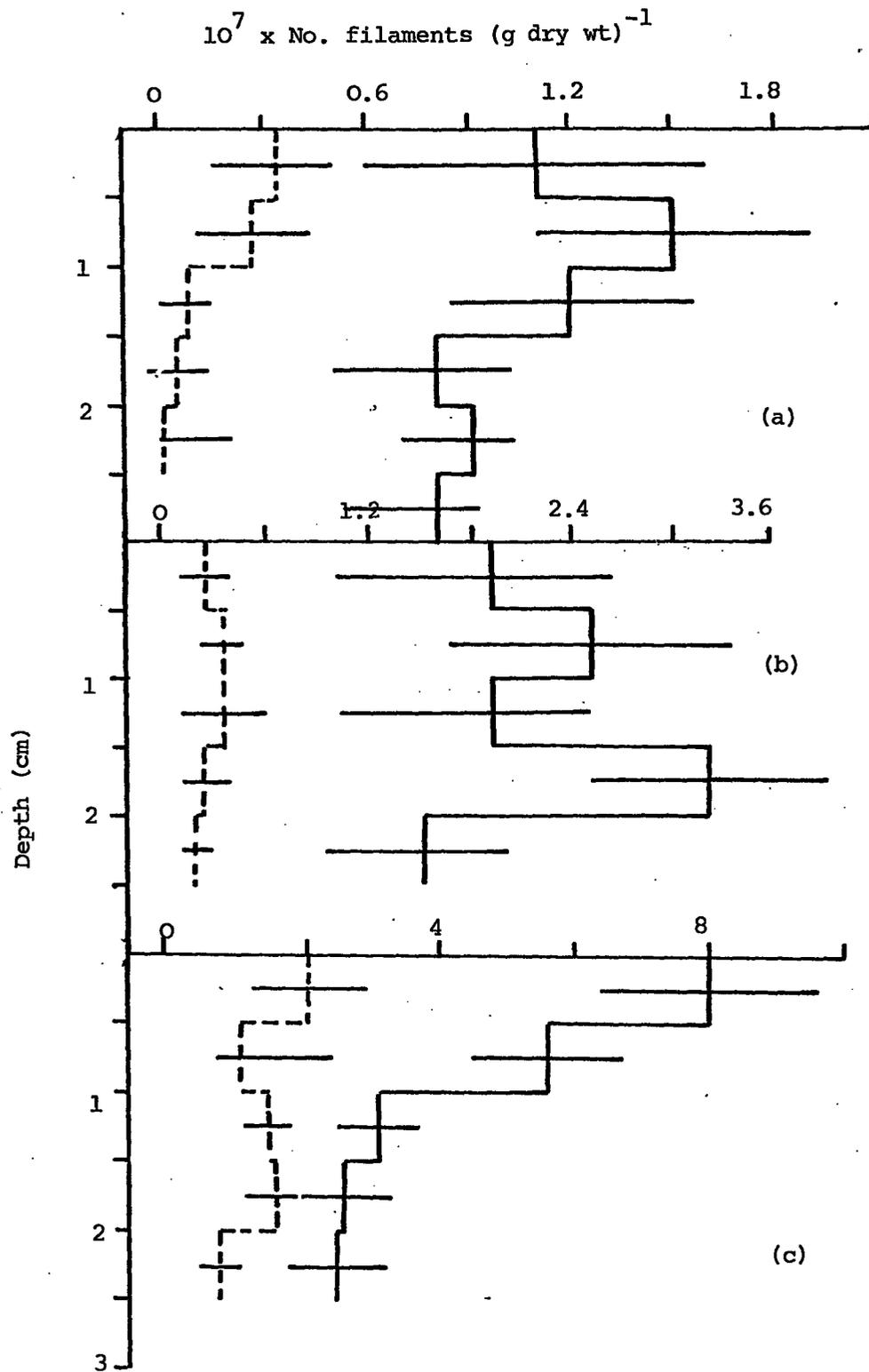


Fig. 51. Vertical distribution of filamentous bacteria using marked slide technique in the sediments of Wastwater (a), Windermere South Basin (b) and Blelham Tarn (c) before the onset of stable thermal stratification. (—) profundal sediments; (----) littoral sediments.

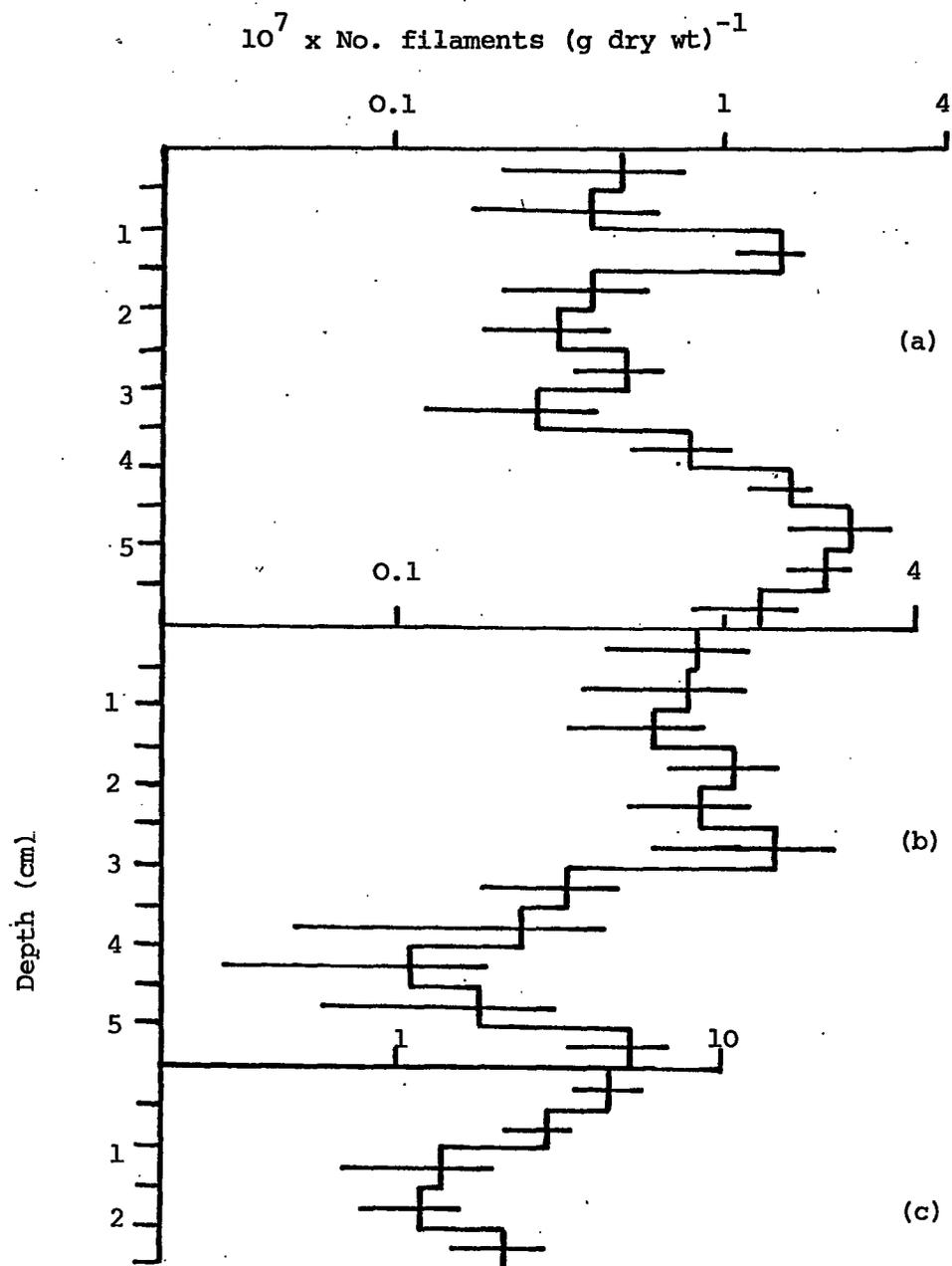


Fig. 52. The vertical distribution of filamentous bacteria, using marked slide technique, in the profundal sediments of Wastwater (a), Windermere South Basin (b) and Blelham Tarn (c) sampled in late summer after prolonged thermal stratification.

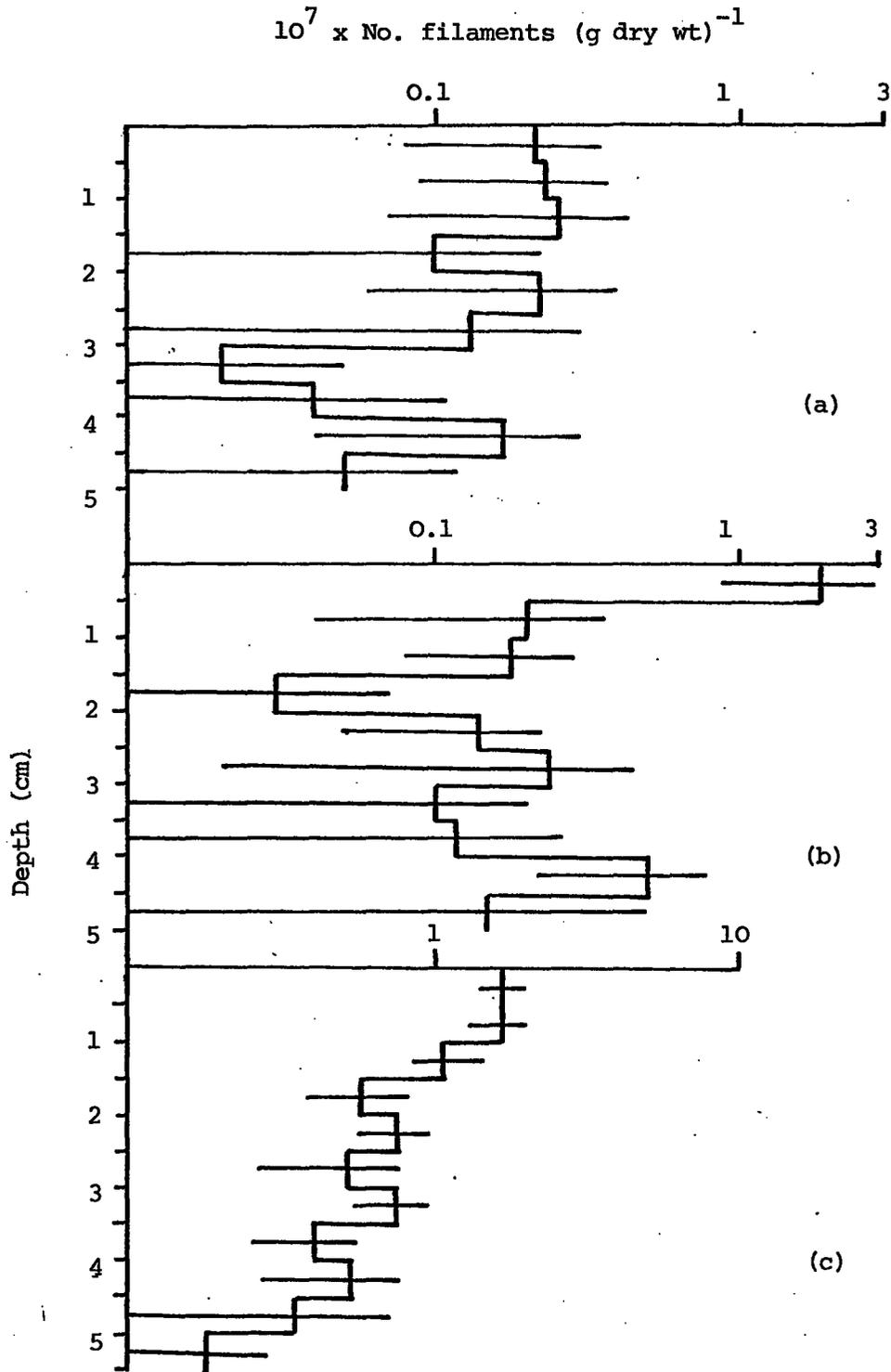


Fig. 53. The vertical distribution of filamentous bacteria using marked slide technique in the littoral sediments of Wastwater (a), Windermere South Basin (b) and Blelham Tarn (c) sampled in late summer after prolonged thermal stratification.

Table 10. Number of filamentous bacteria estimated by marked slide technique expressed as percentage of total counts - AO of bacteria.

Sampling zone	Wastwater	Windermere South Basin	Blelham Tarn
	1/6/78	12/5/78	22/5/78
Profundal	0.040	0.030	0.070
Littoral	0.008	0.004	0.020
	18/9/78	18/8/78	4/9/78
Profundal	0.012	0.011	0.040
Littoral	0.006	0.014	0.020

it was impossible to be absolutely certain that bacteria stained with fluorescein di-acetate and acridine orange were actually alive a technique which encouraged their growth by colonization of a surface would provide valuable additional information. The microcapillary techniques (Perfil'ev & Gabe, 1969) were tried and although some success was achieved, a tendency to select for certain bacterial groups, particularly Beggiatoa was observed. It was also felt that isolation of the bacteria might be more successful from the open surface of the coated slides than from the inner surface of a microcapillary. Preliminary trials were run to determine the effect of the surface coating on the numbers of filamentous bacteria obtained. Slides were coated with agar, gelatin and Ullrich's adhesive. The results are presented in Tables 11 and 12 and graphically in Figs 54, 55 and 56. The agar and the gelatin coated slides gave best results when incubated for a period of one week whereas the ones coated with Ullrich's adhesive gave higher values after 2 weeks incubation. The profundal sediments of Windermere South Basin gave higher numbers of filamentous bacteria than Blelham Tarn and Wastwater. In the littoral sediments the highest numbers were usually obtained with samples from Blelham Tarn followed by Wastwater and Windermere South Basin in that order. Table 13 shows a comparison of the results obtained by the coated slide technique with those from marked slides and wet mount techniques. Although extremely variable they indicate that a higher count was obtained with the agar coated slides. A greater variety of filamentous forms was also obtained using agar and therefore it was used for all further studies.

3.423 Further analysis of chemical and physical variables of the sediments.

During the initial survey of the lakes, analyses of different variables were made on the surface sediment only, and therefore more information was necessary on the depth distribution of carbon (C), nitrogen (N) and E_h

Table 11. Vertical distribution of viable filamentous bacteria using coated slides incubated for one week at 8 °C in the sediment cores

Lake	Depth (cm)	10 ³ x Number of filamentous bacteria (cm ⁻²)					
		Agar		Gelatin		Ullrich's adhesive	
		Profundal	Littoral	Profundal	Littoral	Profundal	Littoral
	0.0-0.5	0.41 (0.18)	17.1 (4.30)	0.54 (0.53)	0.46 (0.14)	0.29 (0.12)	0.23 (0.10)
	0.5-1.0	0.66 (0.72)	24.7 (5.41)	0.09 (0.06)	0.58 (0.25)	0.14 (0.08)	0.15 (0.11)
	1.0-1.5	0.21 (0.11)	18.5 (3.22)	0.13 (0.07)	0.31 (0.16)	0.10 (0.09)	0.08 (0.07)
	1.5-2.0	0.24 (0.13)	13.2 (3.75)	0.70 (0.35)	0.08 (0.08)	0.80 (0.35)	-
WASTWATER	2.0-2.5	-	10.9 (2.38)	0.24 (0.21)	0.05 (0.05)	0.05 (0.05)	-
	2.5-3.0	-	7.7 (2.79)	0.33 (0.18)	0.03 (0.04)	-	-
	3.0-3.5	-	4.3 (1.25)	0.12 (0.12)	-	-	-
	3.5-4.0	-	2.3 (1.80)	-	-	-	-
	4.0-4.5	-	0.2 (0.24)	-	-	-	-

Table 11 (continued)

	0.0-0.5	15.20 (2.48)	0.10 (0.14)	3.00 (0.91)	0.29 (0.26)	1.40 (0.63)	0.05 (0.10)
	0.5-1.0	13.00 (2.09)	0.19 (0.24)	1.30 (0.77)	0.00	1.20 (0.67)	0.05 (0.10)
WINDERMERE	1.0-1.5	9.57 (4.42)	1.06 (0.41)	2.90 (0.26)	0.34 (0.26)	1.30 (0.77)	-
SOUTH BASIN	1.5-2.0	5.63 (3.00)	0.10 (0.14)	-	0.05 (0.10)	-	-
	2.0-2.5	1.20 (0.65)	-	-	0.10 (0.14)	-	-
	2.5-3.0	0.09 (0.14)	-	-	-	-	-
	0.0-0.5	0.71 (0.28)	1.40 (0.43)	2.06 (0.37)	3.10 (0.41)	0.48 (0.34)	6.60 (1.15)
	0.5-1.0	0.77 (0.19)	0.18 (0.11)	2.70 (0.43)	3.30 (0.59)	0.38 (0.26)	2.30 (0.79)
	1.0-1.5	0.78 (0.28)	0.12 (0.09)	2.90 (0.55)	2.40 (0.44)	0.05 (0.10)	1.70 (0.63)
	1.5-2.0	1.50 (0.36)	-	2.20 (0.38)	1.50 (0.48)	-	1.80 (0.70)
BLELHAM	2.0-2.5	1.50 (0.96)	-	2.90 (0.75)	0.57 (0.17)	-	0.67 (0.46)
TARN	2.5-3.0	2.00 (0.39)	-	0.60 (0.36)	0.13 (0.08)	-	-
	3.0-3.5	1.60 (0.37)	-	0.30 (0.26)	-	-	-
	3.5-4.0	0.99 (0.31)	-	-	-	-	-
	4.0-4.5	0.53 (0.18)	-	-	-	-	-
	4.5-5.0	0.22 (0.12)	-	-	-	-	-

- = not detectable

() = 95% confidence limits

Table 12 Vertical distribution of viable filamentous bacteria using coated slides incubated for two weeks at 8 °C in the sediment cores.

Lake	Depth (cm)	10 ³ x Number of filamentous bacteria (cm ⁻²)					
		Agar		Gelatin		Ullrich's adhesive	
		Profundal	Littoral	Profundal	Littoral	Profundal	Littoral
WASTWATER	0.0-0.5	0.14 (0.11)	0.13 (0.10)	0.18 (0.10)	2.50 (0.99)	0.37 (0.17)	0.57 (0.23)
	0.5-1.0	0.23 (0.13)	0.13 (0.07)	0.05 (0.05)	0.96 (0.53)	0.13 (0.10)	0.49 (0.23)
	1.0-1.5	0.14 (0.08)	0.04 (0.05)	0.05 (0.05)	1.13 (0.36)	0.32 (0.23)	0.09 (0.06)
	1.5-2.0	0.99 (0.49)	0.26 (0.25)	-	0.14 (0.11)	0.23 (0.17)	0.04 (0.06)
	2.0-2.5	0.04 (0.05)	0.03 (0.04)	-	0.05 (0.05)	0.14 (0.08)	-
	2.5-3.0	0.06 (0.08)	-	-	0.03 (0.04)	-	-
WINDERMERE	0.0-0.5	8.50 (3.08)	-	2.60 (1.01)	0.24 (0.31)	7.40 (1.66)	0.20 (0.12)
	0.5-1.0	2.10 (0.84)	-	1.40 (0.89)	0.05 (0.10)	1.06 (0.70)	0.10 (0.14)
	1.0-1.5	2.60 (0.84)	-	0.60 (0.48)	-	0.70 (0.41)	-
SOUTH BASIN	1.5-2.0	0.60 (0.54)	-	-	-	0.18 (0.13)	-
	2.0-2.5	0.80 (0.53)	-	-	-	-	-

Table 12 (continued)

	0.0-0.5	0.09 (0.06)	2.06 (0.48)	1.80 (0.65)	3.70 (0.47)	-	7.40 (0.78)
	0.5-1.0	0.03 (0.04)	2.40 (0.71)	0.48 (0.26)	1.90 (0.35)	-	1.30 (0.31)
	1.0-1.5	0.03 (0.05)	0.50 (0.23)	0.14 (0.17)	1.90 (0.68)	-	0.90 (0.33)
	1.5-2.0	-	0.06 (0.06)	-	1.20 (0.62)	-	1.30 (0.60)
BLELHAM	2.0-2.5	-	0.05 (0.06)	-	0.87 (0.45)	-	1.08 (0.87)
TARN	2.5-3.0	-	-	-	17.70 (13.0)	-	0.04 (0.05)
	3.0-3.5	-	-	-	6.50 (2.33)	-	-
	3.5-4.0	-	-	-	7.90 (7.91)	-	-
	4.0-4.5	-	-	-	0.87 (0.43)	-	-
	4.5-5.0	-	-	-	0.43 (0.36)	-	-

- = not detectable

() = 95% confidence limits

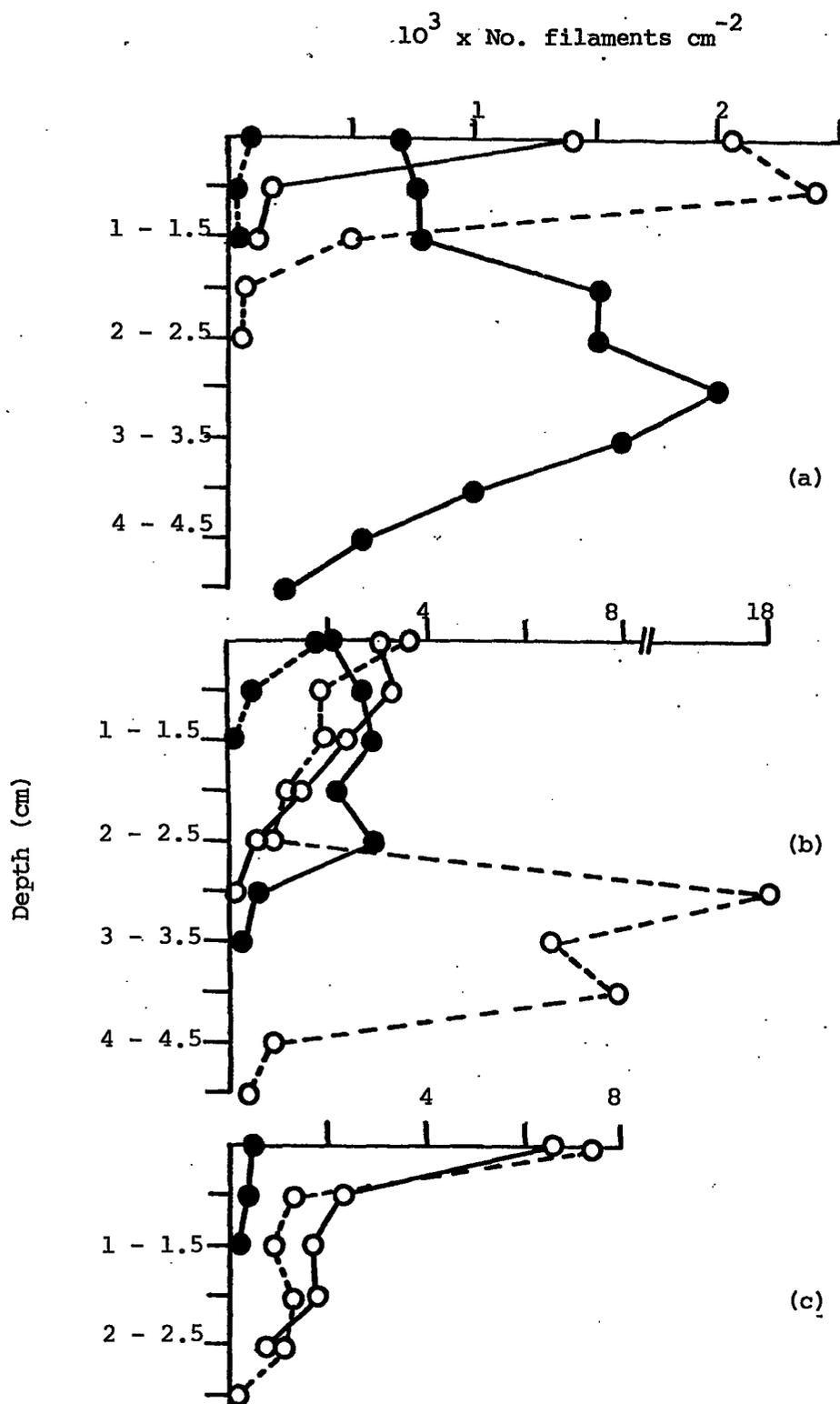


Fig. 54. The vertical distribution of viable filamentous bacteria in Blelham Tarn using coated slides incubated for one and two weeks at 8°C in profundal and littoral sediment cores. (a) agar; (b) gelatin; (c) Ullrich's adhesive (●—●) profundal sediments, one week; (●--●) profundal sediments, two weeks; (○—○) littoral sediments, one week; (○--○) littoral sediments, two weeks. Note break on the abscissa scale.

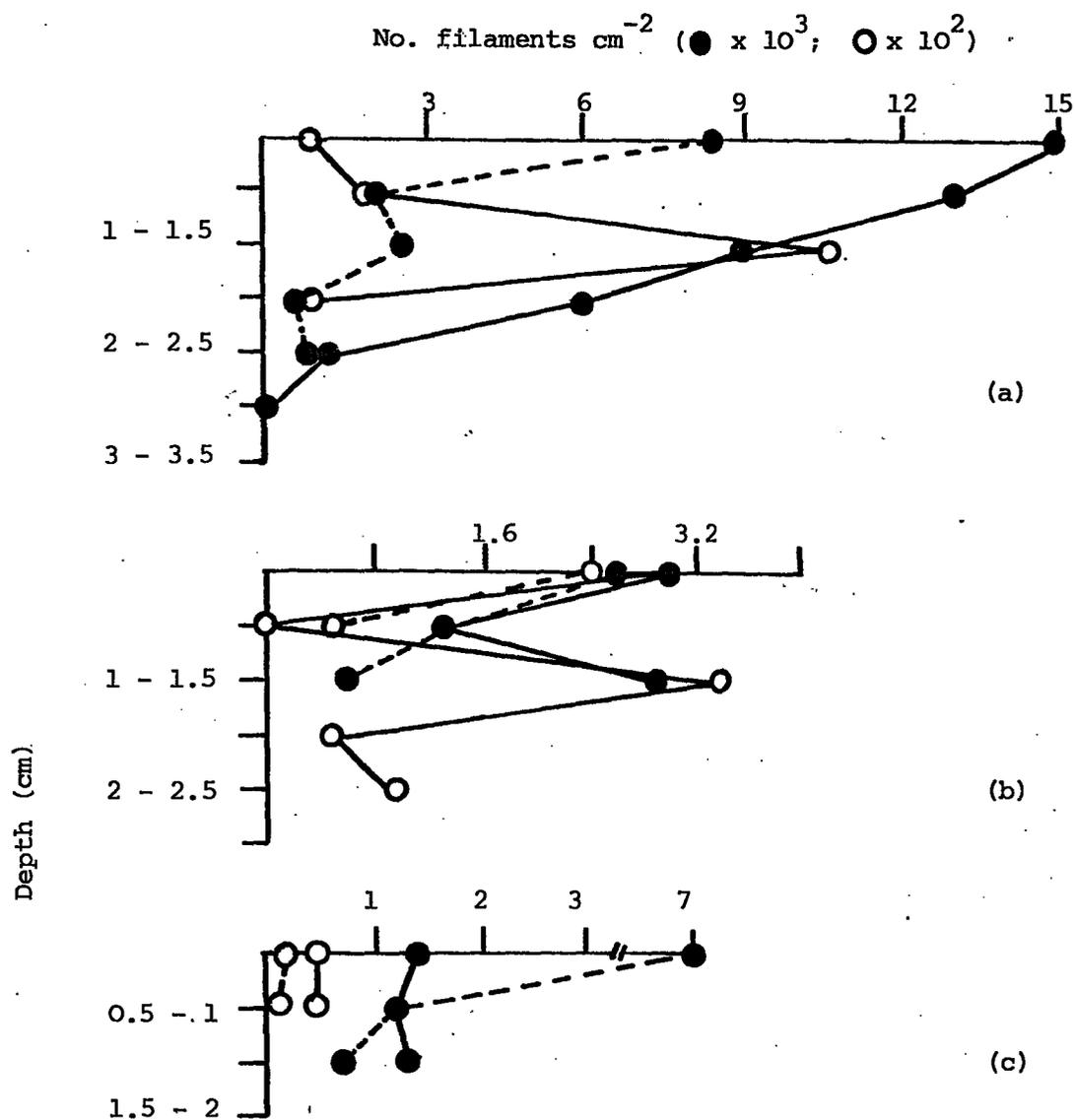


Fig. 55. The vertical distribution of viable filamentous bacteria in Windermere South Basin using coated slides incubated for one and two weeks at 8°C in profundal and littoral sediment cores. (a) agar; (b) gelatin; (c) Ullrich's adhesive ($\bullet\text{---}\bullet$) profundal sediments, one week; ($\bullet\text{---}\bullet$) profundal sediments, two weeks; ($\circ\text{---}\circ$) littoral sediments, one week; ($\circ\text{---}\circ$) littoral sediments, two weeks. Note break on the abscissa scale.

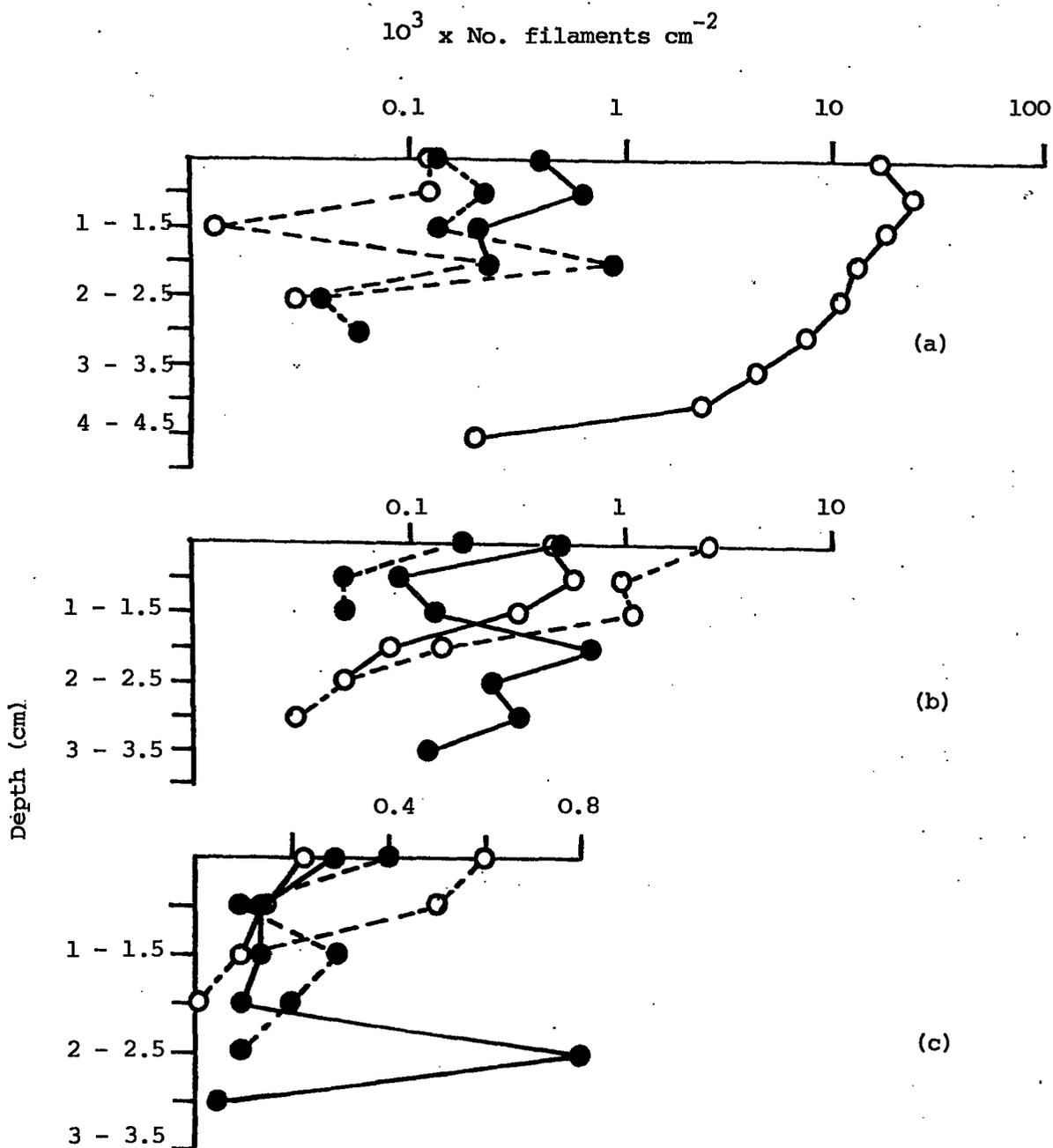


Fig. 56. The vertical distribution of viable filamentous bacteria in Wastewater using coated slides incubated for one and two weeks at 8°C in profundal and littoral sediment cores. (a) agar; (b) gelatin; (c) Ullrich's adhesive. (●—●) profundal sediments, one week; (●---●) profundal sediments, two weeks; (○—○) littoral sediments, one week; (○---○) littoral sediments, two weeks.

Table 13. Numbers of filamentous bacteria obtained with coated slide technique expressed as a percentage of those on marked slides and wet mount.

	Agar		Gelatin		Ullrich's adhesive	
	Profundal	Littoral	Profundal	Littoral	Profundal	Littoral
	as % of marked slides					
Wastwater	0.63	11.30	0.33	1.40	0.16	0.31
Windermere South Basin	81.64	0.18	5.13	0.25	2.28	0.04
Blelham Tarn	0.35	0.47	2.02	3.13	0.16	5.58
	as % of wet mount					
Wastwater	1.67	16.10	0.88	1.98	0.43	0.44
Windermere South Basin	132.70	-	8.34	-	3.71	-
Blelham Tarn	10.62	1.06	61.62	6.98	4.73	12.46

- = no data available

within the sediment. The C and N content of the sediments from the three lakes are shown in Figs. 57 and 58 respectively. Higher values were obtained in the profundal sediments in most cases except for the N data in Wastwater where the littoral sediments had, in 73% of the depths sampled, equal or higher concentrations. The sediments with the highest C content in profundal zone were those of Blelham Tarn; those with the lowest, Wastwater. The highest N contents were also found in the Blelham Tarn sediments whereas the values of Windermere South Basin and Wastwater were similar.

Further detailed E_h Profiles are presented in Figs. 59 and 60. The littoral sediments were usually more oxidized to a greater depth than those from the profundal zone. The arithmetic means of the highest values obtained at the two sampling occasions were +556 mV, +531 mV and +312 mV in the profundal sediments and +630 mV, +501 mV and +511 mV in the littoral sediments for Wastwater, Windermere South Basin and Blelham Tarn respectively. Negative E_h readings observed in Windermere South Basin and Blelham Tarn in May were not observed at all in Wastwater nor in Blelham Tarn in September. The September survey of Blelham Tarn demonstrated the more oxidizing nature of the littoral sediments and therefore the relative values might be considered acceptable. However the absence of negative values in these sediments leads one to question the absolute values and to suspect electrode malfunction.

3.43 Isolation, characterization and measurement of activity of filamentous bacteria

The attempts to isolate the observed filamentous bacteria were first made using CPS, CPS 10%, lake water agar, sediment extract agar, I and A media (section 2.521). Diluted samples, supernatants and washed pellets of agar used to coat slides incubated in the sediment were used to streak plates or as a point inoculum in the centre of the

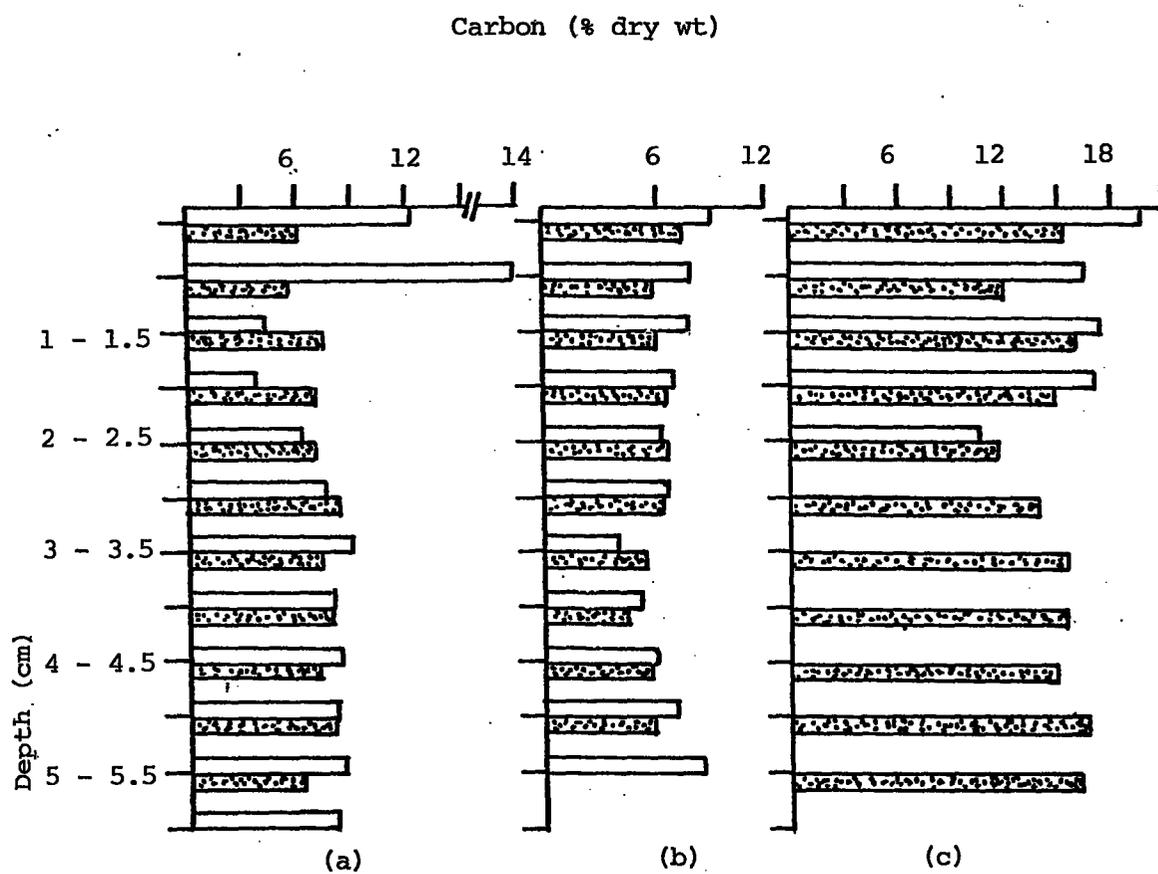


Fig. 57. Carbon content at different depth in the sediment of Wastwater (a), Windermere South Basin (b) and Blelham Tarn (c). (□) profundal sediments; (▨) littoral sediments. Note break on the abscissa scale.

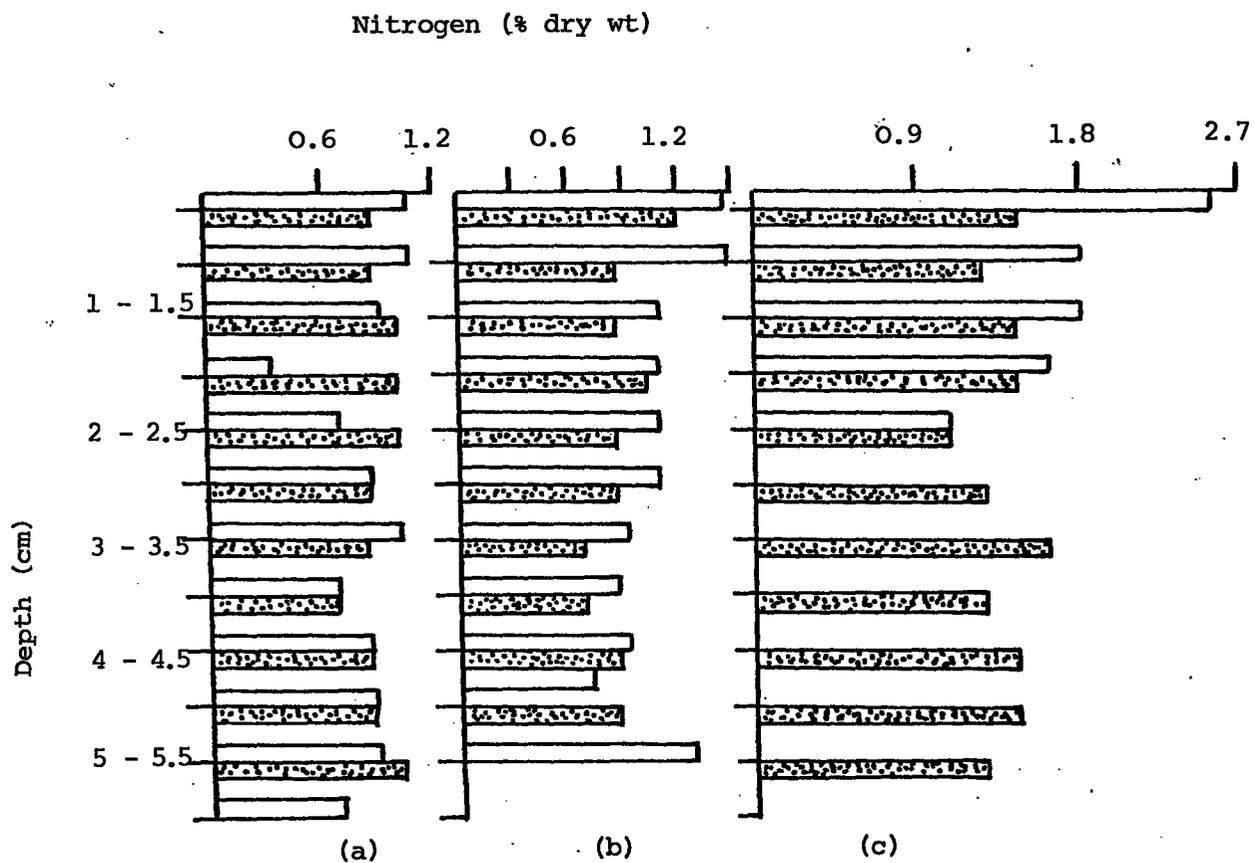


Fig. 58. Nitrogen content at different depth in the sediment of Wastewater (a), Windermere South Basin (b) and Blelham Tarn (c). (□) profundal sediments; (▨) littoral sediments.

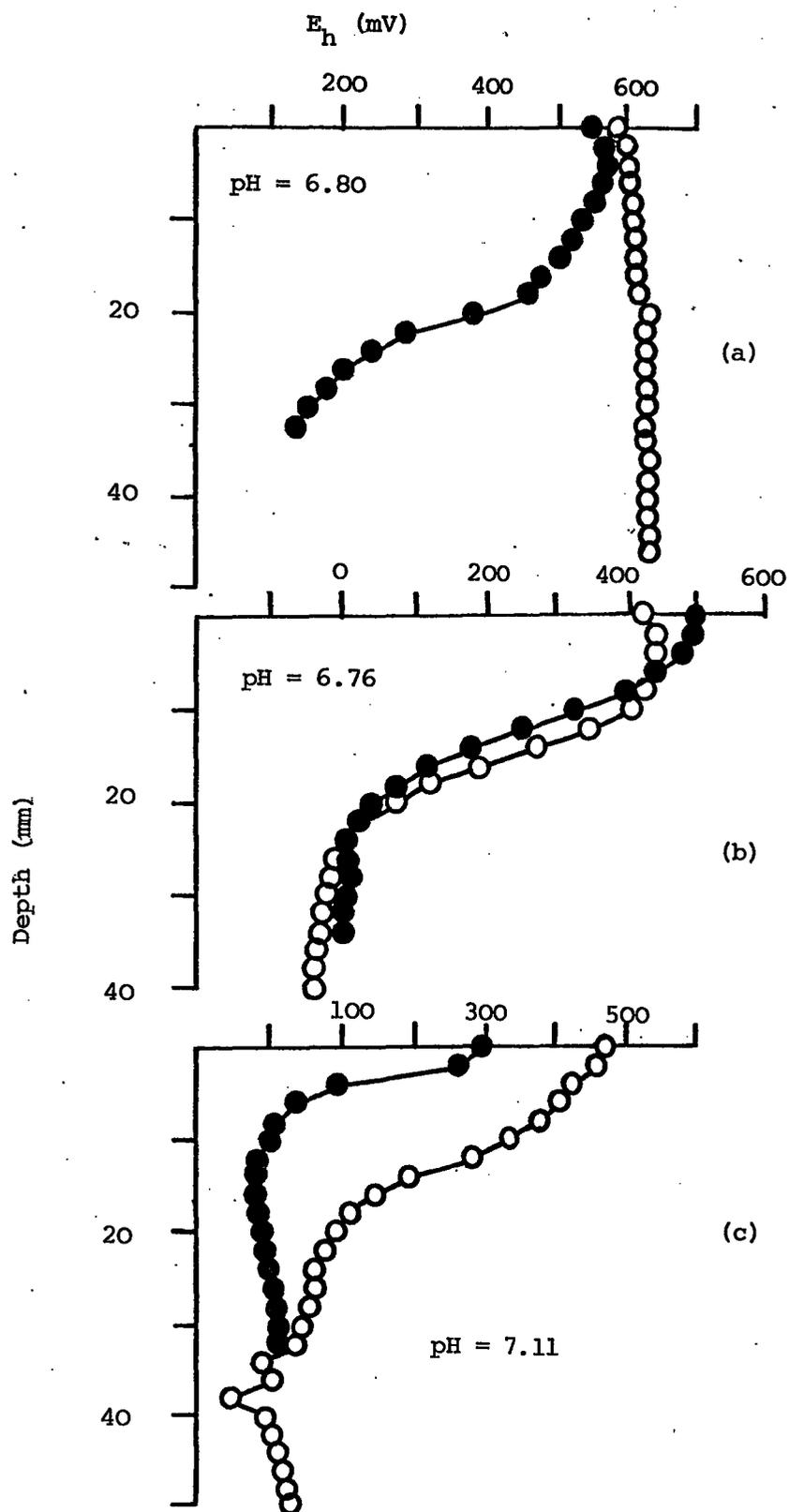


Fig. 59. Individual readings of the electrode potential in the sediments of Wastwater (a), Windermere South Basin (b) and Blelham Tarn (c) taken before the onset of stable thermal stratification. (●) profundal sediments; (○) littoral sediments.

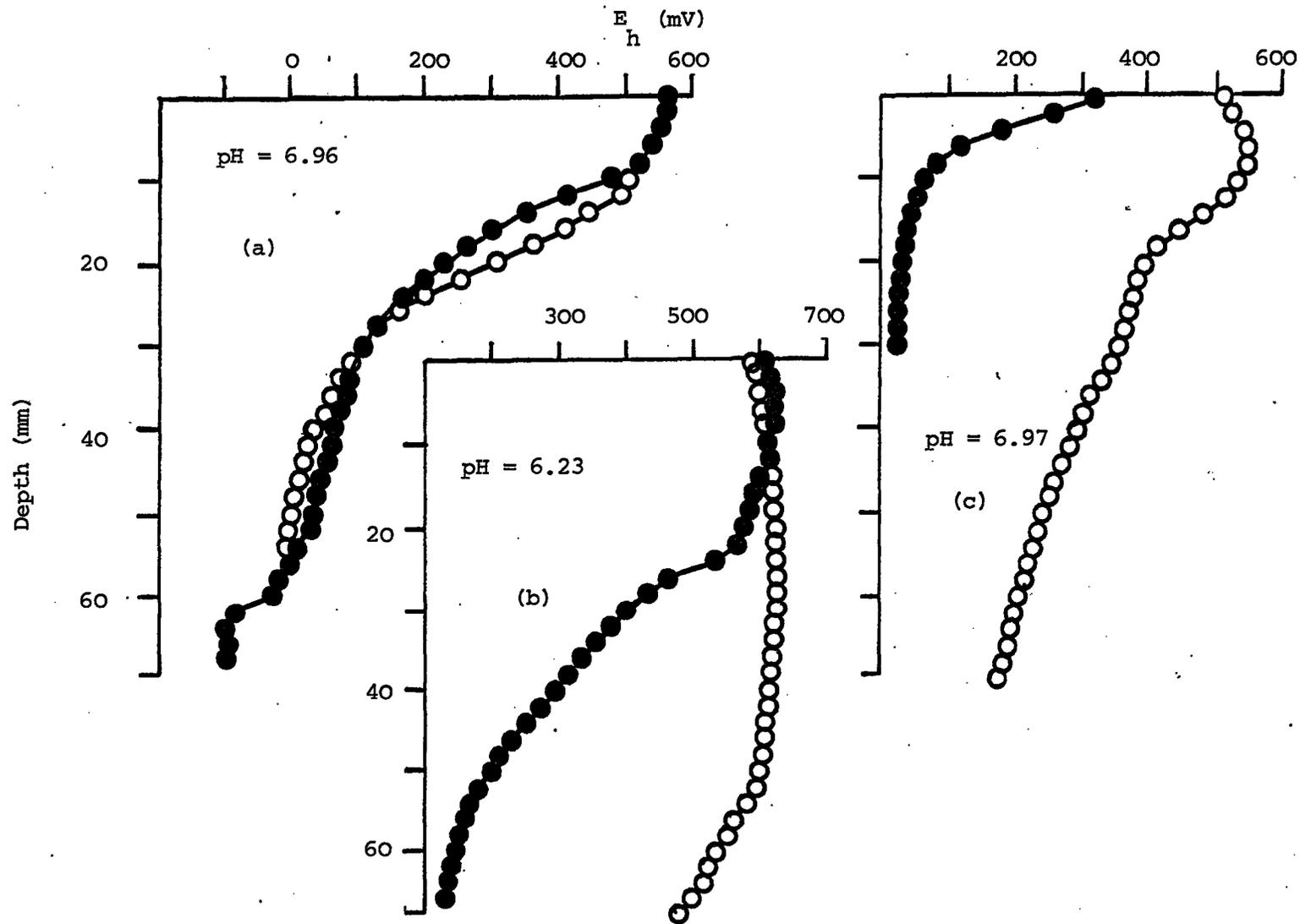


Fig. 60. Individual readings of the electrode potential in the sediment of Windermere South Basin (a), Wastwater (b) and Blelham Tarn (c) in late summer after prolonged thermal stratification. (●) profundal sediments; (○) littoral sediments.

plate. These procedures were not successful. The point inoculum in the centre of the plate was used since gliding motility of certain filamentous microorganisms can be used to separate them from others. It was therefore expected that the filaments would migrate from the edges of the drop. The use of agar plates the surfaces of which had previously been scored with a dry calcium alginate swab providing a path and direction for gliding microorganisms, failed to encourage migration of filamentous bacteria from the area of inoculation. The membrane technique where membrane filters were laid over the agar surface to allow the motile forms to migrate through the membrane to the medium surface proved to be unsatisfactory. In all cases where solid media were used the problem encountered was one of rapid and heavy growth of unicellular bacteria.

The inoculation of liquid media with sediment and the inclusion of a piece of cellophane PT 400 was the most satisfactory technique for primary enrichment of filamentous bacteria and this could also be used to estimate the viable population. However, unicellular bacteria and usually protozoa, were also encountered when the above techniques were used. The composition of the population of filamentous bacteria on the pieces of cellophane was not necessarily identical to that described in later sections although organism of groups 2 and 3 were often observed. The CPS, TSC and SCY culture media encouraged a very heavy growth of unicellular bacteria on the cellophane and the autotrophic medium resulted in algal growth. The heterotrophic medium proved to be the most satisfactory and the pieces of cellophane containing filamentous bacteria were transferred to fresh heterotrophic medium straight from the initial isolation in replidishes, or after being rinsed with sterile water. Although filaments were still observed after a week they did not stand subsequent reinoculation and no further growth was observed. Further enrichments were attempted by adding different substrates to

the liquid medium, and substrates plus inhibitors to the agar used for coating slides to be incubated in the sediment cores. Antibiotics were applied to reduce the number of unicellular bacteria since their presence had hampered the isolation of filamentous bacteria. The individual antibiotics did not, in fact reduce the number of unicells significantly and the use of liquid media containing different substrates was not satisfactory for the same reasons. The effects of the substrates and antibiotics on the total numbers of filamentous bacteria developing in the agar coated slides are shown in Table 14 and the effect on the group composition in Table 15. The numbers of filamentous bacteria were affected by the treatments. Generally higher control counts were obtained with glutamic acid, light incubation with NaHCO_3 and CMC agar. Occasionally the use of inhibitors increased the count (e.g. the use of glucose / streptomycin and TSC medium/chloramphenicol) but unfortunately these results were far from reproducible and as mentioned earlier the additional substrates encouraged the growth of unicells to intolerably high population densities. In addition to this the variety of types encountered was usually greater on the control slides. Some morphological changes were observed. On those containing NaHCO_3 and incubated in the light, some multi cellular sheathed filaments exhibited, along their length, sections without cells which represented areas of false branching. Sections were also observed where the filament was no longer straight but undulated in an irregular manner. Filaments possibly belonging to group 1 organisms (described in section 3.51) were observed in bundles, and in the slide containing NaHCO_3 plus streptomycin and incubated in the light, a very thick sheath impregnated with iron (Plate 3) was observed in filaments possibly of group 2 organisms (described in section 3.51). Filaments with unusually long cells, possibly belonging to group 1 organisms were observed on the slides coated with agar plus glucose.

Table 14. The effect of enrichment and use of inhibitory substances on counts of filamentous bacteria in agar coated slides incubated into the sediment core from the profundal zone. The incubation was for a week at 8 °C.

Substrate	$10^3 \times$ Number of filamentous bacteria cm^{-2}					
	Cycloheximide	Streptomycin	Penicillin	Neomycin	Chloramphenicol	control*
$\text{Na}_2\text{SH}_2\text{O}$ (0.2 g l^{-1})	2.56 (0.70)	1.51 (0.61)	3.77 (0.60)	0.22 (0.16)	2.50 (0.55)	1.03 (0.38)
Glucose (1 g l^{-1})	1.91 (0.46)	9.09 (1.85)	0.38 (0.20)	0.48 (0.36)	0.91 (0.26)	2.40 (0.58)
Glutamic acid (1 g l^{-1})	4.93 (1.07)	1.11 (0.45)	0.29 (0.17)	0.69 (0.83)	1.27 (0.48)	7.15 (1.32)
Starch (1 g l^{-1})	1.47 (0.35)	2.46 (0.57)	2.34 (0.65)	-	3.63 (0.71)	1.26 (0.40)
NaHCO_3 (dark) (0.5 g l^{-1})	2.64 (1.02)	1.31 (0.35)	1.07 (0.27)	1.43 (0.54)	0.09 (0.08)	1.55 (0.55)
NaHCO_3 (light) (0.5 g l^{-1})	5.56 (1.10)	3.81 (1.02)	4.67 (0.93)	1.23 (0.36)	2.34 (0.54)	3.98 (1.12)
Lake water	1.41 (0.50)	0.04 (0.06)	0.72 (0.30)	-	6.38 (1.25)	0.87 (0.30)
Filtered lake water	2.01 (0.38)	1.83 (0.47)	1.03 (0.32)	0.62 (0.22)	1.51 (0.45)	3.52 (0.53)
CMC (2 g l^{-1})	5.12 (1.61)	4.48 (1.10)	2.98 (0.98)	1.29 (0.39)	1.31 (0.83)	6.13 (1.03)
TSC medium	0.18 (0.12)	1.25 (0.40)	0.95 (0.38)	3.44 (0.76)	27.35 (2.43)	1.00 (1.36)

* mean of two values

() 95% confidence limits

Table 15. Groups of filamentous bacteria observed on slides coated with agar containing different substrates and inhibitory substances. The uncubation was for a week at 8°C.

	Cycloheximide	Streptomycin	Penicillin	Neomycin	Chloranphenicol	control
NaSH ₂ O	2,5,10	2,5,10	1,2,3,10,B	5,10,B	1,5,B	1,3,10,5,4b,B
Glucose	10,15,B	3,15,B	10,15,B	1,10,15	1,5,B	1,3,10,B
Glutamic acid	1,2,5,10	1,5,10,B	5,B	1,2,10,8,n.i.	1,3,5,B	1,2,5,10
Starch	3,5,10,B	3,5,10,n.i.	1,3,5,B,n.i.	-	1,3,5,n.i.	1,3,5,4,10,15,B
NaHCO ₃ (dark)	1,3,10,B	5,10,B	1,4,10,B	5,10,B	1,10	5,10,15,B
NaHCO ₃ (light)	2c	1,15	2c	1,B	1	1,2,10,Sp,n.i.
Lake water	1,5,10,15,B	5,1	1,3,5,B,n.i.	-	10	1,3,10,n.i.
Filtered Lake Water	1,3,4,10,B,n.i.	1,3,5,10,B,n.i.	1,3,5,10,B,n.i.	1,B,n.i.	1,10,B,n.i.	1,3,5
CMC	1,2c,B	1,10	1,2c,10	5,10,B	1	1,2c,10,B
TSC	10,B,n.i.	10,B	1,3,5,B	1,2,5,10,n.i.	10,n.i.	2,5,10,n.i.

B = Beggiatoa

Sp = Sphaerotilus type

n.i. = not identified

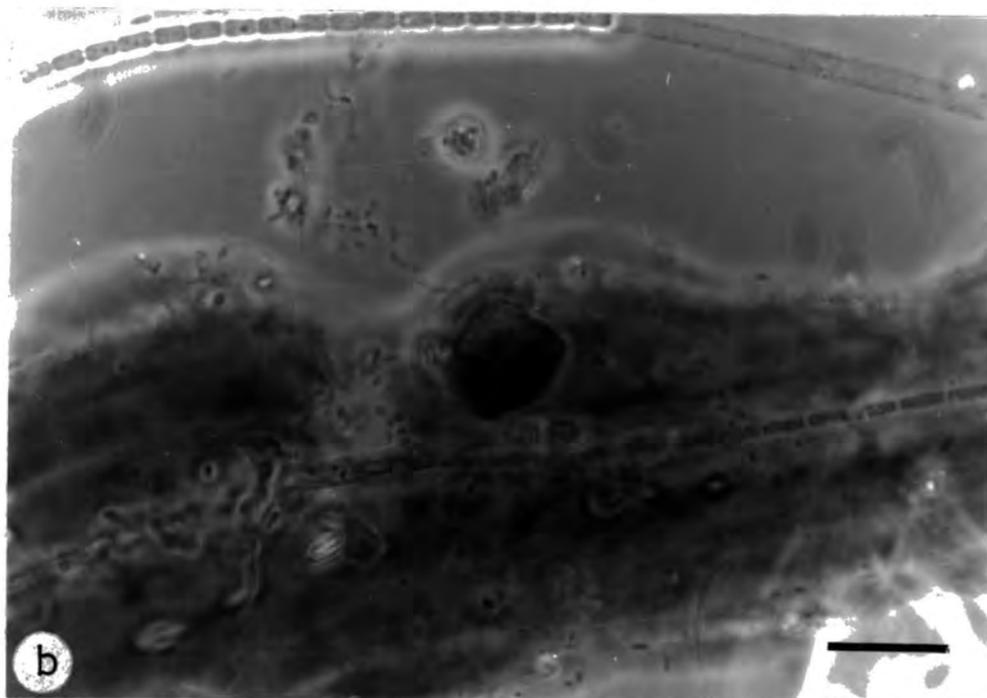
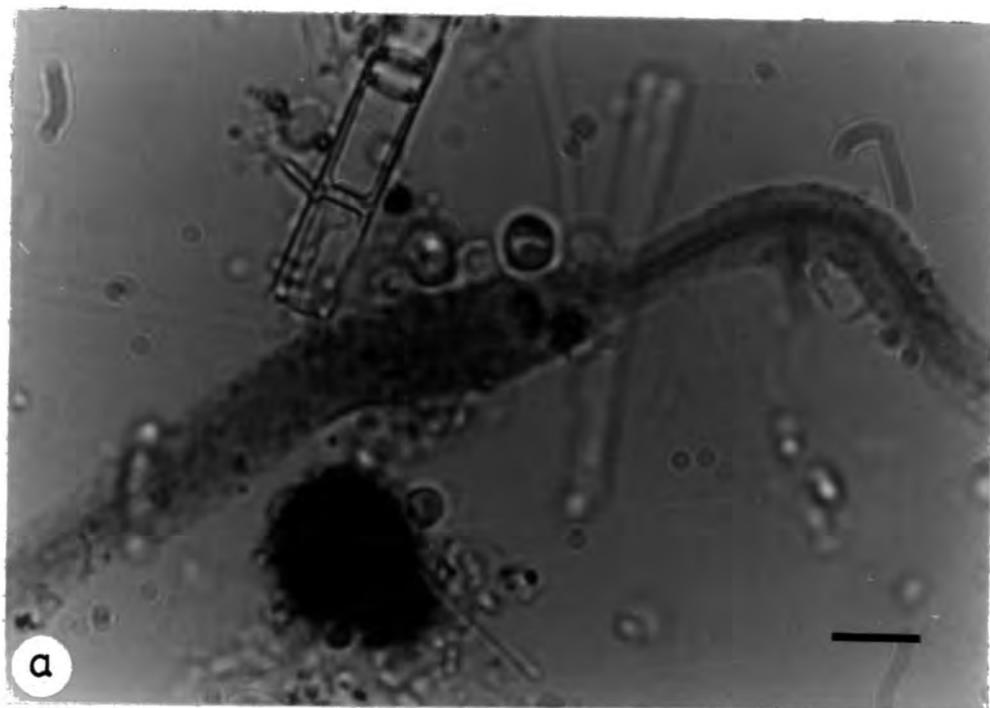


Plate 3. Filamentous bacteria with sheath impregnated with iron observed on slides containing NaHCO_3 plus streptomycin. Bars represent $20\ \mu\text{m}$ (a) and $10\ \mu\text{m}$ (b).

The partition of filamentous bacteria from unicells based on density differences and with subsequent inoculation on solid media did not yield colonies of filamentous bacteria.

The use of microcapillaries was tested since conditions closer to those in the natural environment might be provided with sharper nutrient and oxygen gradients; it was hoped that this might favour the enrichment of filamentous bacteria and possibly be selective for the motile forms. Some success was obtained with capillaries of 0.2 mm internal depth and filaments of Beggiatoa could be observed. Smaller filaments, on the other hand, were more difficult to observe without a long working distance high power objective. The content of the microcapillaries were discharged onto a slide and observed microscopically as a wet mount. This yielded very low numbers and a limited variety of filamentous forms.

The agar sausage technique was used in the hope of selecting filaments which would penetrate the agar. The 0.25% agar concentration selected for one type of filamentous bacterium and this was isolated in pure culture and tentatively identified as Cytophaga.

Membrane holders containing 0.45 μ m and 0.7 μ m cellulose membranes and filled with liquid medium were placed in sediment cores in the hope that motile filamentous bacteria would migrate through the membrane. No filamentous bacteria were observed after the incubation period. Micromanipulation techniques were also used and micropasteur pipettes were drawn out and used to transfer drops of water containing filamentous bacteria to solid media. Growth of filamentous bacteria was not observed. Considerable difficulty was encountered, however, in transferring some of the filaments, particularly gliding bacteria, which stuck firmly to the surface of the agar on the slide.

Relatively few of the filamentous bacteria encountered in the sediment, for which descriptive counterparts may be found in Bergey's

Manual, have been isolated successfully. Of these there have been reports of successful viable counts of actinomycetes in Lake District sediments (Johnston & Cross, 1976). One of the most frequently encountered and dominant genera has been Micromonospora. In an attempt to determine whether this group was an important component in the sediment and on the agar slides a culture of Micromonospora was reinoculated into autoclaved sediment a) to determine whether it would grow successfully b) to determine whether its morphological characteristics were significantly different from those in culture thus hindering its identification by direct observation of the sediment and the slides. Micromonospora grew, its morphological characteristics were retained after the incubation period and it did not resemble any of the filamentous forms normally encountered. This suggested that actinomycetes were a relatively unimportant component of the community under study.

Some attempts were made to determine the biological activity of the filamentous bacteria by measuring electron transport system-ETS-activity (as the reduction of tetrazolium dyes) and autoradiography. The tetrazolium salts are low redox potential indicators and the basis of the method is the measurement of electron movement by providing a tetrazolium dye as an alternative terminal electron transport acceptor. In this case 2,3,5-triphenyltetrazolium chloride was incorporated in the agar which was used to coat slides. The slides were incubated in the sediment cores for a week at 15 °C. No reduction of dye was observed during this period. The microautoradiographic experiments were performed to obtain information on the capacity of the microorganisms to assimilate given substrates. In particular it was hoped to distinguish heterotrophic and autotrophic metabolism in this way using ^{14}C and ^3H labelled organic substrates and ^{14}C bicarbonate. Considerable difficulties were encountered in applying autoradiography to sediments not only due

to high background counts, possibly due to unicellular bacteria, but also due to an apparent loss of filaments during the handling procedure. Application of 5% erythrosine in 5% phenol for 30 seconds, after development, was found to be the best staining technique. The use of a coverslip fixed to a slide with an epoxy resin was affected by retention of emulsion between the coverslip strip and the slide, making microscopic resolution very poor. When the experiment was conducted on slides which had been incubated in the sediment, the agar coating produced a very dark stained preparation compared with slides coated with gelatin and Ullrich's adhesive. Although clear developed grains were observed on some filaments, the high background persisted mainly near the ring of petroleum jelly, probably due to formation of a meniscus. The Ullrich's adhesive proved to be the best fixative even though very few filaments were observed compared with the initial numbers.

Bearing in mind the high failure rate of the tests described above, some modification of the research programme was necessary. Basic population studies on the filamentous bacteria were required but isolation of large numbers in pure culture and determination of metabolic roles was clearly going to be impossible in the time available. It was therefore decided that direct counts under wet mounts and on agar coated slides would be used to determine the distribution of the bacteria, and that the organisms in each sample would be tentatively identified to genus level, using direct observations and cytochemical tests. Preliminary observations had indicated that the key available in Bergey's Manual would not be entirely satisfactory and therefore it was necessary to a) develop and check suitable cytochemical tests to be used on wet mounts and slides incubated in sediment cores, b) devise a new key for filamentous bacteria.

3.44 Cytochemical tests in filamentous bacteria

Uncoated glass slides, incubated in the sediment for different periods of time, were used for the performance of the cytochemical tests. The use of agar coated slides was not possible since the agar interfered with many of the staining procedures used. On the whole the tests applied gave satisfactory results. The test for volutin granules was very effective. For sulphur inclusions the use of pyridine was far more effective than ethanol. The cell wall tests did not always give satisfactory results. The most satisfactory test for the detection of a sheath was the modification of the Farquhar & Boyle (1971) technique. It was observed that pyridine caused the dispersal of gas vacuoles in some filaments examined, and therefore this solvent should be applied only after the pressure test for gas vacuolation. Although some of the tests were not very effective for their intended purpose it was observed that some information could be obtained after their application.

3.5 Biology of filamentous bacteria

3.51 Description of the groups observed

Based on the observations made with agar coated slides, wet mount preparations and cytochemical tests done on uncoated slides, the filamentous bacteria were described and assigned into groups. After the description of the different groups a table is presented (Table 20, p 231) which summarizes the differences between them. The description of each group is given below.

Group 1 Composed of single filaments in most cases, with clearly delimited cells; motile by gliding and characteristically stick to surfaces. The cells were bluish under phase-contrast microscopy. This group is commonly observed in the sediments of Windermere South Basin and in Blelham Tarn. The filaments differ from one another mainly in the width of their trichomes and the length of their cells. The speed of gliding varies ($1 \mu\text{m s}^{-1}$ is commonly observed for the forms present in Blelham Tarn). The filaments observed in Blelham Tarn usually move in a straight line while those from Windermere South Basin are mainly curved and irregularly wavy threads which often change shape. The length of the filaments ranged from $16.5 \mu\text{m}$ to $590 \mu\text{m}$; the widths of the cells ranged from $1.2 \mu\text{m}$ to $4.7 \mu\text{m}$ and the length from $1.7 \mu\text{m}$ to $11.8 \mu\text{m}$. The length of the cells may vary along the trichome. Some idea of the frequency and range of sizes encountered in this group may be seen in Table 16. The results of cytochemical tests were as follows:

Lipid inclusions : positive (doubtful): the cells stained red and some black spots are occasionally observed on the periphery of the cells.

Volutin granules : positive. Discrete granules were always observed.

Sulphur inclusions : negative. Some filaments turned black when pyridine was added while others shrank but remained bluish under phase-contrast.

Table 16 Measurement of individual filaments of group 1 observed at different sampling occasions. Observations on slides incubated into the sediment at 8 °C

Filament length (µm)	Cell	
	width (µm)	length (µm)
587.5	1.2	1.7
43.5	1.5	N.M.
87.0	1.9	4.2
246.8	1.9	4.5
16.5	2.1	3.5
464.0	2.3	2.9 - 5.8
N.M.	2.3	7.3 - 10.2
464.0	2.3	8.7
105.8	2.4	4.7
216.2	2.4	5.9
141.0	2.4	9.4
103.5	2.7	4.5
69.6	2.9	4.9
N.M.	3.2	4.1
75.6	3.5	3.6
225.6	3.5	4.7
49.4	3.5	11.8
47.0	4.7	5.2

N.M. = not measured

Iron deposition : negative.

Sheath : negative

Cell wall stain : positive

Spore stains : negative

Gas vacuoles : negative

Flagella stain : negative

Gram stain : negative

Chlorophyll fluorescence : negative.

The filaments of this group present in Windermere South Basin were normally in the smaller size range when compared with the ones observed in Blelham Tarn. Although the ones observed in Blelham Tarn varied in their dimensions, the most commonly observed have cells with 2.9 μm to 3.2 μm in width and length of 4.0 μm to 4.9 μm ; the length of the trichome was about 69.6 μm . Also, in Blelham Tarn, the filaments could be seen with less marked cell wall constrictions. It was observed that some filaments belonging to this group resemble very closely the genus Pseudanabaena.

Examples of Group 1 organisms are illustrated in Plates 4 to 7.

Plate 4. Group 1 organisms (Vitreoscilla) observed in
Windermere South Basin. (a) motility not observed
(b), (c) motile. Bar represents 20 μ m.

Note: Unless otherwise specified all photomicrographs
were taken under phase-contrast microscopy.

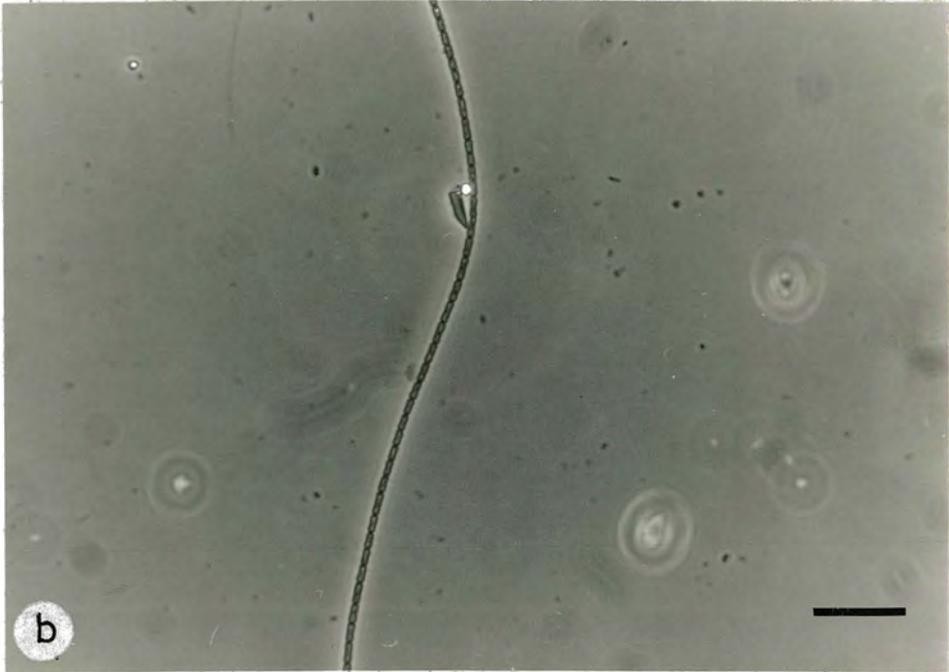
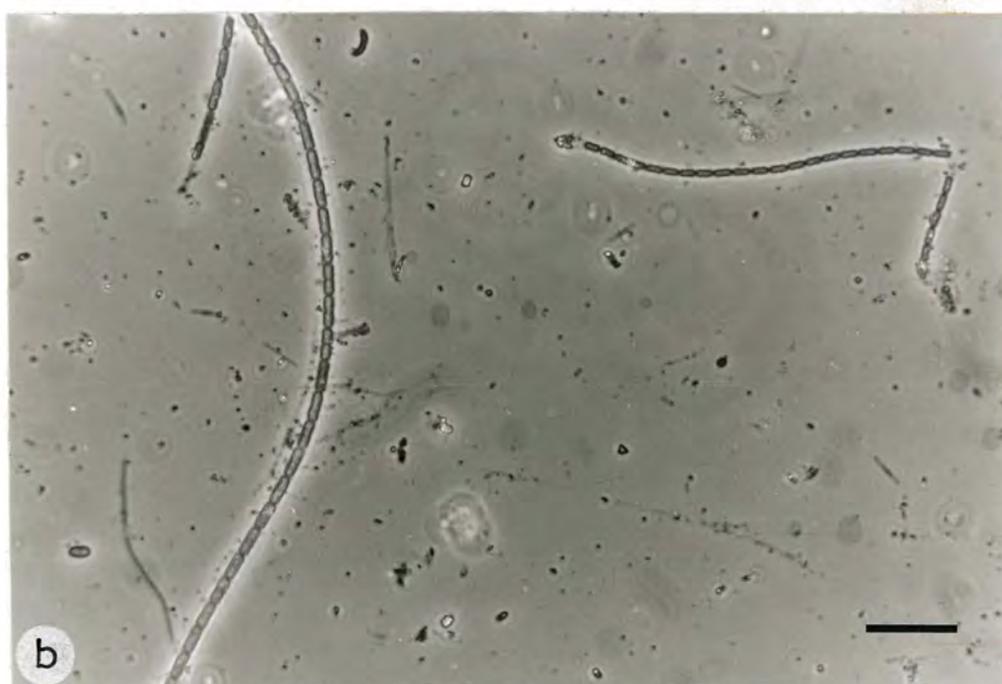
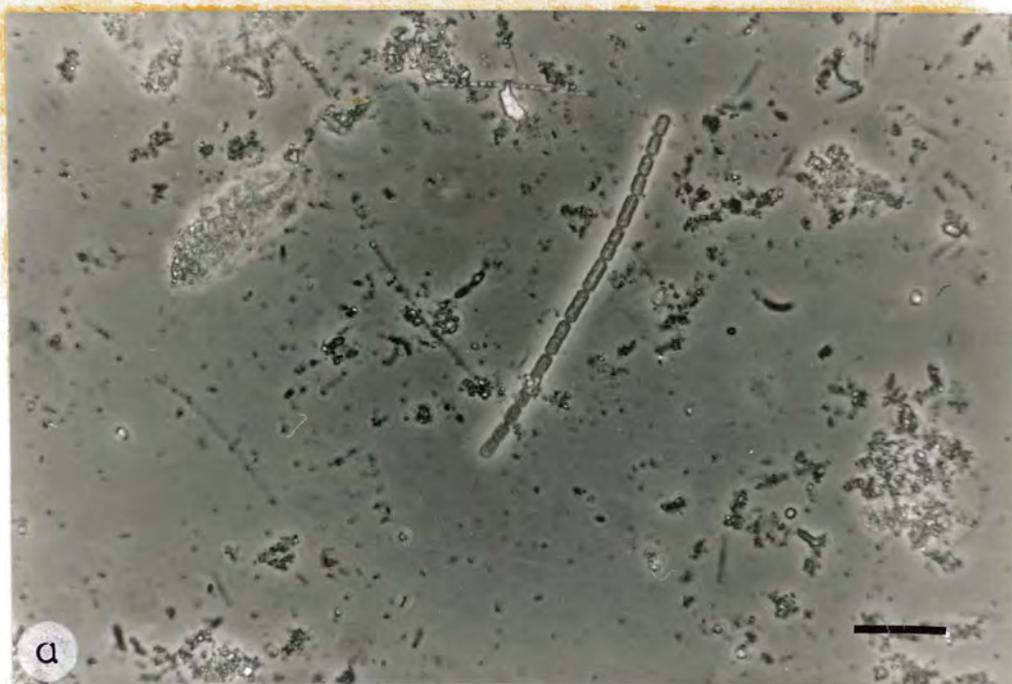


Plate 5 Group 1 organisms (Vitreoscilla) observed in Blelham
Tarn (a) motile (b) motility not observed (c) motile
filament showing parts with attached particles. Bar
represents 20 μm .



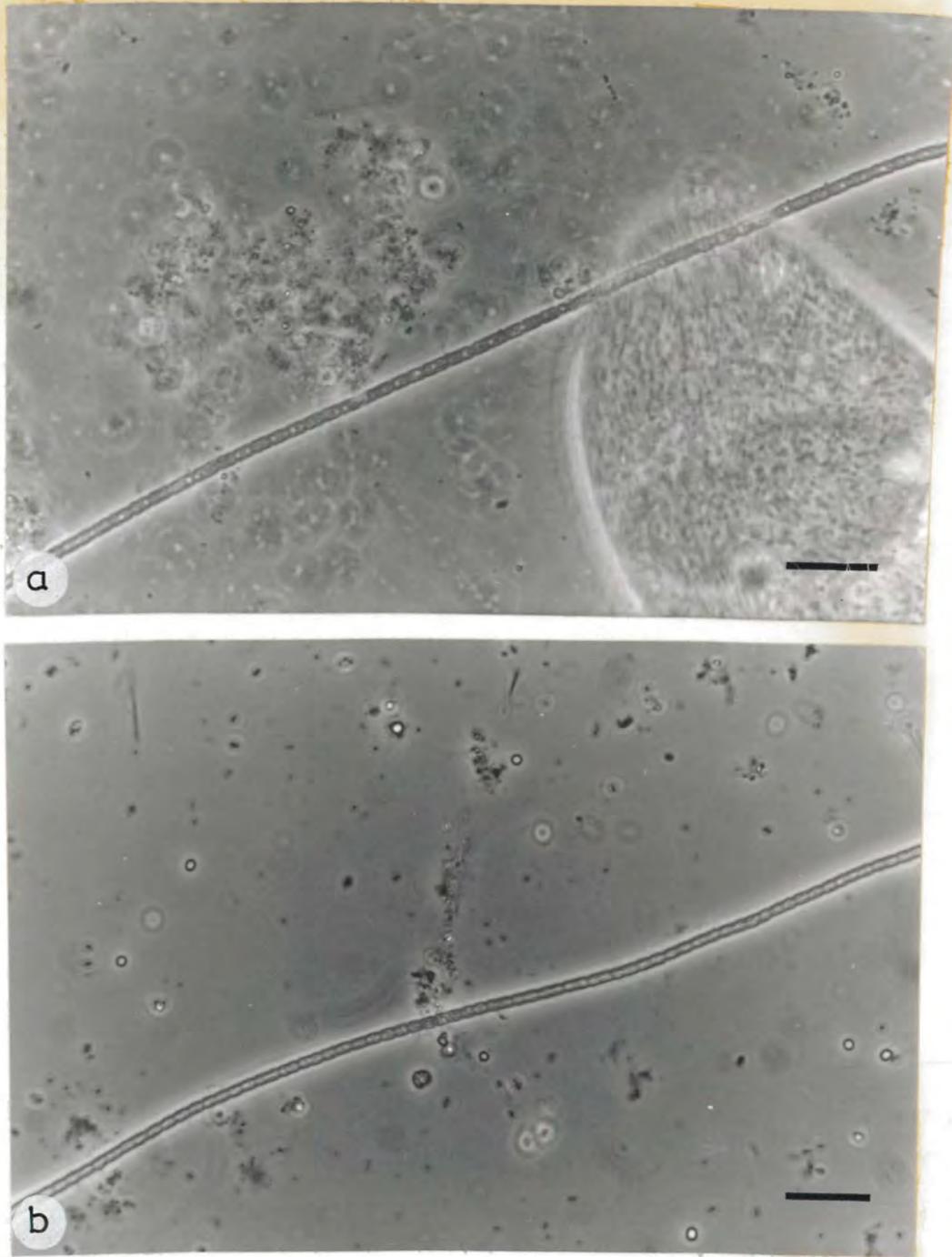
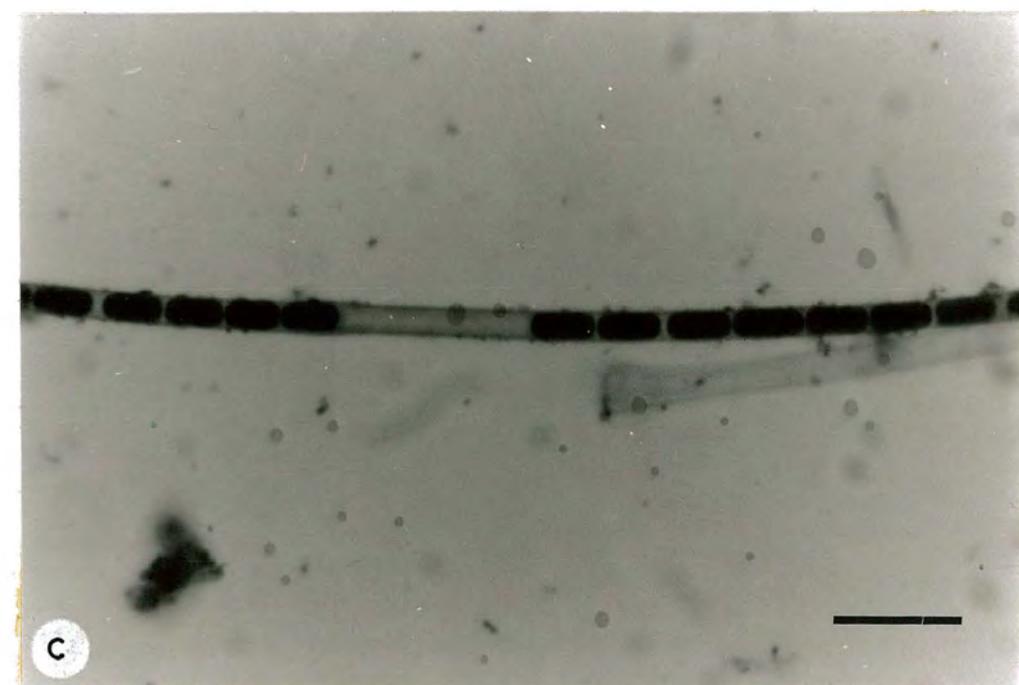
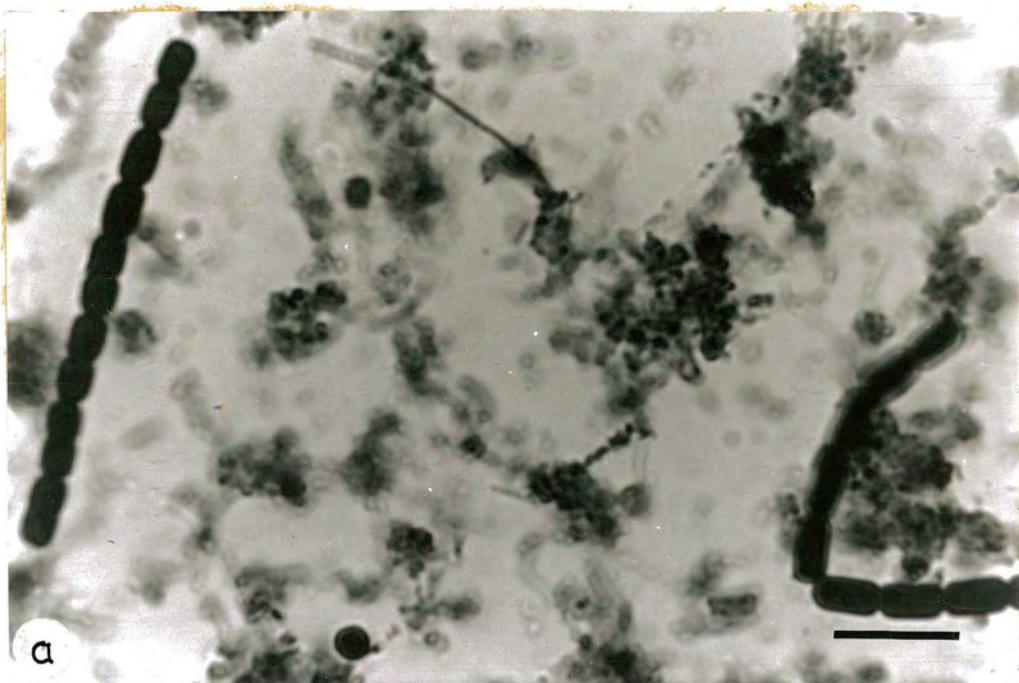


Plate 6. Group 1 organisms (*Vitreoscilla*) observed in Blelham Tarn showing filaments with cell constrictions not as marked as the ones in Plate 4. These filaments were observed with (a) and without refractile inclusions (b). Bar represents 20 μm .

Plate 7 Group 1 organisms (Vitreoscilla) observed in Blelham Tarn. The filaments were treated with 3% NaCl and stained with Tyler's crystal violet. (a) motile filament without sheath. Thick cell walls and cell inclusions were observed. (b), (c) non motile filaments showing sheath and cell inclusions. Bar represents 10 μ m. Photographs taken under bright field optics.



Group 2: Single filaments composed of clearly delimited cells within a sheath. The sheath was yellow or dark in colour under phase-contrast illumination. The cells were bluish or may exhibit refractive areas due to gas vacuoles. The filament has been observed leaving the sheath at a speed of $0.07 \mu\text{m s}^{-1}$ (difficulties are likely to be encountered in correctly distinguishing some organisms that leave their sheath from the unsheathed gliders). These organisms occur mainly in Windermere South Basin. The lengths of the filaments ranged from $75.4 \mu\text{m}$ to $752 \mu\text{m}$; the cells were between $0.9 \mu\text{m}$ and $2.6 \mu\text{m}$ wide and $1.9 \mu\text{m}$ to $4.7 \mu\text{m}$ long. Table 17 provides some idea of the size range encountered. The length of the cells may vary along the same trichome. The results of the cytochemical tests were as follows:

Lipid inclusions : negative. On rare occasions a doubtful positive result was obtained.

Volutin granules : positive.

Sulphur inclusions : negative. In several cases, after adding pyridine the cells were completely dissolved leaving empty sheath. This was more common when the cells were vacuolated.

Iron deposits : positive.

Sheath : positive.

Spore stain : negative

Cell wall stain : positive

Gas vacuoles : variable

Flagella stain : negative

Gram stain : negative

Chlorophyll fluorescence : negative

Examples of Group 2 organisms are illustrated in Plates 8 to 13 and in Fig. 61.

Table 17. Measurement of individual filaments of Group 2 observed at different sampling occasions. Observations on agar coated slides incubated in the sediment at 8 °C.

Filament length (µm)	Cell	
	Width (µm)	length (µm)
N.M.	0.9	1.9
752.0	1.9	7.1
423.0	2.4	2.8
235.0	2.4	2.4 - 4.7
75.4	2.6	2.7

N.M. = not measured

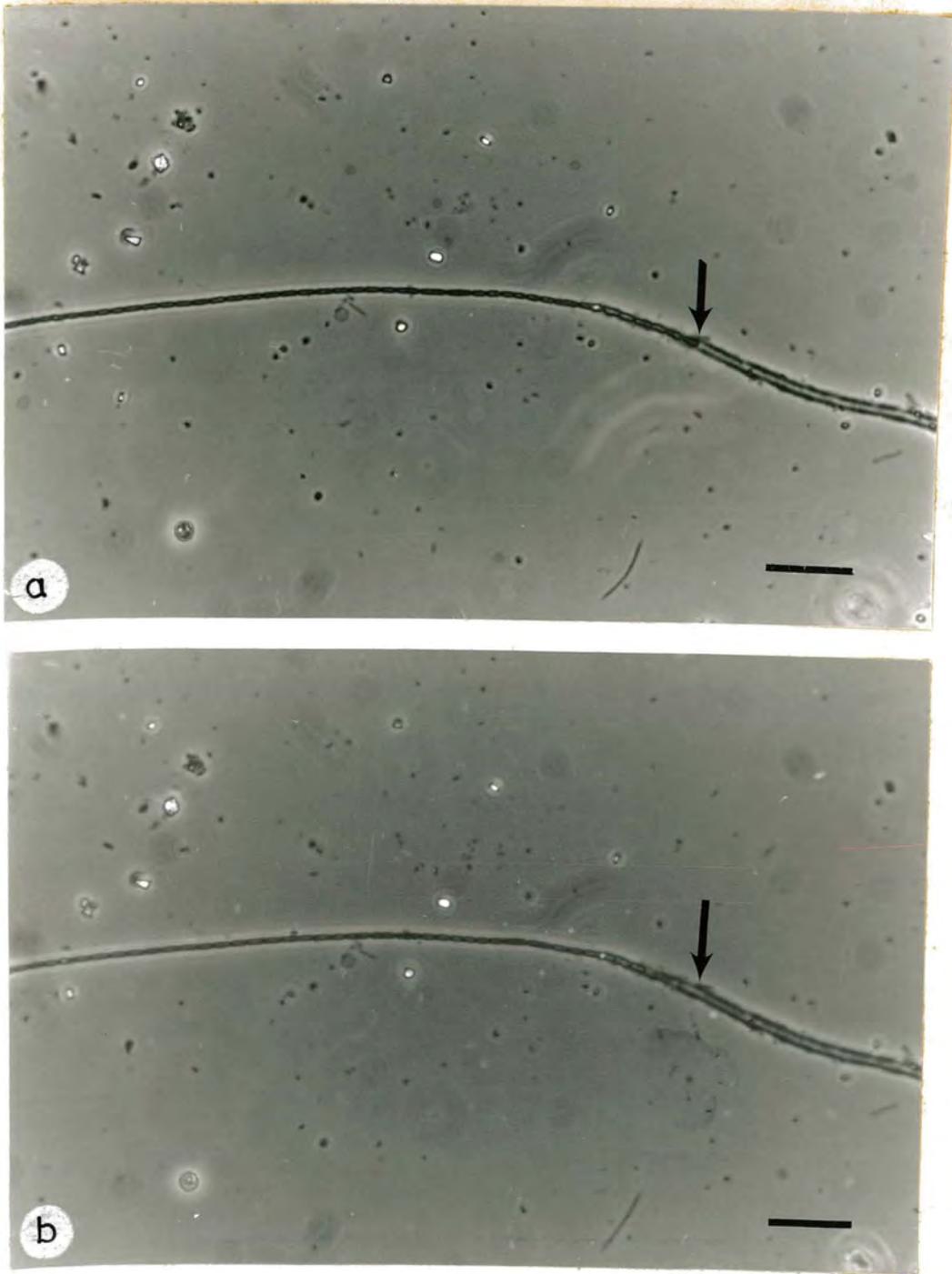


Plate 8. Group 2 organisms (Leptothrix) observed gliding out of their sheath at $0.07 \mu\text{m s}^{-1}$ (a) and (b) show the different position of the filament inside the sheath. Bar represents $20 \mu\text{m}$.

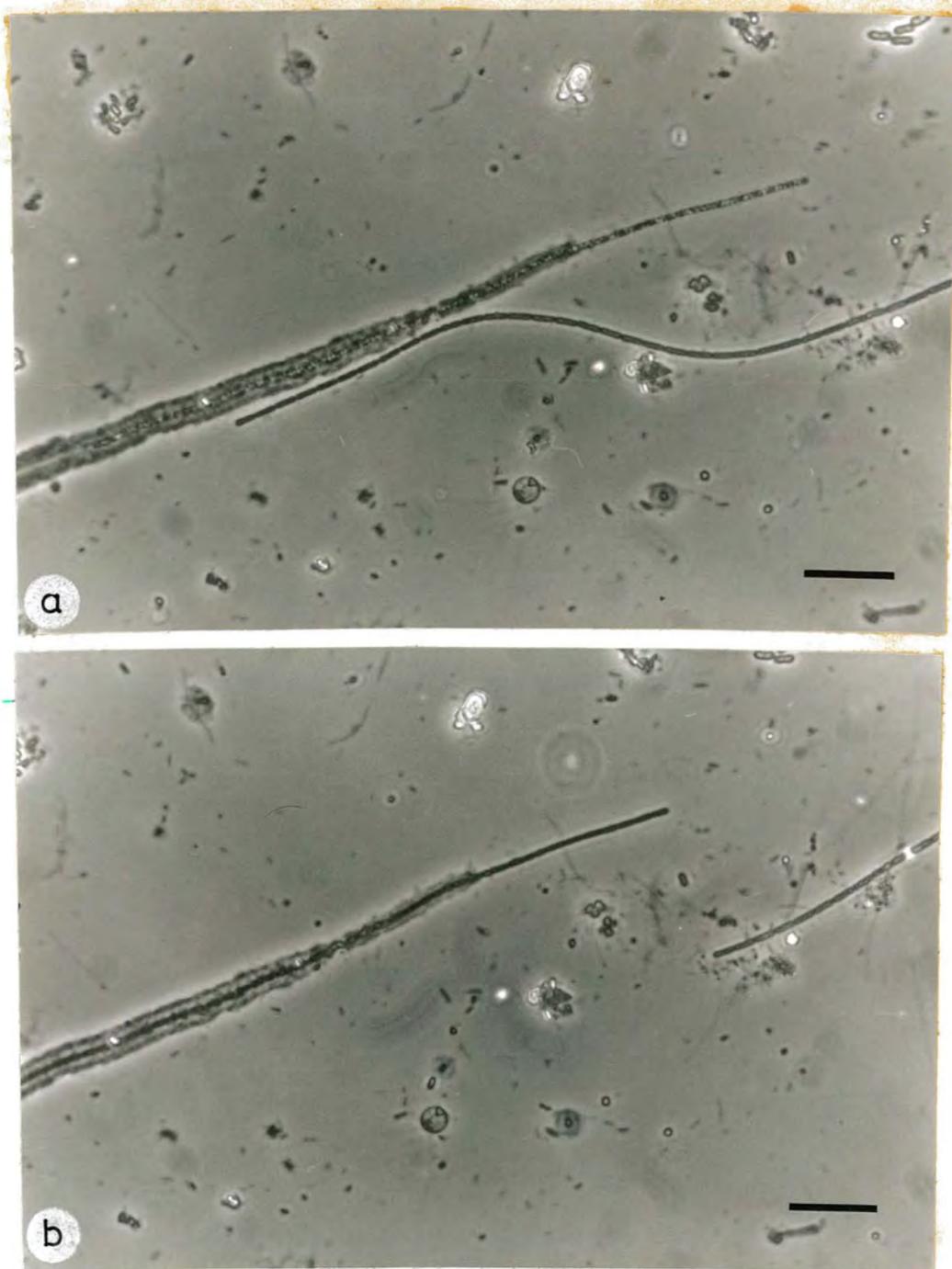


Plate 9. Group 2 organisms (Leptothrix). Filament before (a) and after (b) collapsing the gas vacuoles under pressure. Bar represents 20 μm .

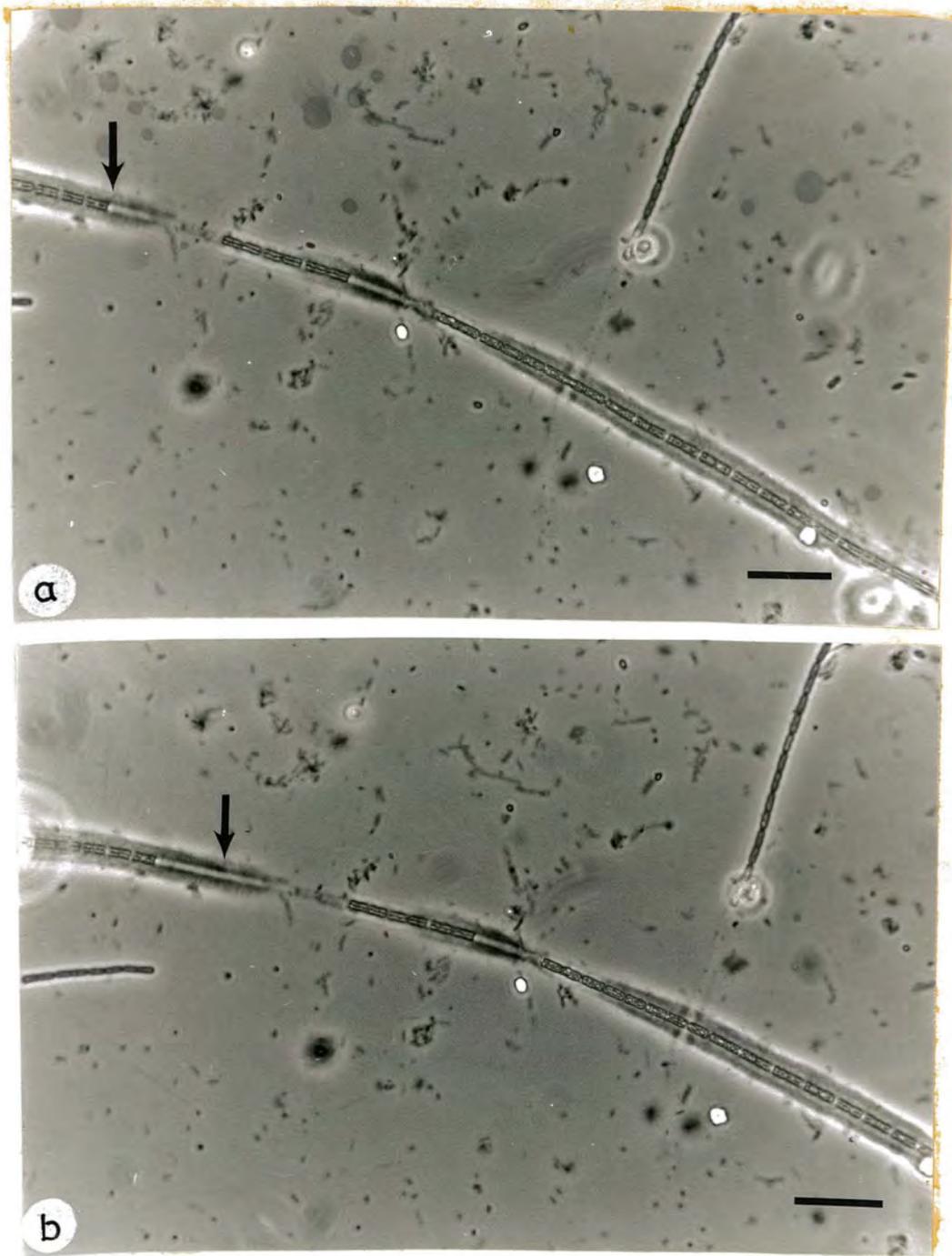


plate 10. Group 2 organisms (Leptothrix). Filament moving inside the sheath observed in slides incubated in cores from Mitchell Wyke, Windermere (Fig. 2) (a) and (b) show the filament at different position inside the sheath. Bar represents 20 μ m.

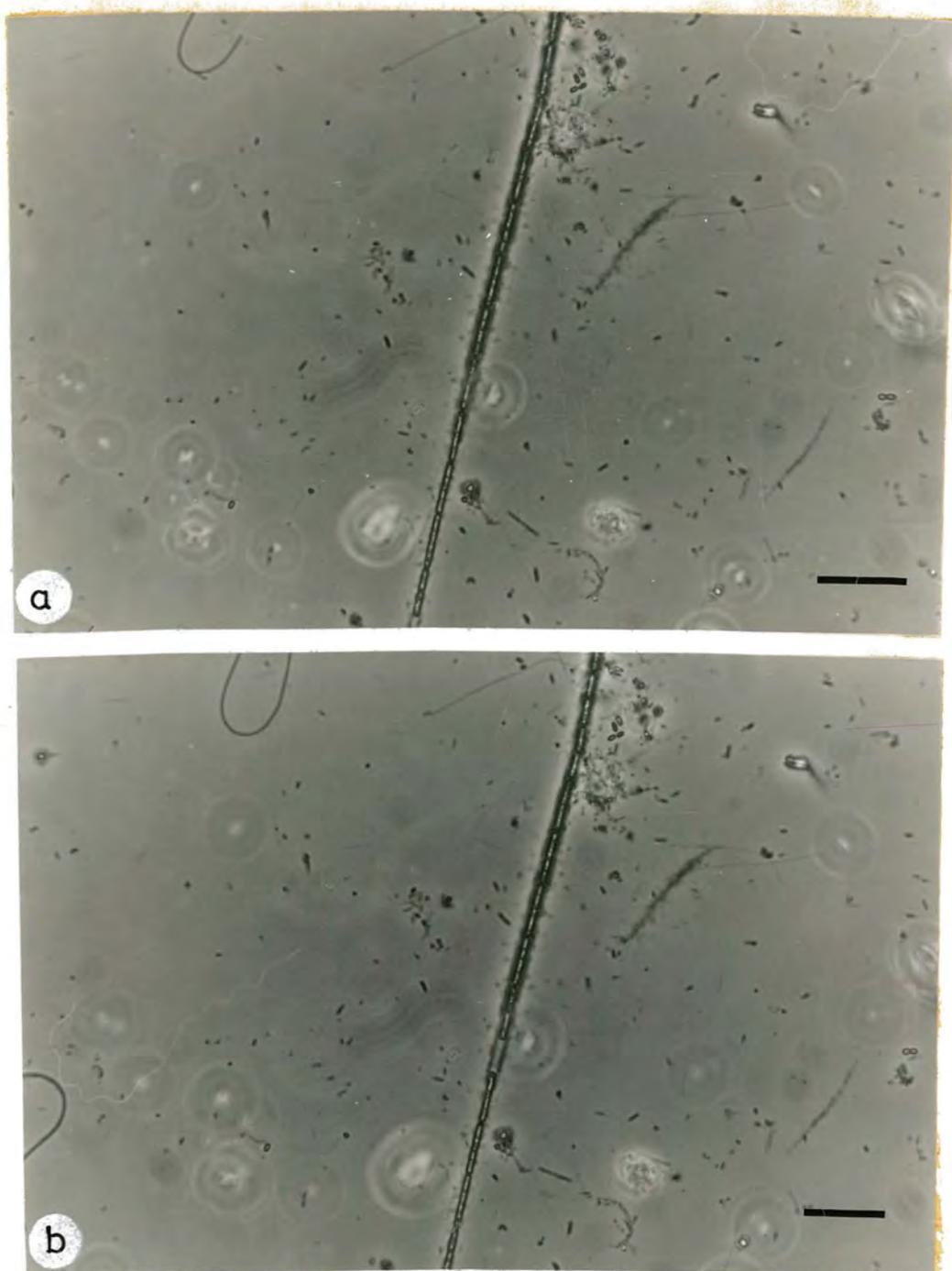


Plate 11. Group 2 organisms (Leptothrix). Filament observed moving inside the sheath and exhibiting gas vacuoles. (a) filament before and (b) after splitting and started gliding. Bar represents 20 μm .

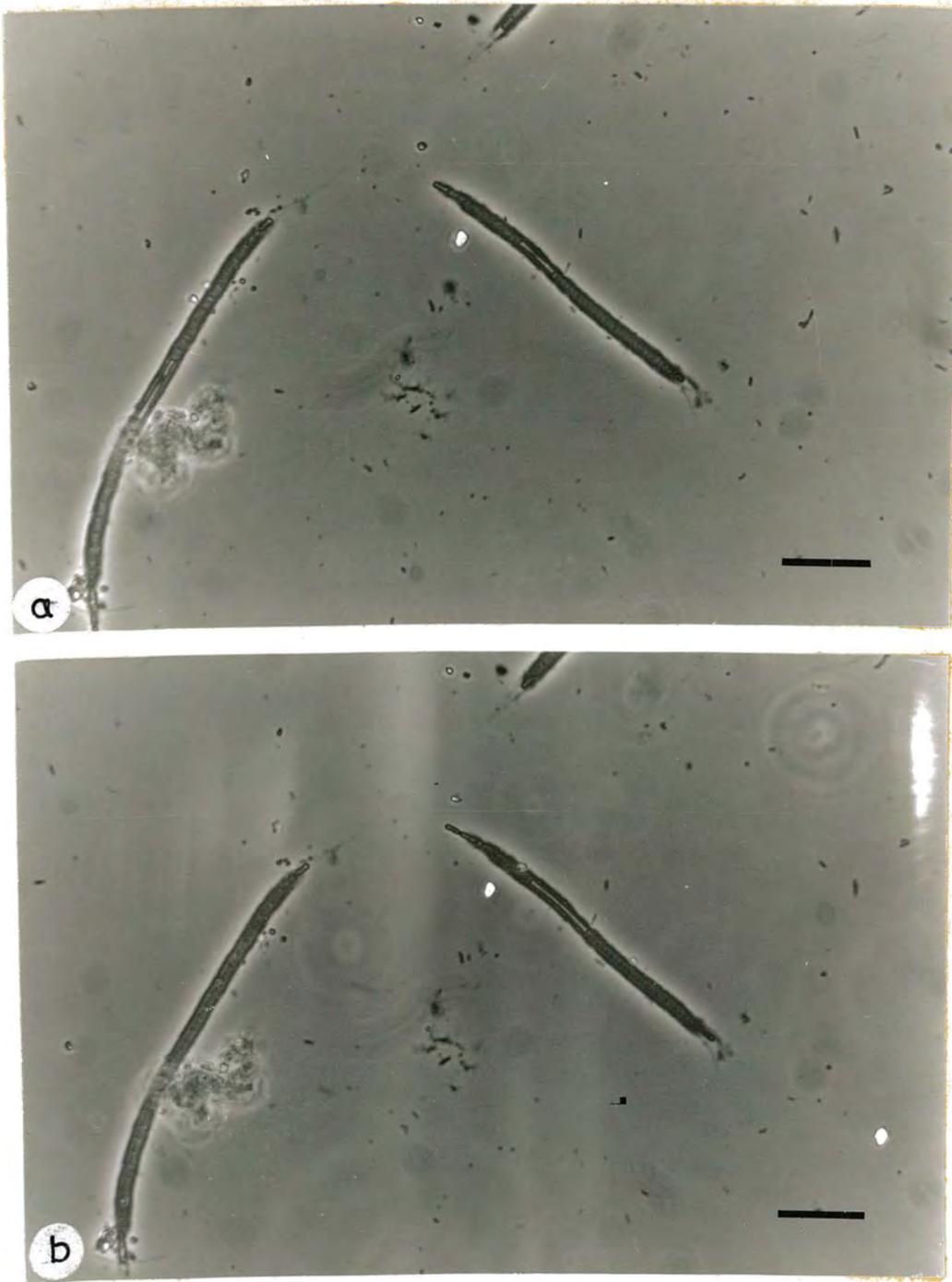


Plate 12. Group 2 organisms (Leptothrix) observed gliding out of the sheath. (a) and (b) shows the filament at different position inside the sheath. Bar represents 20 μm .

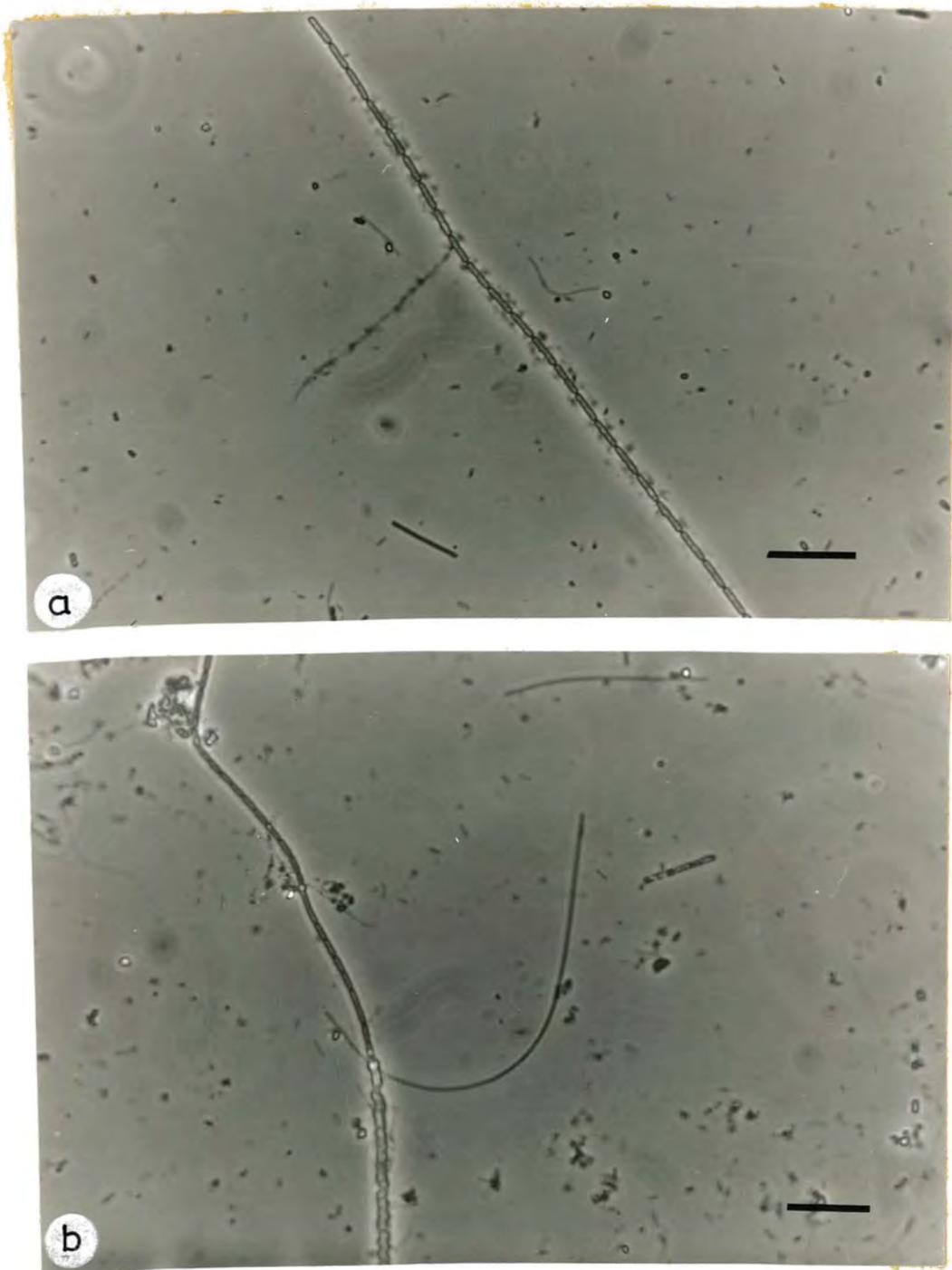


Plate 13. Group 2 organisms (Leptothrix) (a) illustrating the possible early stages of sheath impregnation with iron (b) filament which has part of its sheath encrusted with iron. Bar represents 20 μm .

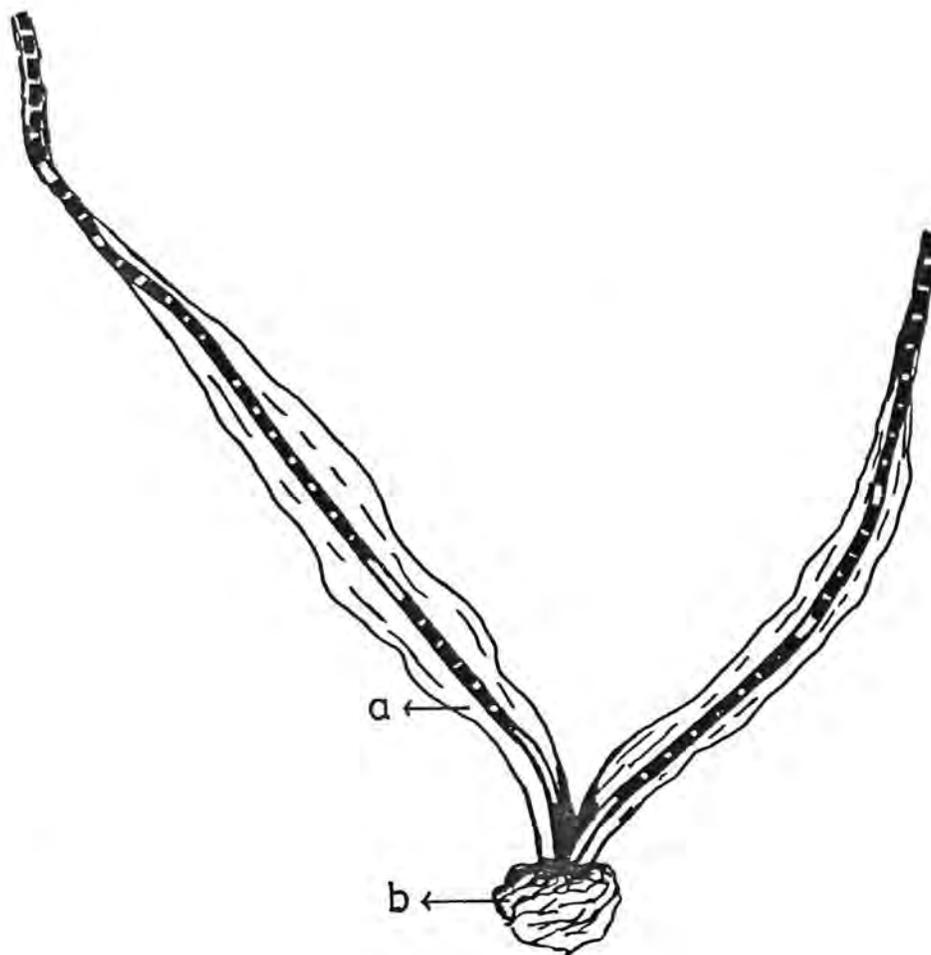


Fig. 61. Group 2 organisms (Leptothrix) growing on uncoated slide incubated in the sediment core from Windermere South Basin after 3 weeks at 15°C. (a) sheath incrustated with iron (b) sediment particles. Approximately x 500.

Group 3. Single filaments whose main characteristic was the gliding motility. The filaments may glide long distances in one direction without changing shape but on other occasions they alternate a forward and backward movement followed by an almost instantaneous change in shape. The filaments adhered to surfaces, and were black under phase-contrast microscopy. The maximum speed of gliding observed was $4.5 \mu\text{m}$ to $5.8 \mu\text{m s}^{-1}$. The filament width ranged from $0.70 \mu\text{m}$ to $1.0 \mu\text{m}$ and the length from $35.3 \mu\text{m}$ to $165 \mu\text{m}$. Table 18 gives the size range observed.

The cytochemical tests were as follows:

Lipid inclusions : negative.

Volutin granules : positive.

sulphur inclusions : negative. The addition of pyridine made the filaments break into several pieces of similar size.

Iron deposition : negative.

Sheath : negative.

Spore stain : negative.

Cell wall stain : negative (doubtful)

Gas vacuoles : negative.

Flagella stain : negative.

Gram stain : negative.

Chlorophyll fluorescence : negative.

After prolonged observation under the microscope the filaments sometimes break into pieces. When NaCl 3% was added to the slides some of the filaments shrank immediately and the very thin ones broke into pieces.

Examples of Group 3 organisms are illustrated in Plates 14 to 16 and in Fig. 62 and 63.

Table 18. Measurement of individual filaments of Group 3 observed at different sampling occasions. Observations on slides incubated into the sediment at 8 °C.

Filament width (μm)	Filament length (μm)
0.7	35; 47; 96
0.7	86; 144
0.9	31; 92; 261
1.0	86
1.0	165

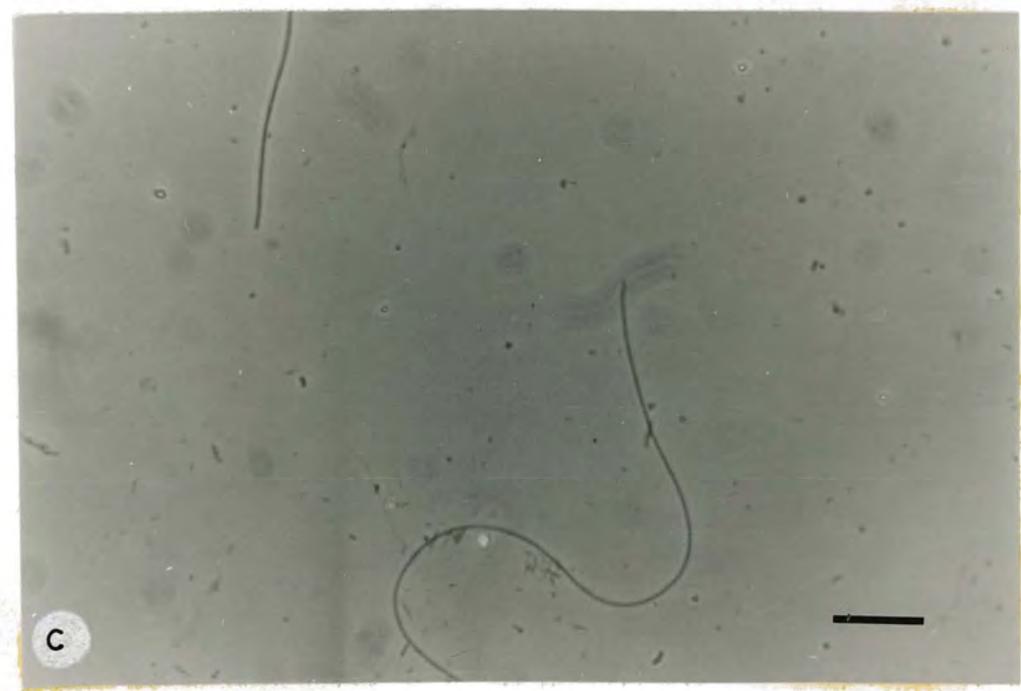
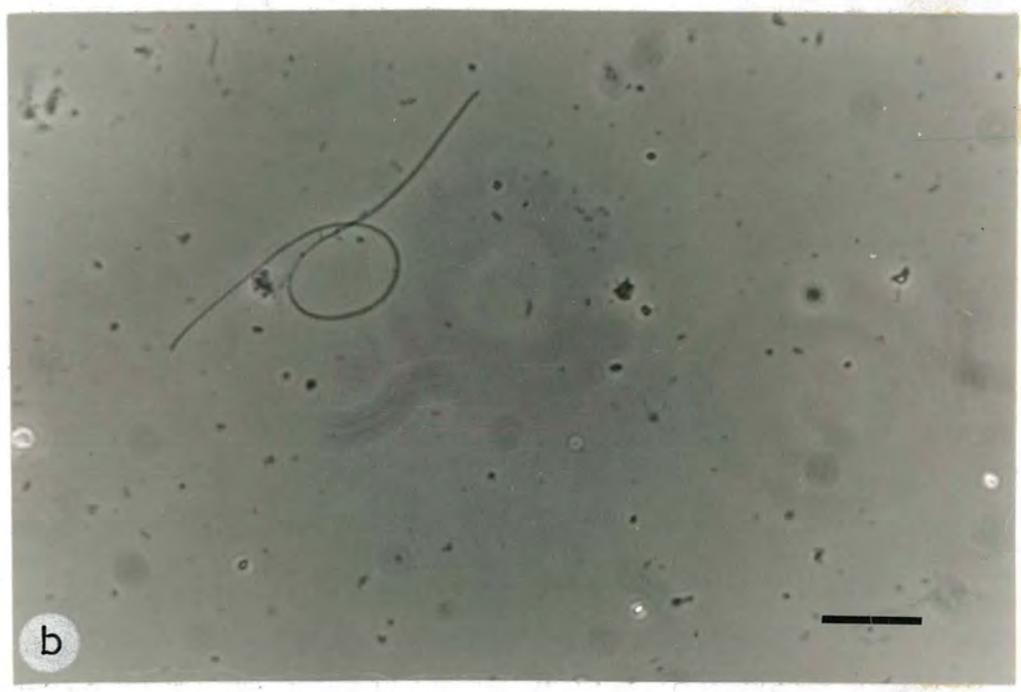
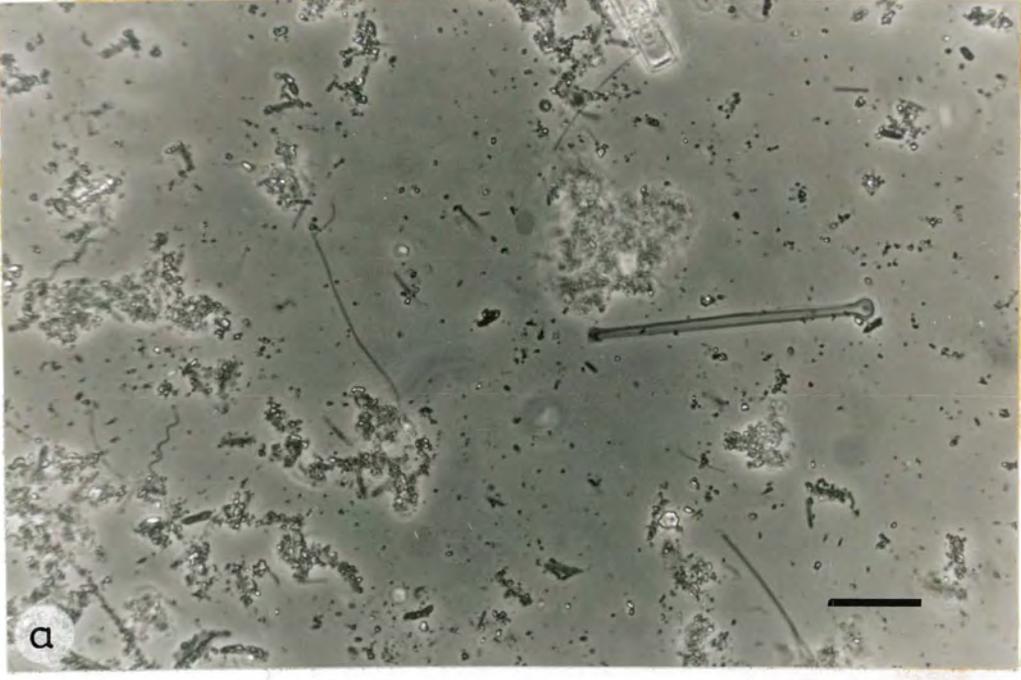
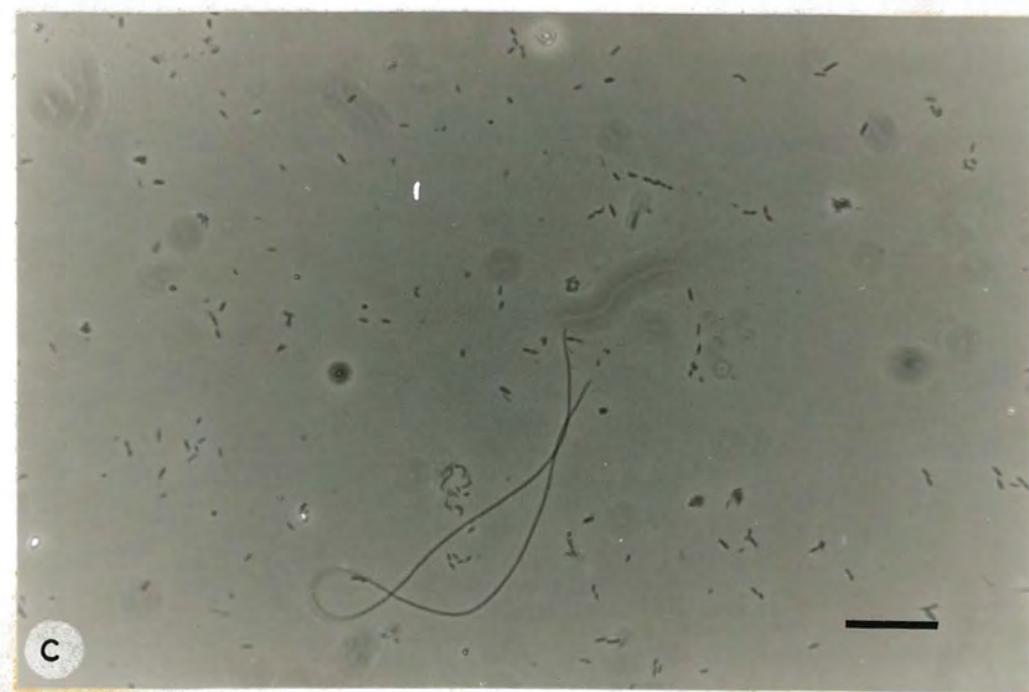
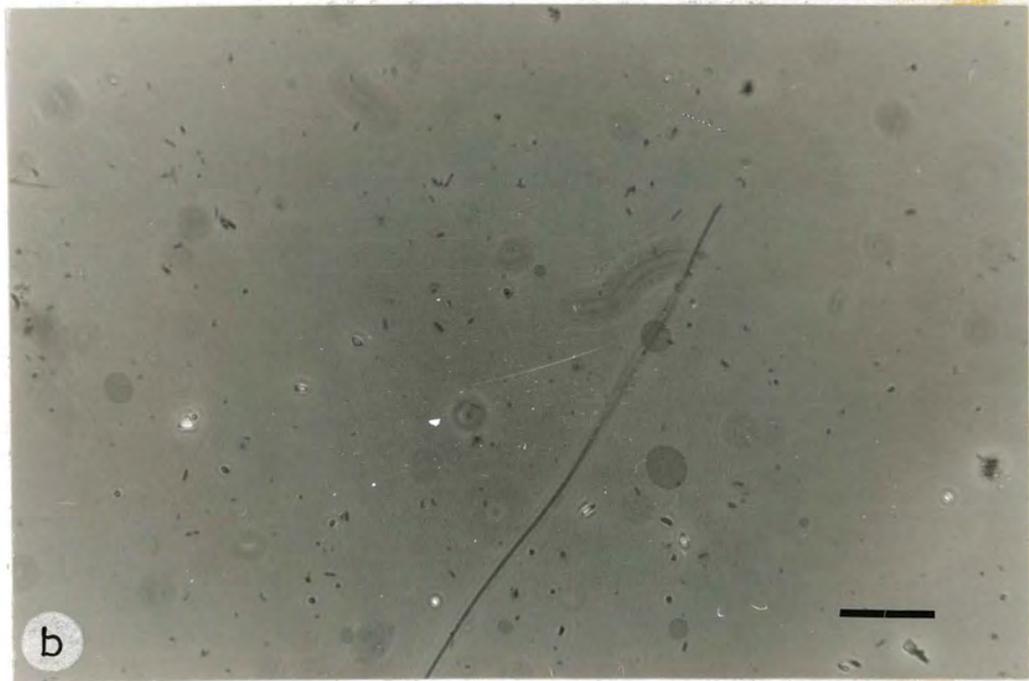
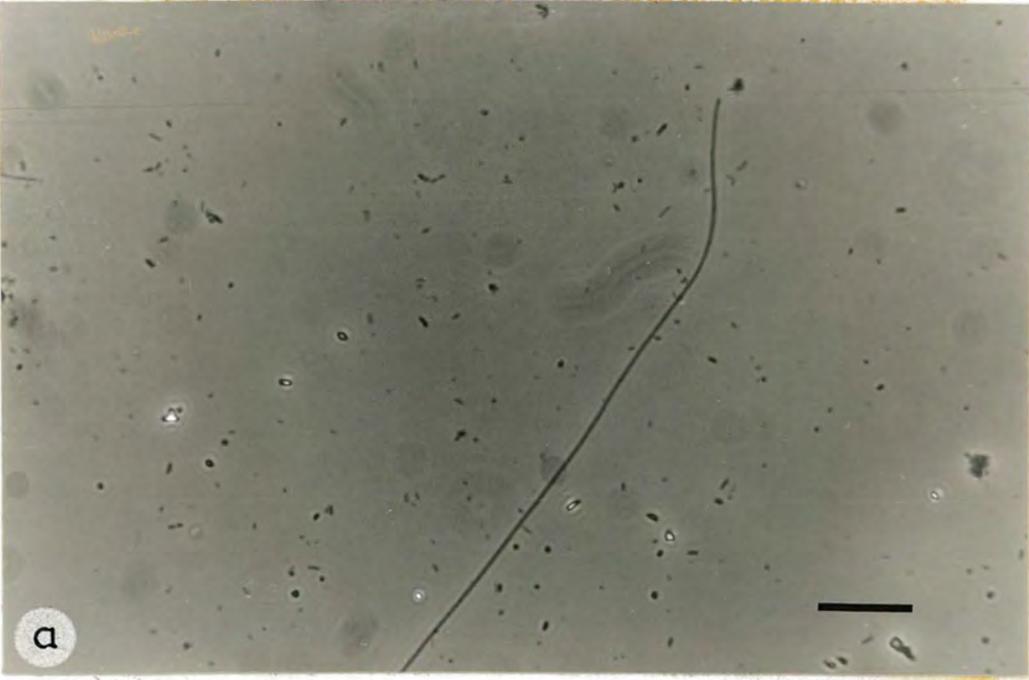


Plate 15 Group 3 organisms (Flexibacter) (a), (b) and (c) show different forms of the filament during its movement. Bar represents 20 μm .



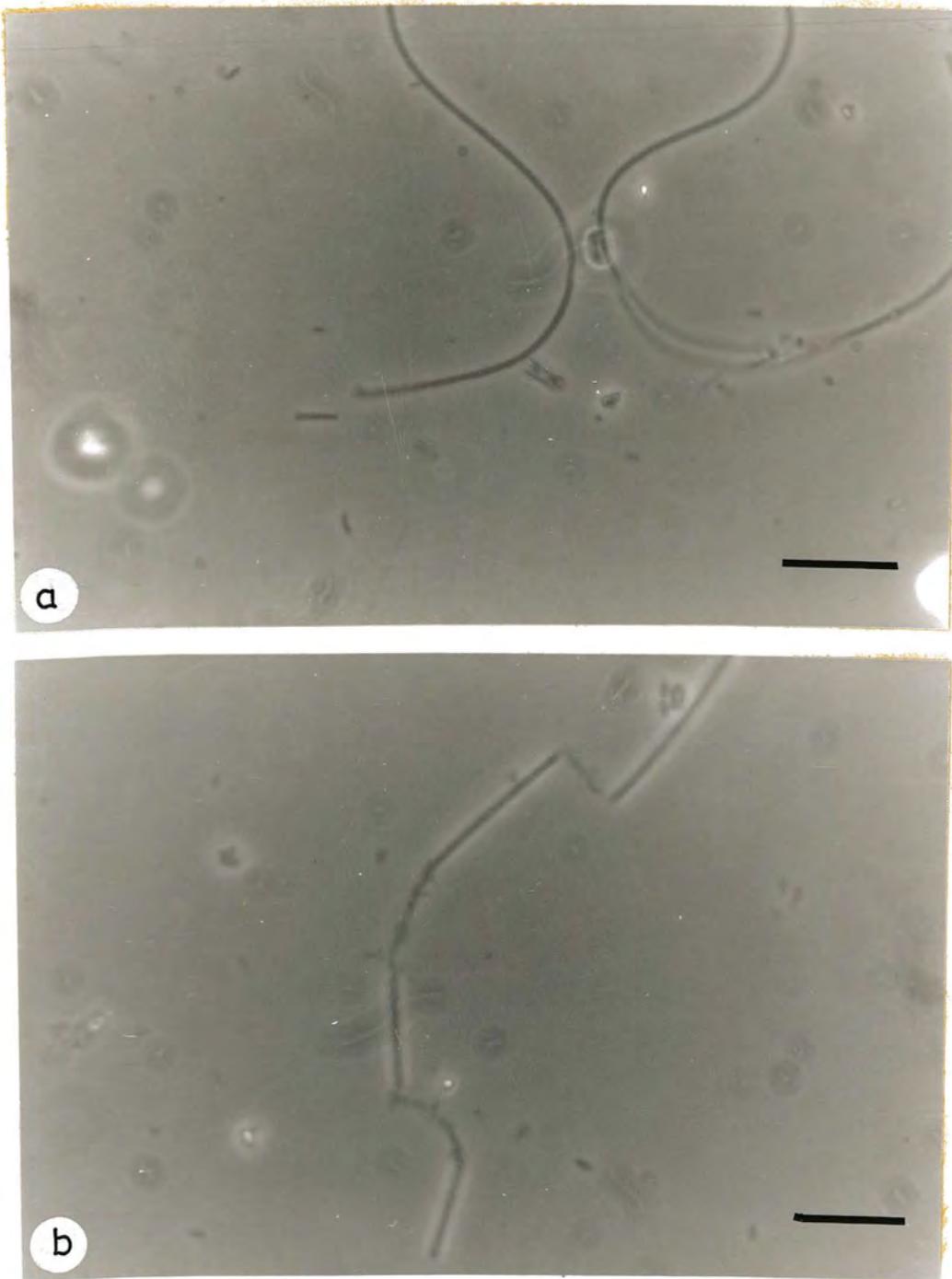


Plate 16. Group 3 organisms (Flexibacter). Filament before (a) and after (b) fragmentation during microscopic observation. Bar represents 10 μm .

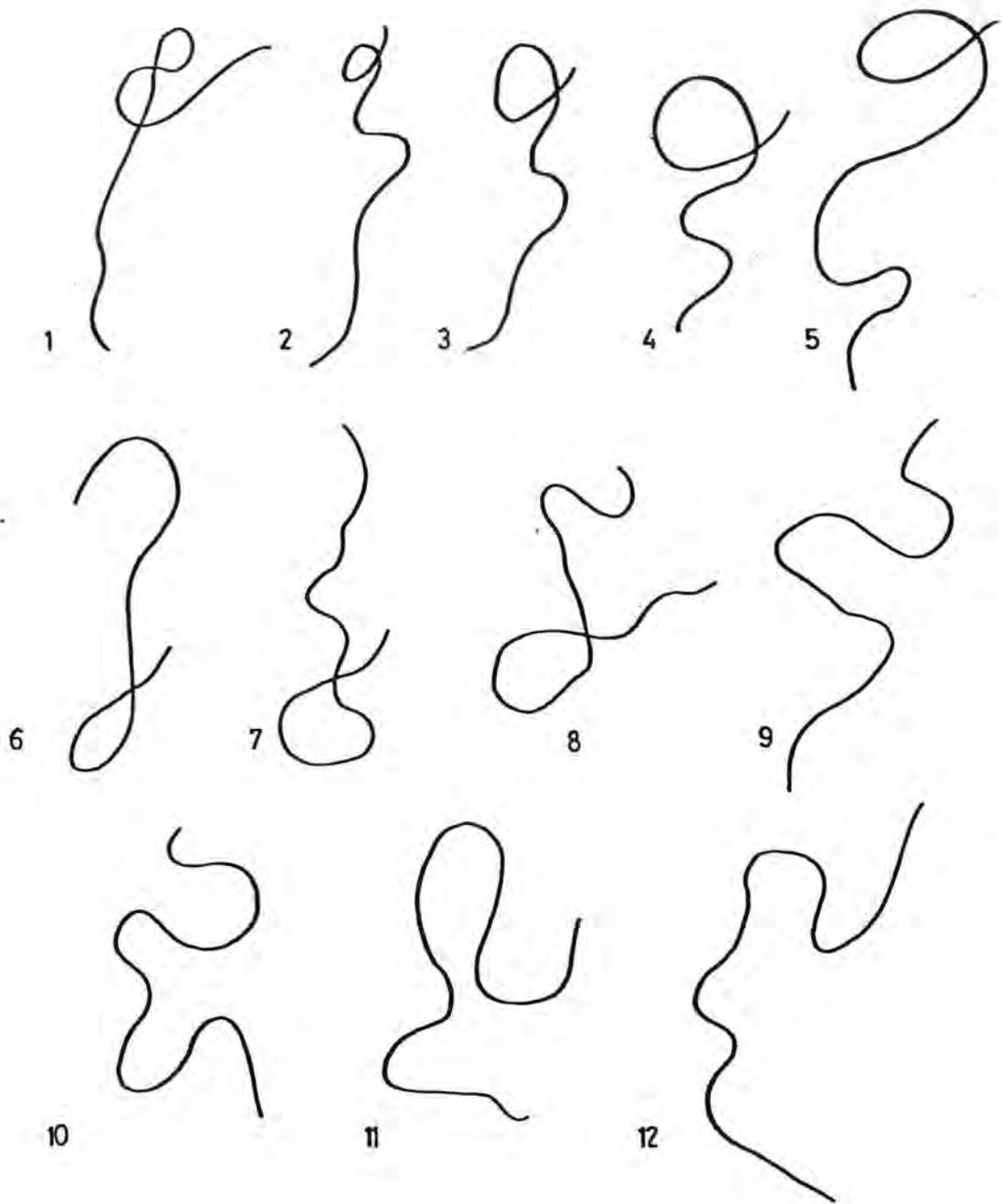


Fig. 62. Sequence of movements of Group 3 organisms (*Flexibacter*) growing in the piece of cellophane plus heterotrophic medium inoculated with 0.5 ml of sediment from Blelham Tarn, incubated for a week at 15°C. Approximately x 500.

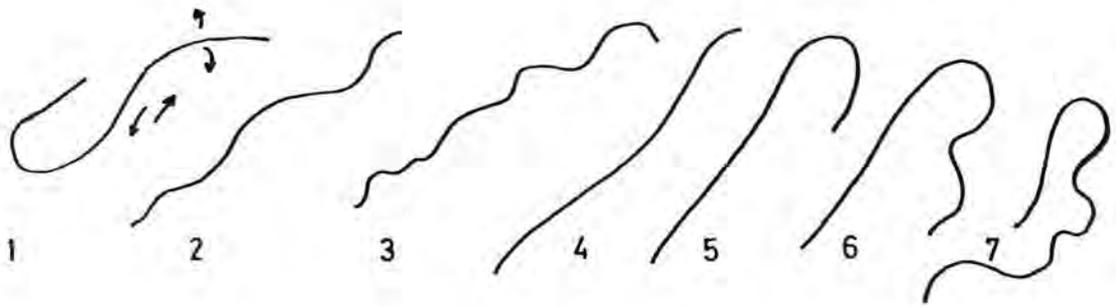


Fig. 63. Sequence of movements of Group 3 organisms (Flexibacter) observed on slide incubated for 9 days at 8°C in sediment core from Windermere South Basin. The above sequence was taken in 20 seconds. The organism was moving at $4.5 \mu\text{m s}^{-1}$. Approximately $\times 500$.

Group 4a. These organisms occur mainly in Blelham Tarn; a single filament which glided in a similar way to Beggiatoa. It was dark blue under phase contrast-microscopy and most of the time contained inclusions of various sizes. The length of the filament ranged from 352 μm to 435 μm ; the cell width ranged from 1 to 2 μm and the length from 7 μm to 11 μm (this variation in cell length could be observed in a single filament). The results of the cytochemical tests were as follows:

Lipid inclusions : positive.

Volutin granules : positive.

Sulphur inclusions : positive.

Iron deposition : negative.

Sheath stain : negative.

Spore stain : negative.

Cell wall stain : positive.

Gas vacuoles : negative.

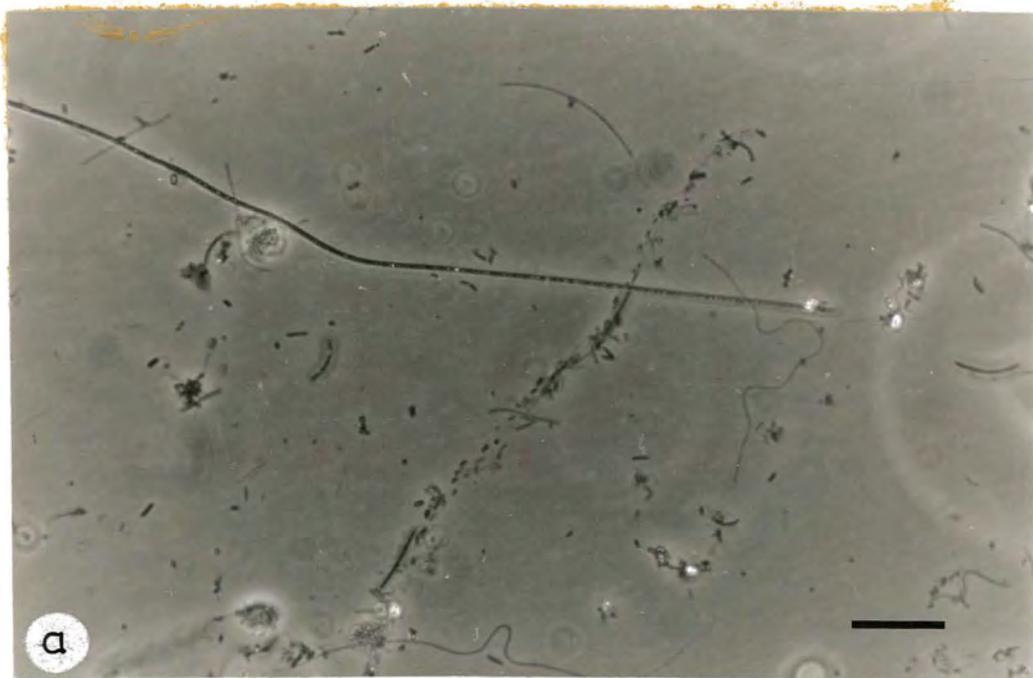
Flagella stain : negative.

Gram stain : negative.

Chlorophyll fluorescence : negative.

Examples of Group 4a organisms are illustrated in Plates 17 and 18 and in Fig. 64.

Plate 17. Group 4a organisms (Beggiatoa leptomitiformis)
observed in Windermere South Basin. The cell
inclusions observed in (a), (b) and (c) are sulphur
granules. Bar represents 20 μm .



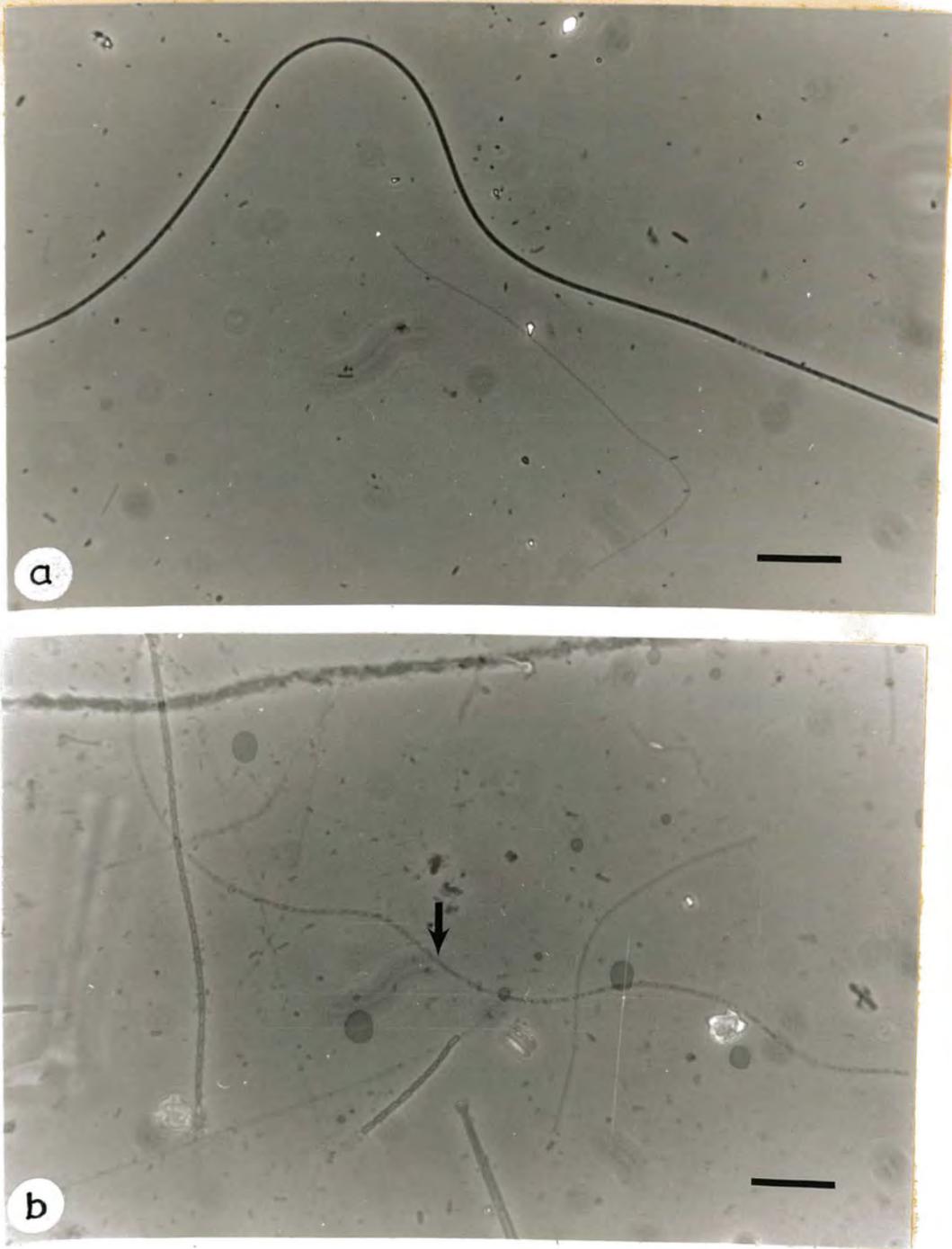


Plate 18. Group 4a organisms (Beggiatoa) (a) showing sulphur inclusions (b) the arrow shows a filament in which the sulphur granules have been dissolved with pyridine. Bar represents 20 μm .

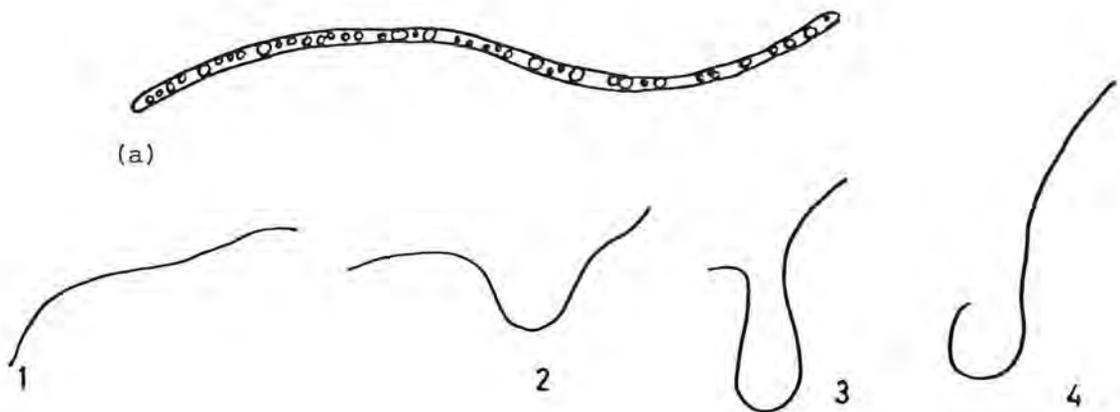


Fig. 64. Sequence of movement of Group 4a organism (Beggiatoa) observed on agar coated slides incubated in sediment cores from Windermere South Basin for a week at 8°C. The filament was 1 by 176 μm . (a) large scale drawing. 1 - 4 approximately $\times 250$.

Group 4b. A single filament with a gliding movement similar to Beggiatoa. It was bluish under phase-contrast microscopy and cell walls not evident. The length of the filaments ranged from 76.5 μm to 940 μm , the cells had width ranging from 1.6 μm to 2.4 μm and length ranging from 8.4 μm to 11.6 μm . Table 19 provides some values for the range of dimensions encountered. Occur mainly in Blelham Tarn. The results of the cytochemical tests were as follows:

Lipid inclusions : positive.

Volutin granules : positive.

Sulphur inclusions : negative. On one occasion some observed non refractile inclusions were dissolved after the pyridine test. The addition of pyridine did not alter the colour nor cause the filament to shrink.

Iron deposition : negative.

Sheath stain : variable.

Spore stain : negative.

Cell wall stain : positive.

Gas vacuoles : negative.

Flagella stain : negative.

Gram stain : negative.

Chlorophyll fluorescence : negative.

Examples of Group 4b organisms are illustrated in Plates 19 and 20 and in Fig. 65.

Table 19. Measurements of individual filaments of Group 4b observed at different sampling occasions. Observations on agar coated slides incubated in the sediment at 8 °C.

Filament length (μm)	Cell	
	width (μm)	length (μm)
76.5 - 940	N.M.	4 - 6
80	2	9 - 12
356	2.5	8.5 - 10.5

N.M. = not measured

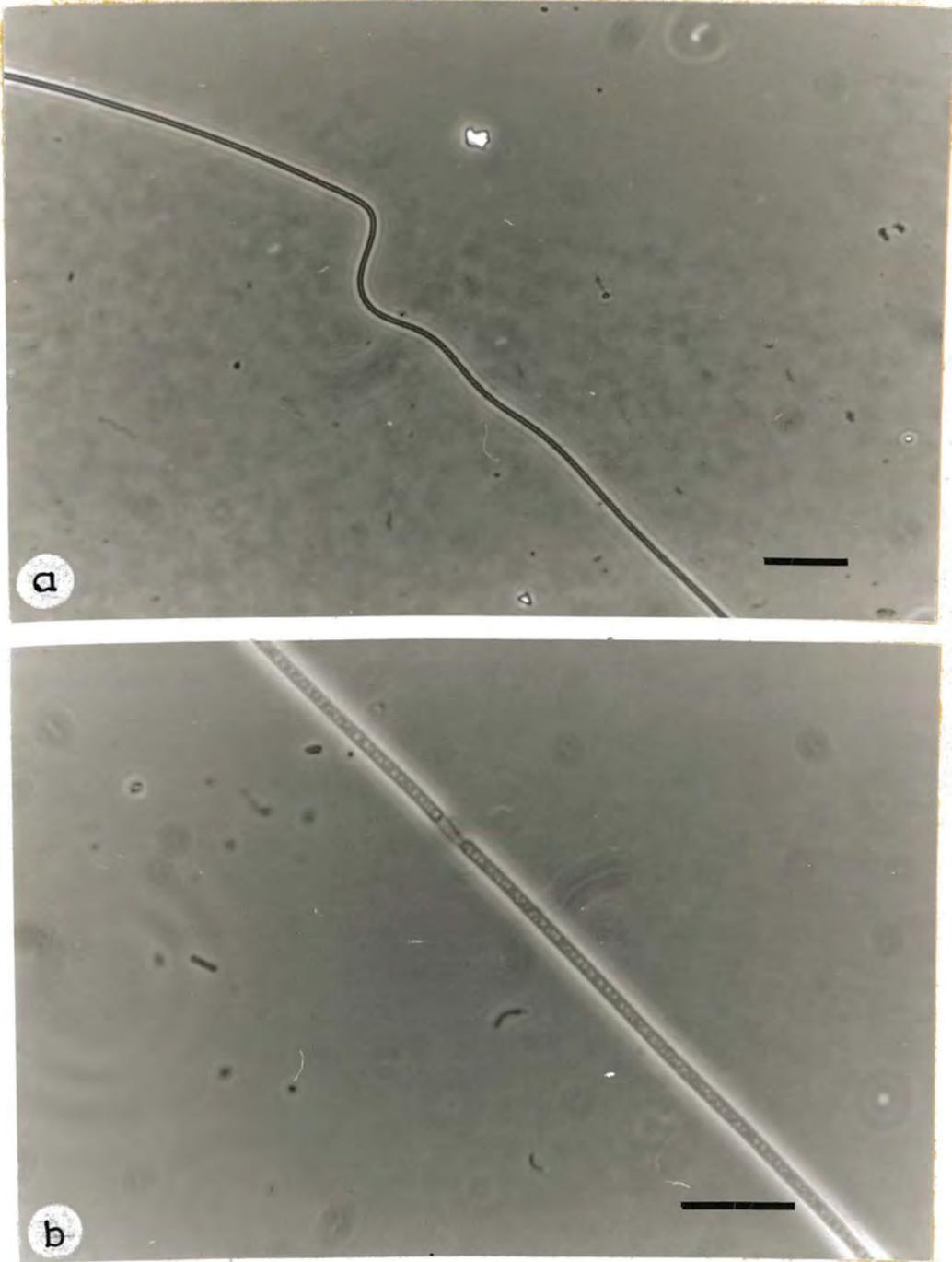


Plate 19. Group 4b organisms (Achromonema) (a) and (b) filaments observed in 0.3% agar sausage incubated in the sediment core from Blelham Tarn at 15 °C for 7 days. Bars represent 20 μm (a) and 10 μm (b)

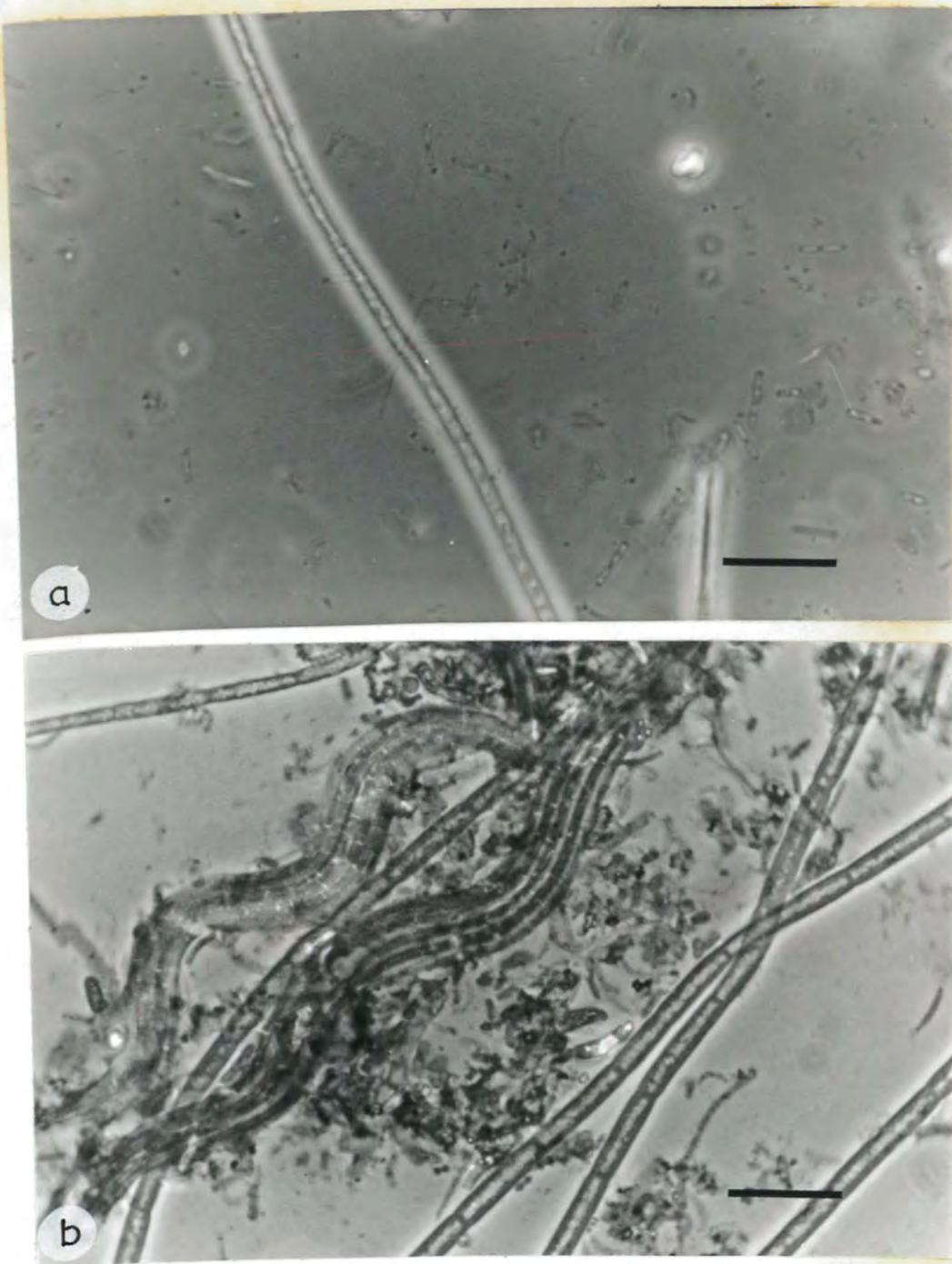


Plate 20. Group 4b organisms (Achronema) (a) filament observed on agar coated slides (b) filaments after cell wall staining. Bar represents 10 μ m.

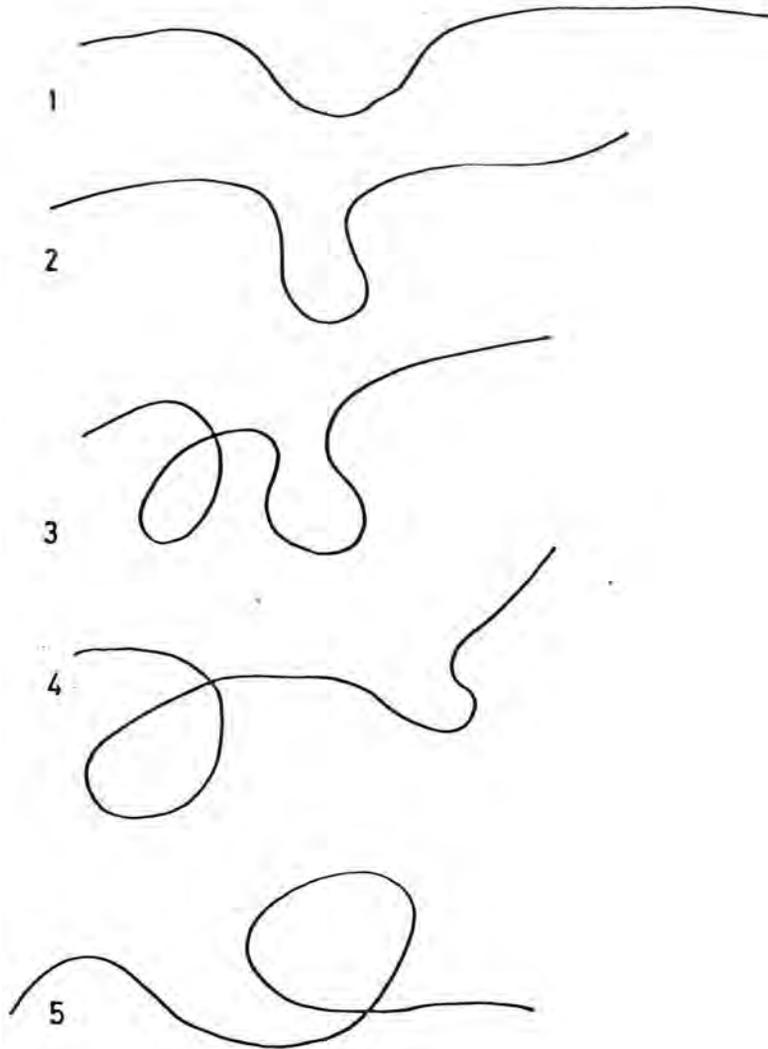


Fig. 65. Group 4b organisms (Achronema) observed on agar coated slides incubated in the sediment core from Mitchell Wyke, Windermere (Fig. 2) incubated for a week at 15°C. The changes of form of this group are much slower than Group 3 organisms. Length approximately x 500.

Group 5b: Single gliding filaments, black under phase-contrast microscopy with clear non refractile granules along the filament. The filaments showed a characteristic gliding action very often forming "loops". The organisms occur in Windermere South Basin and Blelham Tarn. The filaments were 1.2 μm - 2.4 μm by 26 μm - 226 μm . The cytochemical characteristics were as follows:

Lipid inclusion : positive (sometimes weak).

Volutin granules : positive.

Sulphur inclusions : negative. When pyridine was added no empty spaces typical of sulphur granules dissolution were observed. However, the whole filament exhibited a uniform black colour as the granules were no longer visible.

Iron deposit : negative.

Sheath stain : positive (doubtful).

Spore stain : negative.

Cell wall stain : negative.

Gas vacuoles : negative.

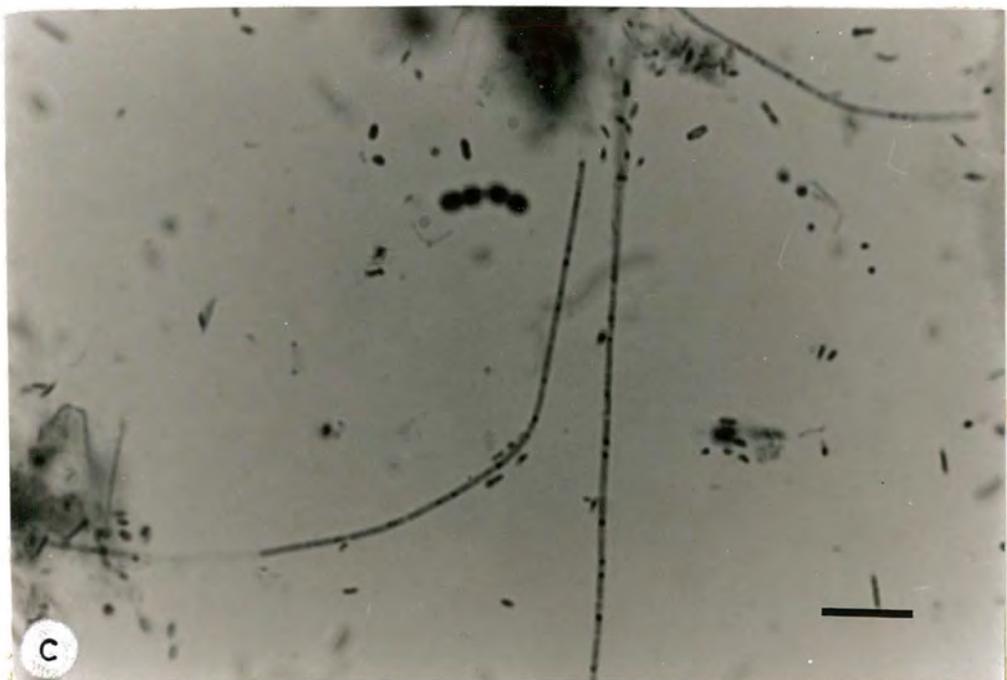
Flagella stain : negative.

Gram stain : not tested.

Chlorophyll fluorescence : negative.

Examples of Group 5b organisms are illustrated in Plate 21 and Fig. 66 and 67.

Plate 21. Group 5b organisms (Flexibacter?) (a) and (b) show the different forms exhibited during gliding action (c) filaments treated with 3% NaCl and stained with Tyler's crystal violet. Bars represent 20 μm (a, b) and 10 μm (c).



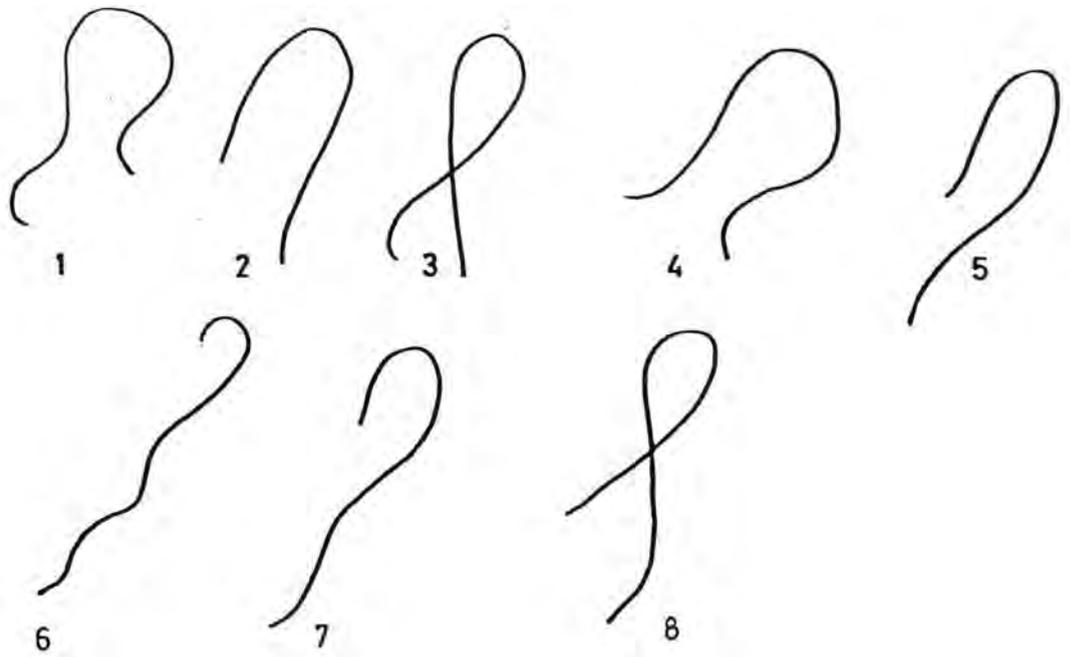


Fig. 66. Sequence of movement of Group 5b organisms (Flexibacter?) observed on uncoated slides incubated in sediment core from Windermere South Basin. The sequence was taken during 1 min. Length approximately $\times 500$.

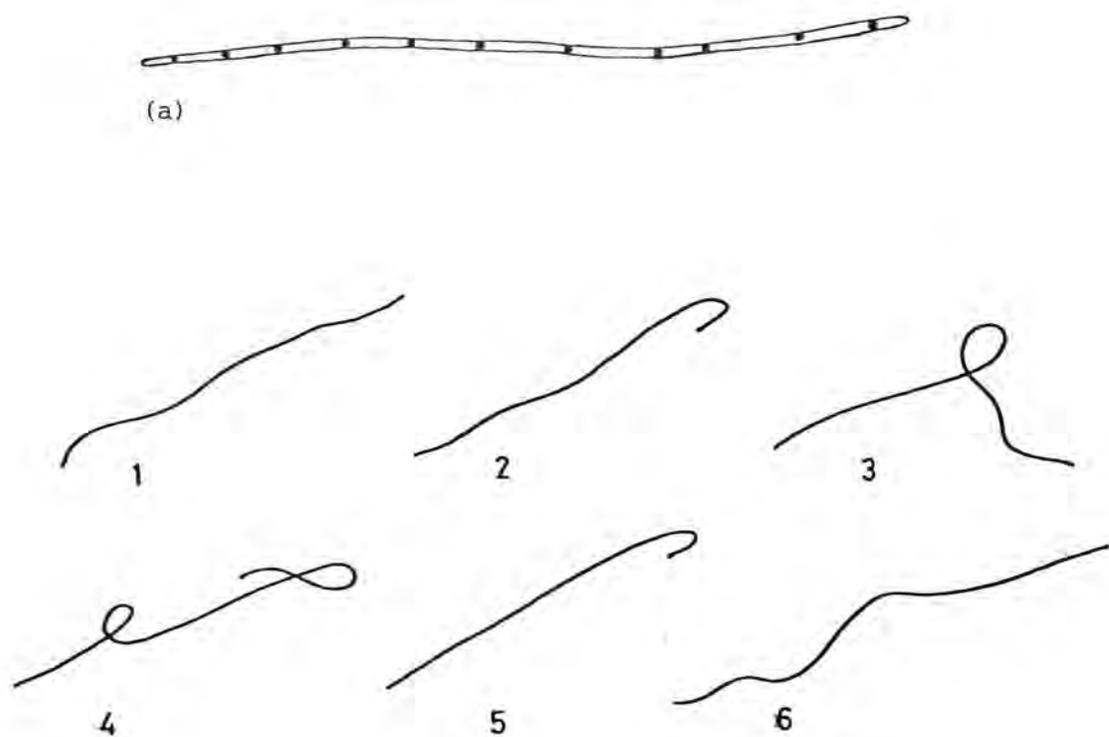


Fig. 67. Organisms observed on one single occasion on chitin medium coated slide incubated for 7 days at 8°C in sediment core from Mitchell Wyke, Windermere (Fig. 2). It was very similar to Group 5b but the length (3 mm) and speed ($13 \mu\text{m s}^{-1}$) had not been observed before for this group. 1 to 6 show the sequence of movements. (a) large scale drawing. 1 - 6 approximately x 250.

Group 6b: Single filaments, non motile although a very slow change of shape has been observed. The filaments were black under phase-contrast microscopy and $0.72\ \mu\text{m} - 1.2\ \mu\text{m}$ by $9.5\ \mu\text{m} - 60\ \mu\text{m}$. The cytochemical tests were as follows:

Lipid inclusions : negative.

Volutin granules : positive.

Sulphur inclusions : negative.

Iron deposit : negative.

Sheath stain : negative.

Spore stain : negative.

Cell wall stain : negative.

Gas vacuoles : negative.

Flagella stain : negative.

Gram stain : negative.

Chlorophyll fluorescence : negative.

Examples of Group 6b organisms are illustrated in Plates 22 and 23.

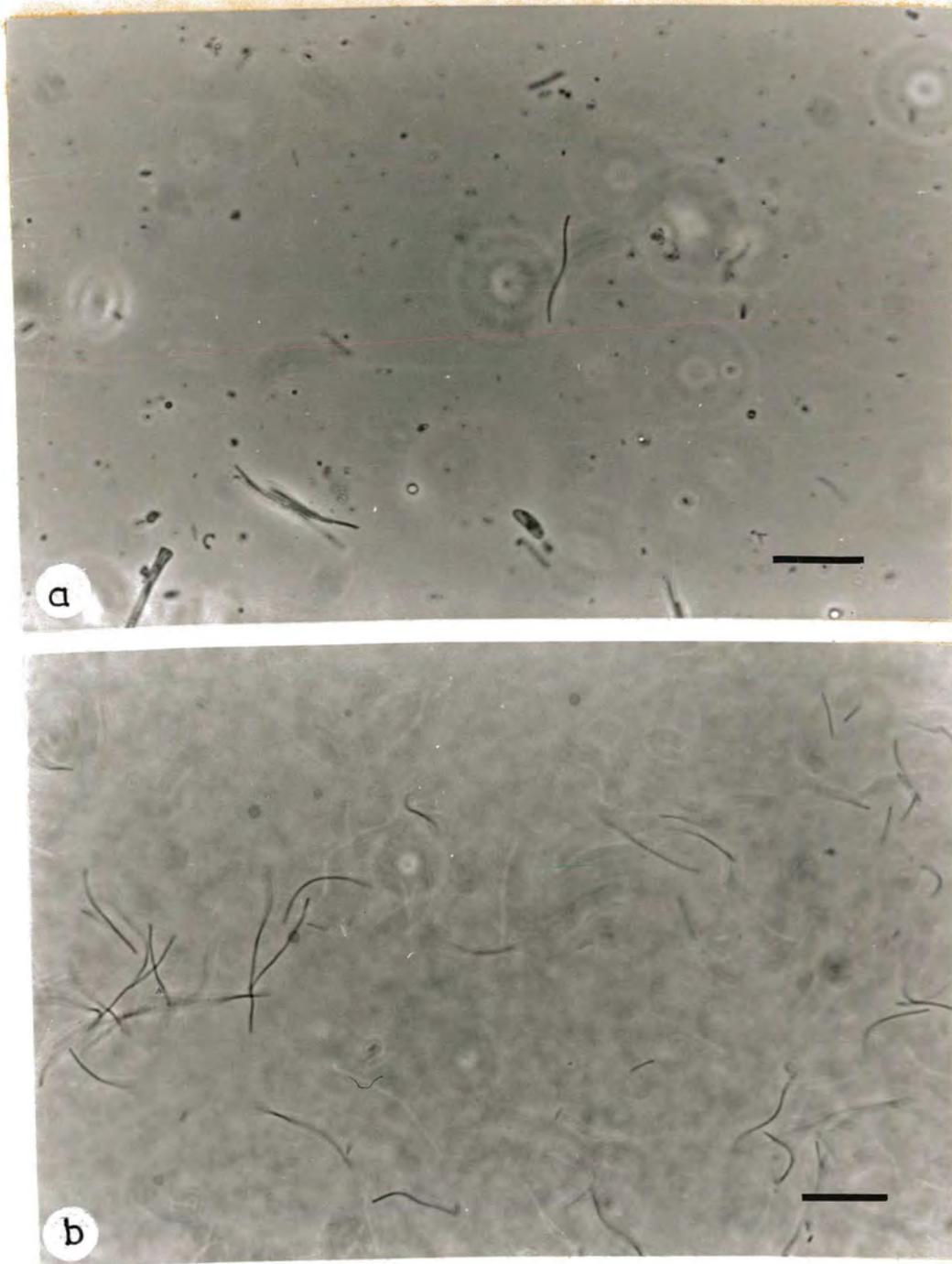
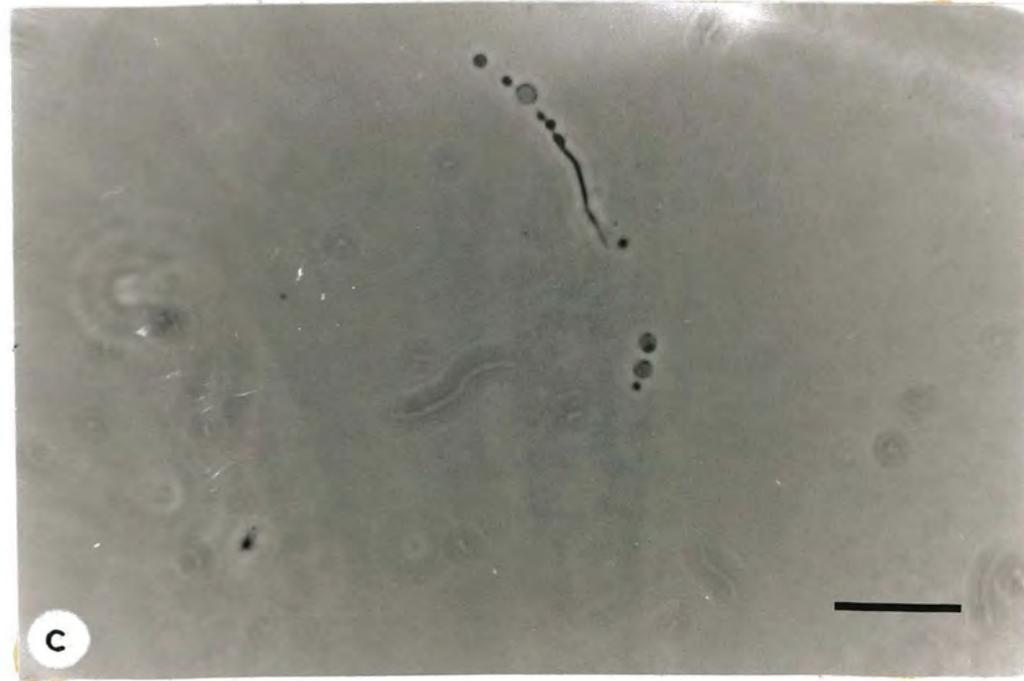
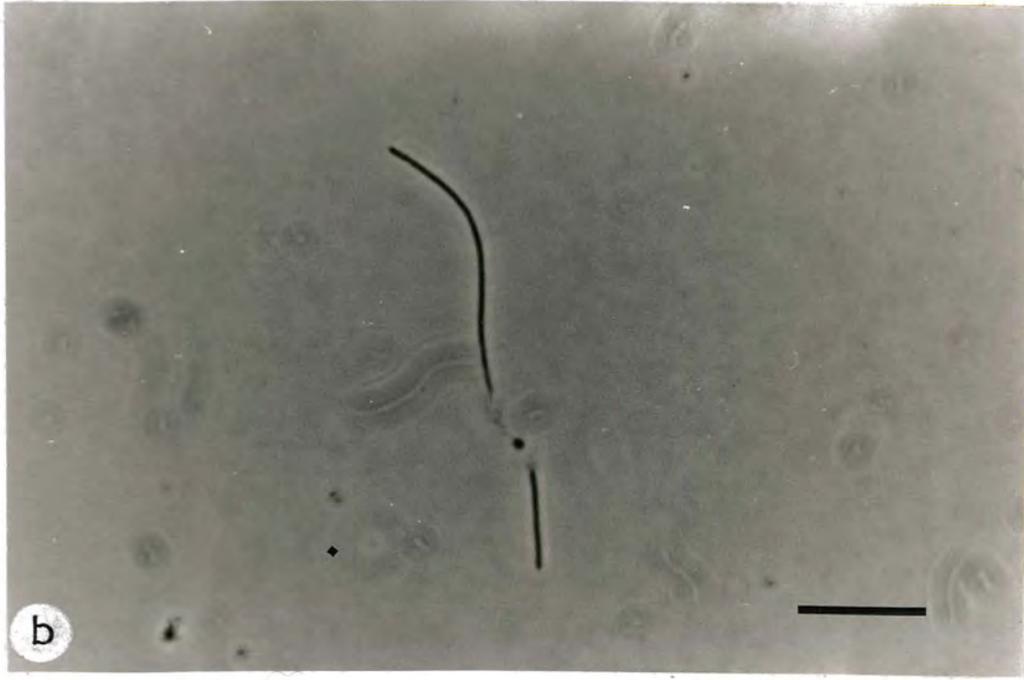
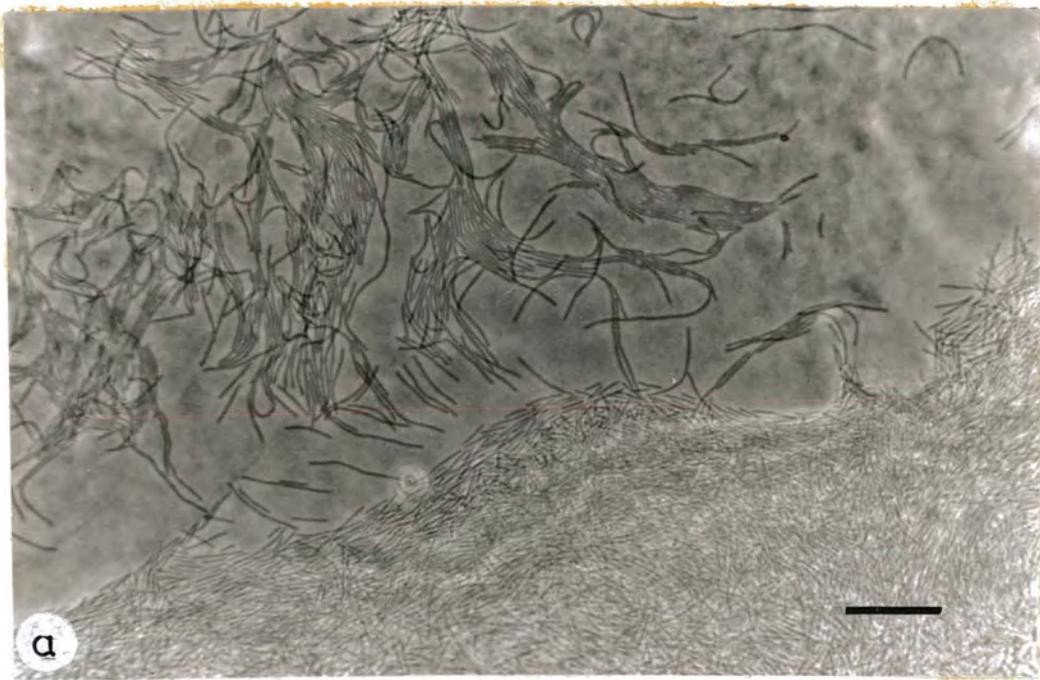


Plate 22. Group 6b organisms (Cytophaga). (a) observed on agar coated slide incubated on sediment core. (b) growing on 0.3% agar sausage incubated for 7 days at 15 °C. Bar represents 20 μ m.

Plate 23 Group 6b organisms (Cytophaga) (a) filaments growing on 0.25% lake water agar; (b), (c) disintegration of the filament during microscopic observation. Bars represent 20 μm (a) and 10 μm (b), (c).



Group 10: Slender filaments within a sheath encrusted with iron, commonly observed in Windermere South Basin. The filaments were straight but not consistently so; non motile although a very gentle almost imperceptible movement along the filament was observed on those which on rare occasions did not have sheath encrusted with iron. The width ranged from 0.2 μm to 0.5 μm and the length from 12 μm to 261 μm . It was not possible to fit this organisms into any of the genera described in Bergey's Manual (1974). The cytochemical tests were as follows:

Lipid inclusions : negative.

Iron deposit : positive.

Sheath stain : positive.

Spore stain : negative.

Cell wall stain : negative.

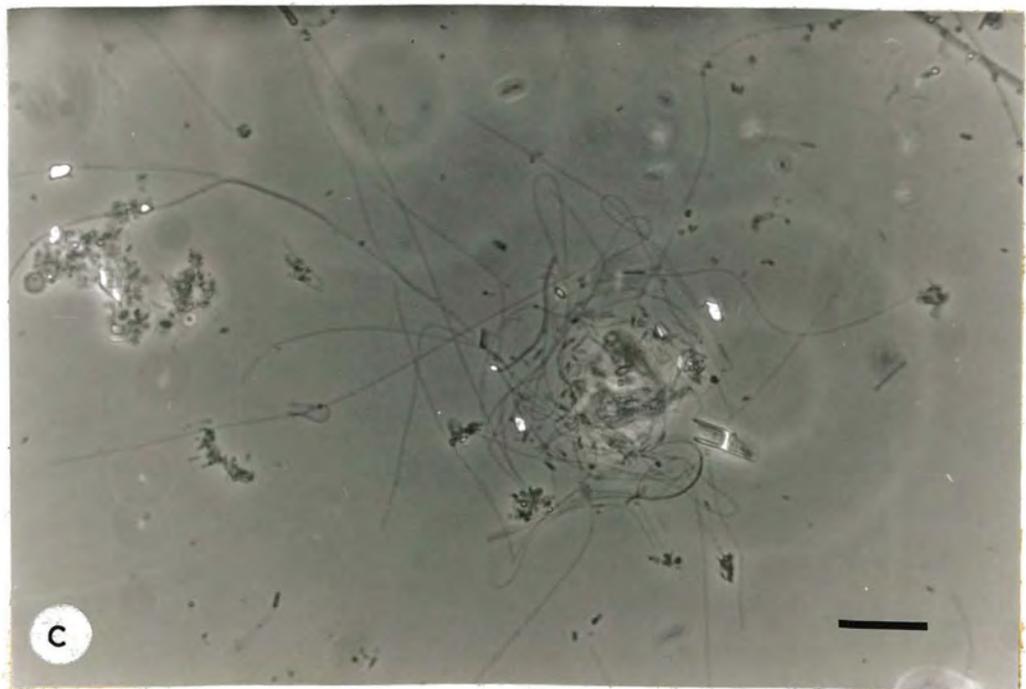
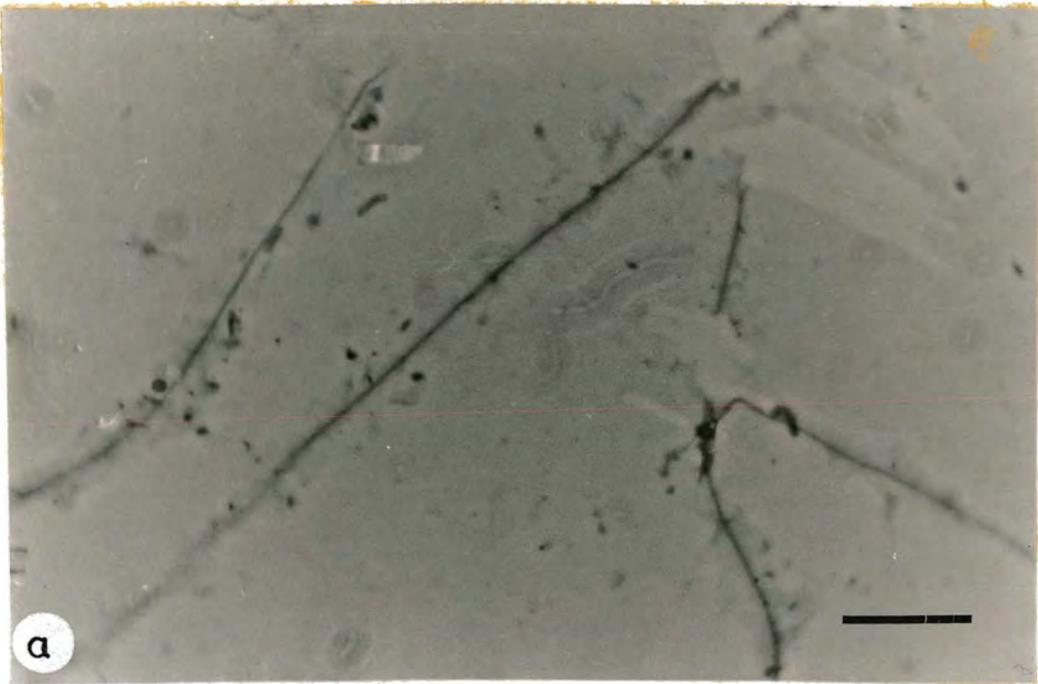
Gas vacuoles : negative.

Gram stain : negative.

Chlorophyll fluorescence : negative.

Examples of Group 10 organisms are illustrated in Plate 24 and Fig. 68.

Plate 24. Group 10 organisms (a) filaments after cell wall staining (b) the arrows show filaments with iron sheath (c) filaments without iron sheath. Bars represent 10 μm (a) and 20 μm (b), (c).



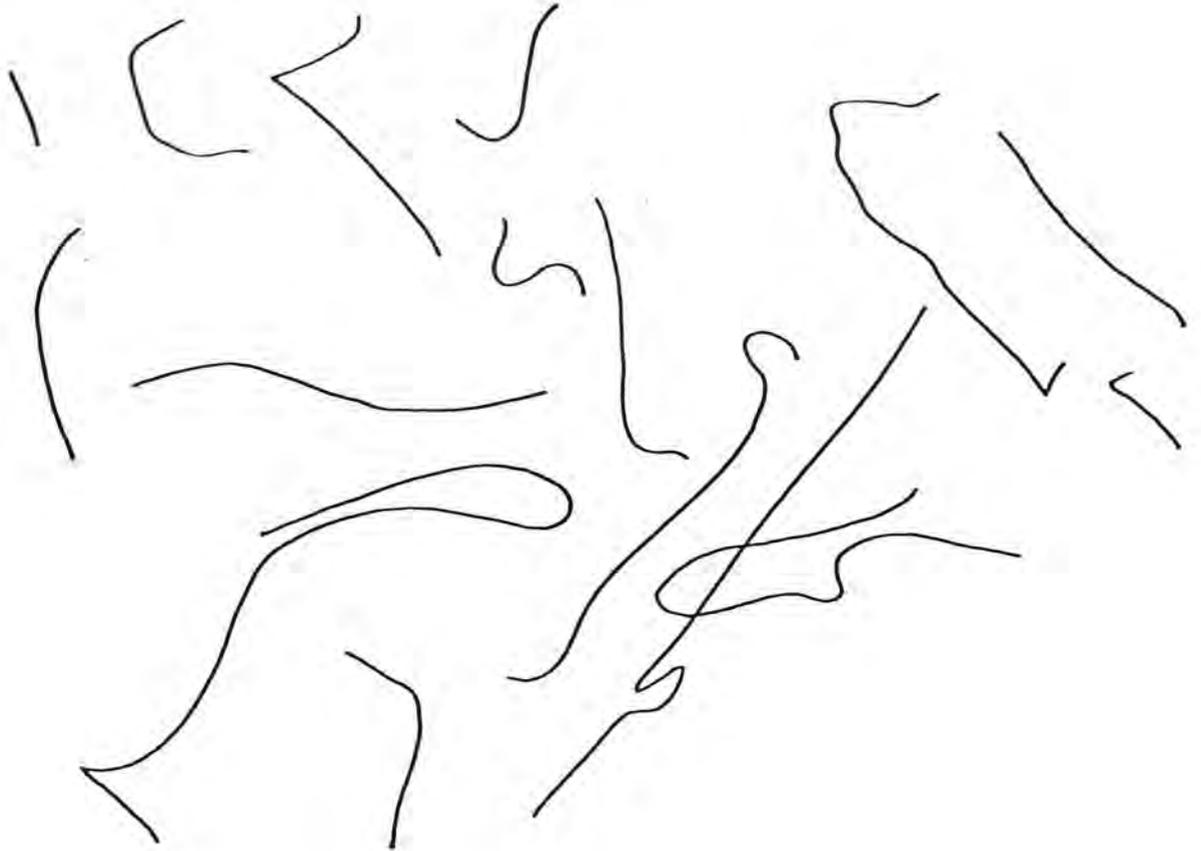


Fig. 68. Group 10 organisms observed in uncoated slides incubated for 6 days at 8°C in sediment cores from Windermere South Basin. Approximately x 500.

Group 15: Single filaments, non motile although on very rare occasions a very slow gliding motion could be observed. The main characteristic is the presence of gas vacuoles. Observed only in Blelham Tarn. The filament length ranged from 26 μm to 632 μm ; The cell width ranged from 0.7 μm to 3 μm and the length from 3.6 μm to 6 μm . The results of the cytochemical tests were as follows:

Lipid inclusions : negative.

Volutin granules : variable.

Sulphur granules : negative. The addition of pyridine collapsed the gas vacuoles leaving an empty black "tube".

Iron deposit : positive although the deposition of iron was not often observed.

Sheath stain : positive. Although the lysozyme test was not performed sheath was observed on several occasions under phase-contrast microscopy.

Spore stain : negative.

Gas vacuoles : positive.

Gram stain : not tested.

Chlorophyll fluorescence : negative.

Examples of Group 15 organisms are illustrated in Plates 25 to 28.

Plate 25. Group 15 organisms (Pelonema). (a), (b) and (c) show sheathed filaments. The filament shown in (c) was left at 4°C overnight and shows the contraction of the gas vacuoles. The arrows show parts of the filament where sheath is evident. Bar represents 20 μm.

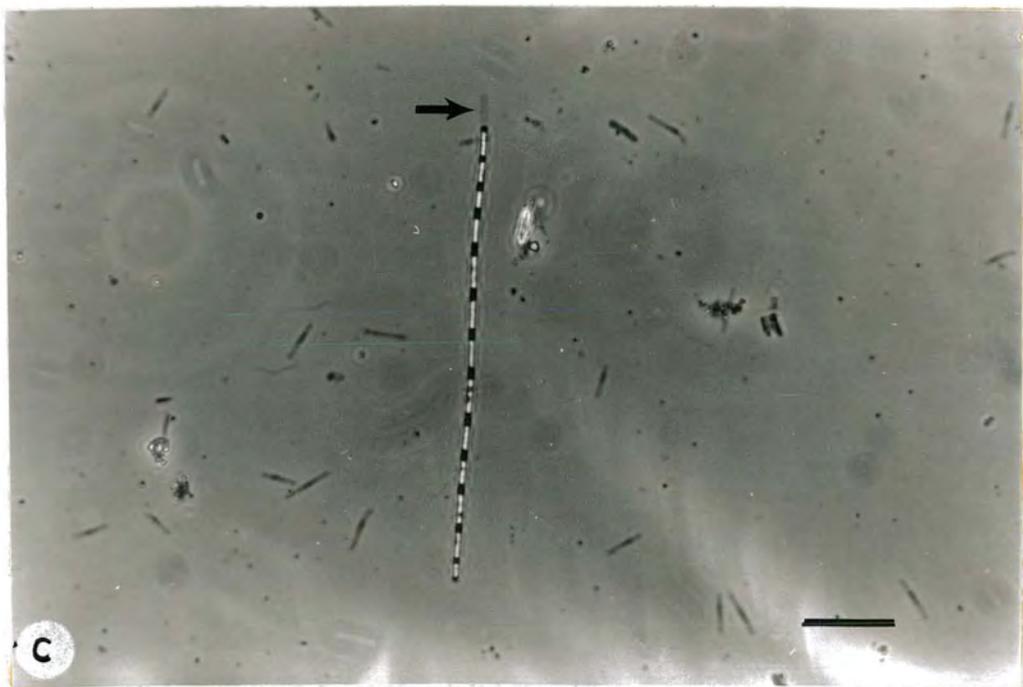
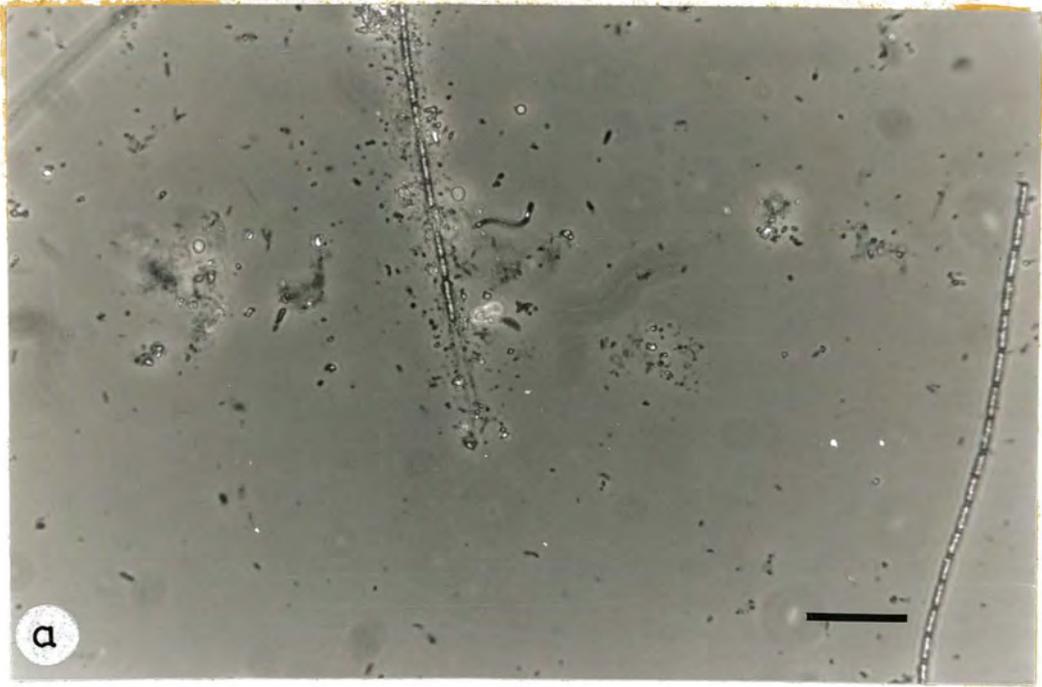
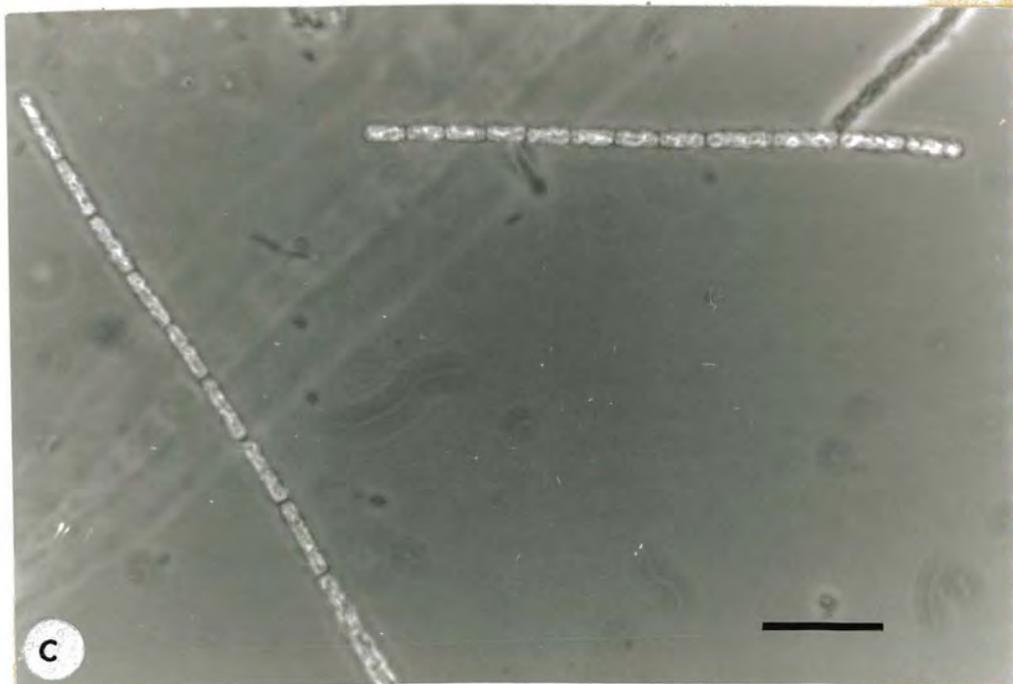
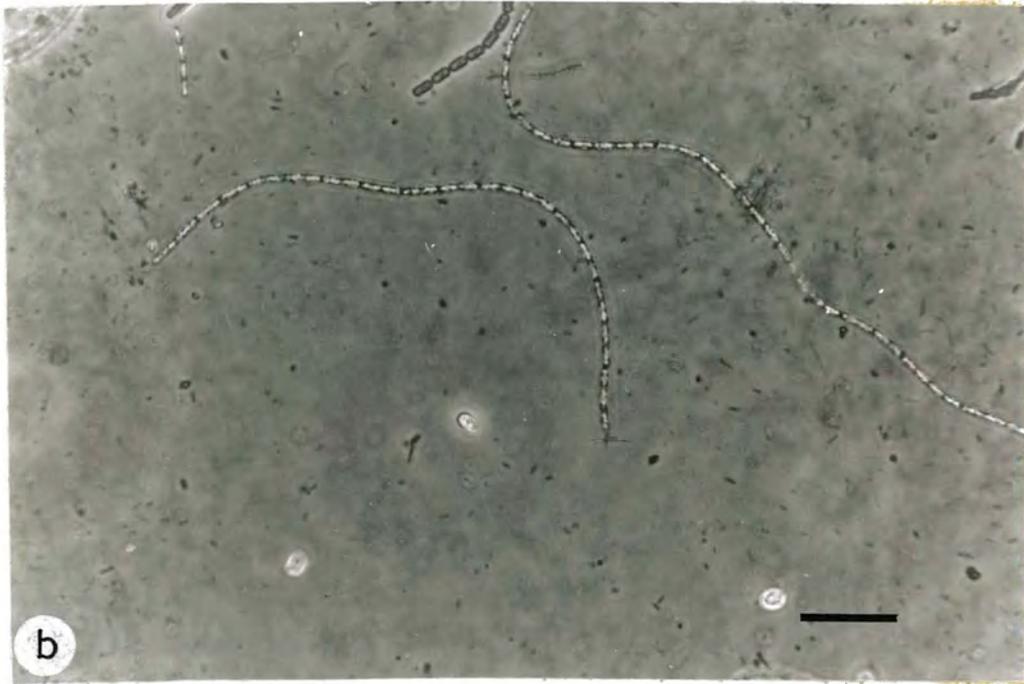
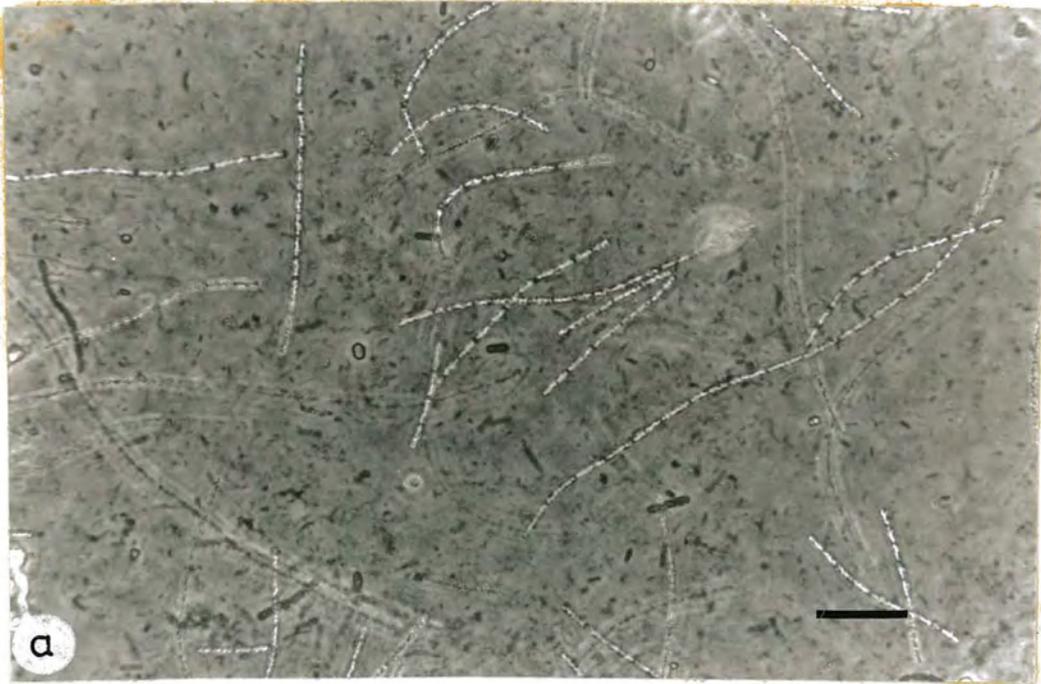


Plate 26. Group 15 organisms (Pelonema) (a) (b) and (c) filaments
observed in 0.3% agar sausage incubated at 15 °C for
7 days. Bars represent 20 μm (a), (b) and 10 μm (c).



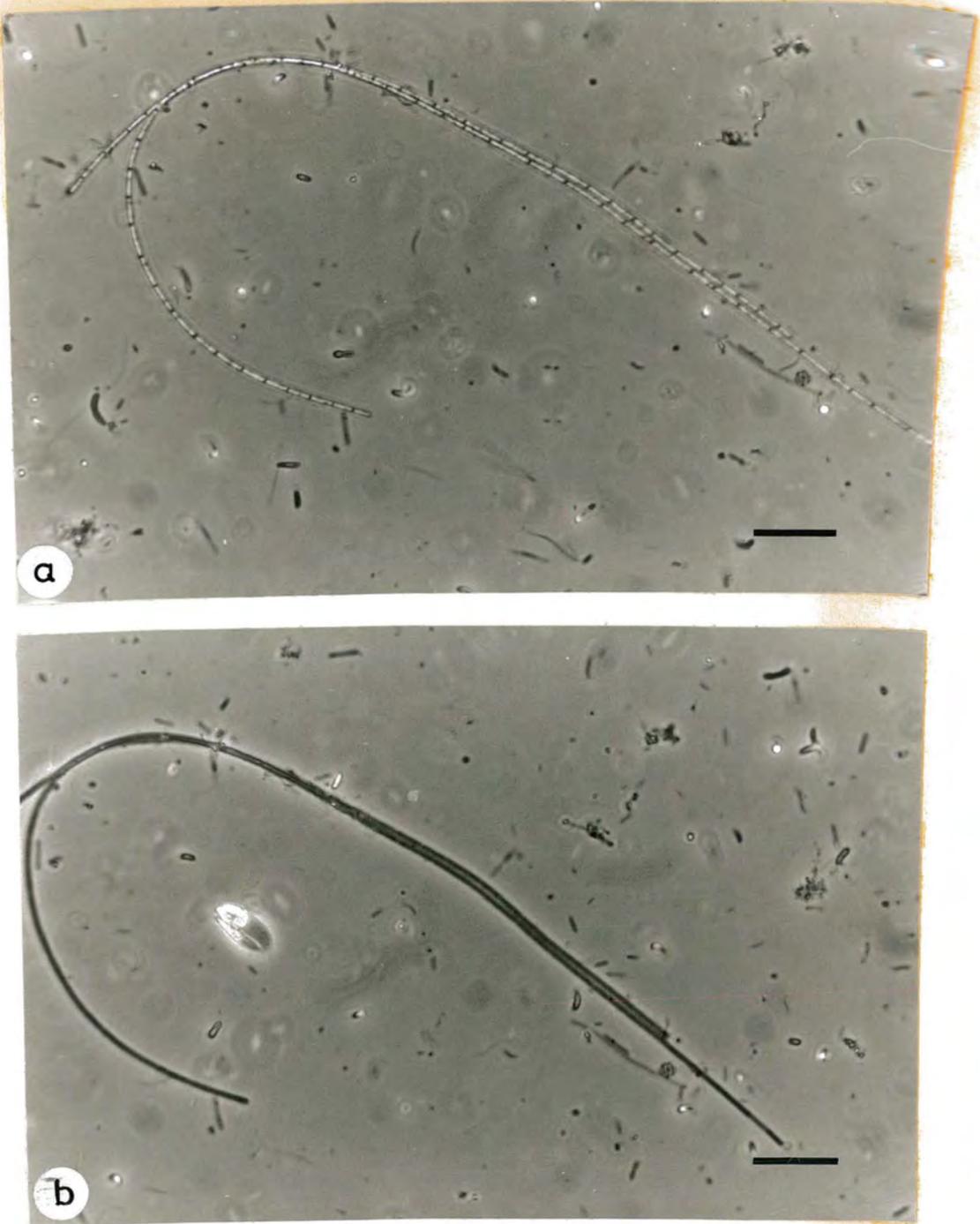


Plate 27. Group 15 organisms (Pelonema). Filaments before (a) and after (b) collapsing gas vacuoles under pressure. Bar represents 20 μm .

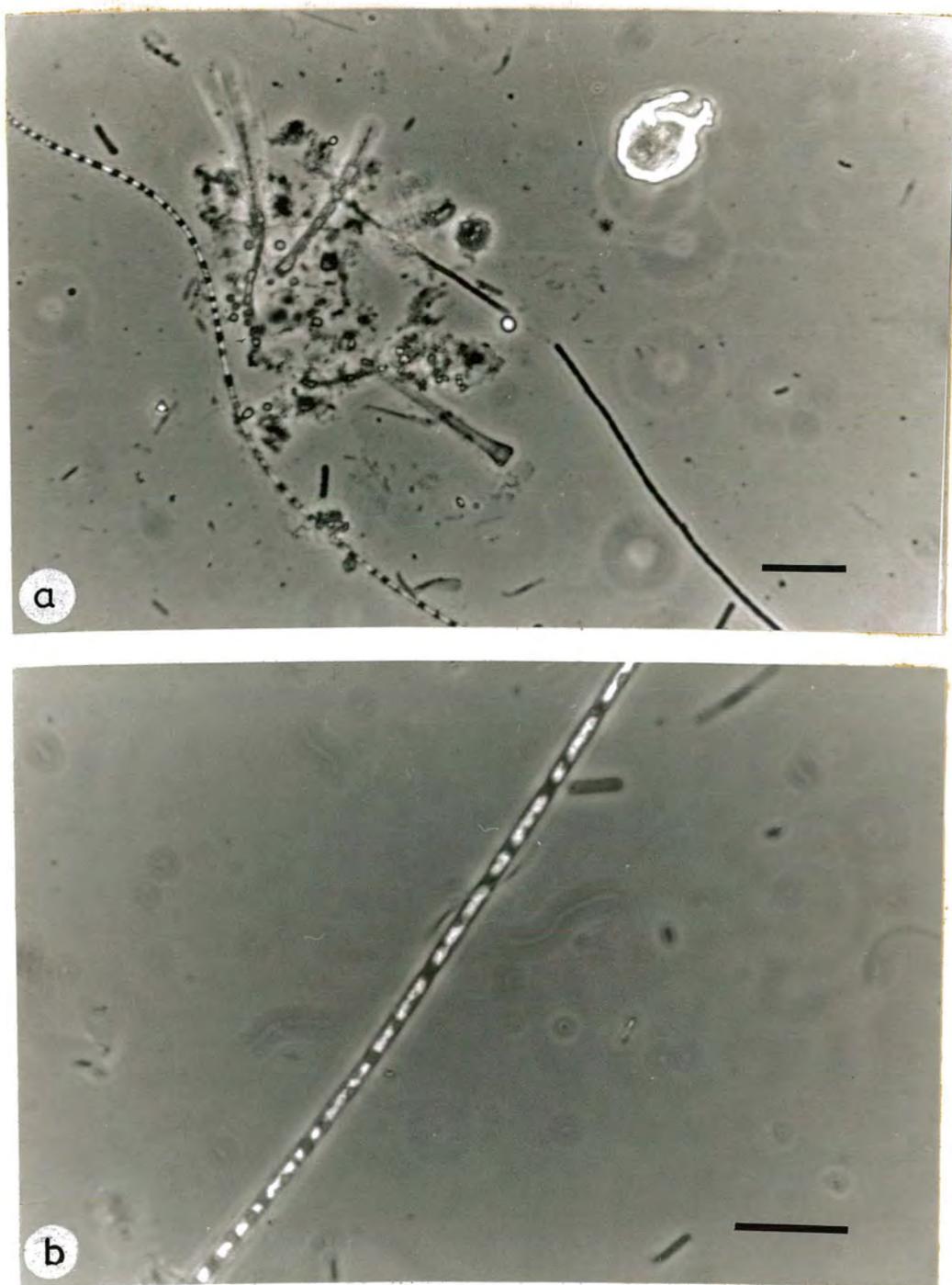


plate 28. Group 15 organisms (Pelonema). (a), (b) Filaments
observed on uncoated slides. Bars represent 20 μm (a)
and 10 μm (b)

Group 16: Gliding single filaments composed of clearly delimited cells. The length of the filament was up to 374 μm and the cells were 0.7 μm by 3.6 μm . The filament was black under phase-contrast microscopy frequently observed forming a "U" shape and an uneven undulation along the filament during the movement was also sometimes observed. Occur mainly in Windermere South Basin. After prolonged microscopic observation the cells may burst. This organism is similar to Bactoscilla nom. prov. described by Pringsheim (1951). The genus Bactoscilla is regarded as incertae sedis in the family Beggiatoaceae (Bergey's Manual, 1974).

The results of the cytochemical tests were as follows:

Lipid inclusions : negative.

Volutin granules : positive, showing one granule at each end of the cell.

Sulphur granules : negative.

Iron deposit : negative.

Sheath stain : negative.

Spore stain : negative.

Cell wall stain : positive.

Gas vacuoles : negative.

Flagella stain : negative.

Gram stain : not tested.

Chlorophyll fluorescence : negative.

Examples of Group 16 organisms are illustrated in Plates 29 and 30.

Plate 29. Group 16 organisms (Bactoscilla?) observed in Windermere South Basin (a) and (b) showing changes in form during the slow gliding motility. (c) filaments observed on slide incubated in the sediment core for 10 days at 8 °C. Bar represents 20 μm.



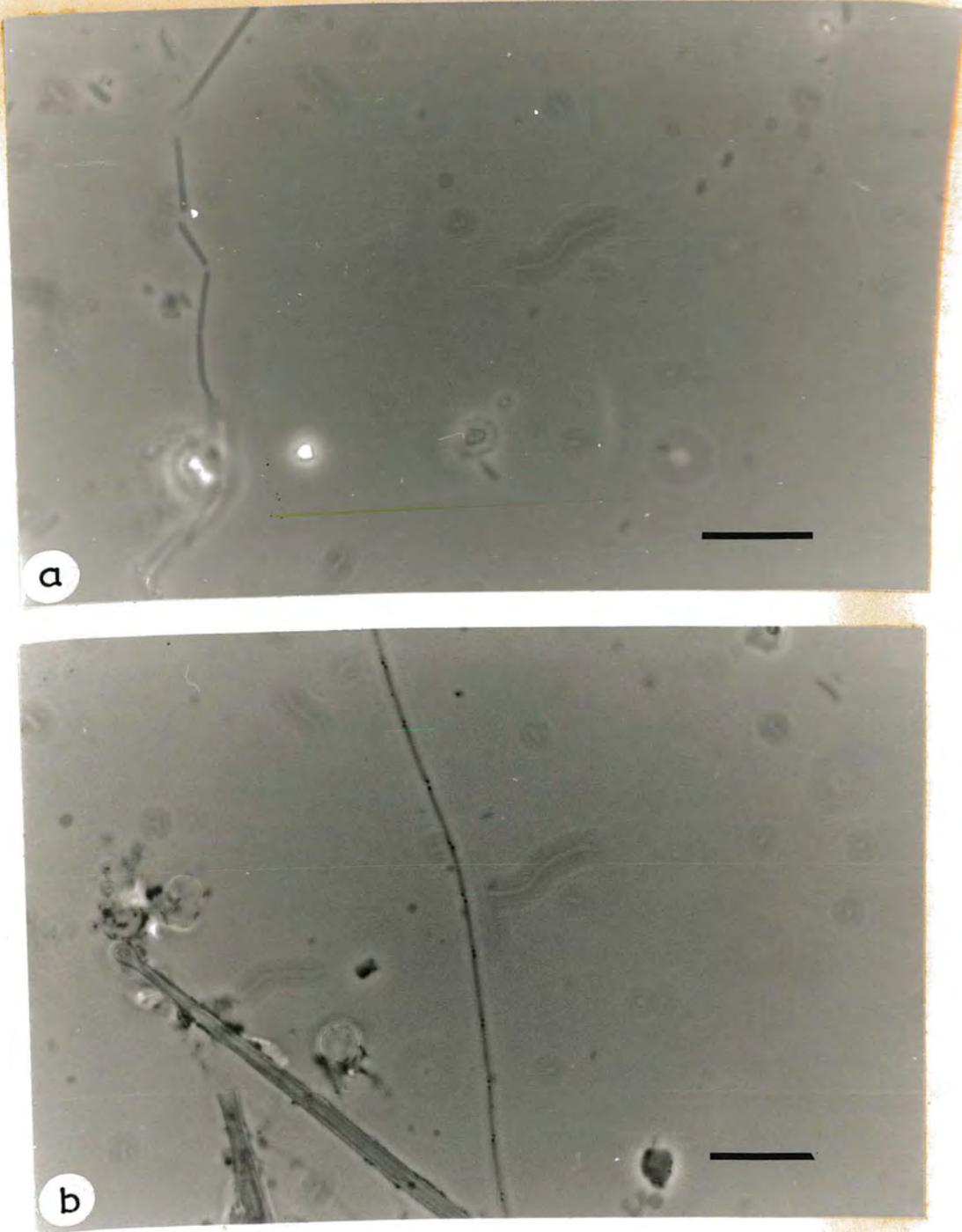


Plate 30. Group 16 organisms (a) disintegration of the filament during microscopic observation (b) filament showing volutin granules (one at each end of the cell). Bar represents 10 μm .

Peloploca: bundles of filaments undulated or in spiral form in which the cells contained gas vacuoles. The width of the bundles ranged from 4.5 μm to 8.1 μm and the length from 40 μm to 140 μm . The cells were 0.9 μm wide by 1.8 - 2.8 μm long. These filaments occurred only in Blelham Tarn.

The cytochemical tests were as follows:

Lipid inclusions : negative.

Volutin granules : positive.

Sulphur inclusions : negative.

Iron deposit : negative.

Sheath stain : not tested.

Spore stain : not tested.

Cell wall stain : positive.

Gas vacuoles : positive.

Flagella stain : negative.

Gram stain : not tested.

Chlorophyll fluorescence : negative.

Peloploca is illustrated in Plates 31 and 32. Plates 33 and 34 illustrate the genus Beggiatoa.

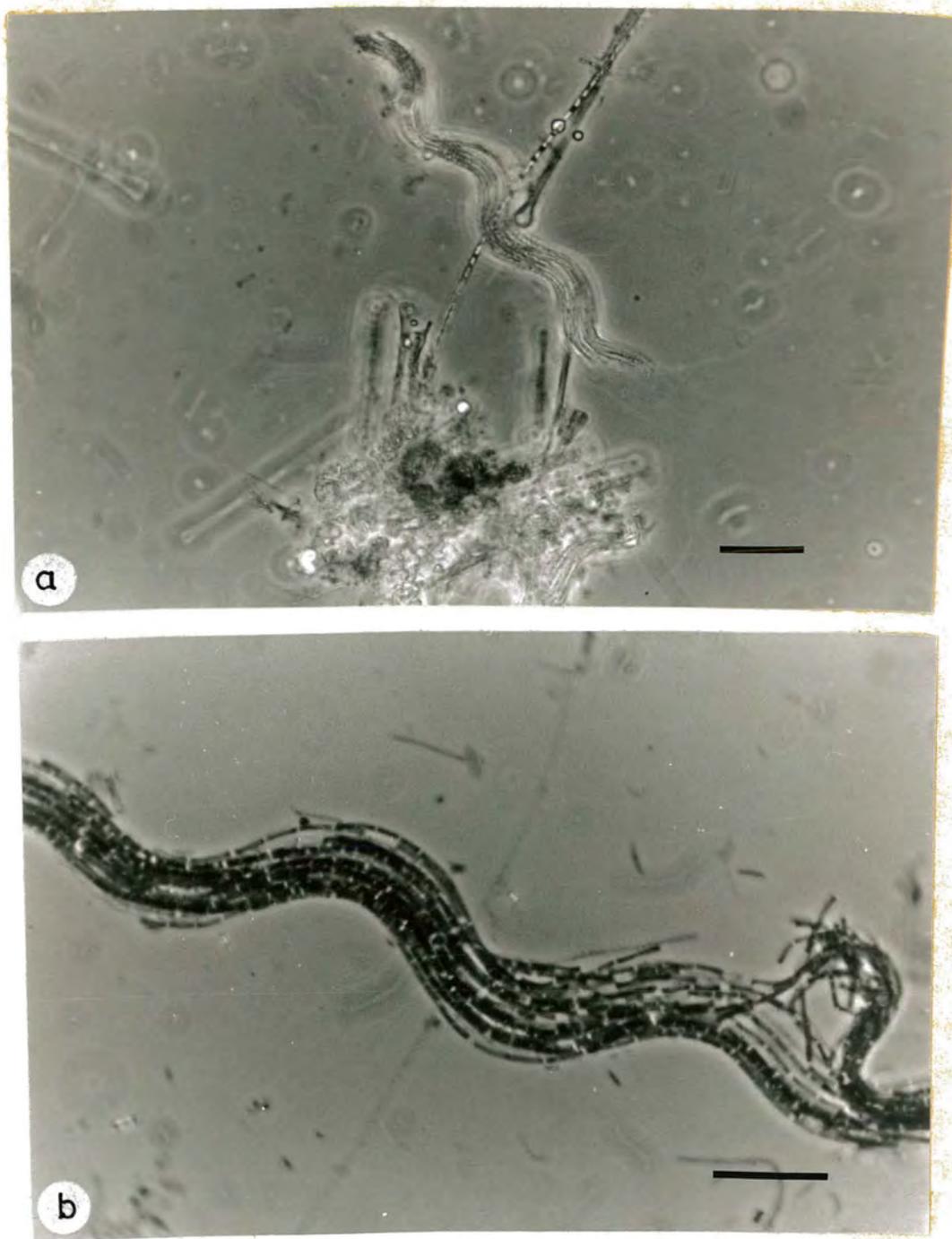


Plate 31. Peloploca (a) organism showing gas vacuoles (b) after staining for volutin granules which made evident the cell walls. Bars represent 20 μ m (a) and 10 μ m (b)

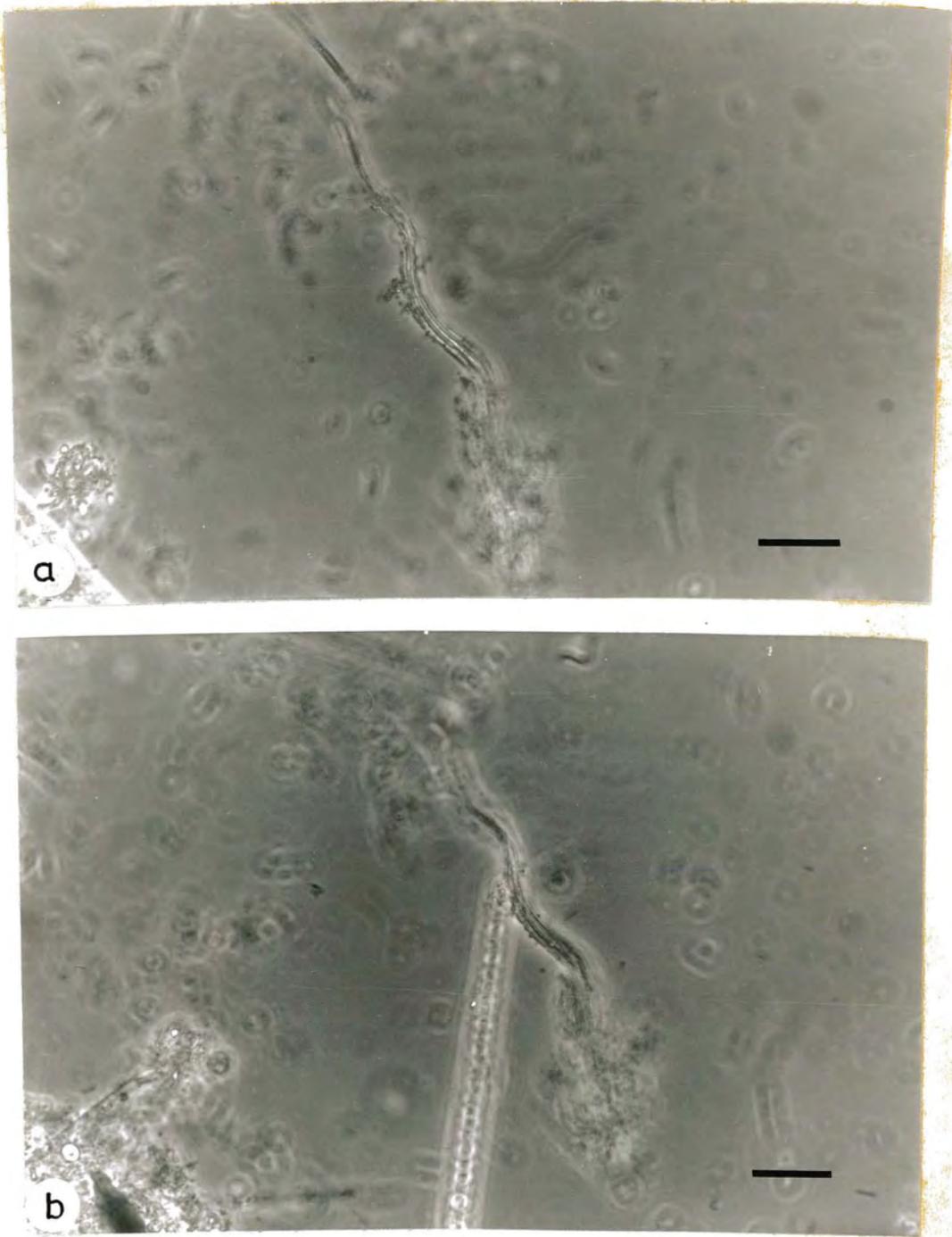
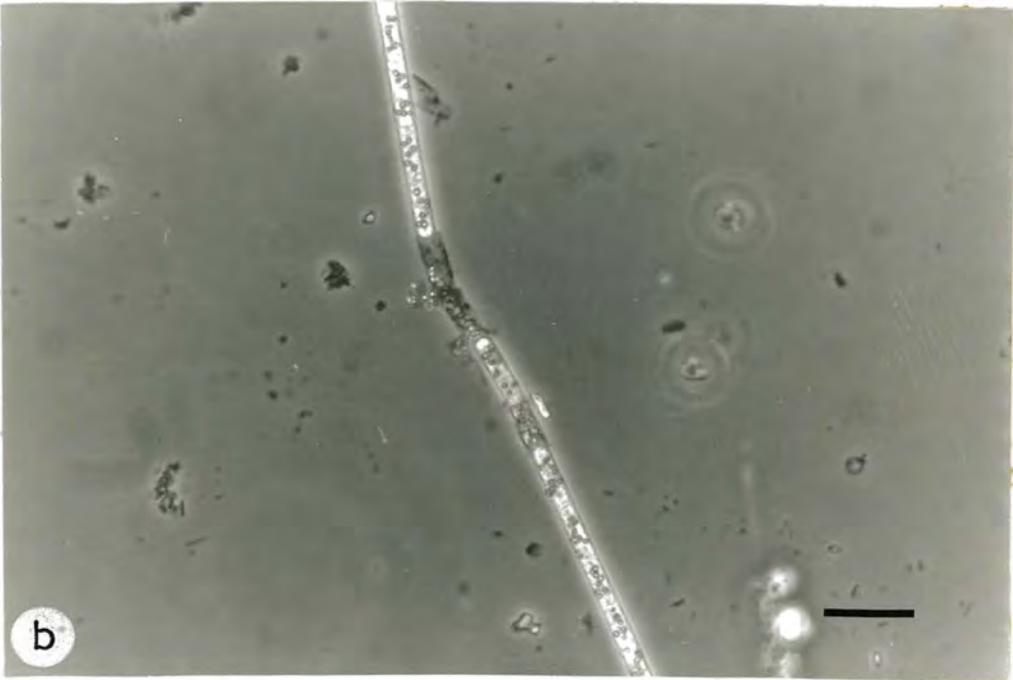
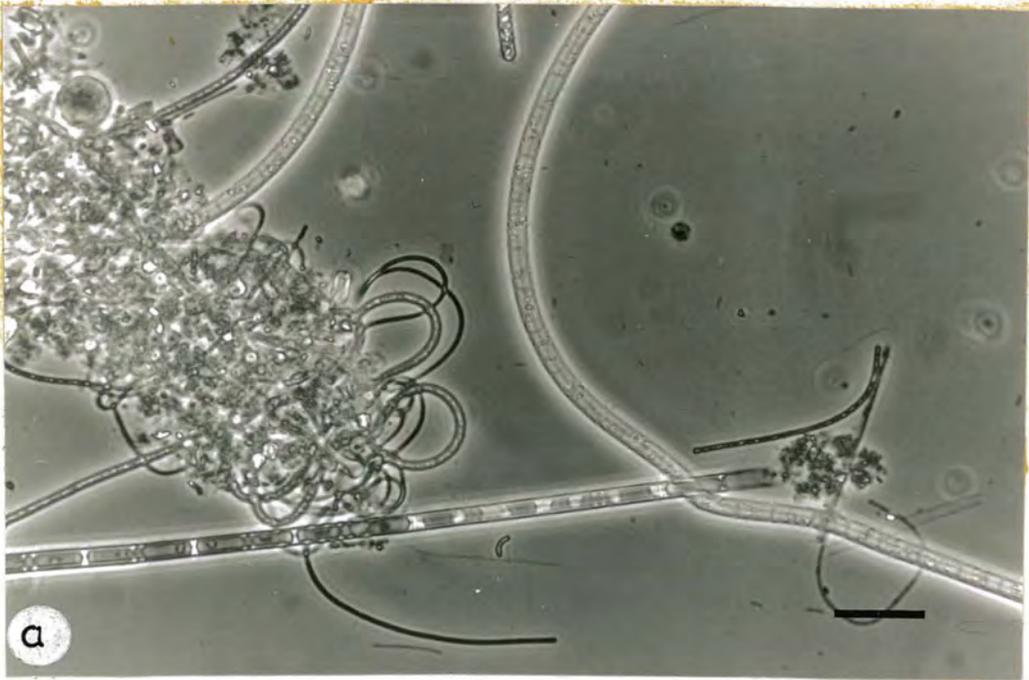


plate 32. Peloploca. (a) before and (b) after collapsing the gas vacuoles under pressure. Bar represents 20 μm .

Plate 33. Beggiatoa (a) gliding filaments (b) filament just after being broken (c) filaments growing in an agar depression, ca 1 cm below sediment-water interface. Bar represents 20 μm .



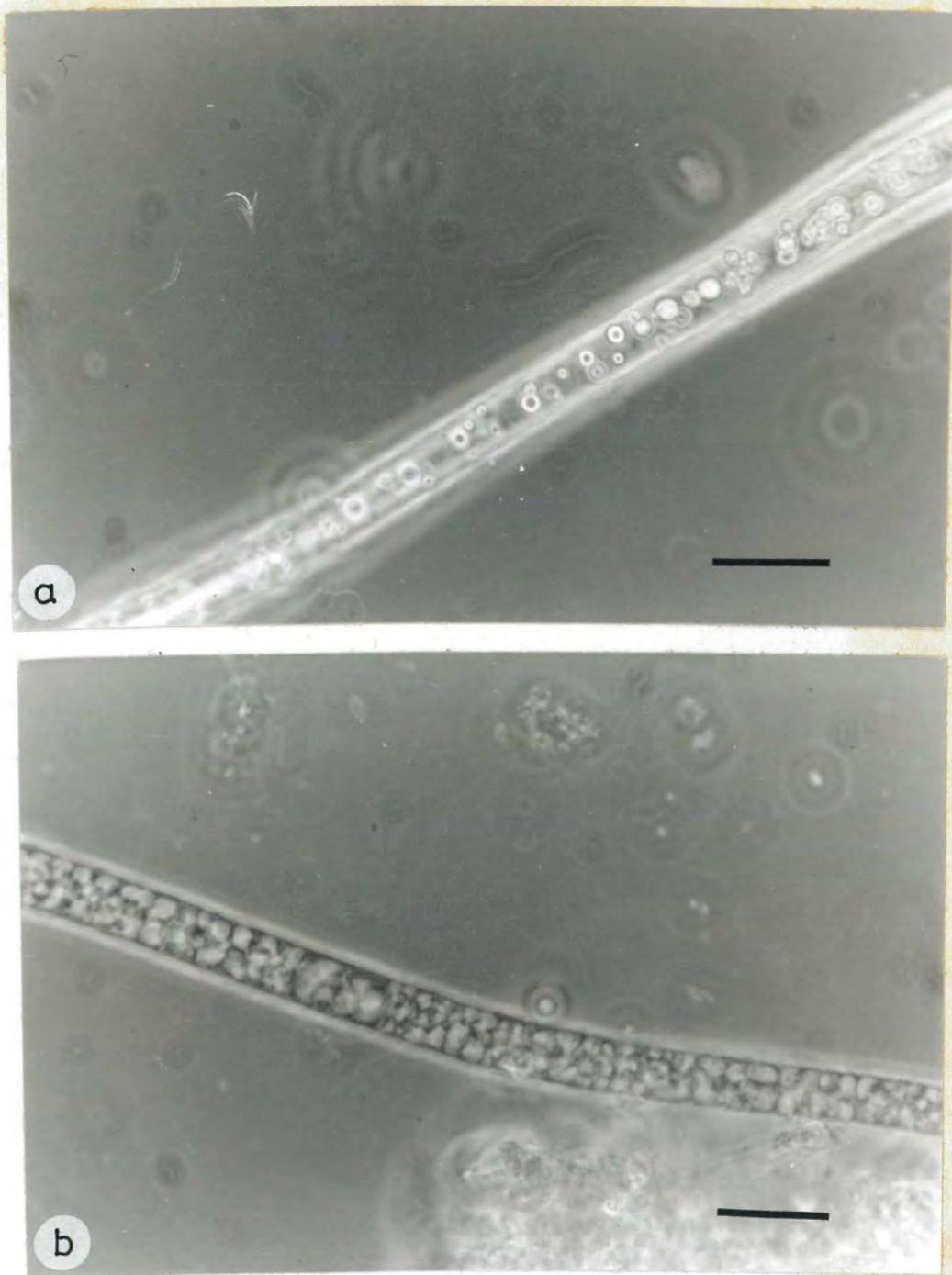


plate 34. *Beggiatoa* showing sulphur granules before (a) and after (b) treatment with pyridine. Bar represents 10 μm .

Cyanobacteria. Under certain circumstances the smaller filamentous cyanobacteria may be confused with non-photosynthetic filaments. The most frequently encountered form which may cause such confusion was identified as a member of the genus Pseudanabaena a description of which follows.

Single filaments were clearly delimited cells containing polar gas vacuoles and motile by gliding. The filaments were up to 103.5 μm long and the cells were 2.7 μm wide by 4.5 μm long. One filamentous cyanobacterium has been isolated in ASM medium and is probably that described above but in culture the dimensions changed somewhat. The gliding trichomes were normally short; the length ranged from 14.5 μm to 32 μm . The cells were 1.45 μm by 2.3 - 3.5 μm . The culture obtained grew forming a pellicle in the bottom of the flask. The filaments were usually enveloped in mucilage but free forms were always observed in the liquid medium. The cytochemical tests were as follows:

Lipid inclusions : negative.

Volutin granules : positive.

Sulphur inclusions : negative

Sheath stain : negative.

Spore stain : negative.

Cell Wall stain : positive.

Gas vacuoles : positive.

Flagella stain : negative.

Gram stain : negative.

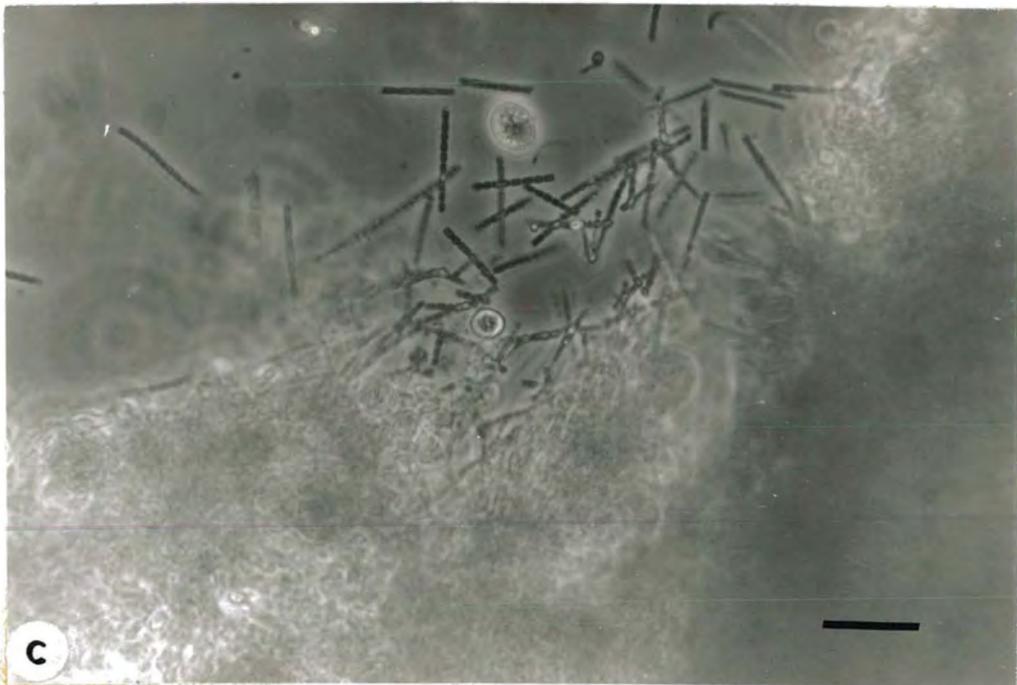
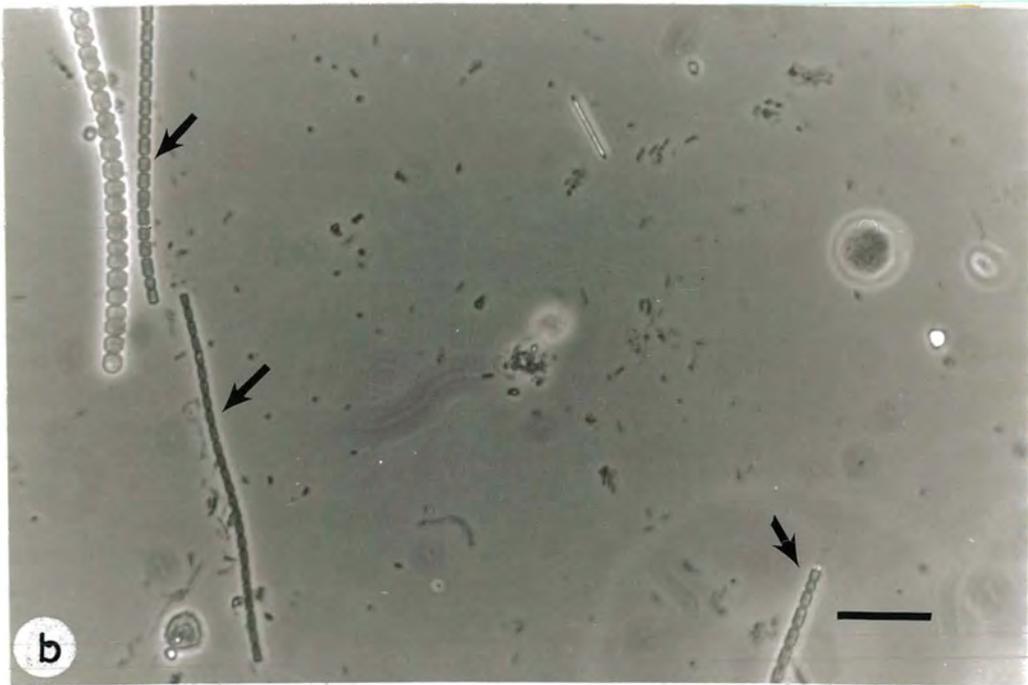
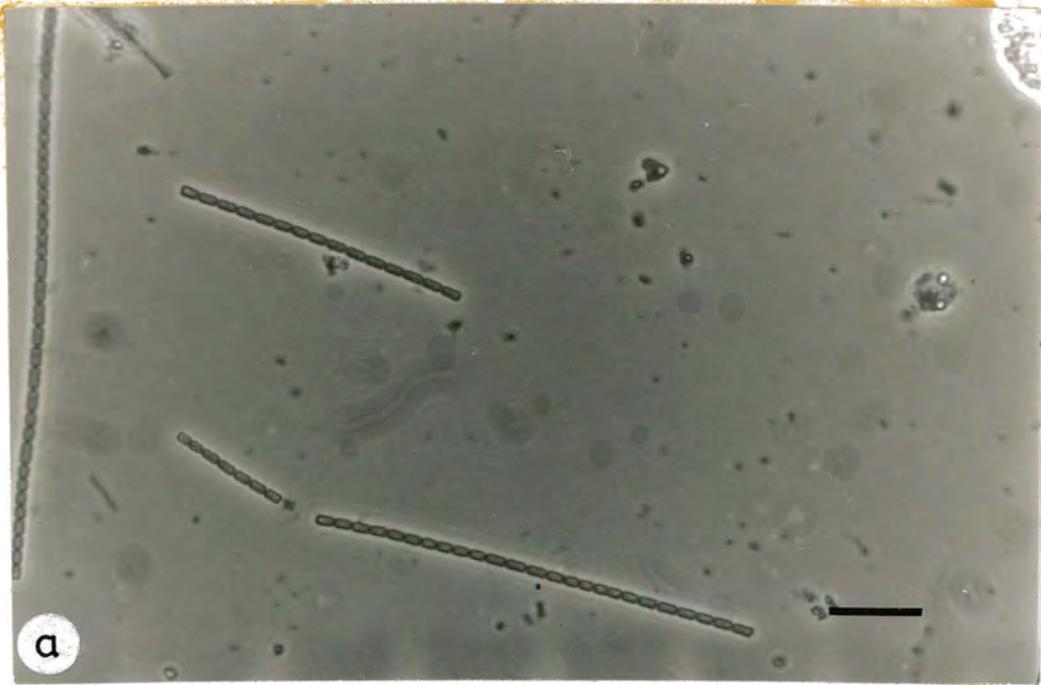
Chlorophyll fluorescence : positive.

Apart from the above described organisms which were observed frequently, two other filamentous bacteria, morphologically very distinct, were observed in Windermere South Basin. One of these was a false branched filament, attached by a holdfast (plates 37 - 38).

The organism was observed only once, on uncoated slides incubated in the sediment core and is similar to the genus Clonothrix. The other filament was observed once in the littoral zone of Windermere South Basin, on agar coated slides. On this occasion, it was the predominant organism on the slide and no motility was observed. The same organism was observed again in Mitchell Wyke (Fig. 2) in which case a continuous gliding motility was noted (Plate 39).

Pseudanabaena is illustrated in Plates 35 and 36.

Plate 35. Pseudanabaena (a) and (b) (arrowed) filaments growing on slides incubated in sediment core from Mitchell Wyke, Windermere (Fig. 2). (c) filaments growing on ASM medium. Bar represents 20 μm .



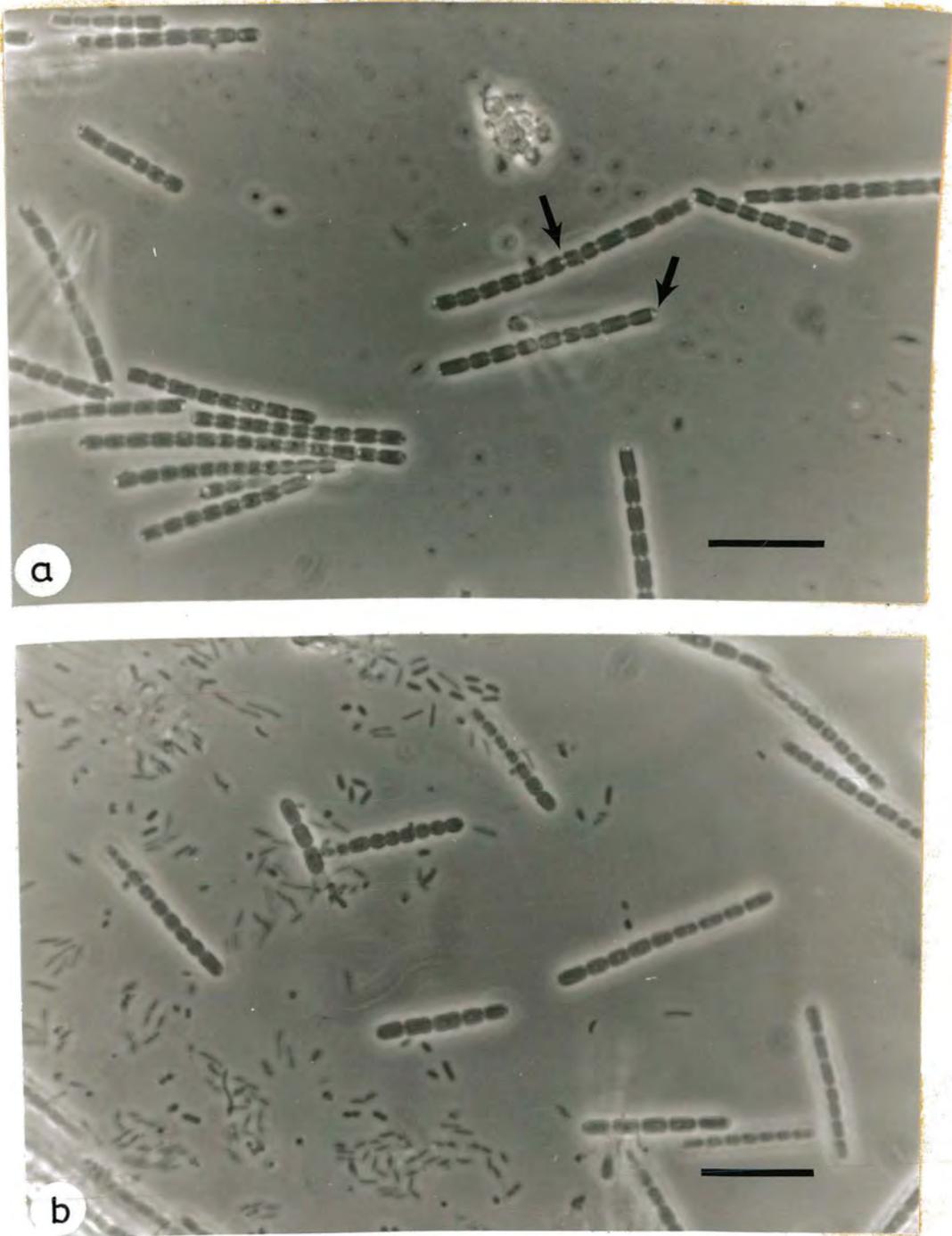


Plate 36. *Pseudanabaena* growing on ASM medium. (a) the arrows show the polar gas vacuoles. (b) filaments after being submitted to pressure. Bar represents 10 μm .

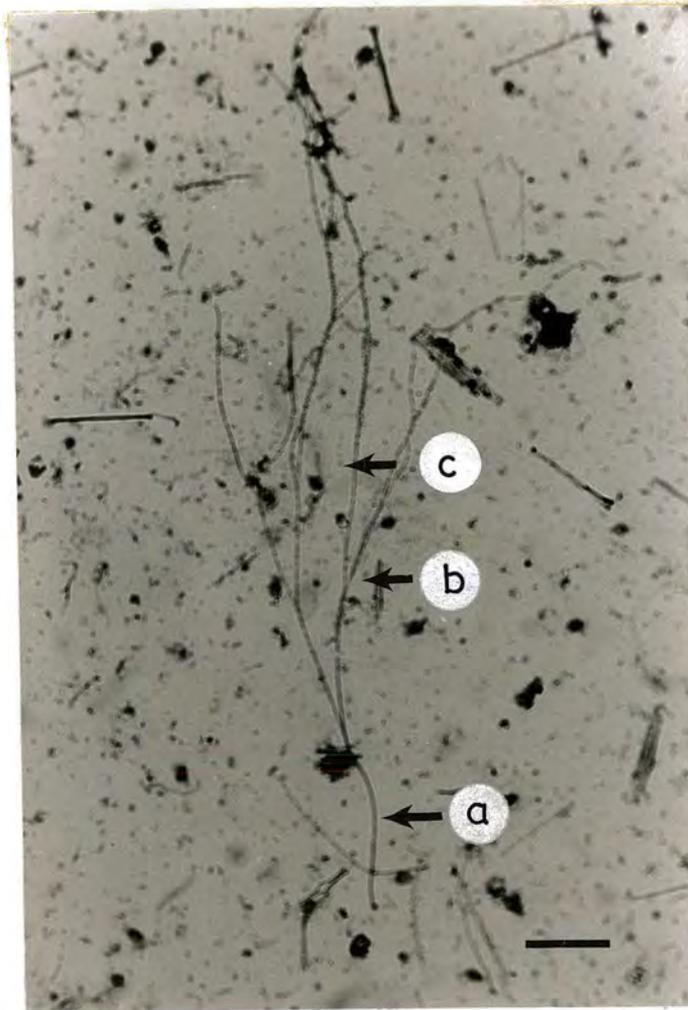
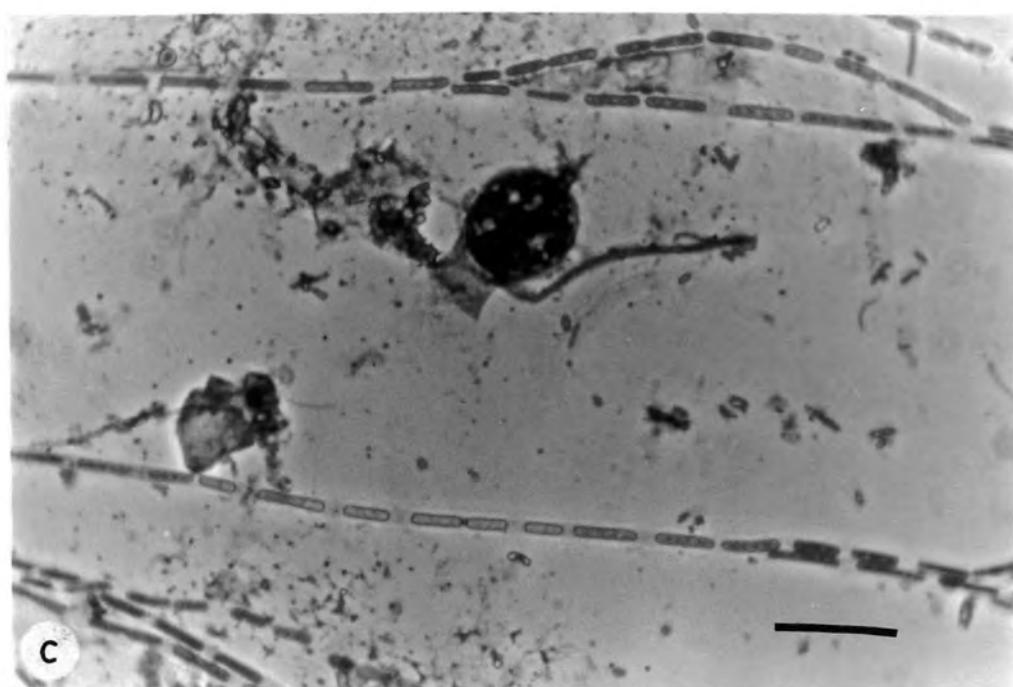
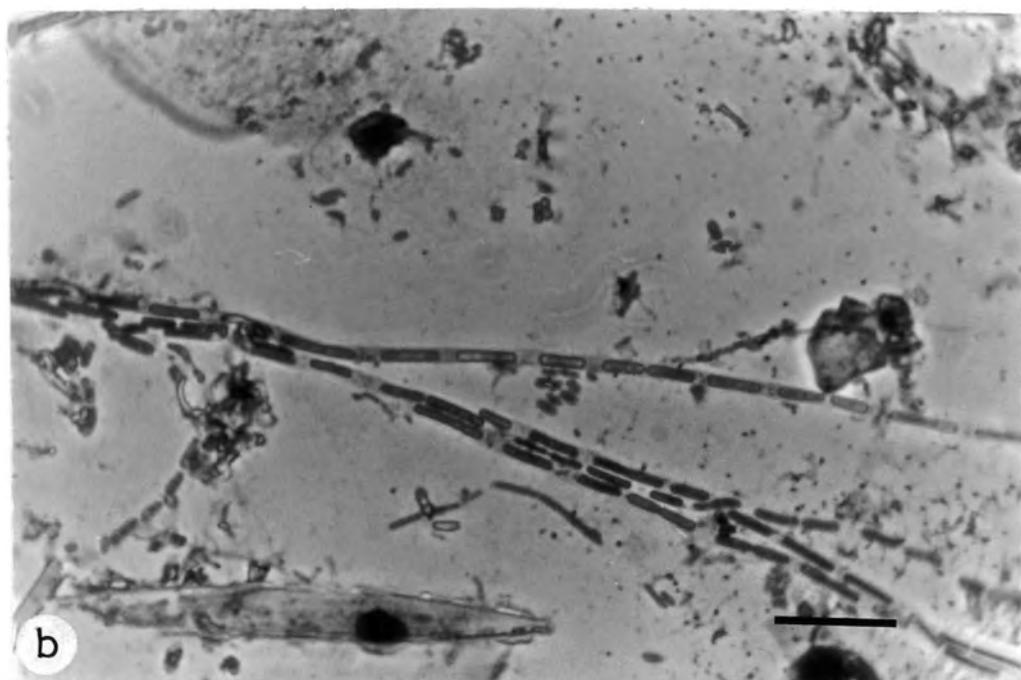
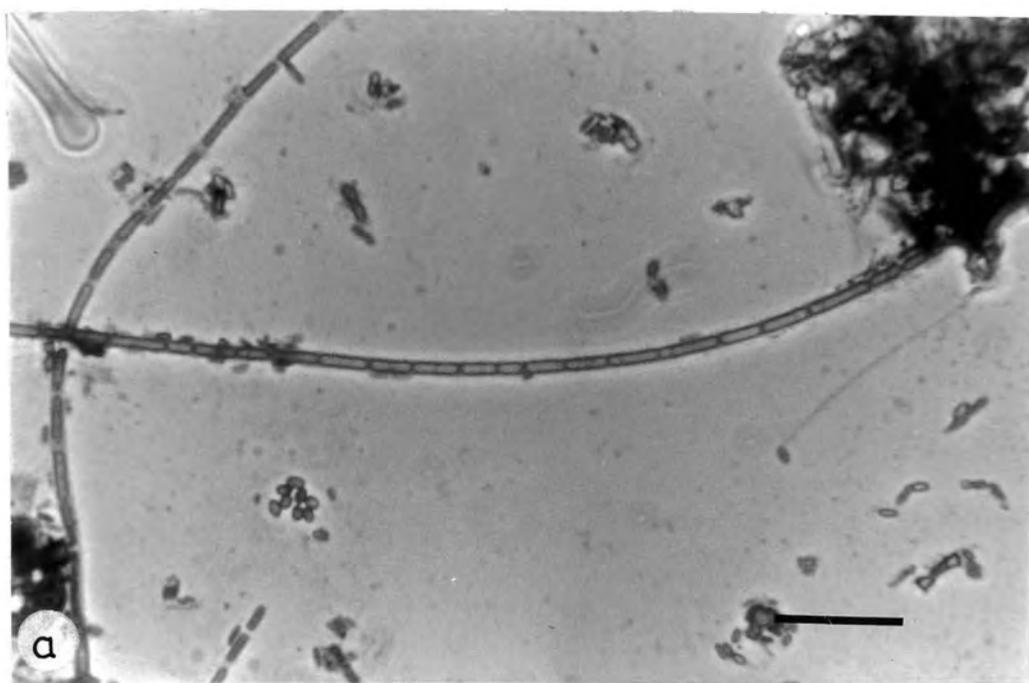


Plate 37. Organisms observed in Windermere South Basin
(Clonothrix?). a, b and c are areas shown at higher
magnification in the facing page. Bars represent
50 μm (above) and 10 μm (facing page).



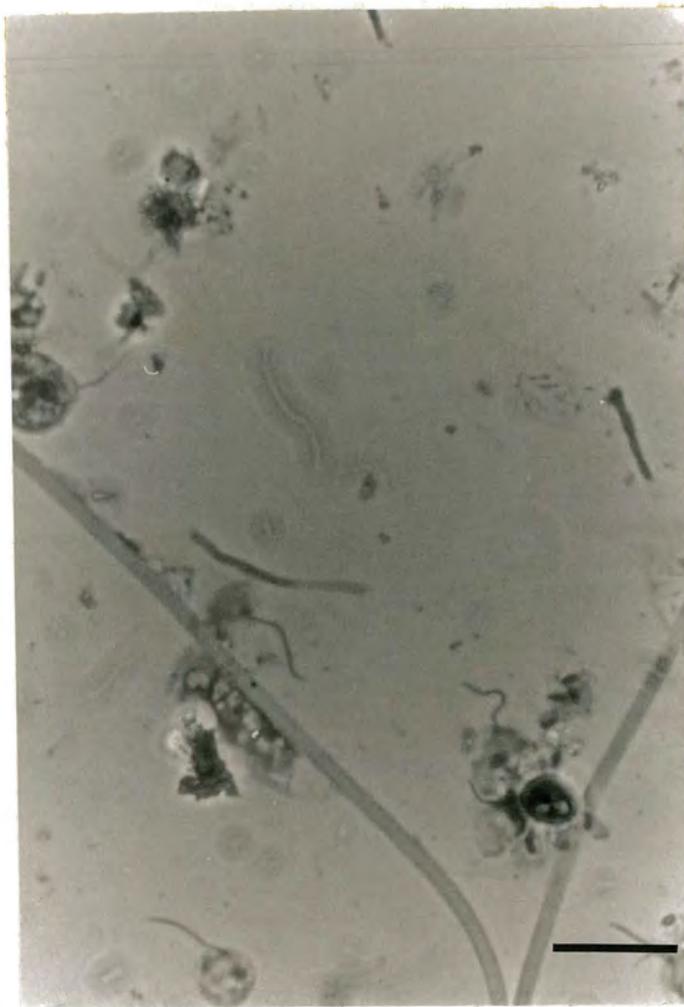


Plate 38. Sheath of the organism shown in Plate 37, after the lysozyme-detergent treatment. Bar represents 10 μm .



Plate 39. Organisms (Lieskeella?) observed in Mitchell Wyke (Fig. 2).

Bar represents 20 μ m.

Table 20. Cytochemical tests applied to the various groups of filamentous bacteria described under phase-contrast microscopy by wet mount technique.

Cytochemical tests	1	2	3	4a	4b	5b	6b	10	15	16	<u>Peloploca</u>	<u>Beggiatoa</u>	<u>Pseud-anabaena</u>
Lipid inclusions	+	d	-	+	+	+	-	-	-	-	-	+	-
Volutin granules	+	+	+	+	+	+	+	+	+	+	+	+	+
Sulphur granules	-	-	-	+	-	-	-	-	-	-	-	+	-
Iron deposition	-	+	-	-	-	-	-	+	+	-	-	-	-
Sheath stain	-	+	-	-	+	d	-	-	+	-	N.T.	+	+
Spore stain	-	-	-	-	-	-	-	-	-	-	N.T.	-	-
Cell wall stain	+	+	d	+	+	-	-	-	+	+	+	+	+
Gas vacuoles	-	+	-	-	-	-	-	-	+	-	+	-	+
Gram stain	-	-	-	-	-	N.T.	-	-	N.T.	N.T.	N.T.	-	-
Chlorophyll fluorescence	-	-	-	-	-	-	-	-	-	-	-	-	+
Flagella stain	-	-	-	-	-	-	-	-	-	-	-	-	-

N.T. = not tested

+ = variable

d = doubtful.

3.52 A tentative key to filamentous bacteria

Filamentous bacteria. The definition of a filament given by Bergey's Manual (1974) as "any long threadlike form which may or may not be segmented and includes the forms sometimes referred to as trichomes" is adopted here.

- 1 (a) Multicellular or unicellular organisms (sheathed or not sheathed), including helical forms, Gram-negative or Gram reaction not reported. Do not contain chlorophyll a 2
- (b) Gram negative organisms containing chlorophyll a 29
- (c) Gram positive organisms, non sporing or may resemble *actinomy-*
cetes (with exospores) or may contain endospores 30

- 2 (a) Multicellular filaments individually or collectively surrounded by a sheath 3
- (b) Unicellular or multicellular filaments without sheath 16
- (c) Helical organisms 25

- 3 (a) Filaments individually surrounded by a sheath, attached to a substrate, unbranched and presenting multiple septation (cells within the sheath divide transversely and longitudinally and may also divide obliquely to produce large numbers of non motile spherical cells); tapering may be observed 4
- (b) Various filaments enclosed in a common gelatinous sheath 5
- (c) Not as above 6

- 4 (a) Filaments up to 2 cm long surrounded by a very thin and colourless sheath except in the basal portion where iron or manganese may be present; the width of individual filaments may vary from 1-6 μm at the base to 6-9 μm at the tip when swollen; cells are 1.2 - 2.4 μm by 1.2 - 5.2 μm ; the diameter of "microgonidia" varies from 0.6 - 2 μm ; the "macrogonidia" may be up to 5 μm in diameter;

filaments tapering to the base and may taper to the apex;
 Gram negative and may contain polyphosphate granules; macroscopic
 appearance of the colonies, as reported, may be brownish-
 beige or colourless

Crenothrix

Plates 40-42

Note: Germination of released reproductive cells, on the
 exterior portion of the sheath, may simulate false branching.

(b) Filaments up to 100 μm long without tapering, the
 cell width may be variable along the filament; the diameter
 varies between 3 and 6 μm ; the basal cells are wider than
 long, the diameter being 4-6 times the length of the cell;
 sheath very delicate and colourless

Phragmidiothrix

Plate 43

5 (a) Filaments normally containing sulphur inclusions,
 occurring in parallel or braided bundles enclosed by a
 common sheath; sheaths width range from 50 μm to 160 μm and the
 length may be up to several cm; filaments from 0.8 μm to
 9 μm wide and the individual cells 1-8 μm long; up to 20 or
 more filaments inside the sheath; the sheath may be colour-
 less, yellowish or dark, frequently encrusted with detritus
 and may contain iron and manganese; poly- β -hydroxybutyrate
 granules may be present; filaments within the sheath glide
 independently of each other; gas vacuoles not reported

Thioploca

Plates 44-45

Note: Occurrence of a single filament inside the sheath
 has been reported (Maier & Preissner, 1979).

(b) Bands of 7-10 filaments with the basal portion enclosed in a common hyaline gelatinous sheath and attached by a holdfast; filaments 50-160 μm long being 0.5 μm wide at the top and up to 1.5 μm wide at the base; bundles 6-8 μm thick at sheathed base; filaments at the top are free and divergent; gas vacuoles are not formed

Desmanthos

Plate 46

(c) Organisms characteristically bound together in rigid bundles or flat ribbons which may be straight, undulate in one plane or spirally wound

Peloploca

For full description of this genus see key no. 10.

- | | |
|-------------------------------------------------|----|
| 6 (a) Sheaths may contain iron and/or manganese | 7 |
| (b) Sheaths may contain iron only | 8 |
| (c) Not as above | 11 |

7 (a) Filaments commonly false branched up to 1.5 cm long; cells are 1.5 - 2.5 by 4.5 - 18 μm being much longer than wide in the basal region; filaments free or attached and may taper to the tip; ampoule-like bodies may be formed from cells of certain filaments; older parts of the filament show sheaths containing iron or manganese and in younger ones which are also more slender, sheaths are colourless and hardly distinct

Clonothrix

Plate 47-48

(b) Chains of straight rods of uniform size and shape, 0.5 - 1.5 by 3 - 12 μm , within a sheath in most cases containing hydrated ferric or manganic oxides; may be free or attached by a holdfast; Gram negative and may contain globules of poly- β -hydroxybutyrate; chains of cells may leave the sheaths; free cells motile by means of one polar flagellum

Leptothrix

Plates 49-51

Note: 1) It was observed during the present study the whole filament of Leptothrix gliding out of the sheath, making it indistinguishable from Vitreoscilla.

2) Gas vacuoles have been observed in the sheathed filaments.

8 (a) Organisms motile by gliding 9

(b) Not as above 10

9 (a) Straight or helical filaments usually 150 - 250 μm long surrounded by a sheath which may be heavily impregnated with iron oxides; the filaments show slow gliding motility; the cells are 2 - 2.5 by 3.5 - 4.5 μm and contain bacteriochlorophyll d, chlorobium vesicles and large gas vacuoles; filaments may glide out of their sheath

Chloronema

Plate 52

(b) Filaments formed by two chains of rod shaped cells intertwined in the form of a spiral usually masked with heavy deposits of ferric hydroxide; cells rod-shaped 0.6 by 2-3 μm ; has a slow but incessant motion; filaments extraordinarily unstable and does not tolerate the use of usual fixatives; explosive disintegration of the cells occur after the addition of reagents

Lieskeella

Plate 53

Note: This organism is not commonly observed partially due to its ability to bury itself in sediment particles and its pronounced microzonal development.

10 (a) Filaments with cells 0.7 - 2.5 μm by 2 - 10 μm , attached by a holdfast or free; exhibit false branching frequently; very little difference between base and tip; sheath usually thin and pale but may be yellow-brown containing iron hydroxide; numerous small globules or few large globules of poly- β -hydroxybutyrate;

Gram negative; free cells motile by means of a bunch of subpolar flagella

Sphaerotilus

Plates 54-55

(b) Filaments with or without a gelatinous sheath which, if present may be ferruginous; characteristically bound together in rigid bundles or flat ribbons which may be straight, undulate in one plane or spirally wound; cells 0.3 - 1 by 0.3 - 10 μm and in bands 3 - 20 μm by up to 4mm; non motile; gas vacuoles may be present

Peloploca

plate 56

Note: Pelonema which has been observed with a sheath impregnated with iron would have keyed out here. For full description see key no. 24.

11 (a) Non motile filaments with delicate sheath usually attached by a holdfast, and the cells containing sulphur granules; cells 1.4 - 2.2 by 3-7 μm ; width of filaments average 1.5 μm and may vary along the length; length range usually up to 200 μm but may be as long as 500 μm ; rosettes formed; filaments may fragment into segments ca 15 μm long ("gonidia"); macroscopic appearance of the colonies, as reported, may be greyish white or milk white

Thiothrix

Plate 57-58

Note: Filamentous appendages along the filaments of this organism have been reported by Morita & Burton (1965).

(b) Not as above

12

12 (a) Single filaments containing gas vacuoles or bundles of filaments which may or may not contain gas vacuoles

13

(b) Not as above

14

13 (a) Free filaments with cylindrical cells usually containing gas vacuoles; filaments straight or spirally twisted

Pelonema

For full description see key no. 24

Note: Bergey's Manual (1974) considered Pelonema to be a sheathless organism.

(b) Filaments characteristically bound together laterally to form rigid bundles or flat ribbons which may be straight or undulate; not motile

Peloploca

For full description see key no.10 .

14 (a) Filaments motile by gliding, colourless with fine granular cytoplasm, sometimes clearly divided into an ecto- and endoplasm.

Achroonema

For full description see key no. 22 .

Note: Achroonema is included at this point because Bergey's Manual (1974) mentions the presence of sheath in some species; most species do not possess a sheath.

(b) Filaments not motile

15

Note: 1) Herpetosiphon as an organisms showing slow gliding motility and with the sheath apparently moving with the filament (Holt & Lewin, 1968) would have keyed out here. For description of this genus see key no. 15.

2) Chloroflexus a thermophilic or mesophilic gliding organism containing bacteriochlorophyll a and c may show a thin sheath in which case it would have keyed out here. For full description see key no. 22.

15 (a) Sheathed, non motile filaments 0.5 by up to 500 μm long; cells 0.3 - 0.5 by 5 - 15 μm ; may show false branching; unsheathed cells 5 - 15 μm long, released from the sheath are motile by gliding; Gram negative

Flexithrix

Plate 59

(b) Flexible filaments not branched and which can attain 500 μm or more in length; cells 0.5 - 1.5 by 5 - 10 μm ; thin and hyaline sheath may be present; unsheathed filaments motile by gliding; Gram-negative

Herpetosiphon

Plate 60-61

(c) Filaments, usually unbranched, up to 100 μm or more in length; cells 0.2 - 0.45 by 2.5 - 5.0 μm ; hardly visible hyaline sheath; cells may contain polysaccharide globules; motility never observed; contain carotenoid pigments; Gram negative

Haliscomenobacter hydrossis

Plate 62

Note: 1) Branching of the filaments may occur in stationary cultures.

2) Haliscomenobacter hydrossis (van Veen et al, 1973) and Streptothrix hyliana (Bergey's Manual, 1974) are considered to be synonymous.

16 (a) Organisms motile by gliding 17

(b) Organisms non motile 23

17 (a) Unicellular organisms with flexible cells 0.3 - 0.7 by 5 - 100 μm or more, not divided by any cross-walls; cells may show a curious twitching in liquids; not branched; Gram-negative 18

(b) Multicellular organisms 19

- 18 (a) Agar, cellulose or chitin attacked Cytophaga
Plate 63
- (b) Agar, cellulose and chitin not attacked Flexibacter
Plate 64

Note: 1) Lewin & Lounsbury (1969) reported that in some strains of Flexibacter the longer filaments exhibited cellular units delimited by cross-walls.

2) The free cells of Flexithrix are indistinguishable from Cytophaga and from Flexibacter aggregans. For full description of Flexithrix see key no. 15.

- 19 (a) Sulphur deposited in the cells 20
(b) not as above 21

20 (a) Filaments less than 1 μm to 55 μm wide, up to 1 cm in length and with cells 1 - 13 μm long; cells contain granules of sulphur or will deposit them in the presence of sulphide; tapering may occur; sometimes ending in a rounded protuberance; granules of poly- β -hydroxybutyrate or polyphosphate may be present; Gram-negative

Beggiatoa
Plate 65-66

Note: Filaments of Thioploca (see key no. 5) may leave the sheath, in which case they are indistinguishable from Beggiatoa.

- (b) Filament usually attached to a solid substrate;
rosettes formed Thiothrix

For full description see key no. 11.

Note: The entry is made at this point since in Bergey's Manual (1974) the genus is included among the gliding bacteria.

21 (a) Flexible, often "U" shaped filaments up to 400 μm long; cells cylindrical, colourless, 0.5 - 0.75 by 3 - 6 μm often exhibiting a dense body on either end; filaments very fragile and explosive disintegration may occur after short periods of laboratory observations; bent filaments rotate while slowly moving forward with the rounded part in the lead; twisted threads and fan shaped structure are formed by excretion of a mucoid substance; the threads (0.2 μm wide) may contain oxidized iron in which case have a yellowish and brittle appearance and diameter of 2.5 μm have been reported

Toxothrix

Plate 67-68

(b) Not as above

22

Note: The genus Bactoscilla described by Pringsheim (1951) would have keyed out here. The description of this organism is as follows. Very slender filaments 0.4 - 0.5 μm wide, composed of rod-shaped cells apparently separated by empty interspaces; motile by gliding with a slow, pronounced bending of the filament at the joints, the individual cells remaining rigid; Gram-negative

Plate 69

22 (a) Colourless filaments 1.2 - 2 by up to 150 μm composed of clearly delimited cylindrical or barrel shaped cells 1.2 - 1.5 by 1.2 - 12 μm ; older trichomes may contain refractive granules; speed of gliding has been reported for some species (0.03-2 $\mu\text{m s}^{-1}$); movement not always regular but intermittent, hesitant with frequent change of direction; Gram-negative

Vitreoscilla

Plate 70-71

(b) Multicellular filaments usually up to 350 μm long but may attain 600 μm or even 5 mm in length; cells 0.3 - 6.8 by 1.5 - 19 μm ; cross walls clearly visible (indistinct in some species); filaments not constricted at the cross walls; fine granular cytoplasm which may be divided into an ecto- and endoplasm; polyphosphate granules may be present; rarely with gas vacuoles; may form arthrospore-like resting cells; speed of gliding has been reported for some species (0.58 - 2.50 $\mu\text{m s}^{-1}$)

Achroonema

Plate 72

Note: Skuja (1948; 1956) in his descriptions of Achroonema included filaments constricted at the crosswalls.

(c) Filaments 0.6 - 0.7 μm wide with indefinite length but generally 30 - 300 μm ; contain bacteriochlorophyll a and c; cross walls are distinguishable with electron microscope and cell length range from 2 to 6 μm ; occur in hot springs but has also been observed in water and sediment of freshwater lakes; on agar surface the filaments glide at 0.01 - 0.04 $\mu\text{m s}^{-1}$; lipid granules and many other inclusions which identities are not certain may be present; Gram-negative

Chloroflexus

Plate 73

Note: 1) Sheathless Herpetosiphon, Leptothrix and Chloronema would have keyed out here. For the full description of the three genera see key numbers 15, 7 and 9 respectively.

23 (a) Filaments greater than 100 μm and 3 - 5 μm wide composed of cylindrical cells averaging 1 - 5 μm in length; unbranched, attached by a holdfast; do not glide but may wave sporadically from side to side; apical cells fragment into gliding "gonidia"; rosette formation observed frequently in culture medium; filaments may taper from base to apex

Leucothrix

Plate 74

Note: 1) Leucothrix is a marine organism and has been tentatively identified in activated sludge by Cyrus & Sladká (1970).

2) Thiothrix (see key no. 11) may lose its sulphur granules in which case resembles very closely Leucothrix

3) Sheathless Sphaerotilus has been observed in cultures and would have keyed out here. For full description of this genus see key no. 10.

(b) Not as above

24

24 (a) Free filaments up to 600 μm or more in length; cells 0.6 - 2.2 by 1.5 - 19 μm ; cells usually contain gas vacuoles; straight or loosely flexuous filaments of uniform thickness; may show a slow gliding motility and may be constricted at cross-walls

Pelonema

Plate 75

(b) Filaments characteristically bound together in rigid bundles or flat ribbons which may be straight, undulate in one plane, or spirally wound

Peloploca

For full description see key no. 10

25 (a) Filaments motile by gliding, up to 500 μm long
 composed of cells 0.8 by 1 - 5 μm ; helix 1.5-2.5 μm ;
 pitch 3 - 17 μm ; cross walls may be indistinct; Gram-
 negative

Saprospira

Plate 76

(b) Filaments motile by gliding with cells containing
large gas vacuoles and bacteriochlorophyll d

Chloronema

For the full description see key no. 9

(c) Unicellular organisms motile by flagella or axial
fibril

26

26 (a) Cells 0.25 - 1.7 by 2 - 60 μm ; polytrichous
 polar flagella usually at both poles; rigid walls;
strictly aerobic or obligately microaerophilic;
 granules of polyhydroxybutyrate present in most species;
 Gram-negative

Spirillum

Plate 77

(b) Highly flexible helical cells 0.2 - 0.75 by 5 - 300
 μm or more; one axial fibril inserted at each end of the
 cell; anaerobic or facultatively anaerobic; Gram-negative

Spirochaeta

Plate 78

(c) Cells 0.1 μm wide by 6 - 20 μm or more long, highly
 flexible, wound in a very fine coil 0.2 - 0.3 μm in overall
 diameter and with a pitch of 0.3 - 0.5 μm ; bent or hooked
at one or both ends; aerobic; axial fibrils present

Leptospira

Plate 79

(d) Not as above

27

27 (a) Cells devoid of pigments, somewhat pointed at the ends, 1.7 - 2.5 by 6.6 - 50 μm ; motile by mono or polytrichous polar flagella; contain sulphur globules Thiospira

Plate 80

(b) Organisms containing bacteriochlorophylls and carotenoid pigments

28

28 (a) Spiral shaped cells motile by means of polar flagella, 0.3 - 1.5 by up to 30 μm ; do not deposit sulphur; Gram-negative

Rhodospirillum

Plate 81

(b) Spiral-shaped bacteria motile by means of tufts of polar flagella; cells 1.5 - 4.0 by 4 - 40 μm ; contain bacteriochlorophyll a and carotenoids; sulphur deposited inside the cells; Gram-negative

Thiospirillum

Plate 82

29 Organisms blue-green to red; prokaryotes; contain chlorophyll a and phycobiliproteins (notably phycocyanin and phycoerythrin); thylakoids present; wall may in turn be surrounded by a gelatinous or fibrous sheath; motility, if present, is by gliding

Cyanobacteria

Note: The genus more likely to cause confusion in the sediments examined during the present work is Pseudanabaena.

This genus consists of filaments usually with 2 to 6 cells in length containing polar gas vacuoles and in most cases exhibiting a rapid gliding motility. The cell width ranges from 2 - 2.5 μm .

Plate 83

30 (a) Organisms resembling actinomycetes, may possess exospores or endospores

31

(b) Not as above

33

31 (a) Unicellular organisms occurring singly and in chains;
true endospores formed Bacillus
Plate 84

(b) Organisms form a rigid, richly branched mycelium-
like structure; no endospore 32

32 (a) Spores formed by fragmentation of the branching
hyphae; mycelium tends to be compact Nocardia
Plate 85

(b) Conidia produced in aerial hyphae in chains
Streptomyces
Plate 86

(c) Conidia produced singly at the ends of special
conidiophores Micromonospora
Plate 87

33 (a) Thin, coiled filaments up to 300 - 500 μm long
containing cells 0.3 - 0.7 by 0.7 - 1.5 μm ; cross walls
not or hardly seen under phase-contrast microscopy; no
sheath; motility not detected; cells usually contain
polyphosphate granules; variation in the width of the
cell give an irregular appearance to the filament
Microthrix parvicella
Plate 88

Note: Some autolyzed cells may show Gram-negative reaction.

(b) Non motile, colourless filaments resembling cyano-
bacteria; up to 200 μm long; cells may extend from 0.3 -
0.6 μm along the longitudinal axis of the filament and are
0.7 - 1.5 μm wide; no sheath; thylakoids, cysts and photo-
synthetic pigments have never been detected; may branch; chains

0.6 - 0.9 mm long may be formed in stationary cultures

Nostocoida limicola

Plate 89

Note: The two organisms above were described by van Veen
(1973).

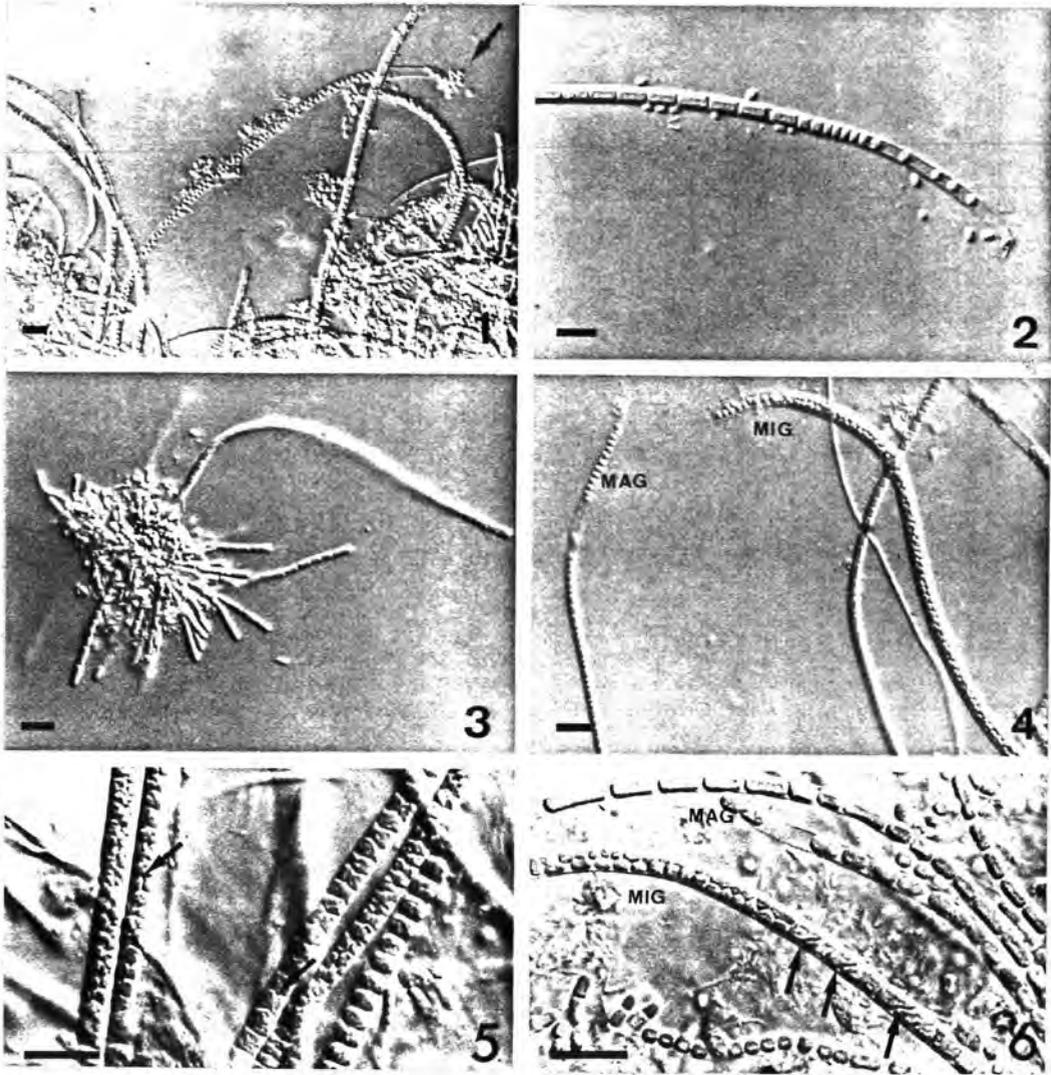


Plate 40 Crenothrix polyspora (Völker et al., 1977)

Bar represents 10 μ m.

1. Two clusters of filaments in various stages of gonidial production. Microgonidia (arrow) are released from the mouth of one sheath.
2. Filaments tip with macrogonidia and cells in various stages of transformation into these propagation forms.
3. Young cluster of growing Crenothrix filaments.
4. Filamental tips producing macro- (MAG) or microgonidia (MIG).
5. Filaments producing microgonidia by cross-septation followed by longitudinal septation (arrows).
6. Filaments with macrogonidia (MAG). Microgonidia (MIG) arise by cross-septation and oblique septation (arrows).

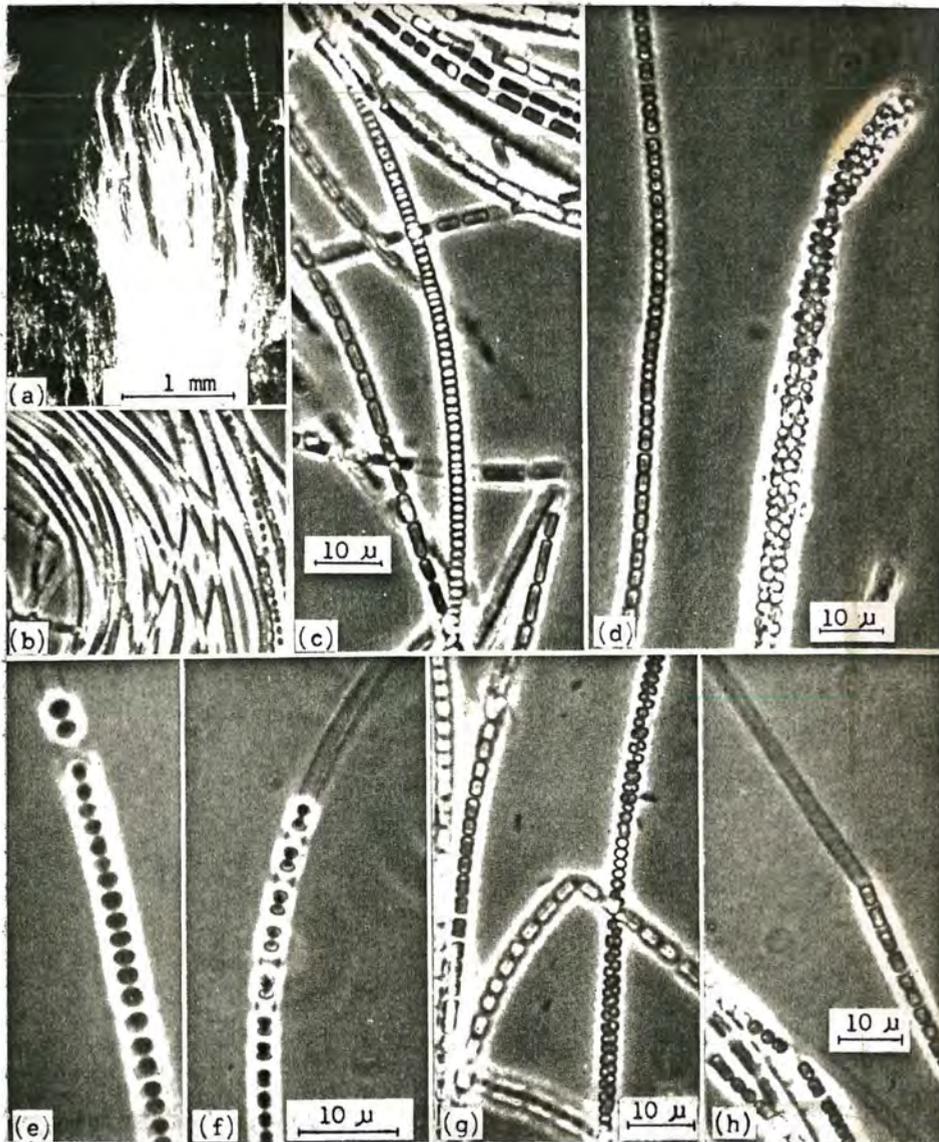


Plate 41 *Crenothrix polyspora*

a - h Wolfe (1960)

a Colony mounted in water

b - h Living specimens mounted in water

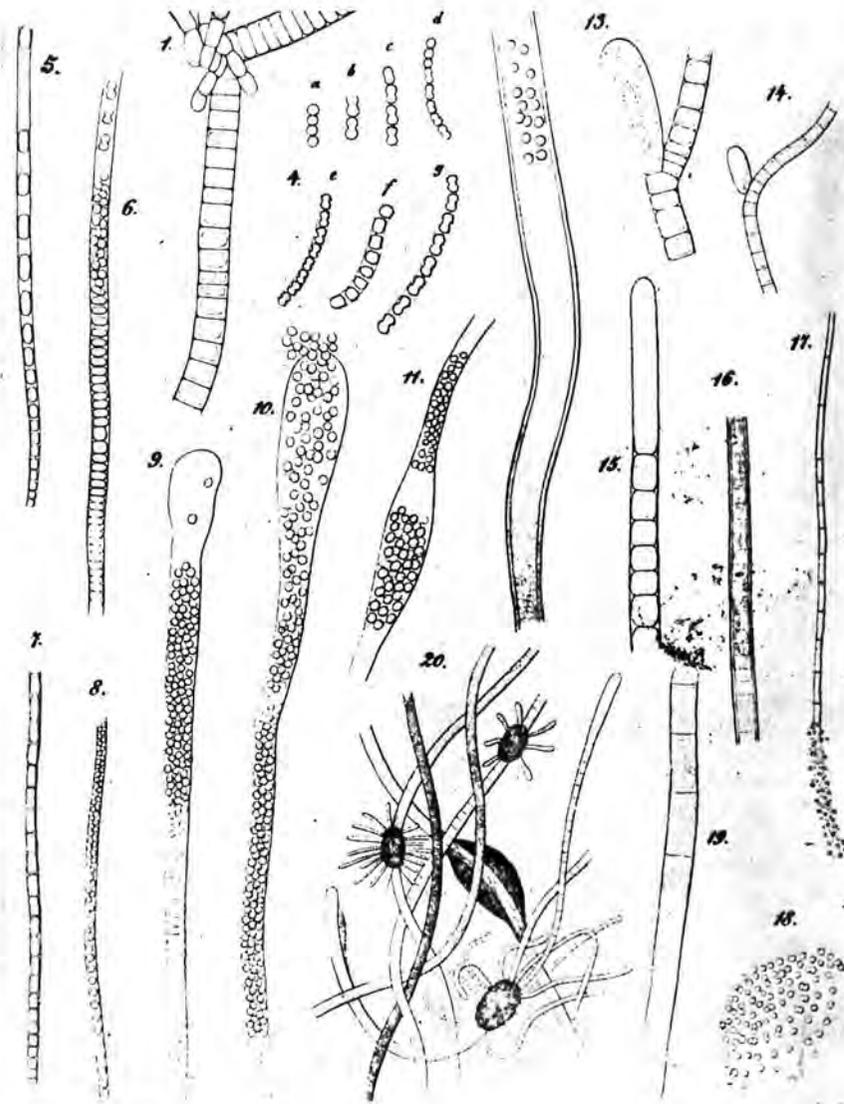


Plate 42. Reproduction of Cohn's original illustration of
Crenothrix polyspora (Wolfe, 1960).

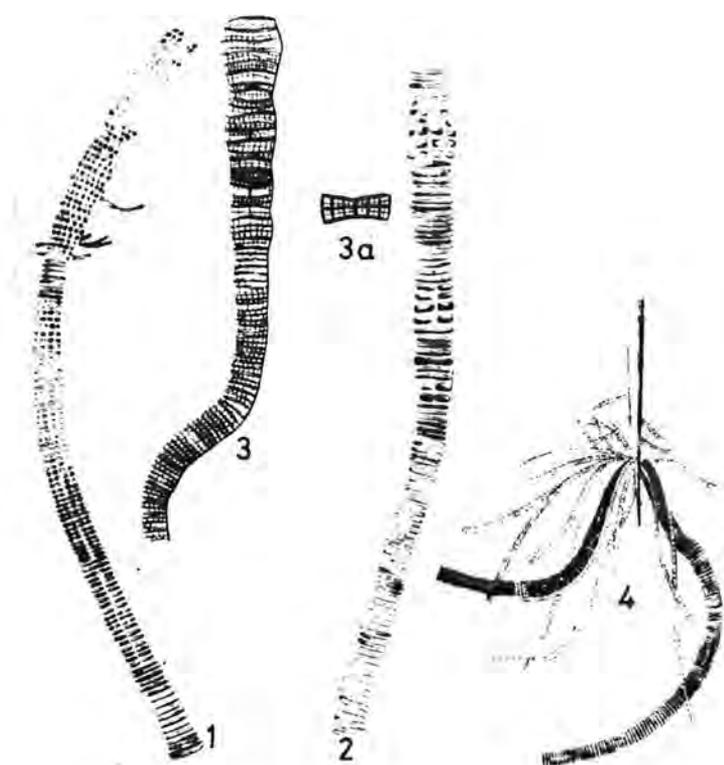


Plate 43. Phragmidiothrix multiseptata

1 - 4 Reproduction of Engler drawings (Bergey's Manual, 1974)

1. Filament showing arrangement and septation of disc-shaped cells.
2. Part of filament showing enlarged end and individual disc-shaped cells of uneven diameter.
3. Part of a filament with much cross- and longitudinal septation.
- 3a. Shows a part at higher magnification.
4. A bristle of Gammarus locusta with two attached filaments of Phragmidiothrix (wide filaments) and several narrow filaments of young "Beggiatoa alba"? Magnification : approximately x 330 (original magnification x 400).

Plate 44. Thioploca

A - C. T. ingrica (Bergey's Manual, 1974).

A. Portion of mass of filaments washed free of mud. Scale 200 μm . b, area enlarged in Fig. B.

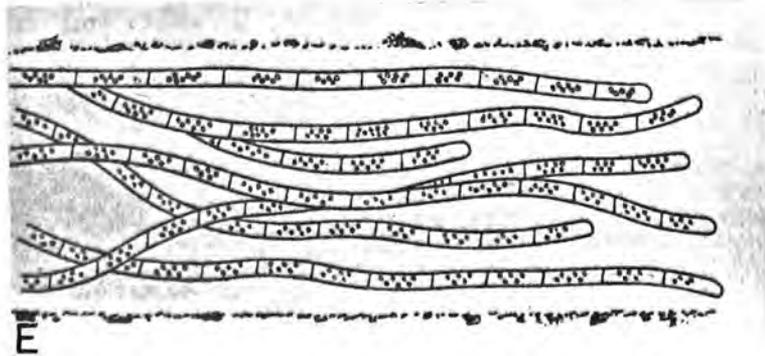
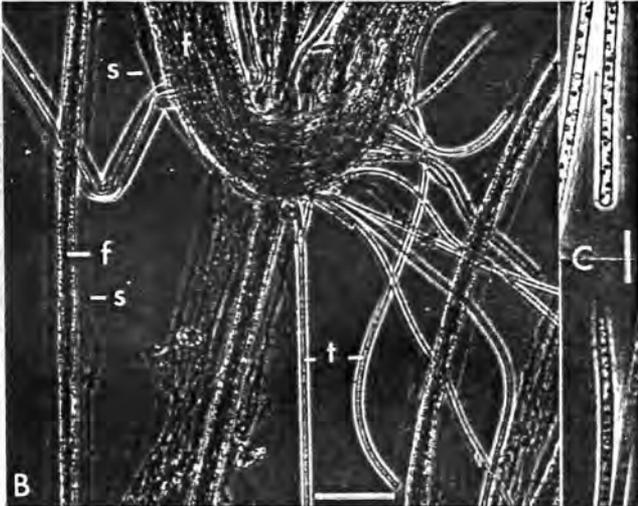
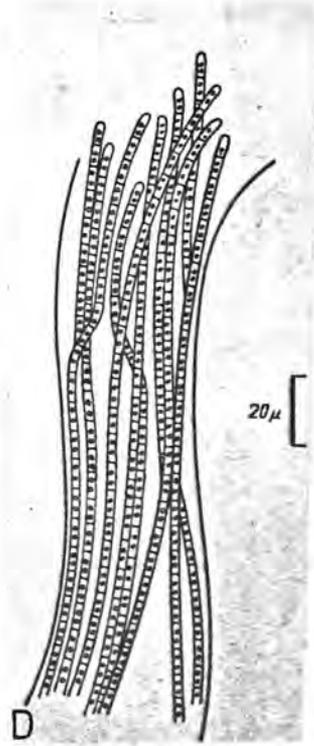
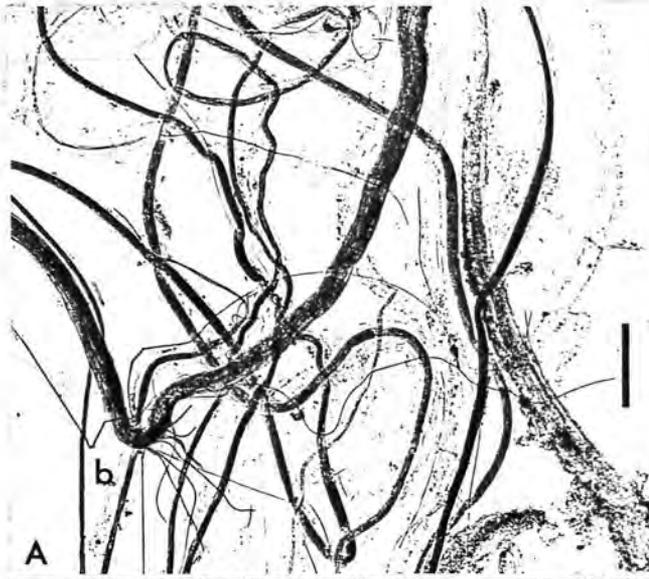
B. Fascicle of filaments (f) enclosed by the sheath (s); free filaments (t) emerged from a break in the sheath. Phase-contrast. Scale 50 μm .

C. Note sulfur inclusions and the obtuse (top) and tapered (bottom) terminal segments. Phase-contrast. Scale 10 μm .

D - E. Fjerdingsstad (1979). Bar represents 20 μm

D. T. ingrica

E. T. schmidlei



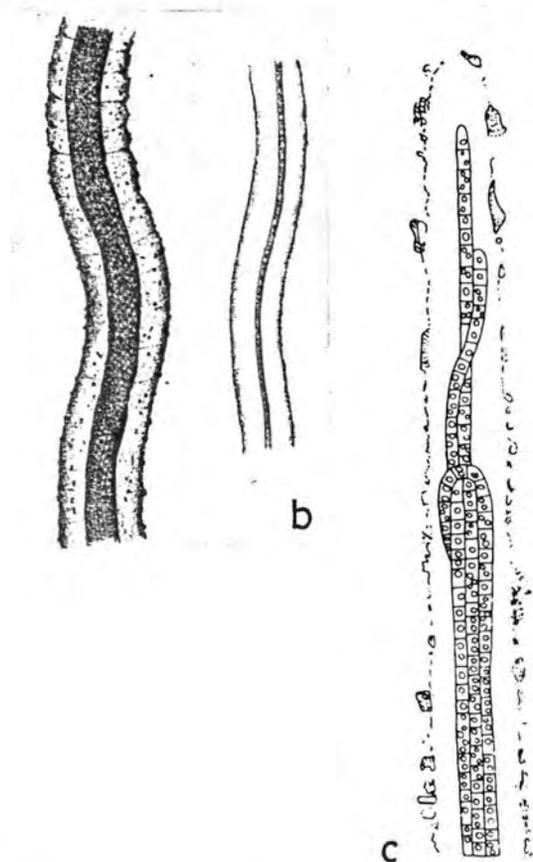
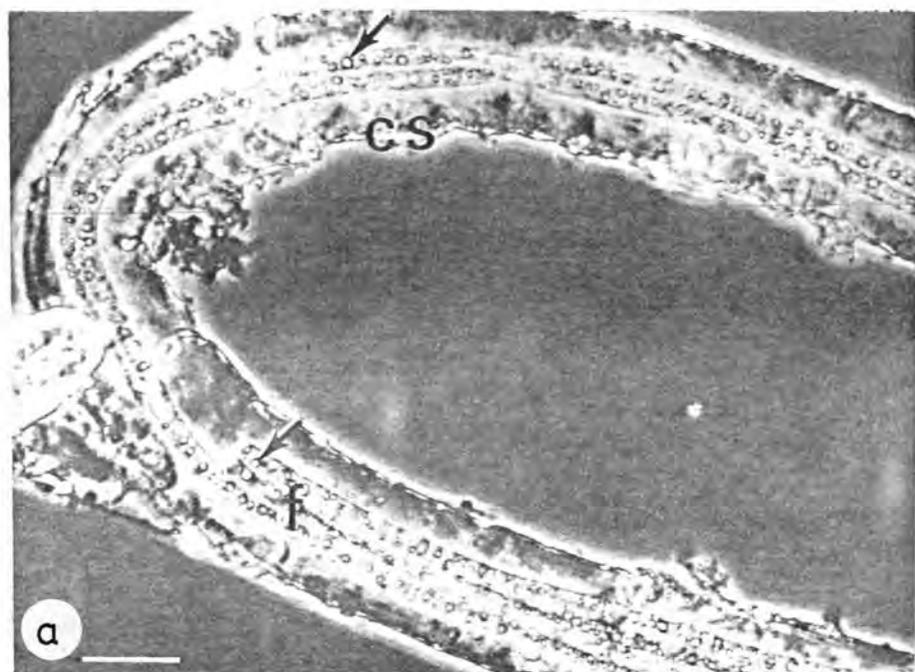


Plate 45. Thioploca

a. T. ingrica (Maier & Preissner, 1979) CS = common sheath.

Bar represents 20 μ m.

b. T. schmidlei (Lauterborn, 1907 In Skerman, 1959).

c. Wislouch (1912) In Perfil'ev et al. (1965)

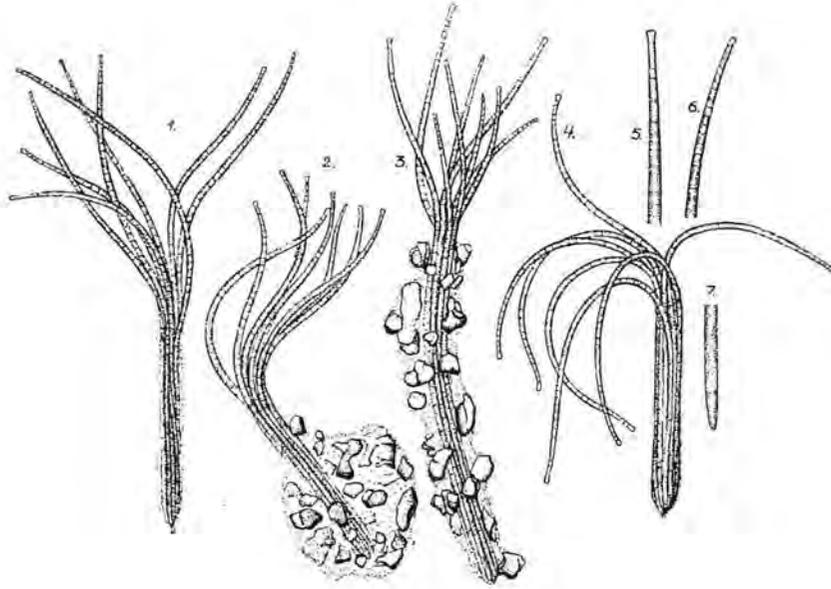


Plate 46 Desmanthos thiokrenophilum (Skuja, 1958).

1. Bundle of filaments with lower part showing thin mucilage sheath; upper part free. 2 - 3. Bundle of filament from bottom mud with sheath containing attached sand particles. 4. As 1. 5 - 6. Terminal portion of a trichome. 7. Basal portion of a trichome. 1 - 4 about x 600; 5 - 7 about x 1200.

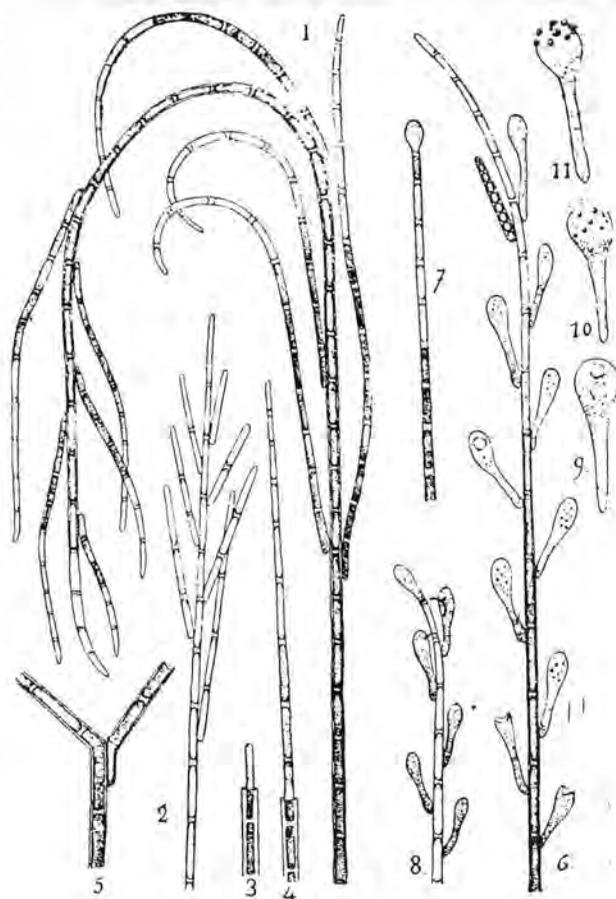


Plate 47 Clonothrix fusca (from Roze 1896 In Bergey's Manual, 1974)

1. End of adult filament with false lateral branches.
2. End of young filament with false lateral branches.
3. Cell emerging from a broken filament.
4. Young filament growing from a broken adult filament.
5. Mode of insertion of two adult filaments, that on right forming a false lateral branch.
6. A filament carrying an ascending series of ampoule-like swellings (buds).
7. End of a filament terminating in an ampoule-like swelling.
8. A filament terminating in swelling but also carrying a series of lateral swellings.
- 9-11. Buds showing vacuoles, and granules.

Magnification: Figs. 1-4, 6-8 approximately x 365. Fig. 5, 9-11 approximately x 650. Original magnification 450 and 800, respectively.

Plate 48 Clonothrix fusca (Kolk, 1938).

1. Tip of filament with branch showing beginning spore formation inside the sheath; oldest spores at base of branch. x 1600. Aceto-carmin stain.
2. Tip of filament showing spore branches; continuation of upper end of filament represented in 8. x 800. Mounted in glycerine, unstained.
3. Tip of filament showing beginning of a false branch at a. At points marked x, after an interval of time, the cells separated, showing the sheath, as indicated above each of these points. Otherwise sheath invisible around vegetative cells and spore branch. Living material, unstained. x 800.
4. Portion from tip of filament observed under oil immersion objective. Sheath around spores distinctly visible, and constrictions evident between spores. Polychrome methylene blue stain. x 1600.
5. Older portion of a filament showing an inner filament emerging from the broken end of the brownish yellow sheath. x 1600. Mounted in glycerin, unstained.
6. Four portions from a single filament; a, the oldest portion where the sheath is brownish yellow; b and c. portions nearer the tip with respectively thinner sheaths; d, tip of the filament where the sheath is difficult to see and where the spore branches present a racemose appearance. Aceto-carmin stain. x 800.
7. Portion of a filament showing branching. x 1600. Polychrome methylene blue stain.
8. A long filament with portions omitted as indicated, showing arrangement of branches alternate and opposite. Unstained, mounted in glycerine. x 800.



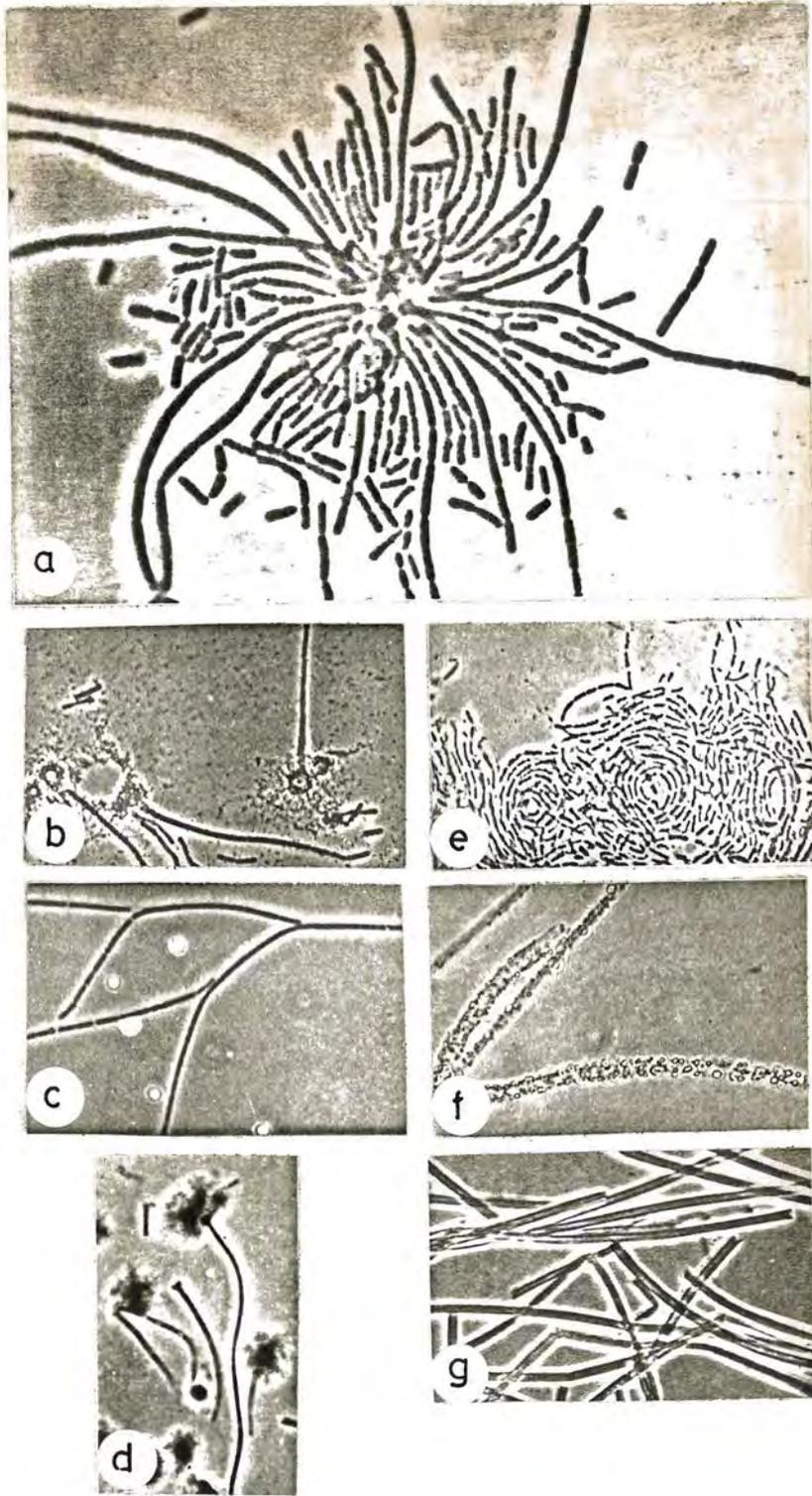


Plate 49 Leptothrix Cultivated.

a. van Veen et al. (1978) x 1625.

b, c, e, f, g. Mulder & van Veen (1963) x 810.

d. Bergey's Manual (1974) x 812.

a - d. L. lopholea.

e - g. L. pseudo-ochracea.

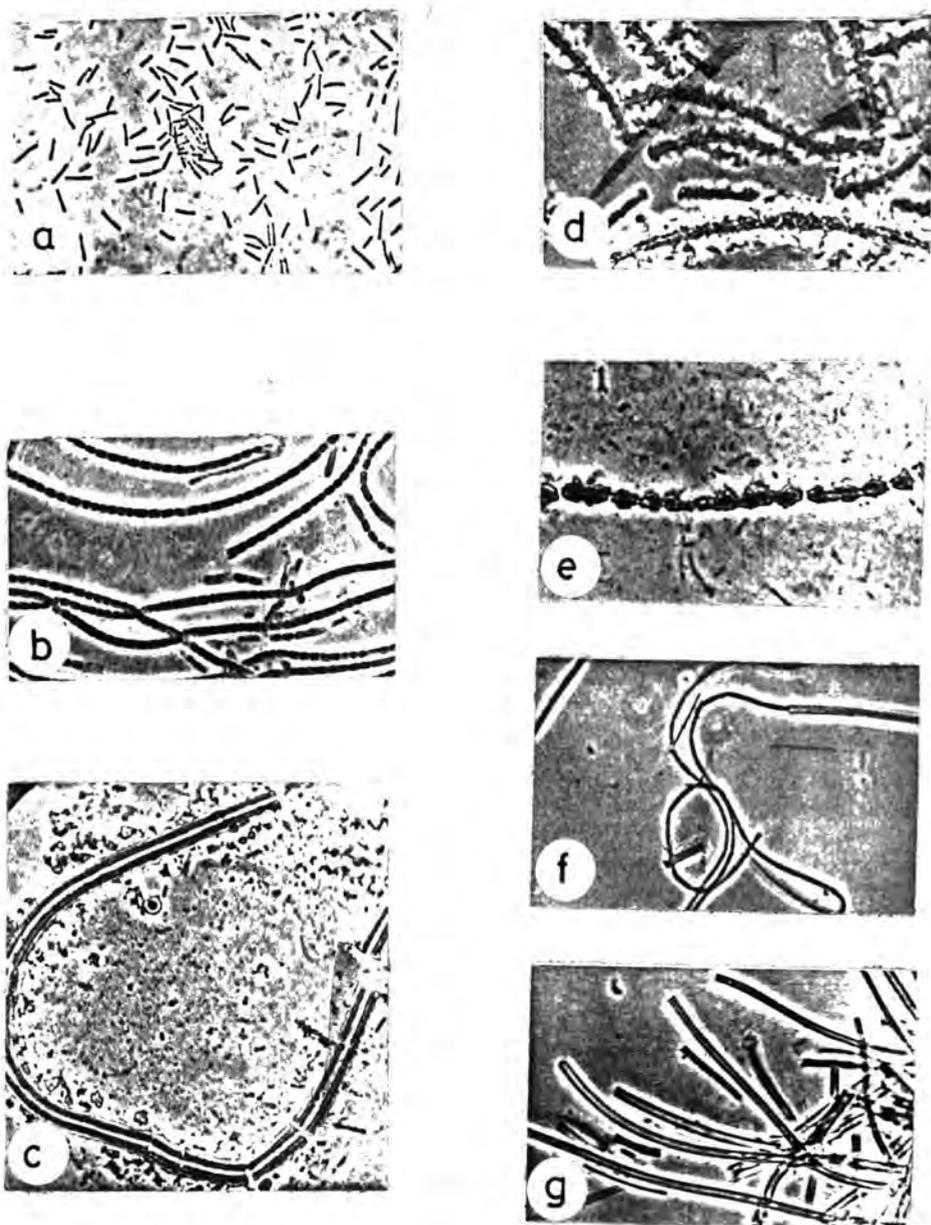


Plate 50 Leptothrix

a - g. Mulder & van Veen (1963) x 810.

a - c. L. cholodnii

d - e. L. discophora

f - g. L. ochracea.

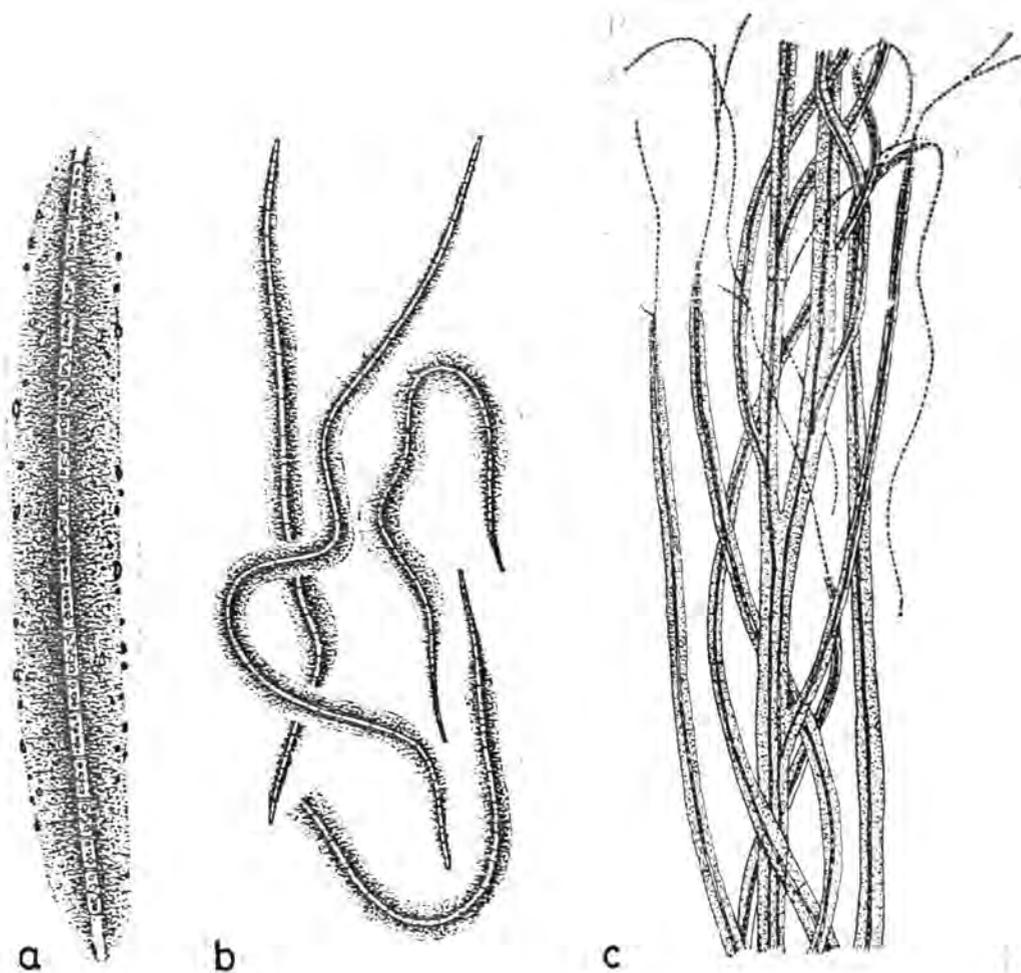


Plate 51 Leptothrix

- a. L. pseudovacuolata (Skuja, 1948) x 900
- b. L. tenuissima (Skuja, 1948) x 1000
- c. L. major (Beger & Bringmann, 1953).

Plate 52 Chloronema giganteum

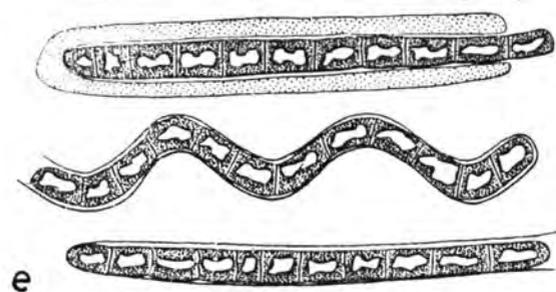
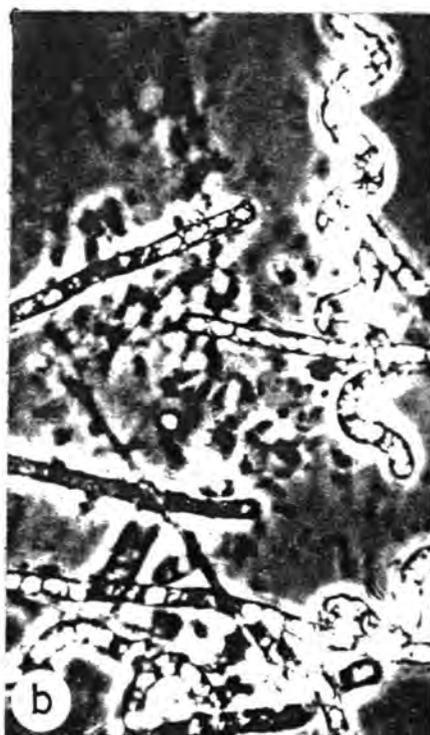
a - c. Dubinina & Kuznetsov (1976).

a. straight filaments x 450; b. filaments on glass slides x 1000 c. ultra-structure of the cell.

cv chlorobium vesicles; gv gas vesicles x 20000.

d - e. Dubinina & Gorlenko (1975). d.x 1600;

e. Schematic diagram



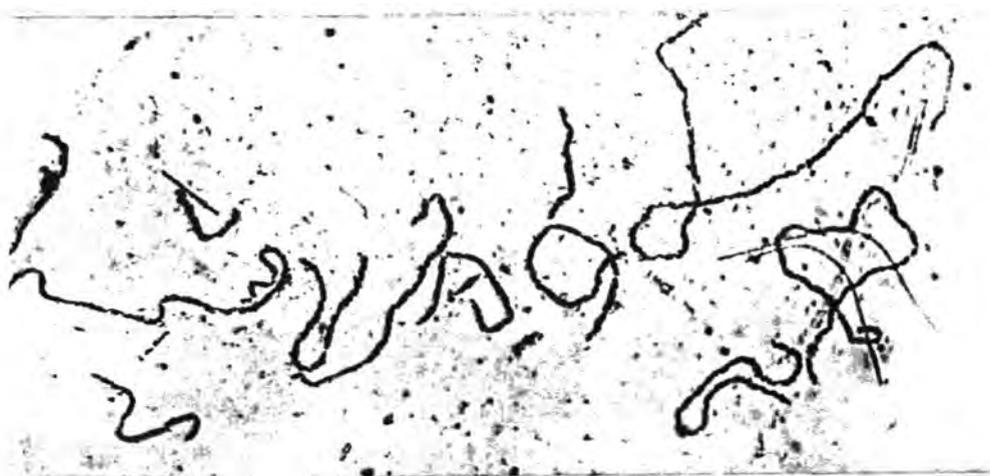


Plate 53 Lieskeella (Perfil'ev & Gabe, 1969) x 450.

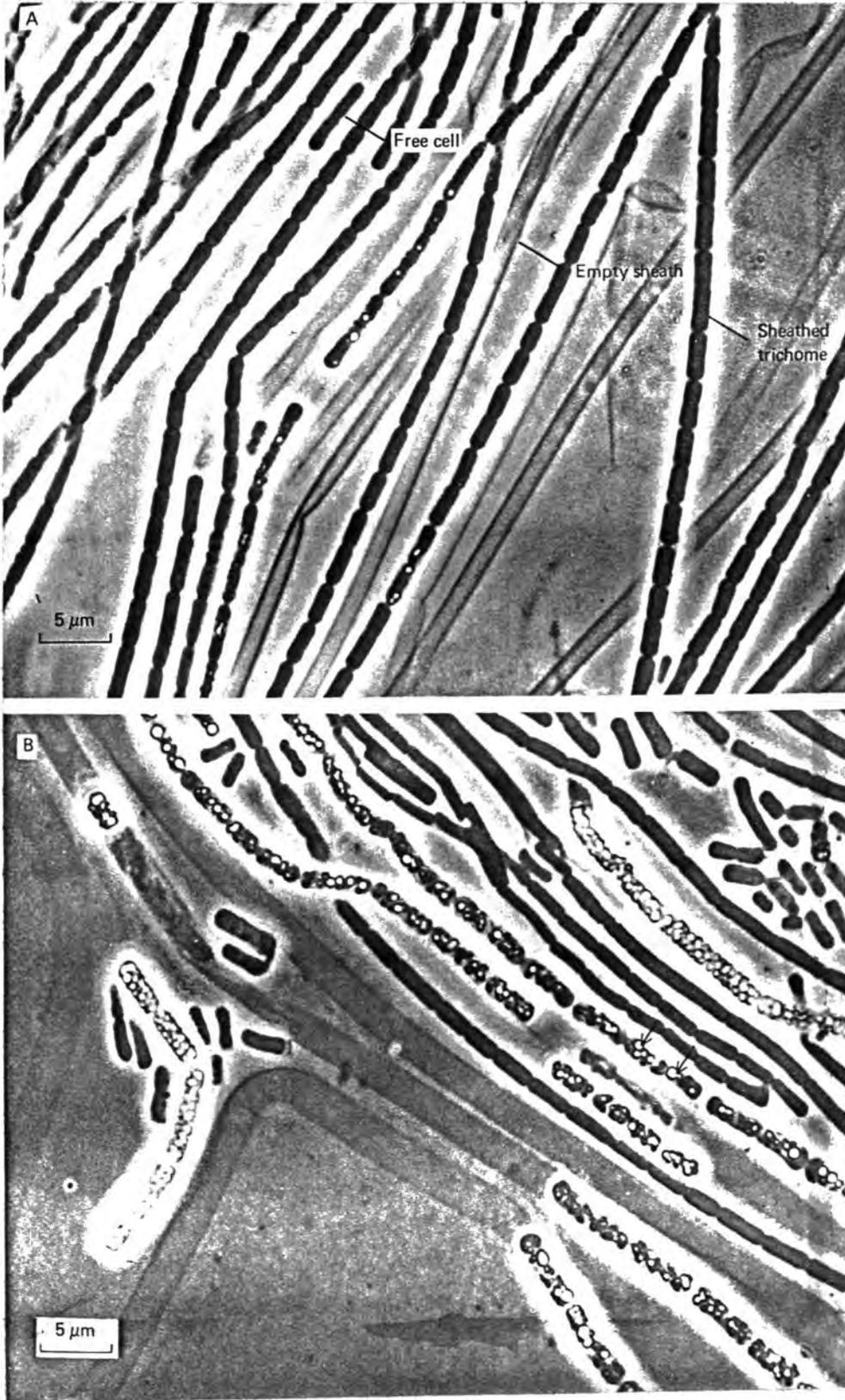
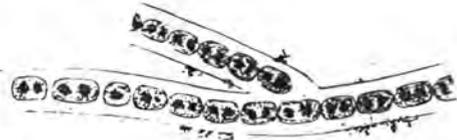
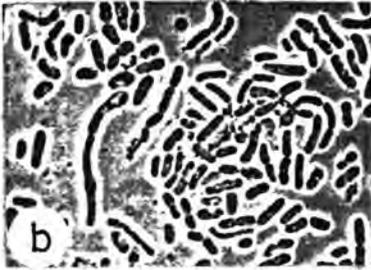
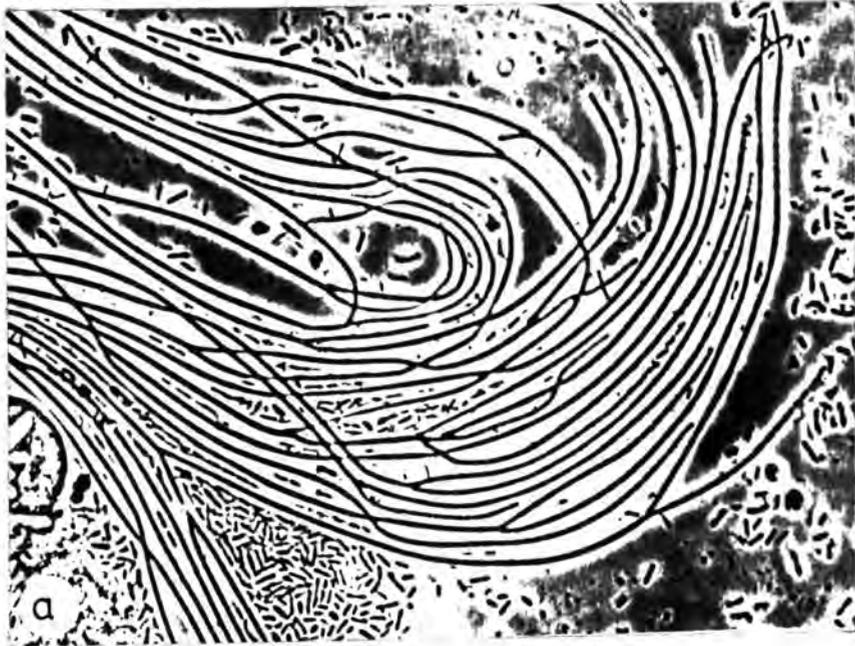


Plate 54 *Sphaerotilus natans* (Poindexter, 1971) Cultivated.



d

Plate 55 Sphaerotilus natans.

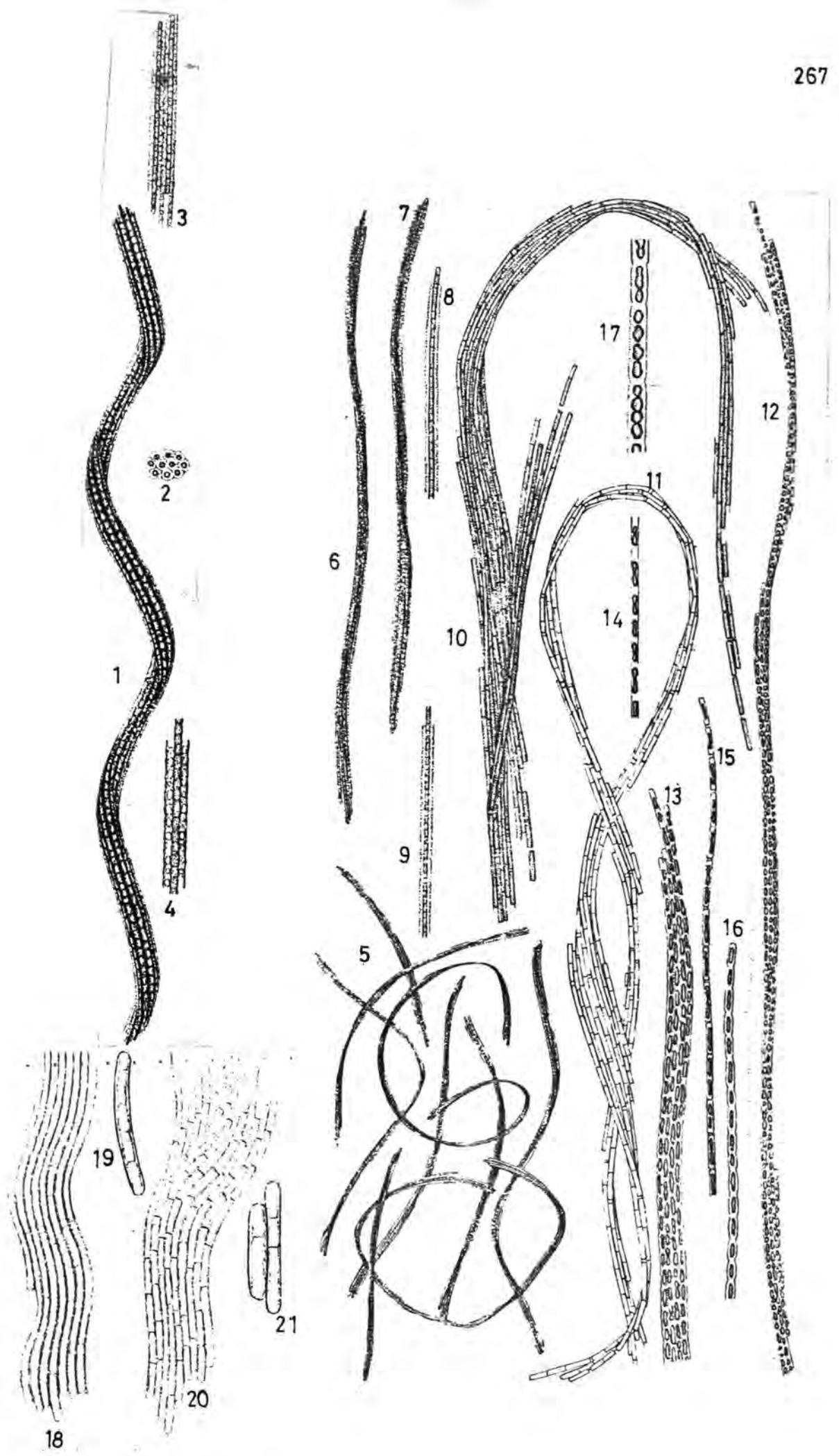
a. In bulking activated sludge (Mulder et al., 1971) x 650.

b - c. Cultivated (Mulder & van Veen, 1963) x 810.

d. Schematic diagram. Eikelboom (1975).

Plate 56 Peloploca

- 1 - 17. Skuja (1956).
18 - 21. Lauterborn (1914-1917).
1 - 4. P. pulchra (1 x 70; 2 - 4 x 2000)
5 - 9. P. ferruginea (5 x 135; 6 - 7 x 470; 8 - 9 x 1000).
10 - 11. P. fibrata x 1340
12 - 17. P. taeniata (12 - 13 x 340; 14 - 17 x 2700).
18 - 19. P. undulata.
20 - 21. P. taeniata.



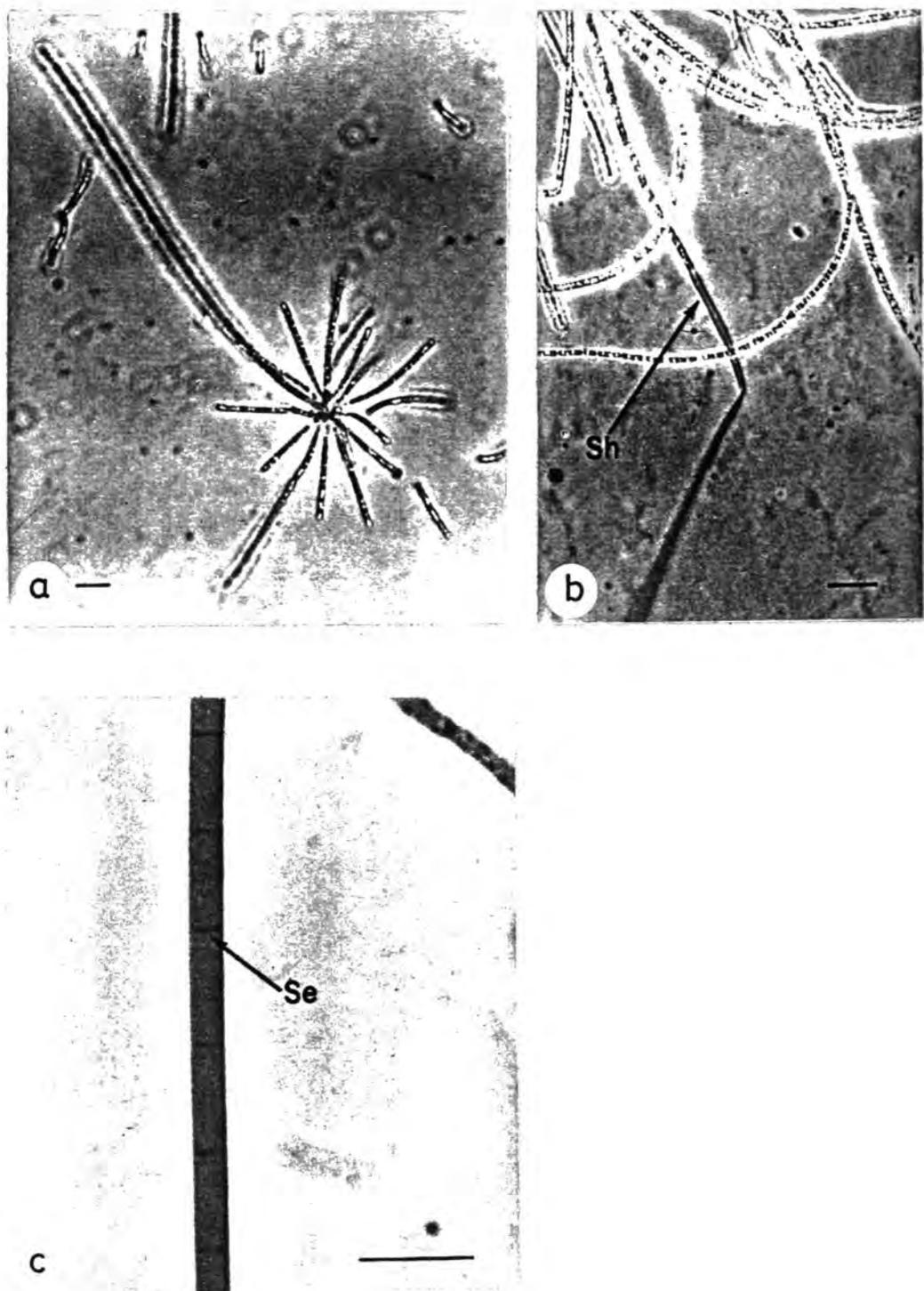


Plate 57 Thiiothrix. (Bland & Staley, 1978).

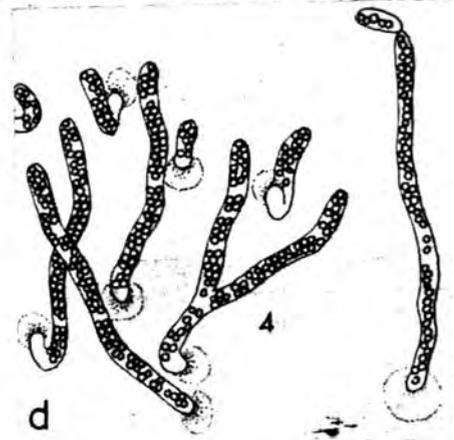
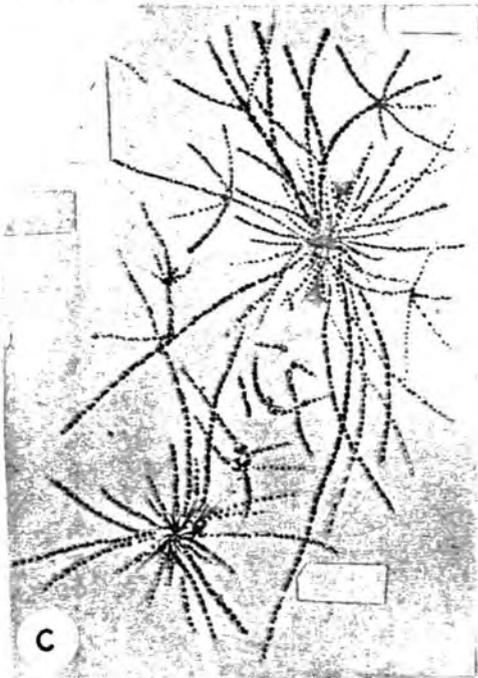
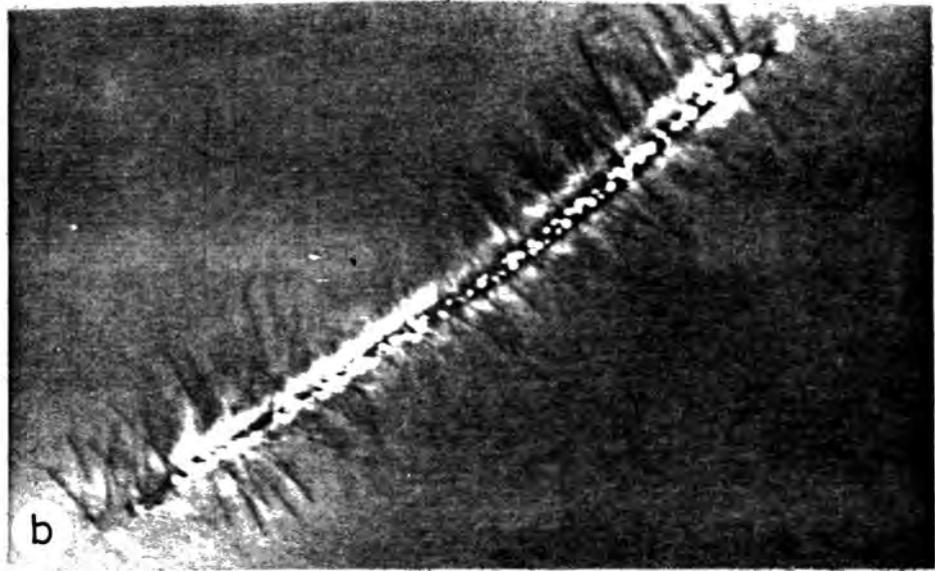
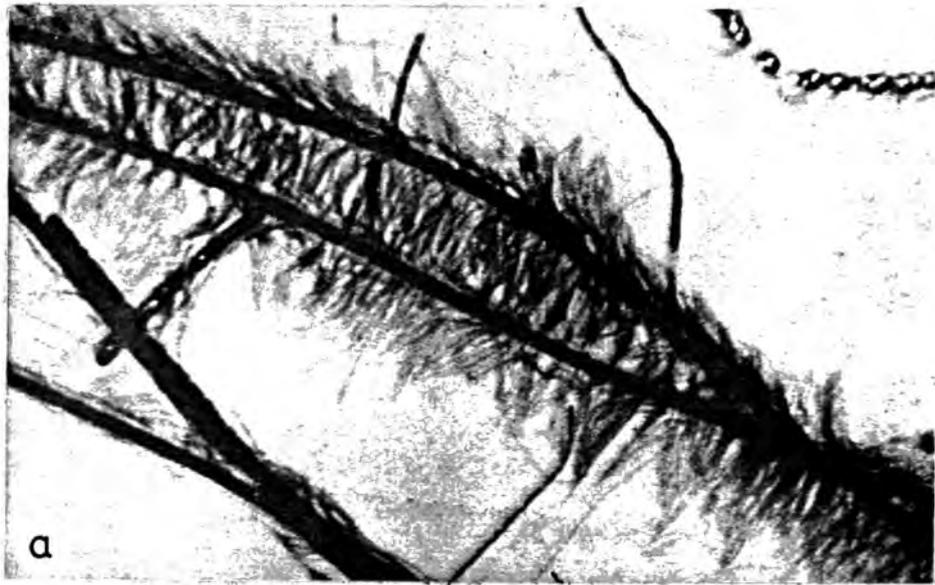
a - b. Filaments with sulphur granules. sh = sheath.

Bar represents 10 μm . c. Filament depleted of sulphur

granules. se = septum Bar represents 5 μm .

Plate 58 Thiothrix

- a - b. Filamentous appendages in Thiothrix (Morita & Burton, 1965).
- c. Filament growing in nature reproduced from Myioshi (Harold & Stanier, 1955).
- d. T. nivea. Reproduced from Winogradsky (Skerman, 1959). x 800



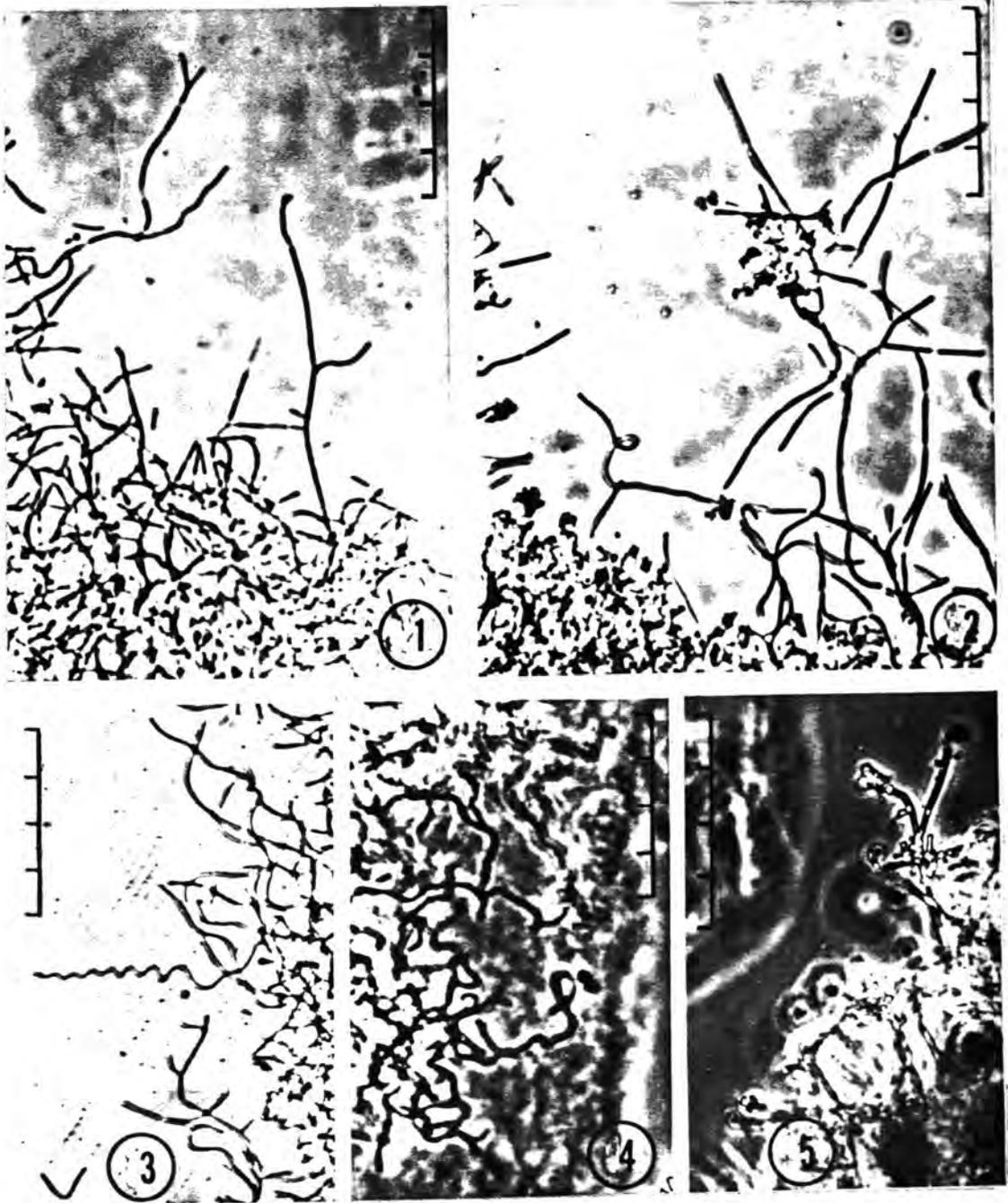


Plate 59 *Flexithrix dorotheae* (Lewin, 1970). Cultivated.

Bar represents 40 μm .

Plate 60 Herpetosiphon. Cultivated

a. Herpetosiphon aurantiacus (Holt & Lewin, 1968).

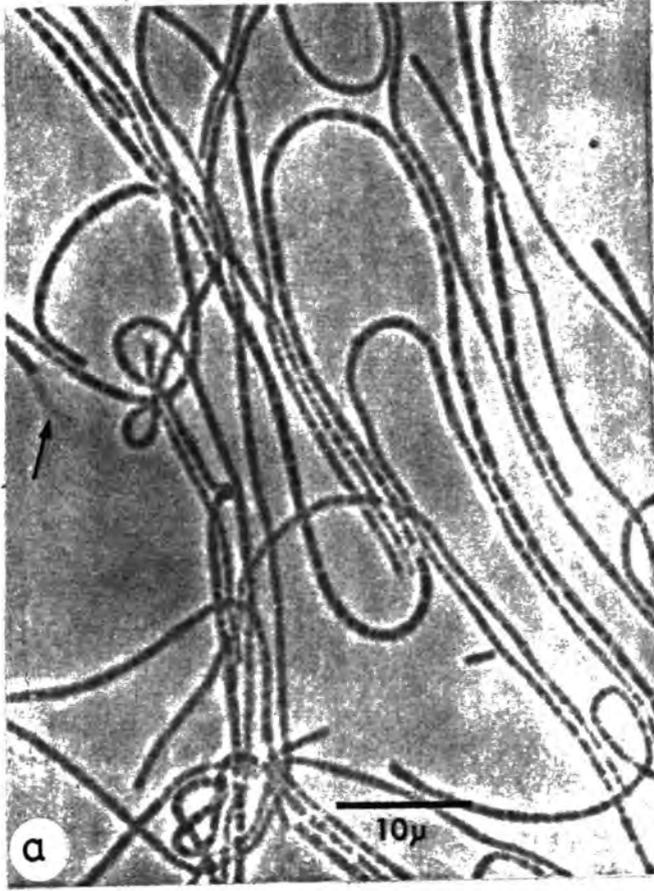
Arrow shows a short extension of the sheath.

b. H. nigricans (Lewin, 1969).

c. H. geysiricolus (Lewin, 1969).

d. H. persicus (Lewin, 1969).

Bar represents 10 μm .



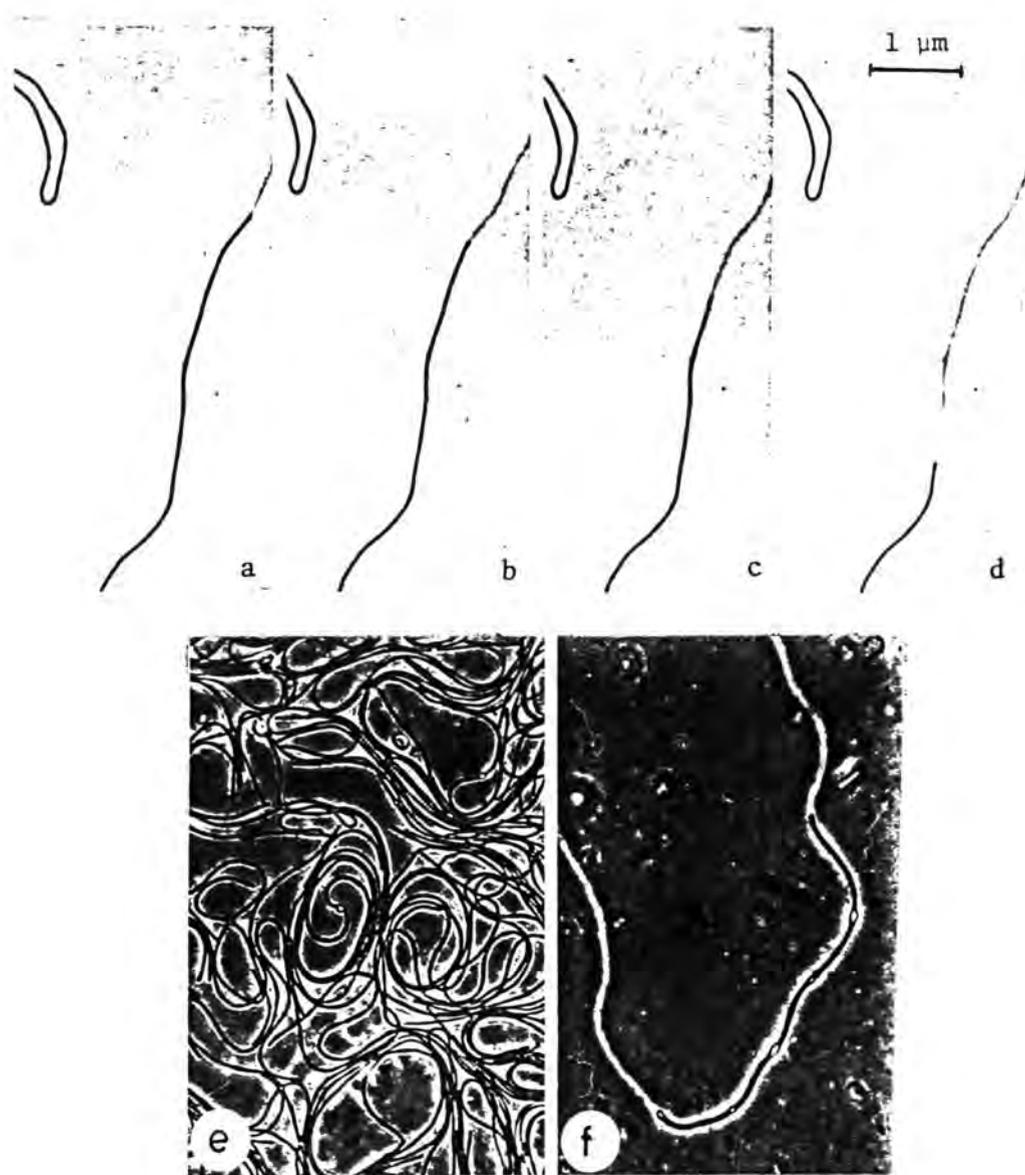


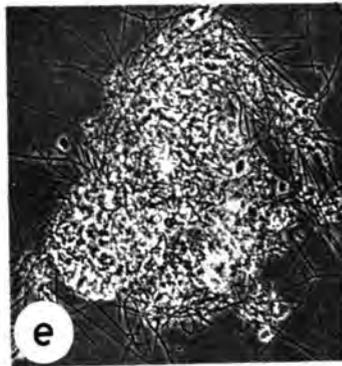
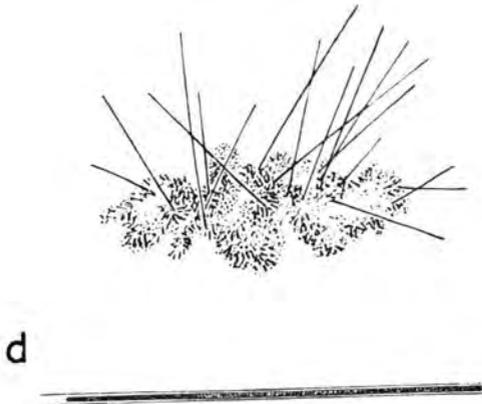
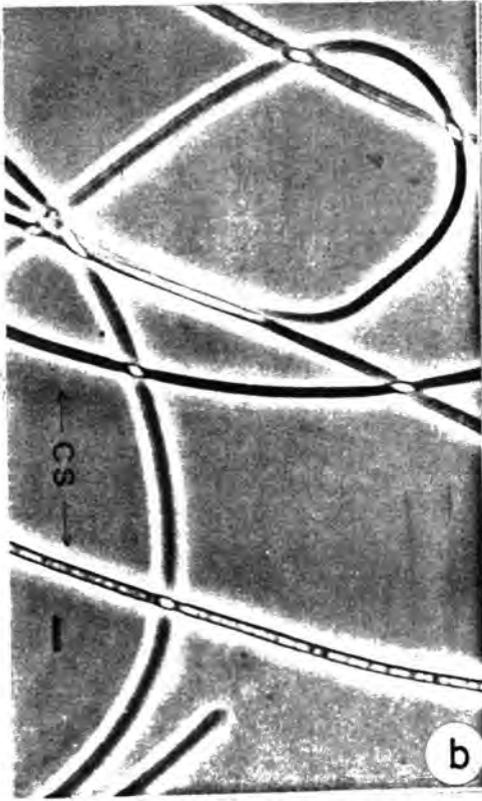
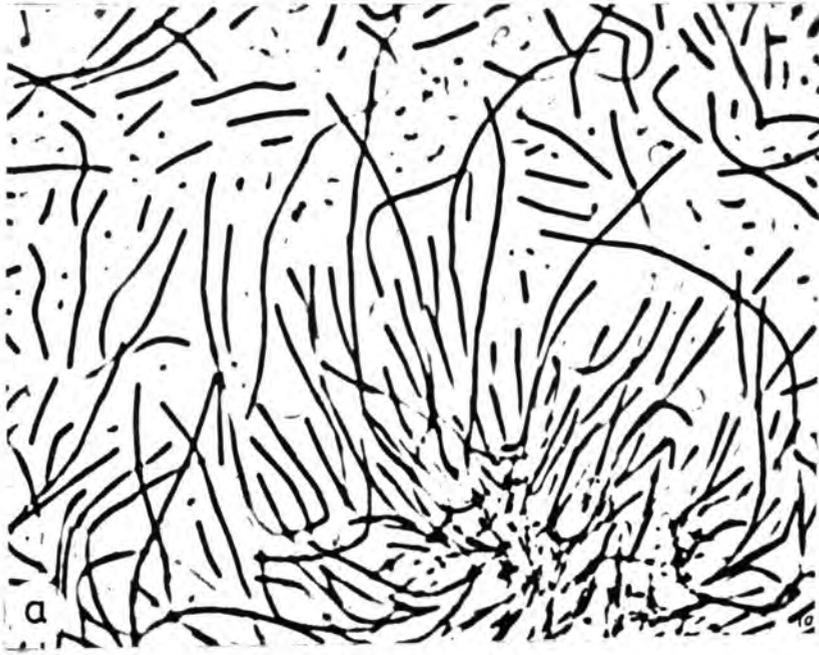
Fig. 61 Herpetosiphon. Cultivated.

a - d. Organisms grown on lake water agar. Micrographs taken over a period of 40 min which show a filament which has migrated inside the sheath (Skerman et al., 1977).

e - f. Filaments on agar surface in a chamber culture in which a few bulbs are visible (the bulbs are swollen sections of filaments, occurring mainly in ageing cultures) Reichenbach & Golechki, 1975) e.x 335; f. x 425.

Plate 62 Haliscomenobacter hydrossis. Cultivated.

- a. H. hydrossis (Streptothrix hyalina). Mulder et al.
(1971). x 1650.
- b - c Deinema et al. (1977). cs = cell septa. Bar
represents 5 μ m.
- d. Schematic diagram (Eikelboom, 1975).
- e. van Veen et al. (1973) x 325



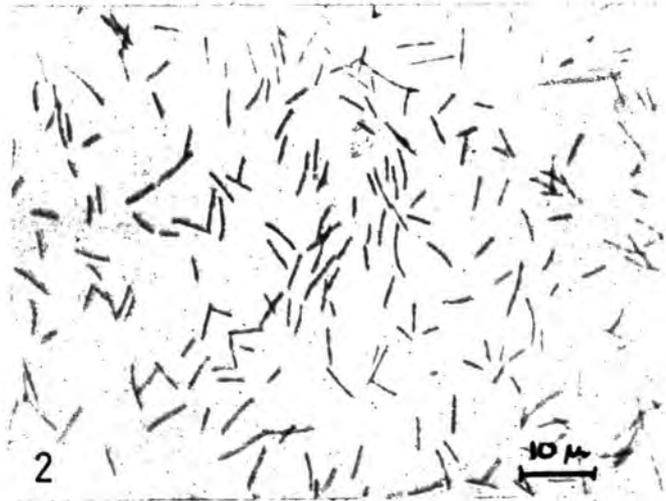
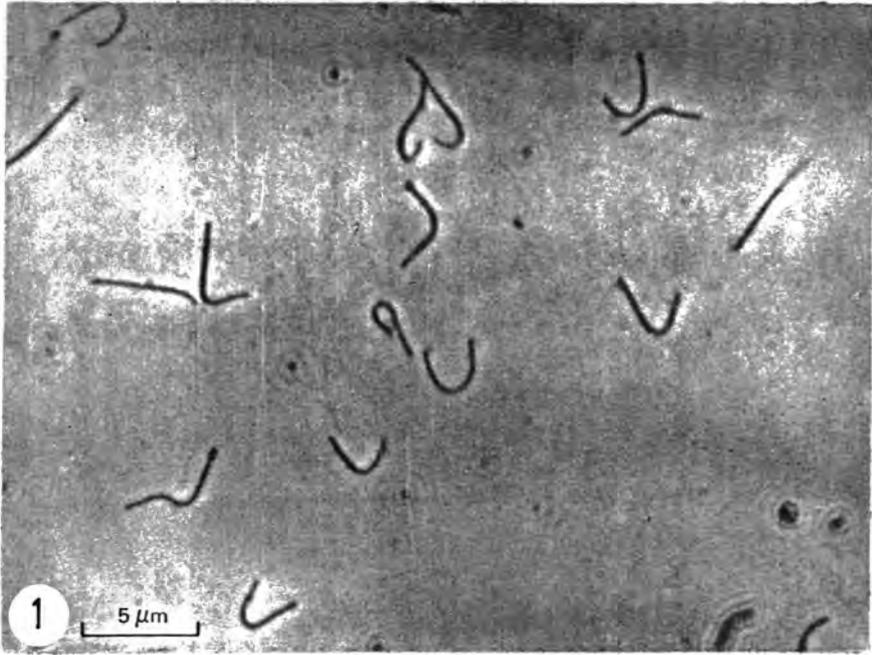


Plate 63 Cytophaga. Cultivated.

1. Poindexter (1971).

2 - 3 C. johnsonae (Stanier, 1947).

Plate 64 Flexibacter. Cultivated.

1 - 5 Soriano (1947) x 1000.

1. F. flexilis

2. F. elegans.

3. F. albuminosus.

4. F. aureus.

5. F. giganteus

6. Flexibacter (Microscilla sp) Farquhar (1971).

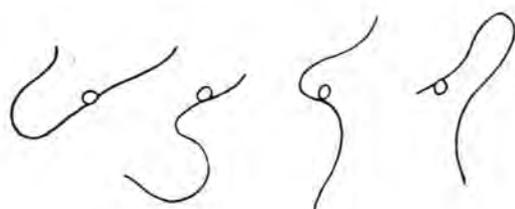
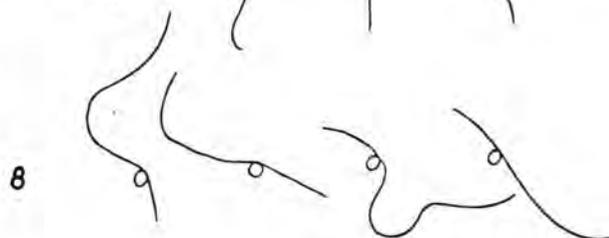
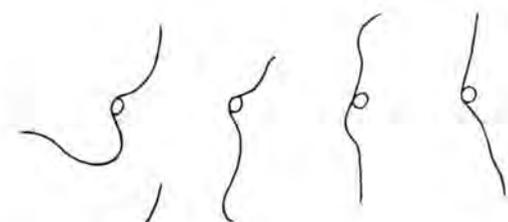
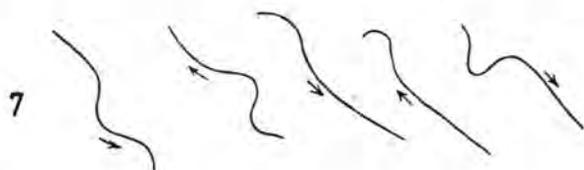
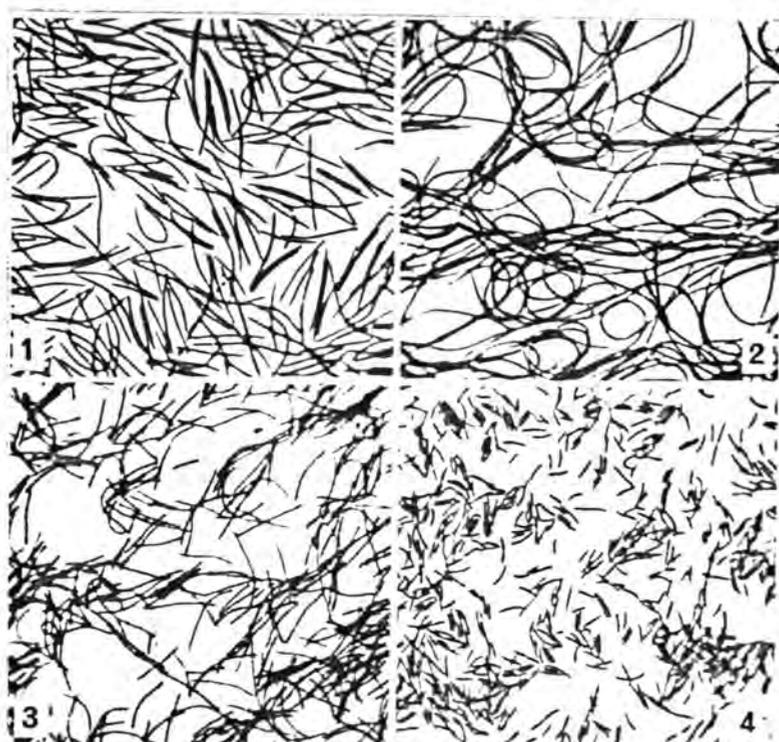
7-8 Flexibacter (Microscilla agilis) Pringsheim (1951)

x 500. 7 Sketches of quick changes in shape and

direction (indicated by arrow). 8. Filament glides

along particle to which remains attached in spite of

translocation.



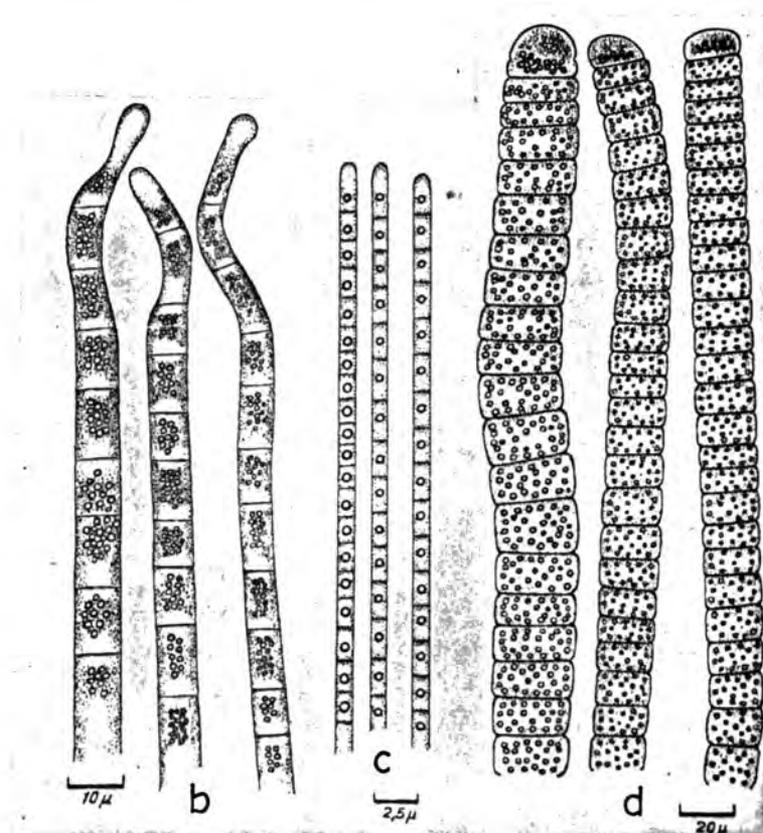


Plate 65 Beggiatoa. Cultivated

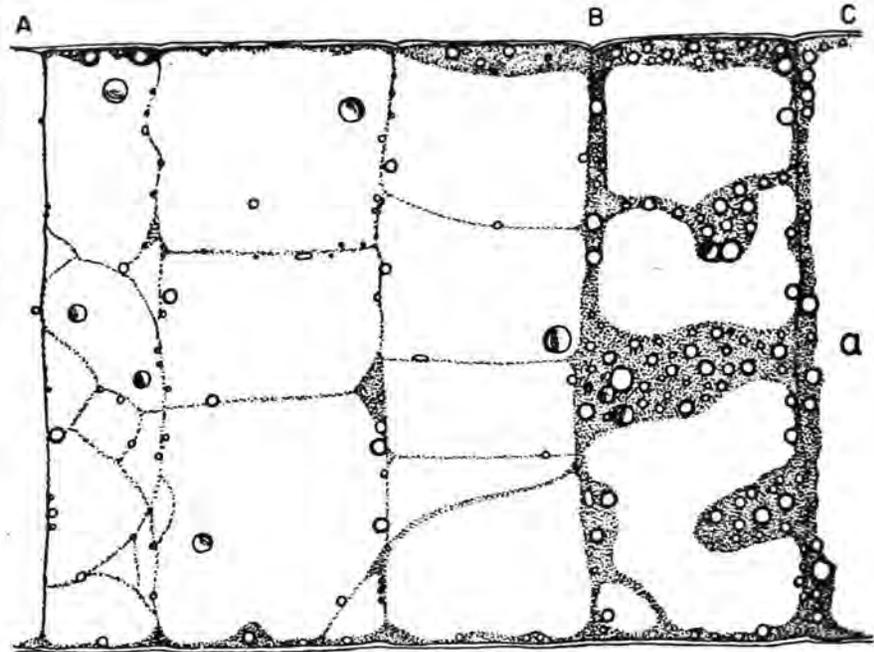
a. Sieburth (1979). cw = crosswalls s = sulphur granules

b - d Fjerdingstad (1979).

b. B. arachnoidea

c. B. uniguttata

d. B. mirabilis



0 5 10 15 20
SCALE IN MICRONS

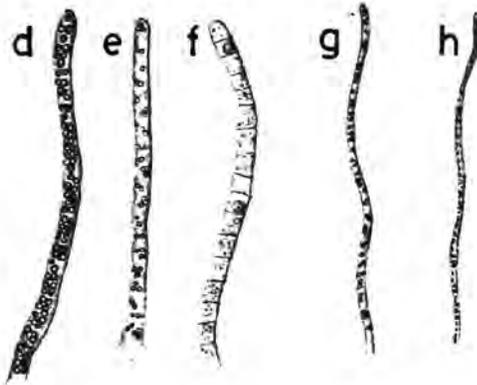
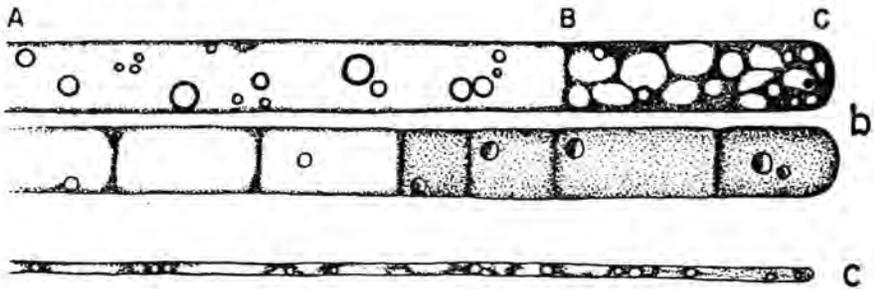


Plate 66 Beggiatoa

a-c. Lackey (1961). d-h. Winogradsky (1949).

a. B. gigantea. b. B. arachnoidea. c. B. leptomitiformis.

A-B Optical cross section

B-C Surface view

d-f. B. alba. g. B. media. h. B. minima.

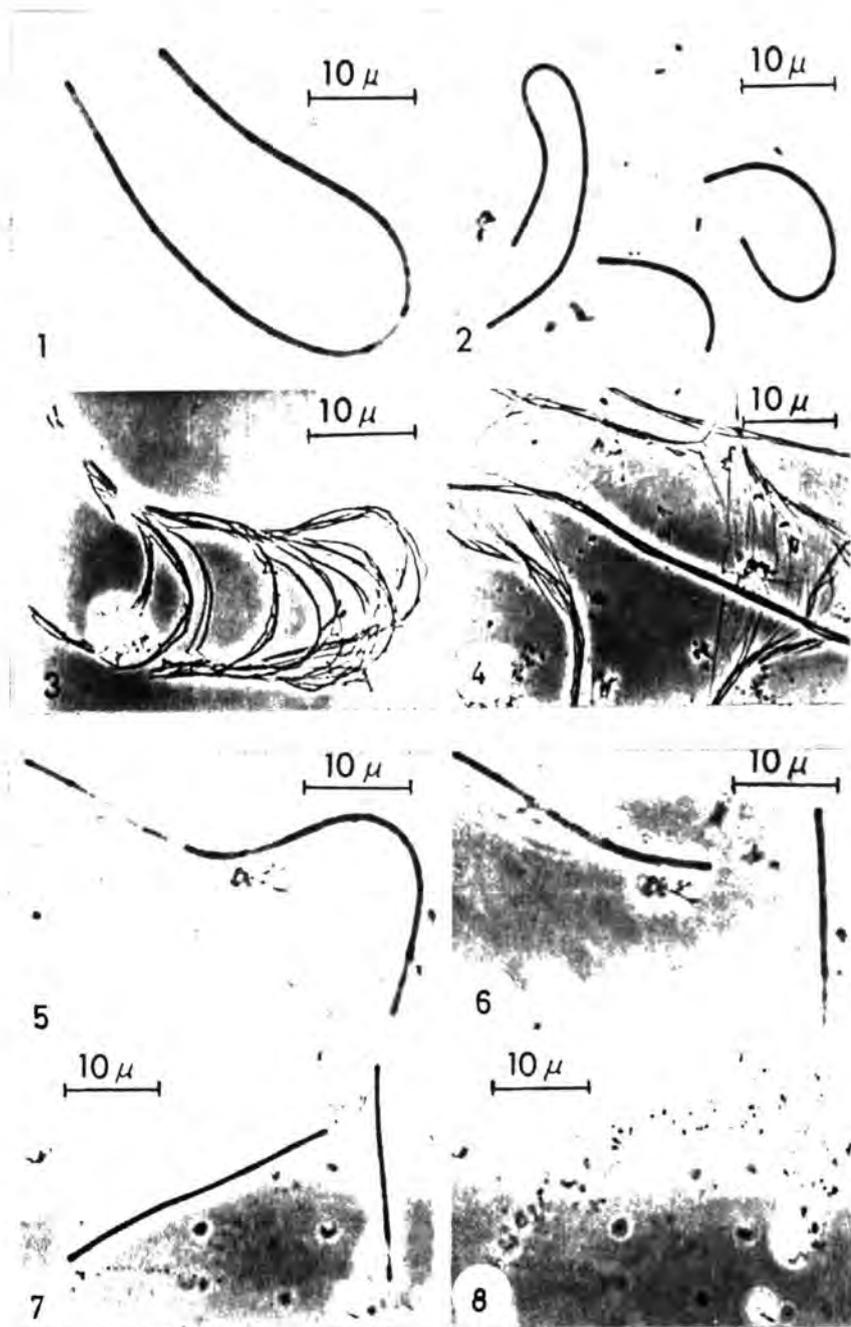


Plate 67 Toxothrix trichogenes (Krul et al., 1970).

1-2 Living filaments

3-4 Excreted material encrusted with iron.

5-8 Breakage of the filament during microscopic observation.

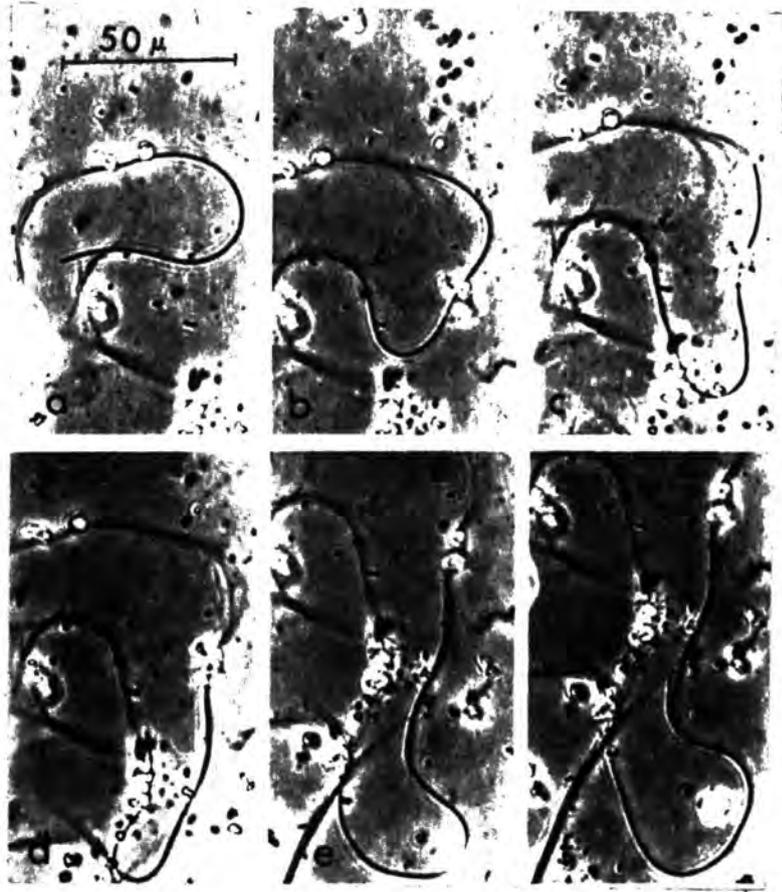


Plate 68 Toxothrix trichogenes (Krul et al. 1970).

a-f Movement of the filament.

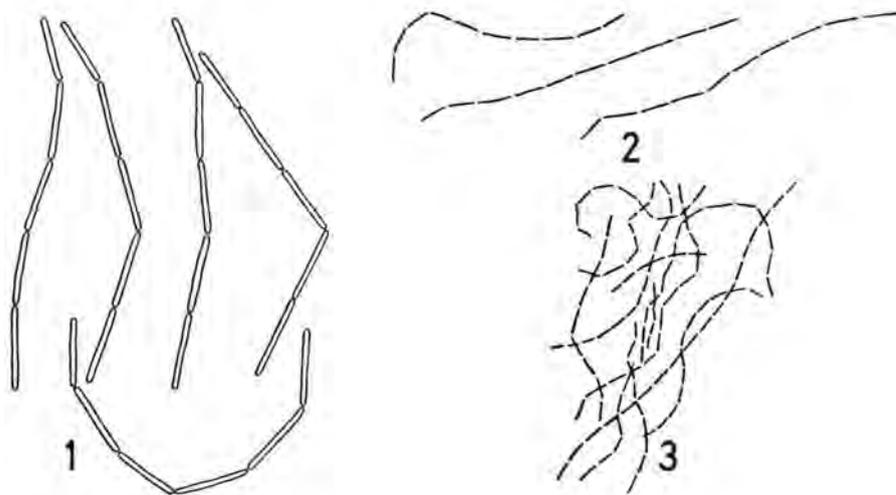


Plate 69 Bactoscilla (Pringsheim, 1951).

1. Trichome of five rods, stages in quick change of position, and trichome of six rods, bending in joints; x 1250.
2. Trichome of ten rods, stages in quick change; x 200.
3. Bundle of Trichomes; x 500.

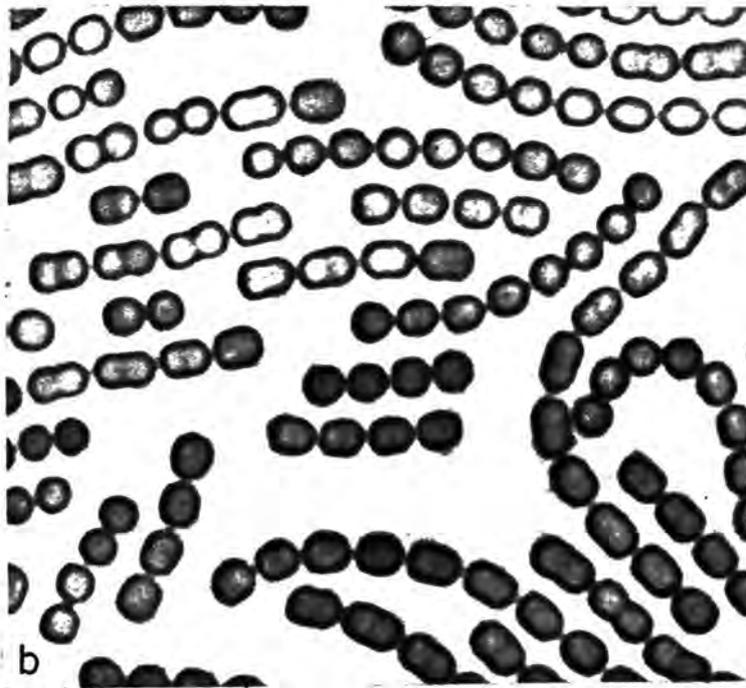
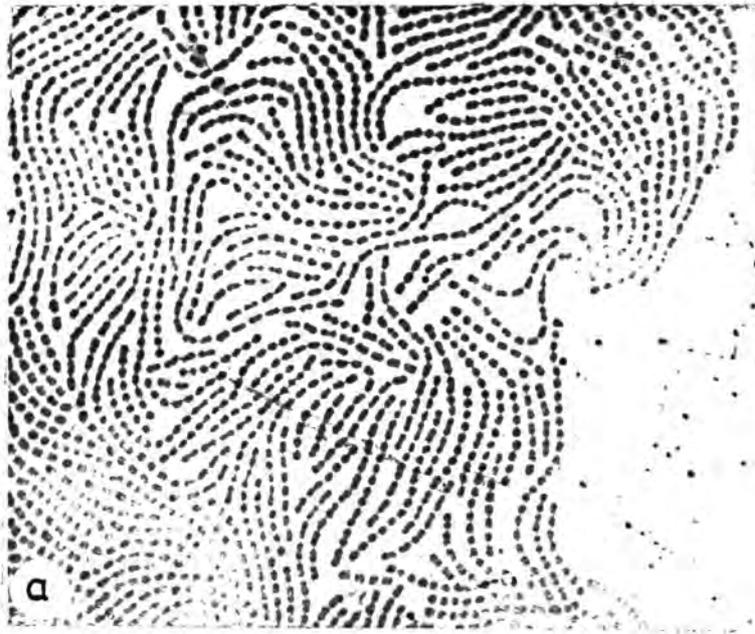


Plate 70 Vitreoscilla (Pringsheim, 1951). Cultivated.

a - b V. moniliformis

a. Edge of living, 3-day-old colony x 720. b. Part of the same colony, Bouin tannic acid + crystal violet. x 3000.

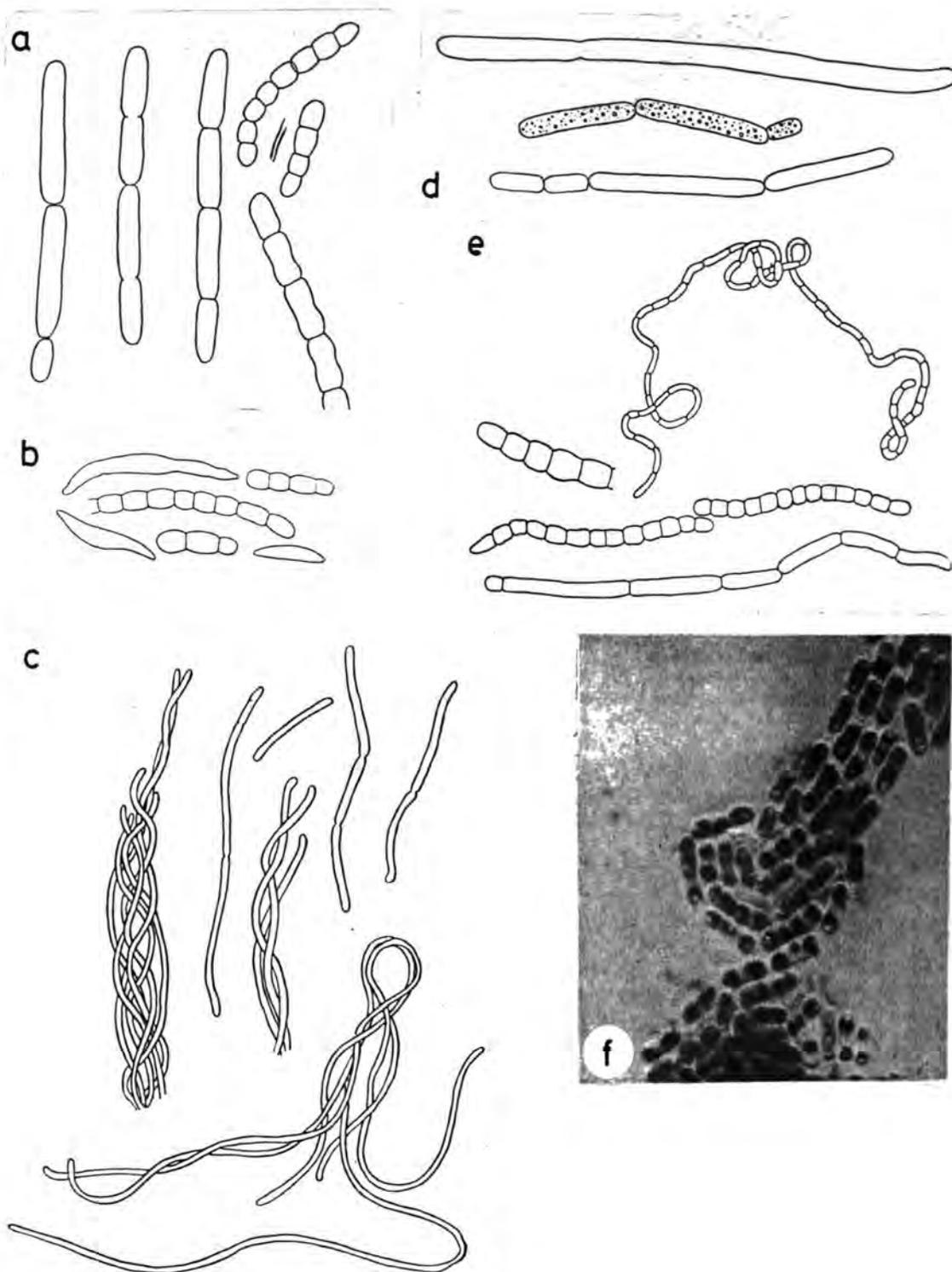


Plate 71 Vitreoscilla (Pringsheim, 1951).

a. V. moniliformis. x 1500. b-c V. catenula b x 1500; c x 400.

d. V. paludosa x 1500. e-f. V. stercoraria. e x 1250; f. yeast

extract agar, 24 hr room temperature, osmic acid HCl Giemsa.

Plate 72 Achroonema

1 - 6 Skuja (1956) 7 - 8 Skuja (1948).

1 - 3 A. splendens 1 x 670; 2 - 3 x 1000

4 - 5 A. splendens var. tenuior 4 x 670; 5 x 1000.

6. A. proteiforme x 670.

7 - 8 A. spiroideum x 900.

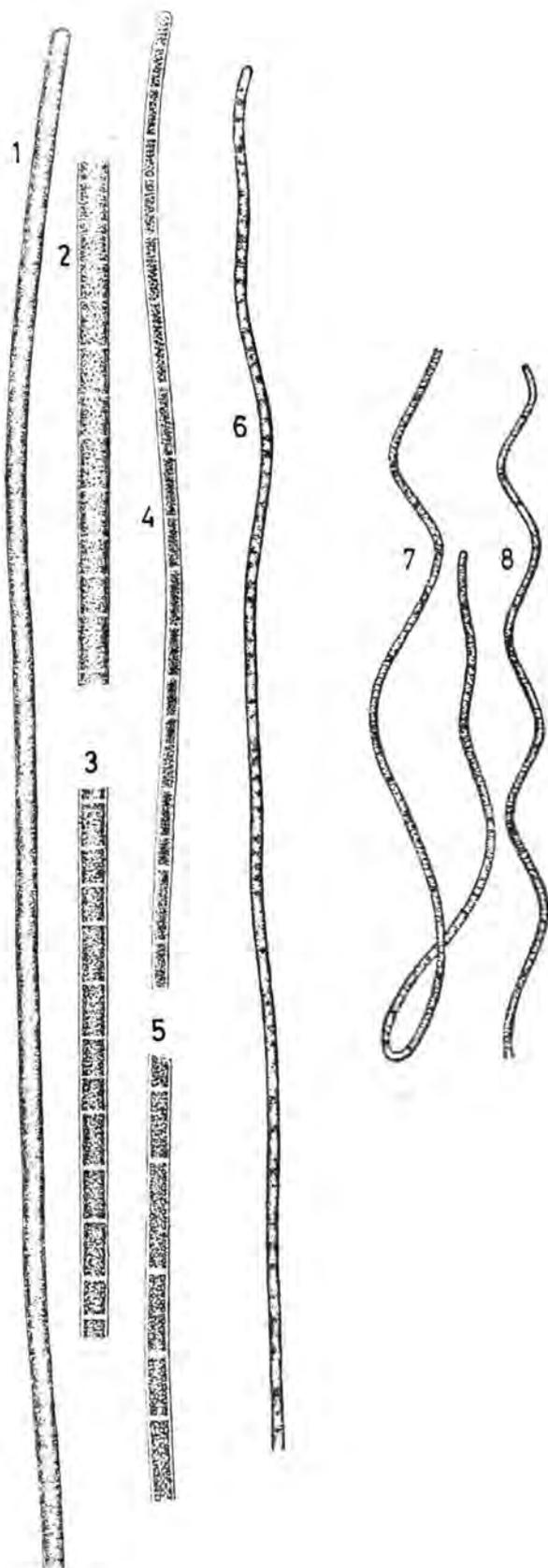
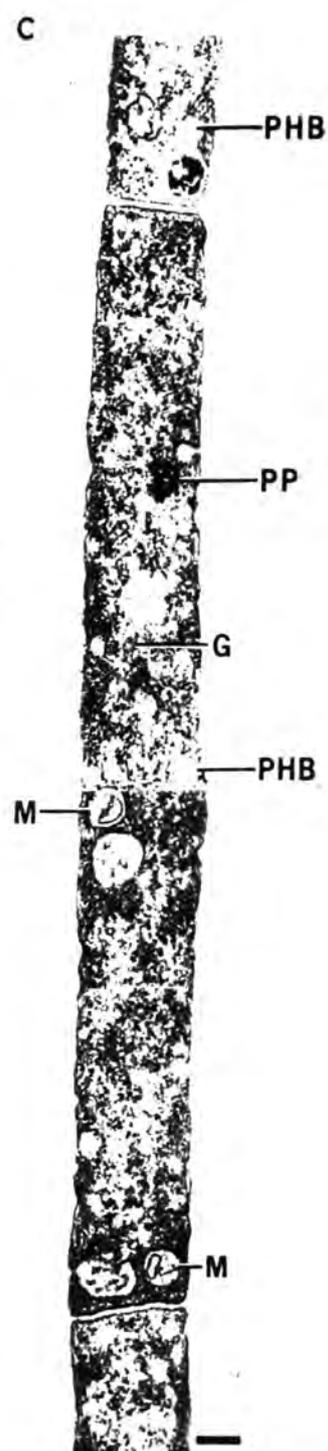


Plate 73 Chloroflexus aurantiacus (Pierson & Castenholz, 1974).

Cultivated a - b x 1000; C. Electron micrograph of thin section of filament; M = meso somes; PP = inclusions tentatively identified as polyphosphate; PHB = poly- β -hydroxybutiric acid granule; G = possibly glycogen.

Bar represents 0.2 μ m.



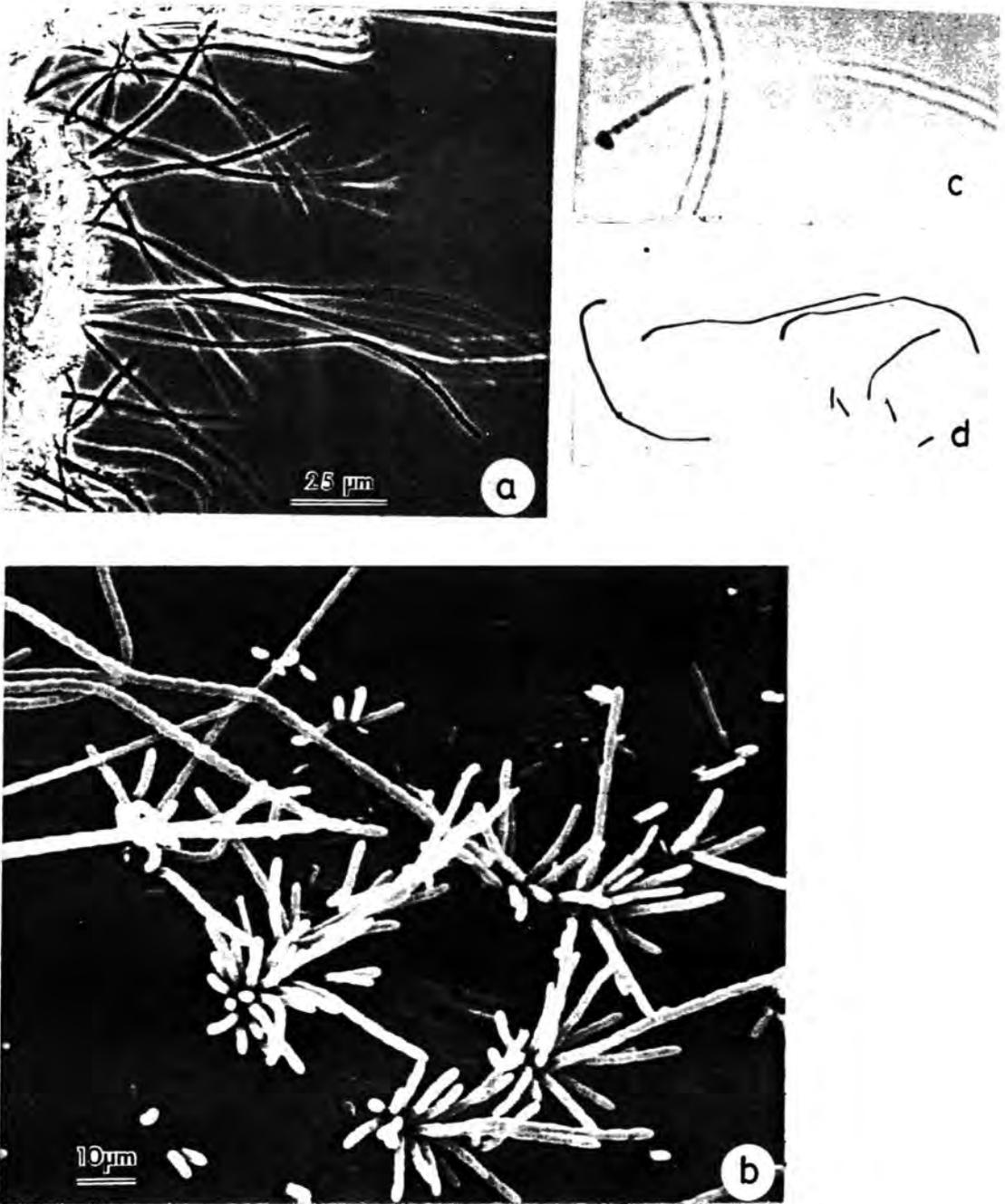


Plate 74 Leucothrix. Cultivated.

a - b. Sieburth (1979)

a. Filaments with terminal gonidia.

b. Formation of rosettes.

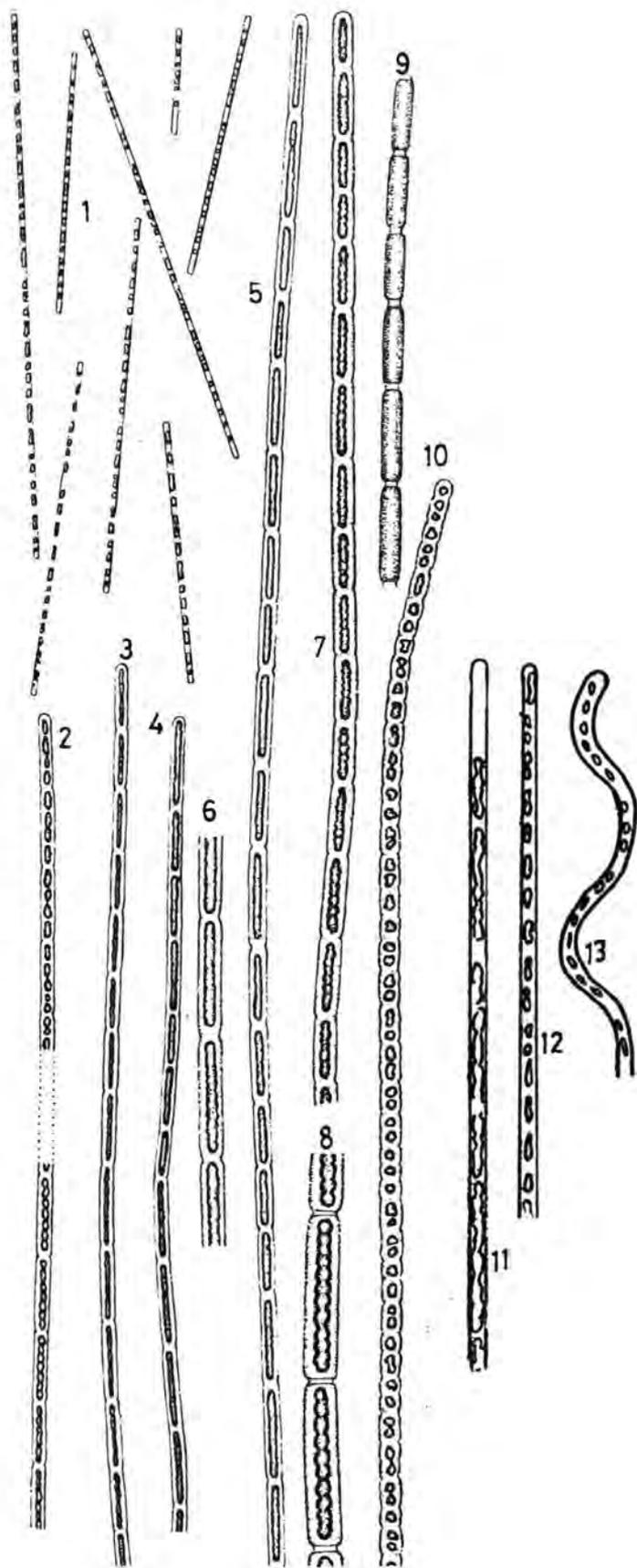
c - d. Harold & Stanier (1955).

c. Holdfast at the base of the filament. x 1500.

d. Isolated filaments developed from "gonidia". x 400.

Plate 75 Pelomema (Skuja, 1956)

1. P. subtilissimum. x 670
- 2 - 6 P. aphanes 2 - 6, x 670; 7. x 1000.
- 7 - 9 P. tenue 8. x 670; 9. x 1000
10. P. pseudovacuum x 670.
- 11-13 Lauterborn (1914-1917).
11. P. tenue.
12. P. pseudovacuum.
13. P. spirale.



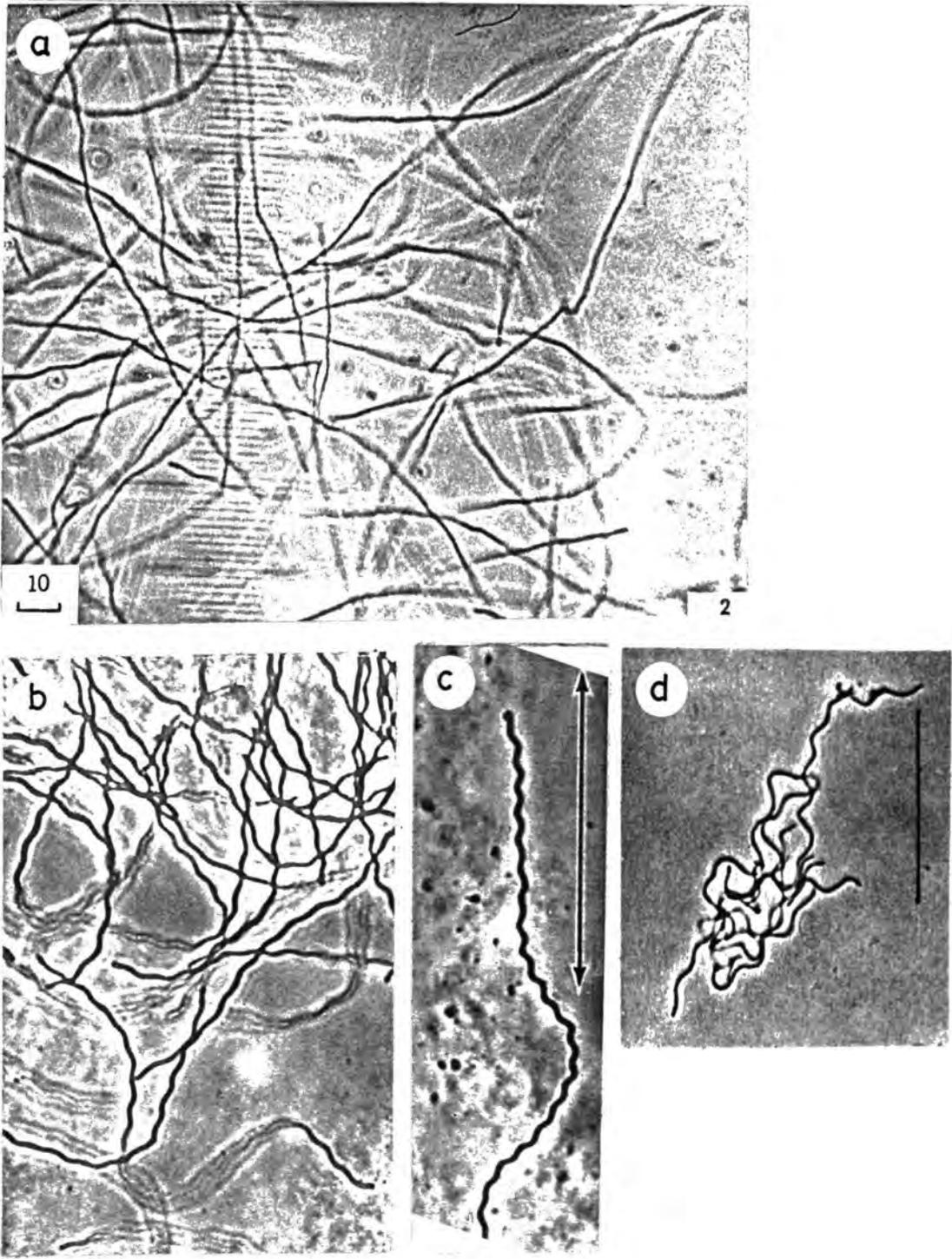


Plate 76 · Saprospira. Cultivated.

- a. S. grandis (Lewin, 1962).
- b. S. albida (Lewin, 1965) x 910.
- c. S. flammula (Lewin, 1965) Bar represents 50 μm .
- d. S. toviformis (Lewin, 1970). Bar represents 40 μm .

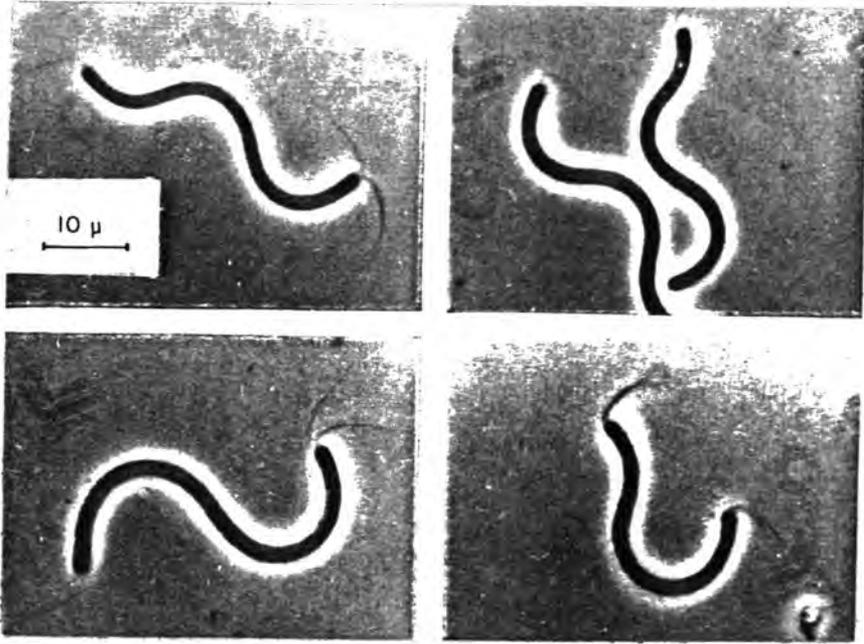


Plate 77 Spirillum volutans (Wells & Krieg, 1965).



Plate 78 Spirochaeta plicatilis (Bergey's Manual, 1974) x 2200.

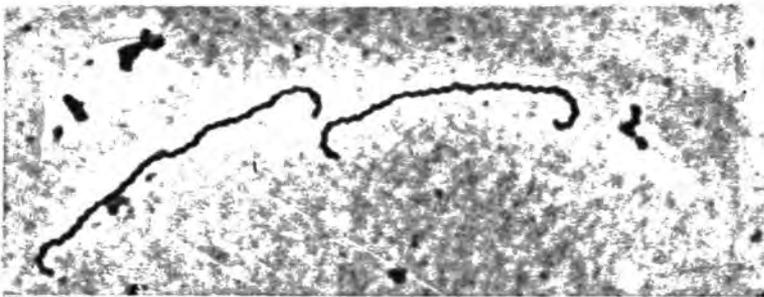


Plate 79 Leptospira interrogans (Bergey's Manual, 1974) x 2200.

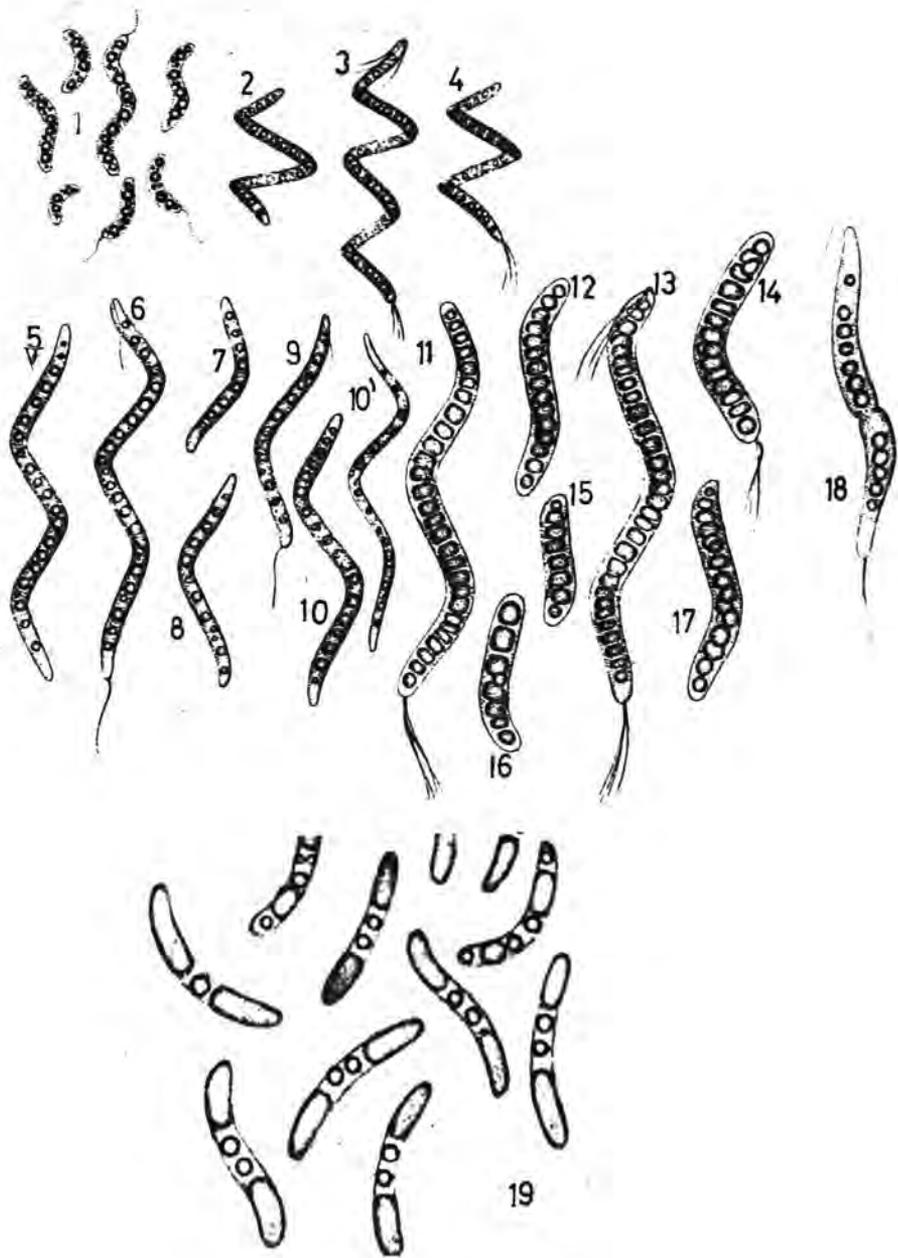


Plate 80 Thiospira (Skuja, 1956) x 1340.

1. Th. agilis.

2 - 4 Th. tenuis.

5 -10 Th. dextrogyra.

10¹ Th. dextrogyra var leptosoma.

11-18 Th. winogradski.

19. Th. bipunctatum (Skerman, 1959) x 1500.

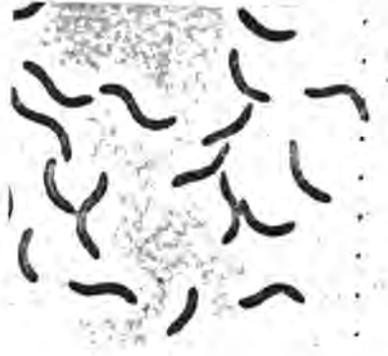


Plate 81 Rhodospirillum molischianum (Bergey's Manual, 1974) x 2000.

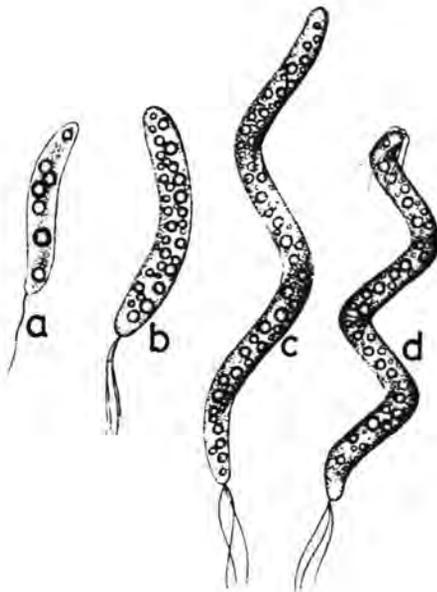


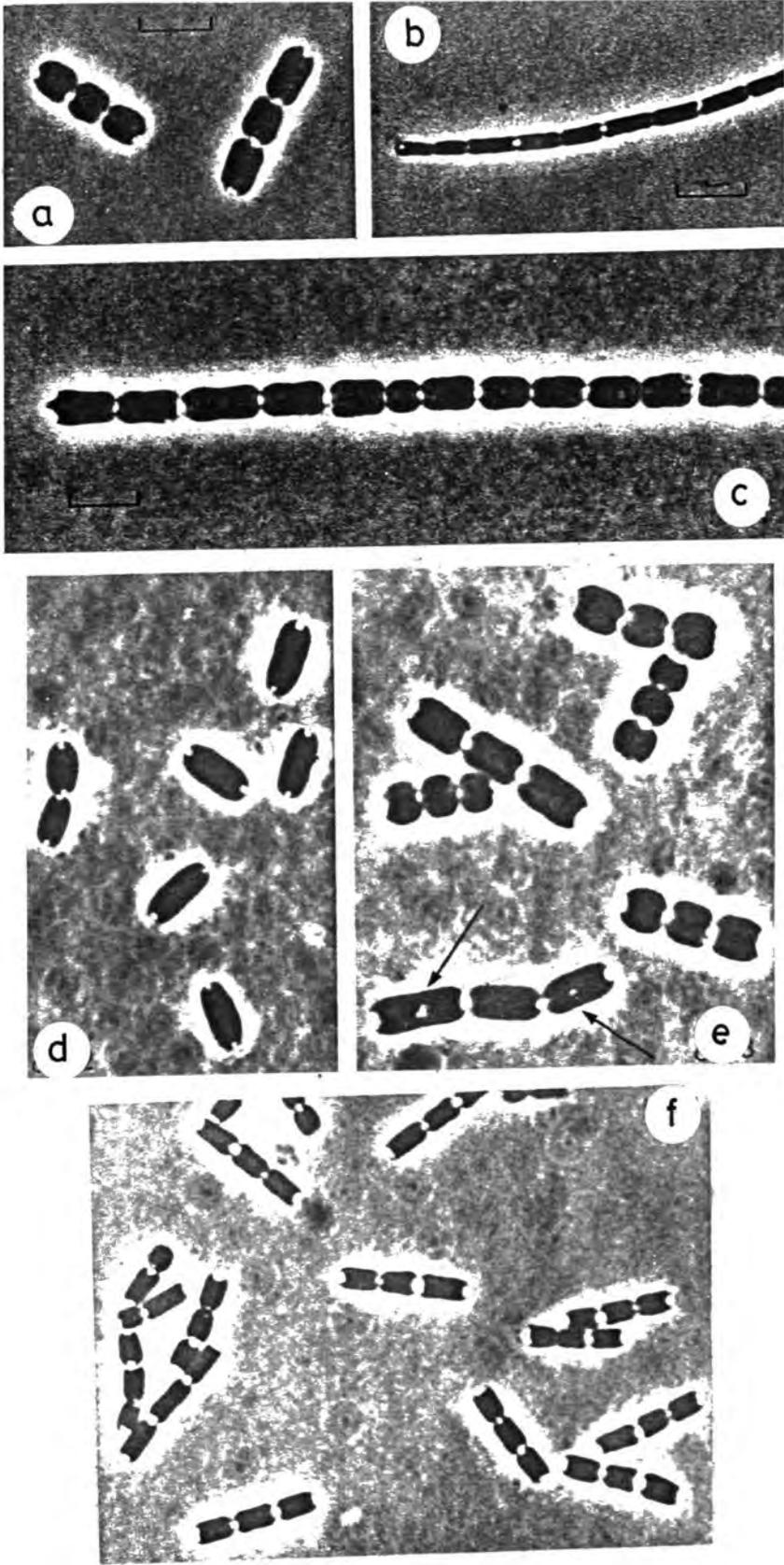
Plate 82 Thiospirillum (Skuja, 1956). x 1340.

a. Th. Rosenbergi

b-d Th. jenense.

Plate 83 Pseudanabaena.

a-c. Rippka et al. (1979). Bar represents 5 μ m. d-f. Stanier et al. (1971). This organism was described in this paper as Synechococcus sp. but assigned as Pseudanabaena by Rippka et al. (1979). The arrows point the newly formed polar vacuoles which are developing at the site of septum formation in two dividing cells.



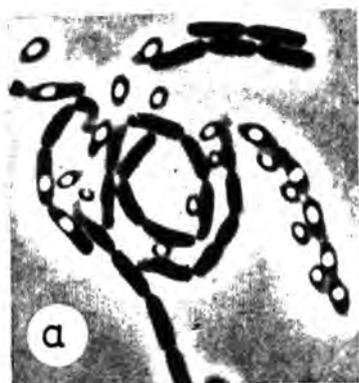


Plate 84 Bacillus.

- a. B. megaterium (Skerman, 1959) x 1800.
b. A chain-forming Bacillus (Stanier et al. 1971) x 2350.

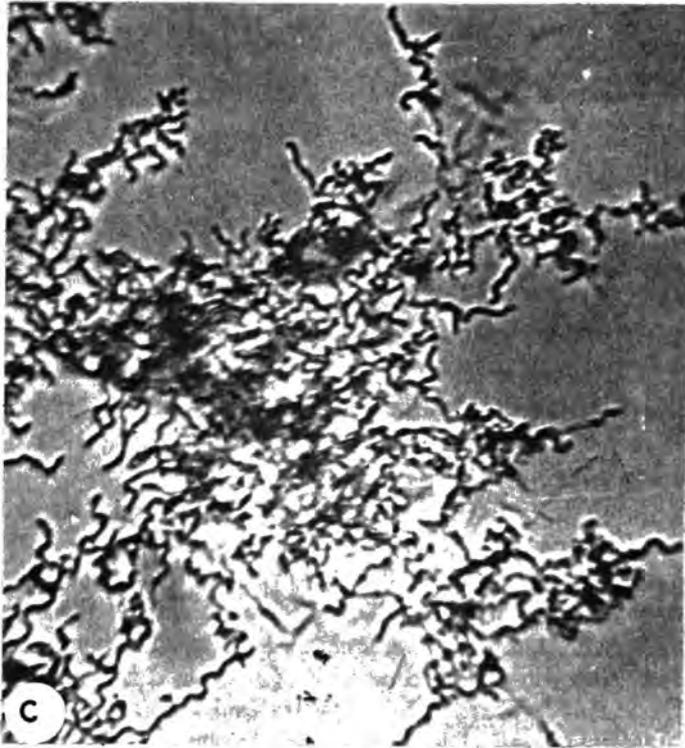
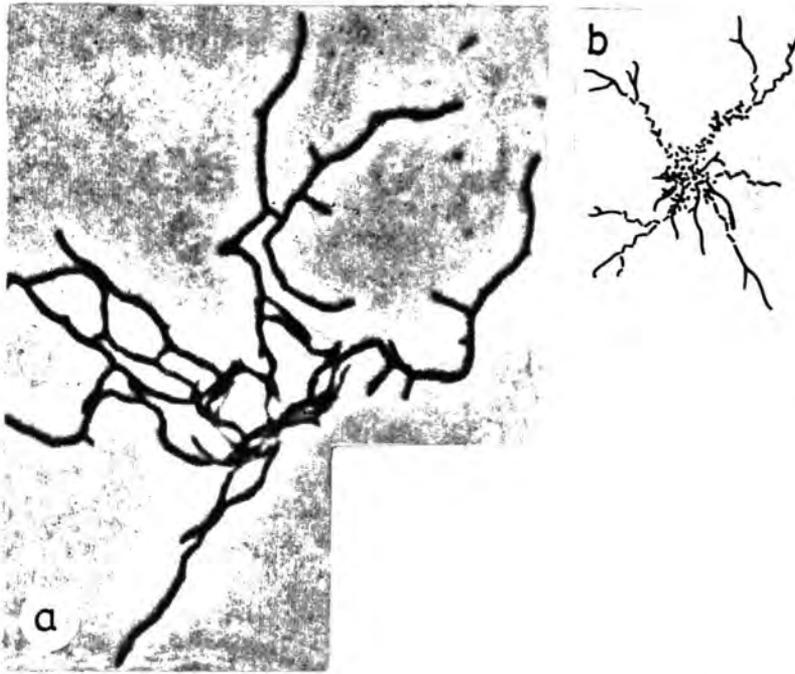


Plate 85 Nocardia

- a. N. asteroides (Skerman, 1959) x 1800
- b. Schematic diagram of the mycelium (Sykes & Skinner, 1973).
- c. Waksman (1967).

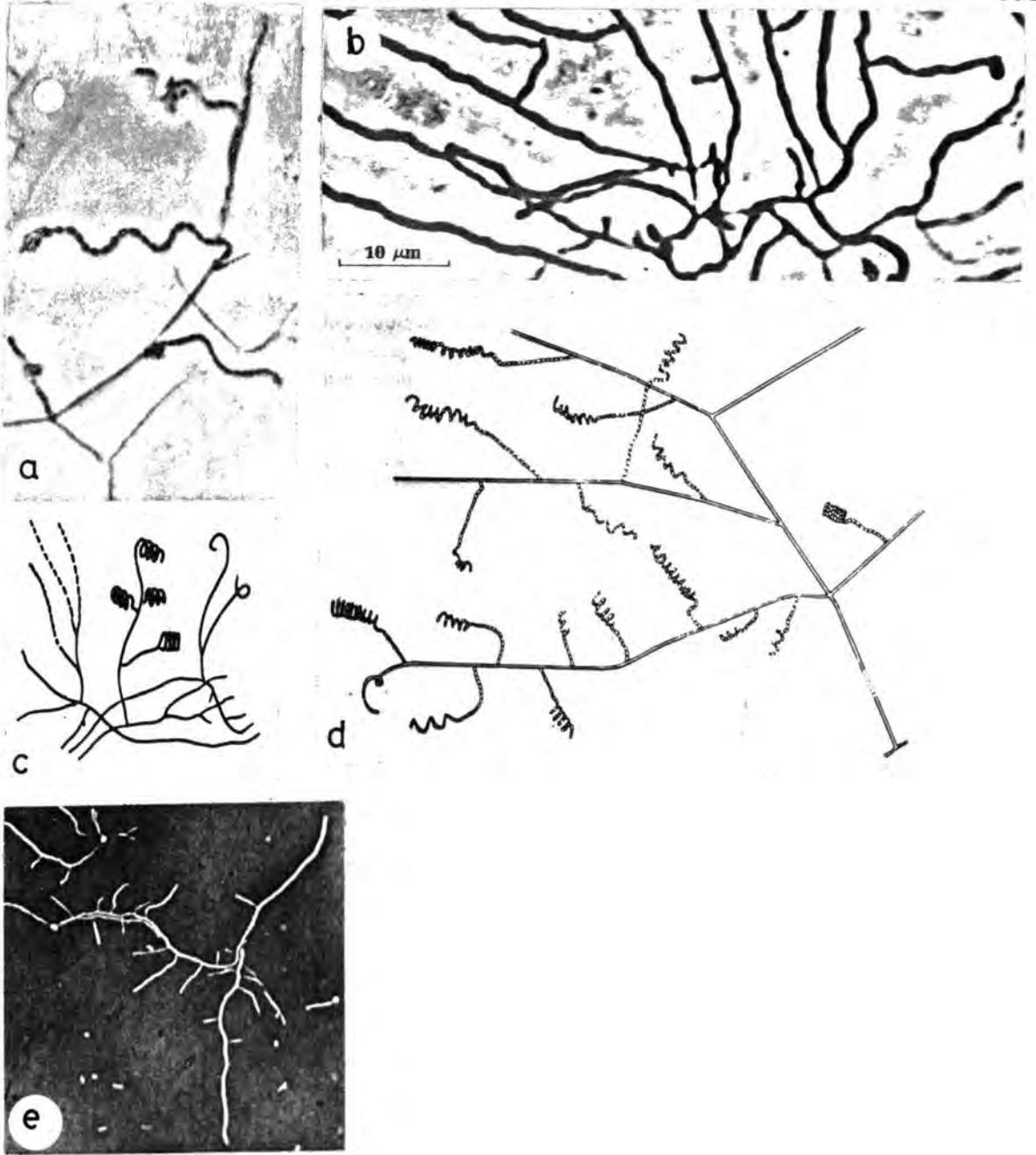


Plate 86 Streptomyces

- a. Slide culture (Skerman, 1959) x 1440.
- b. Stanier et al. (1971) x 1500.
- c. Schematic diagram of the mycelium (Sykes & Skinner, 1973)
- d. Waksman (1967).
- e. Early stages of development (Stanier et al. 1971).

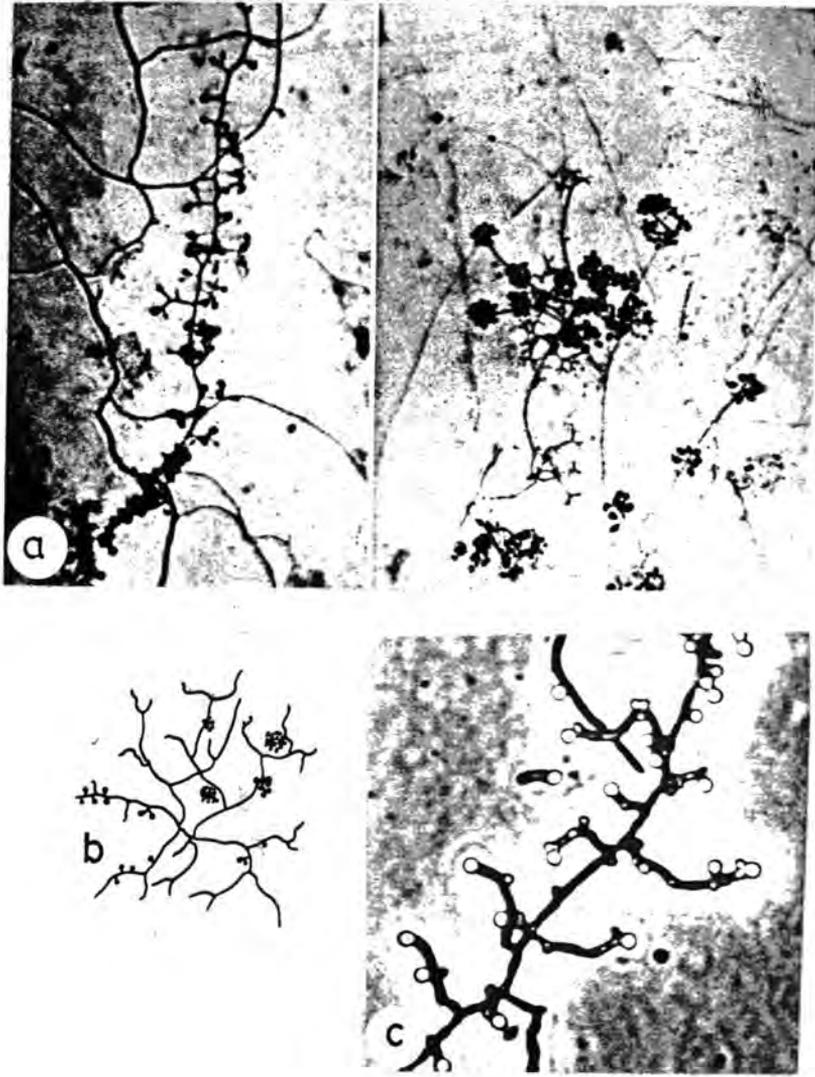


Plate 87 Micromonospora

- a. Growth of two typical strains (Waksman, 1952).
- b. Schematic diagram of the mycelium (Sykes & Skinner, 1973).
- c. M. chalcea, showing spherical spores born singly at the tips of the hyphae (Stanier et al, 1978).

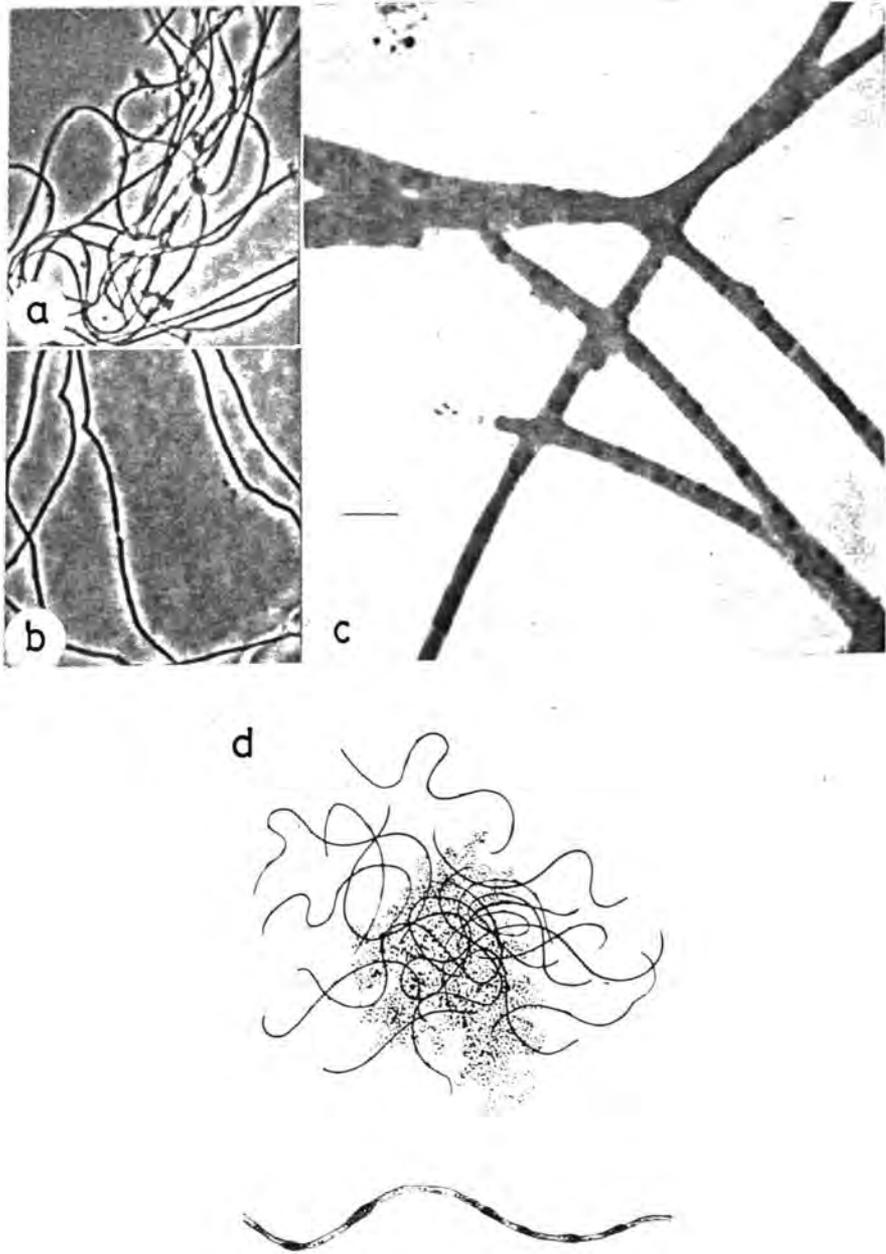


Plate 88 Microthrix parvicella

a - c van Veen (1973) a - b Filaments in activated sludge a. x 1035. b. Isolated strain. x 1035. c. Bar represents 1 μm .

d. Eikelboom (1975). Schematic diagram.

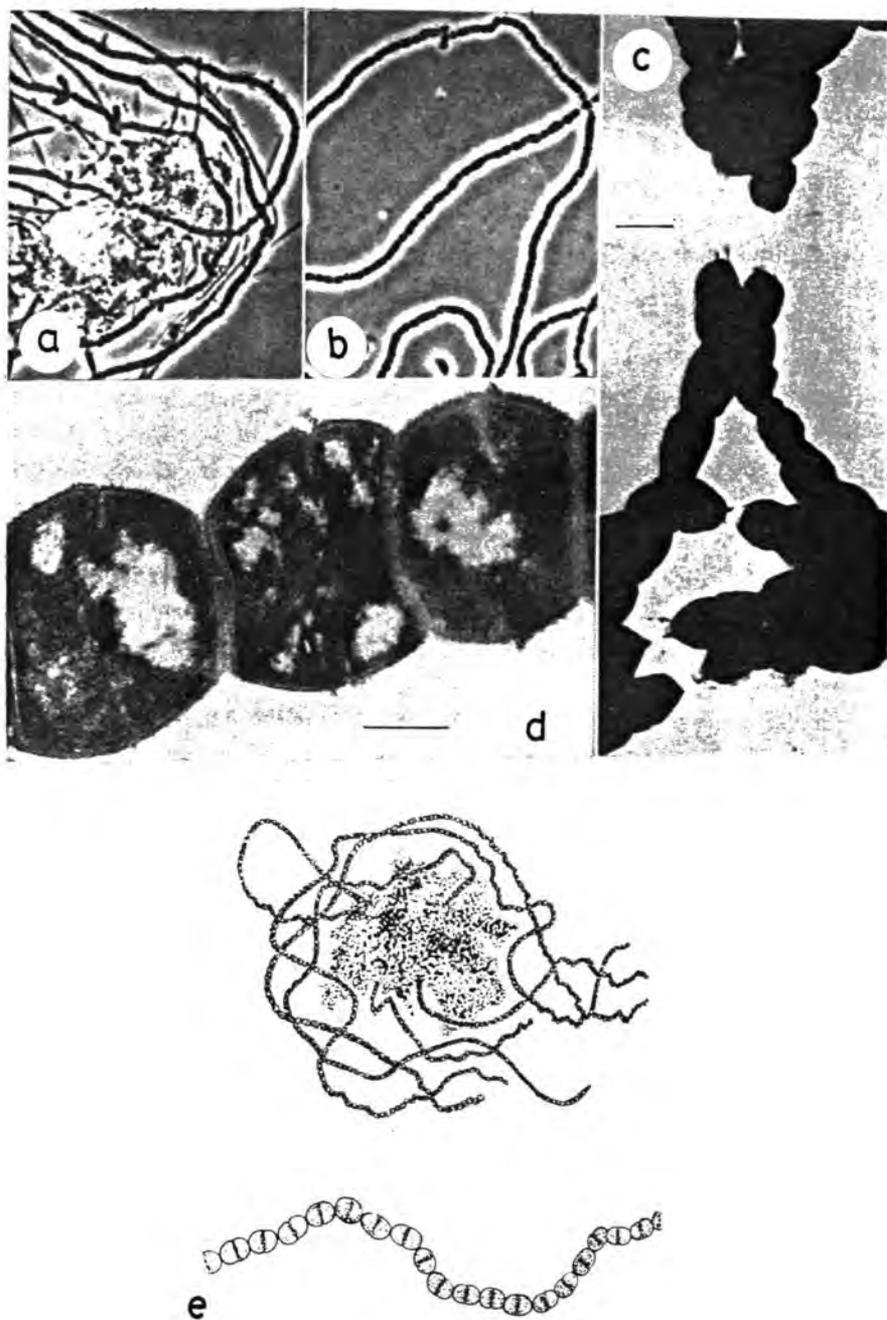


Plate 89 Nostocoida limicola.

a - d van Veen (1973). a. Filaments in activated sludge. x 1035; b. Isolated strain x 1035. c. Bar indicates 1 μ m. d. Ultra-thin section of cells. Bar represents 0.5 μ m.

e. Eikelboom (1975). Schematic diagram.

3.53 Quantitative estimates of the populations of filamentous bacteria in the sediments of Wastwater, Windermere South Basin and Blelham Tarn.

3.531 Direct counts with phase-contrast microscopy of wet mounts.

Wastwater, Windermere South Basin and Blelham Tarn were sampled during July 1979 to estimate the populations of the different groups of filamentous bacteria. The counts, made with wet mount preparations, have shown that in the majority of the cases the numbers of filamentous bacteria decreased with increasing sediment depth. Considering the different groups of filaments observed in the profundal and littoral zones it was found that in 57% of the cases the higher counts were obtained between 0 and 4 mm depth. The results obtained are shown in Figs. 69 to 74. The vertical distribution of filamentous bacteria in Wastwater showed that Group 2, (Leptothrix) was distributed between 0 mm and 16 mm with a peak at 2 - 4 mm and 0 - 2 mm depth in the profundal and littoral zone respectively. High numbers of empty sheaths encrusted with iron were observed; they were found between 0 mm and 35 mm with a peak at 6 - 8 mm (profundal zone) and 2 - 4 mm (littoral zone) depth. Pseudanabaena was observed only in the littoral zone and distributed between 0 mm and 20 mm with the highest value at 0 - 2 mm depth. In Windermere South Basin Group 1 (Vitreoscilla) was observed between 0 mm and 16 mm. In the profundal zone the peak was at 0 - 2 mm depth and in the littoral at 8 - 10 mm depth. Group 2 was observed between 0 mm and 12 mm whereas Group 3 (Flexibacter) had a shallower and more limited depth range. Groups 4a (Beggiatoa), 5b (Flexibacter?) and Pseudanabaena were found only in the littoral zone whereas Groups 4b (Achroonema), Group 10 and Beggiatoa were observed in the profundal zones. The distribution patterns of the organisms were similar in Blelham Tarn with some exceptions. Lower numbers of Group 2 (Leptothrix) organisms were

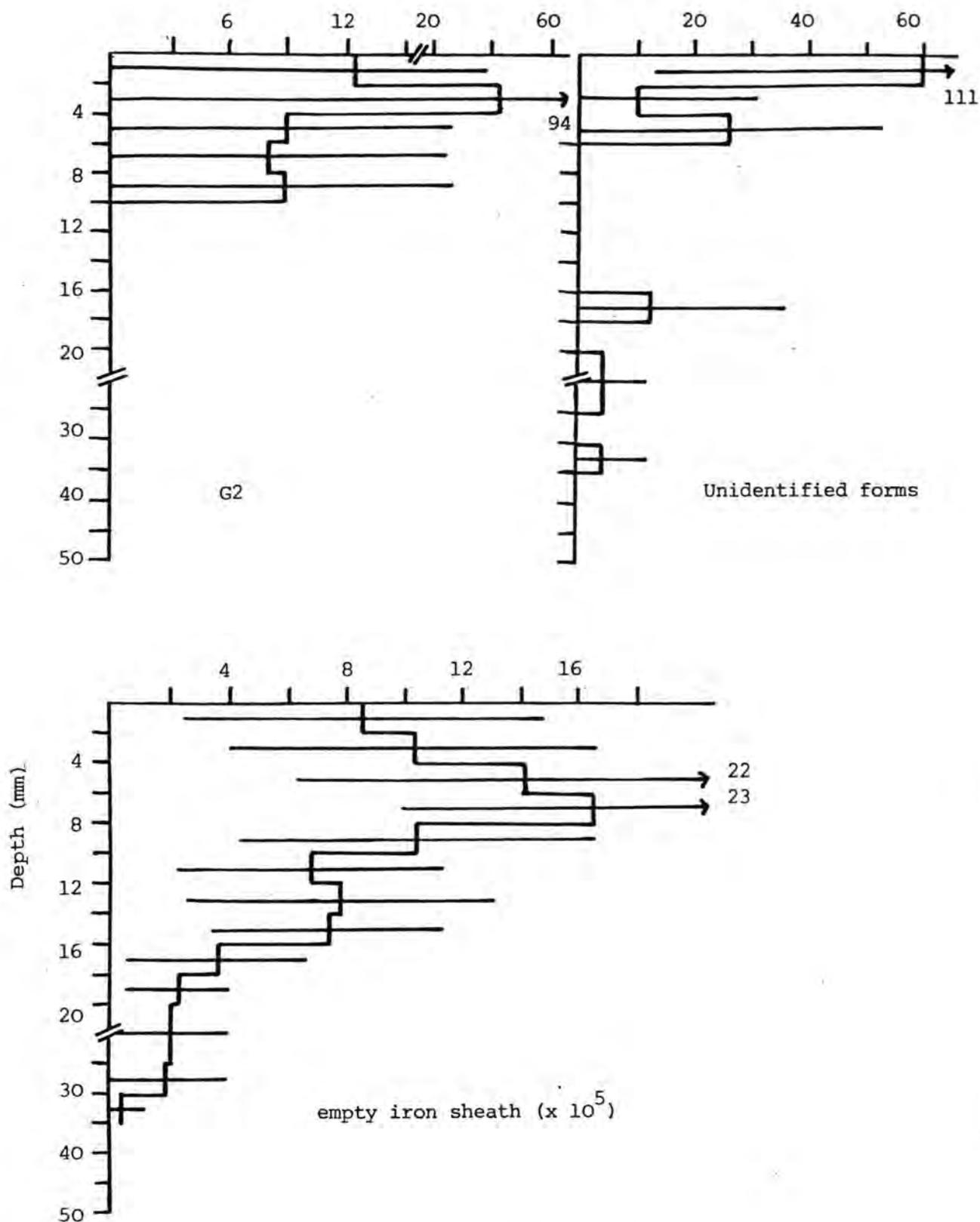


Fig. 69. The vertical distribution of filamentous bacteria in the profundal zone of Wastwater, estimated with wet mount preparation under phase-contrast microscopy. Note breaks in the ordinate and abscissa scales.

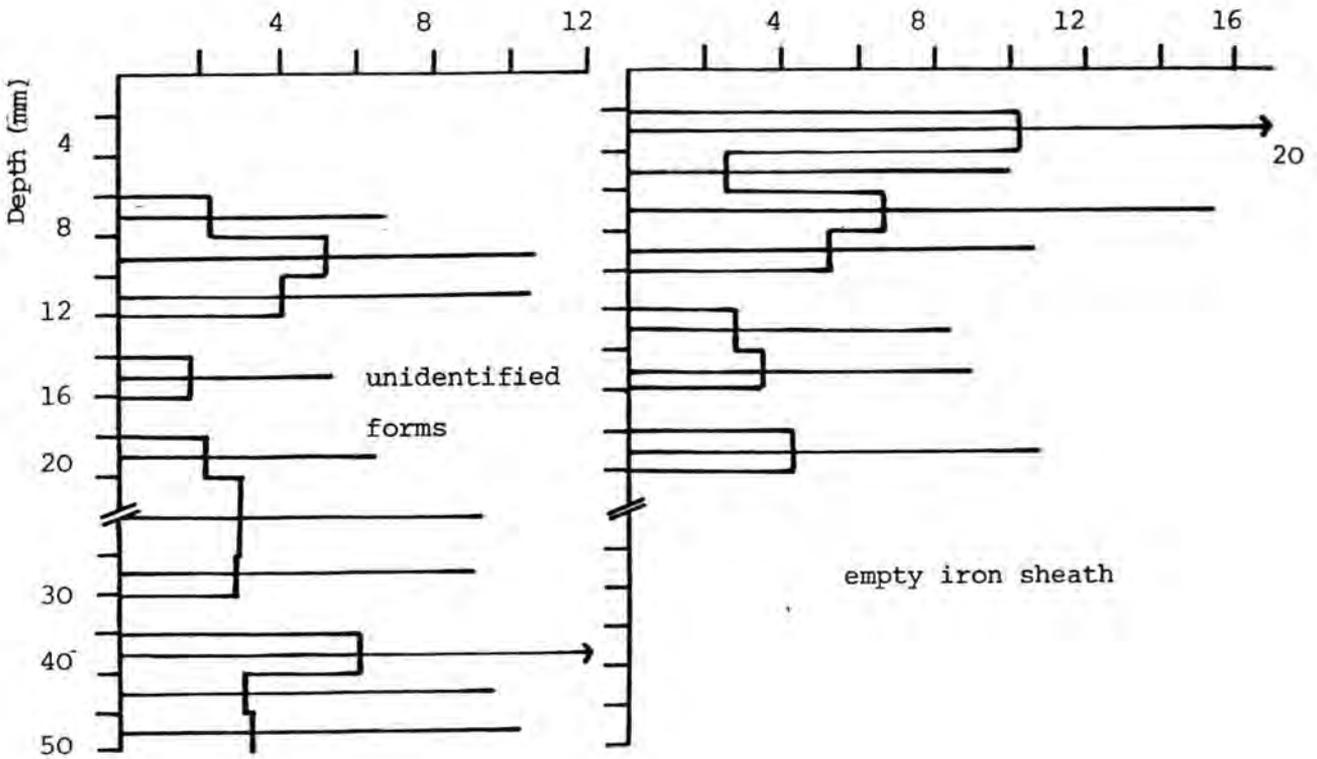
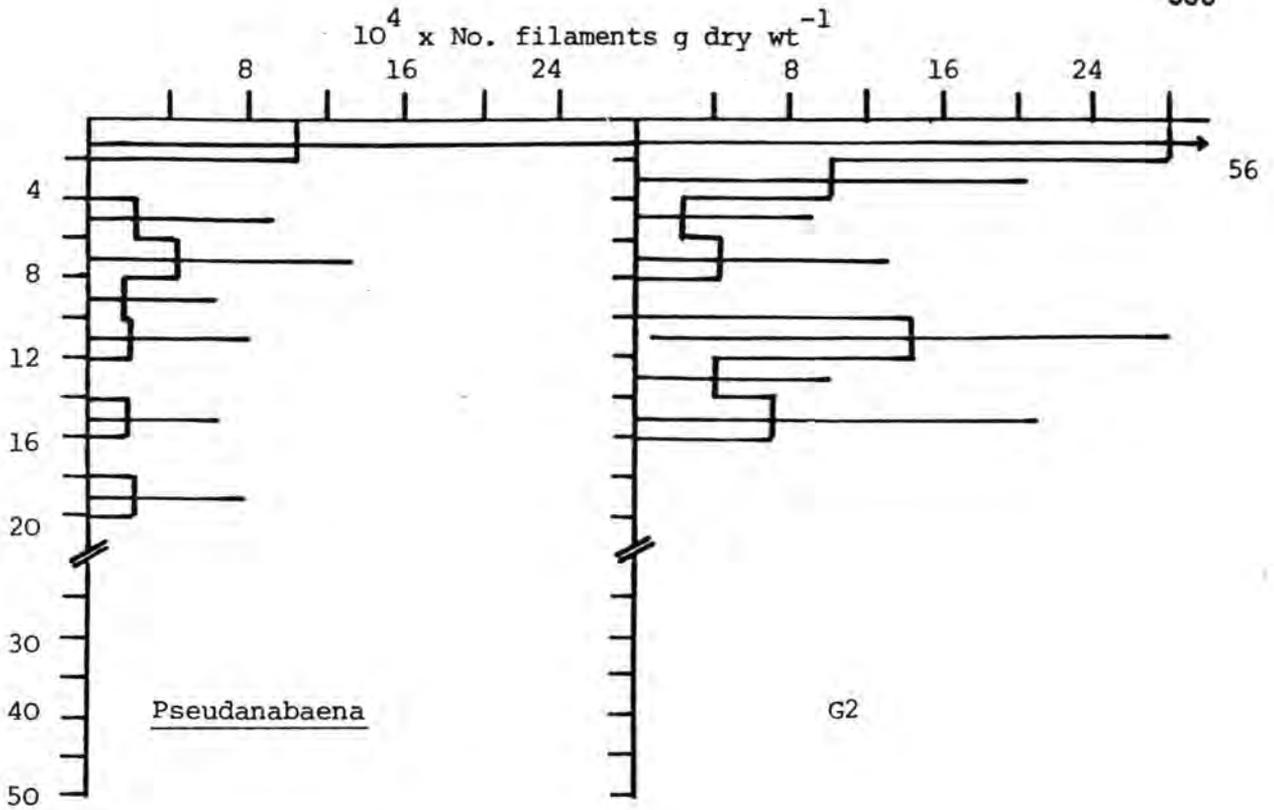


Fig. 70. The vertical distribution of filamentous bacteria in littoral zone of Wastwater. Conditions as Fig. 69. Note break in the ordinate scale.

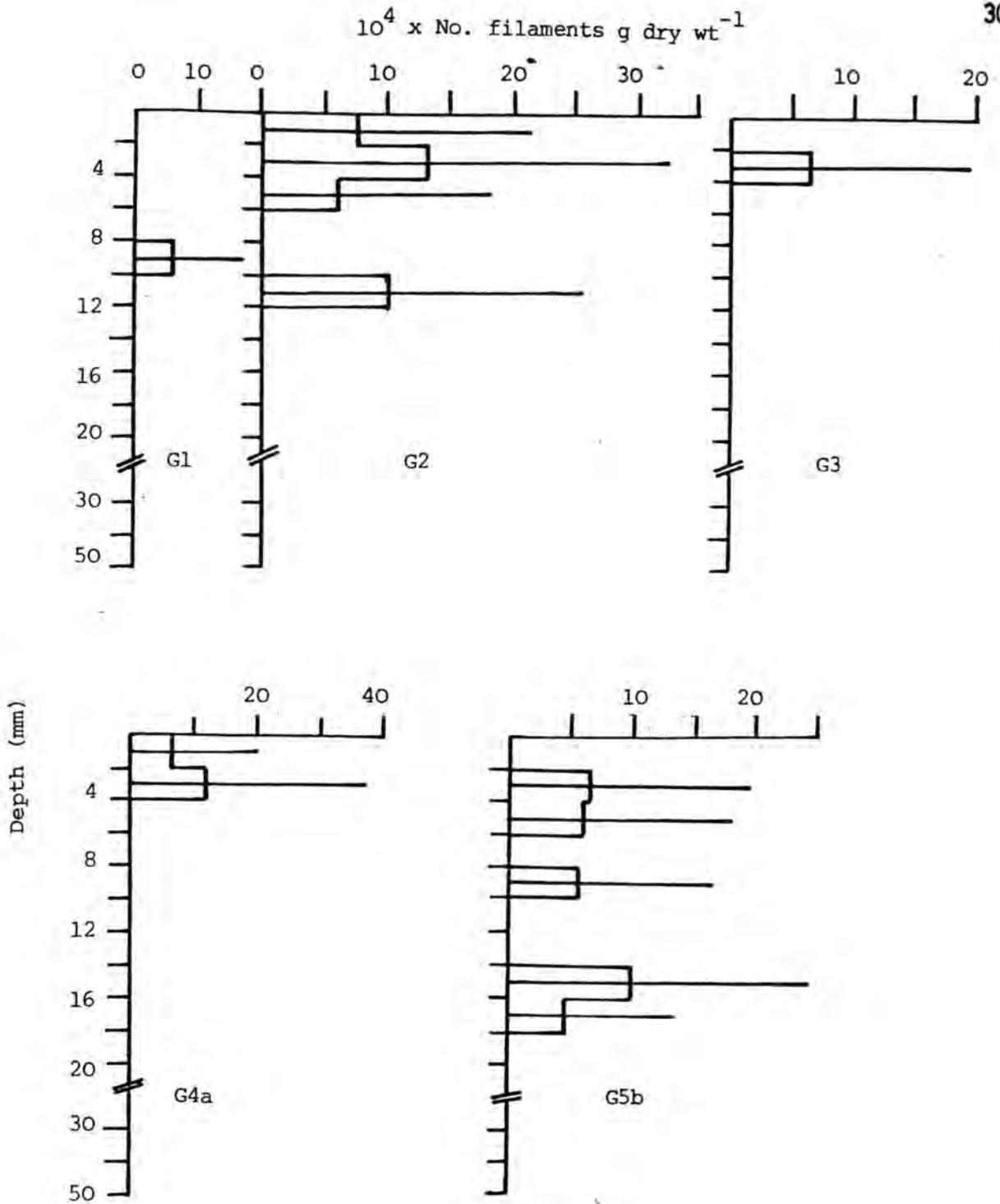


Fig. 71. The vertical distribution of filamentous bacteria in the littoral zone of Windermere South Basin. Conditions as Fig. 69. Note break in the ordinate scale.

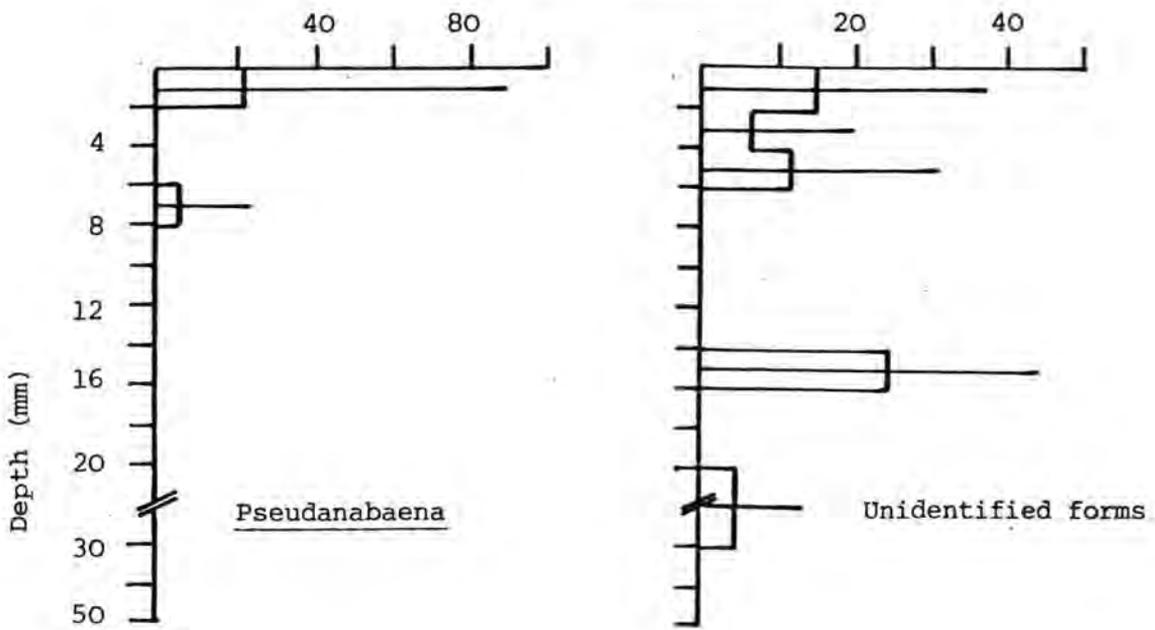


Fig. 71 Cont'd.

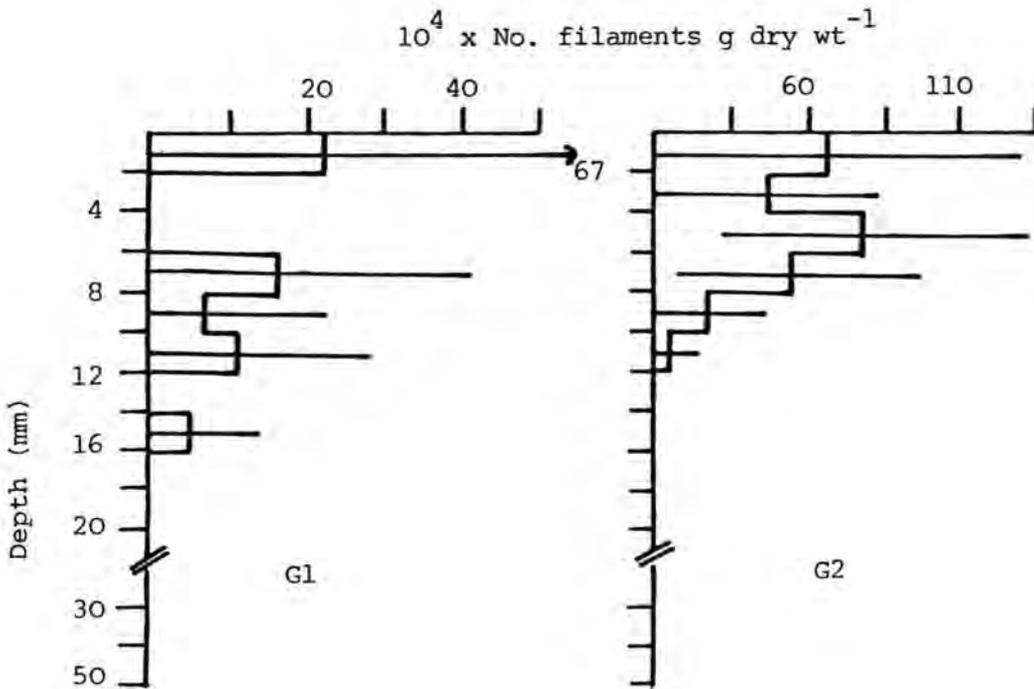


Fig. 72. The vertical distribution of filamentous bacteria in the profundal zone of Windermere South Basin. Conditions as Fig. 69. Note break in the ordinate scale.

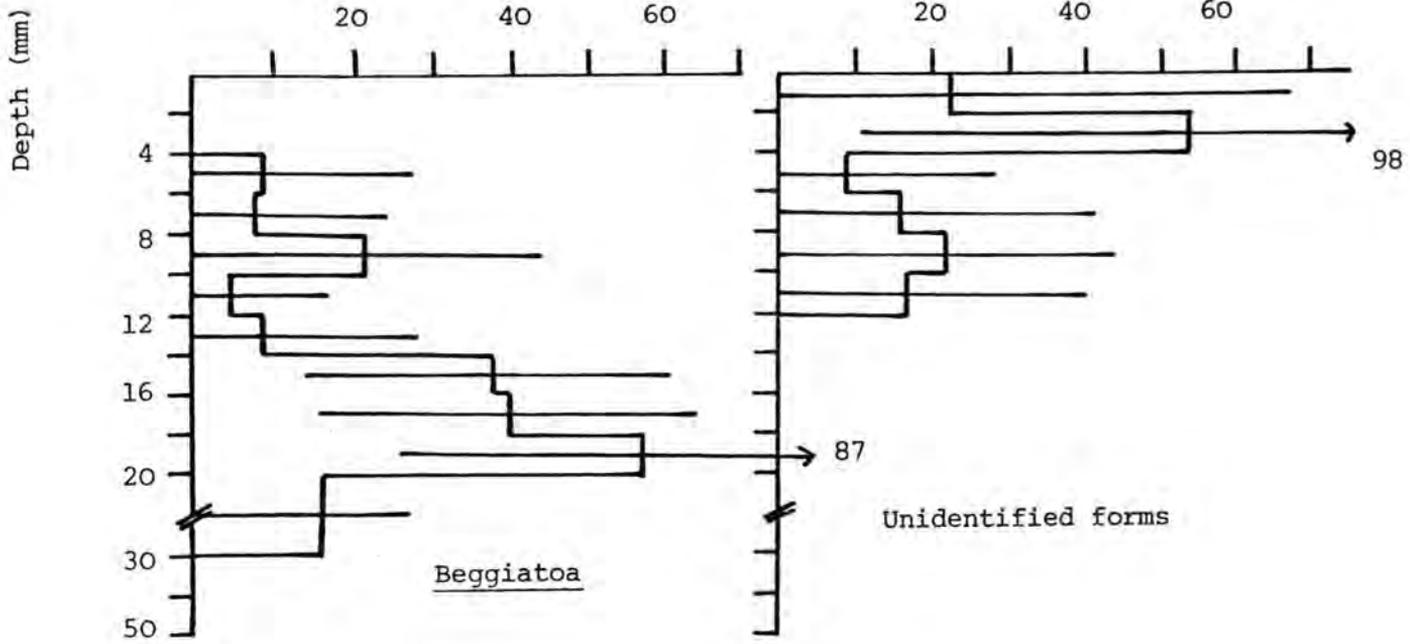
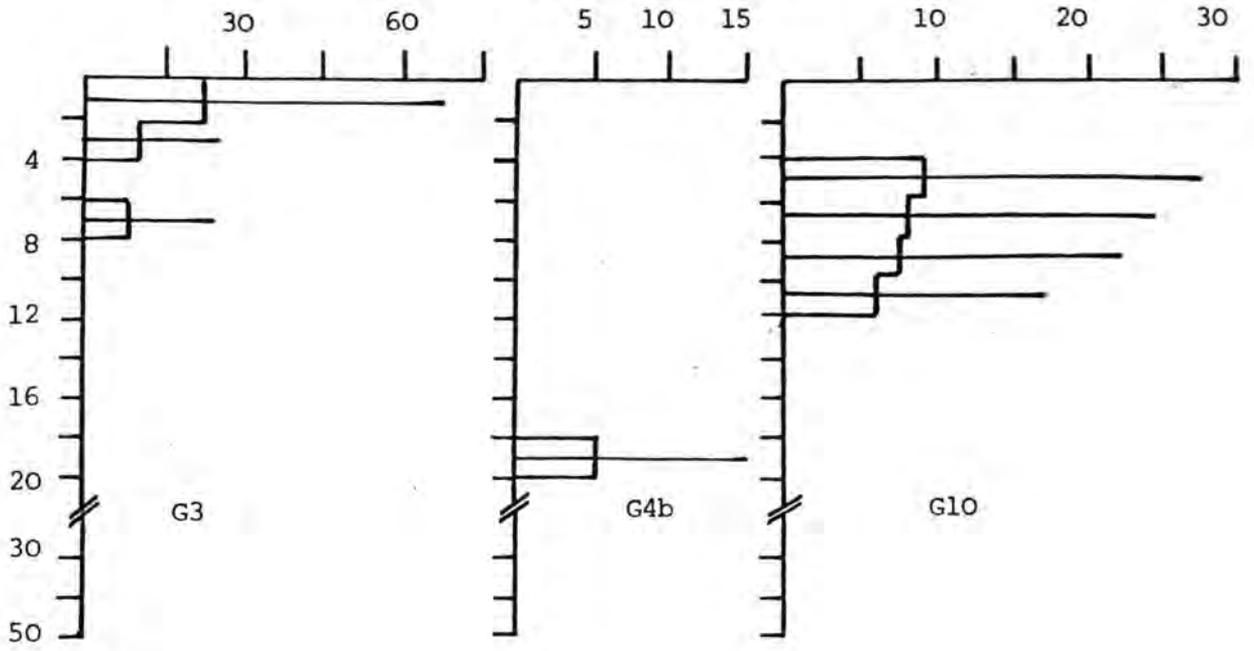


Fig. 72. Cont'd.

$10^5 \times \text{No. filaments g dry wt}^{-1}$

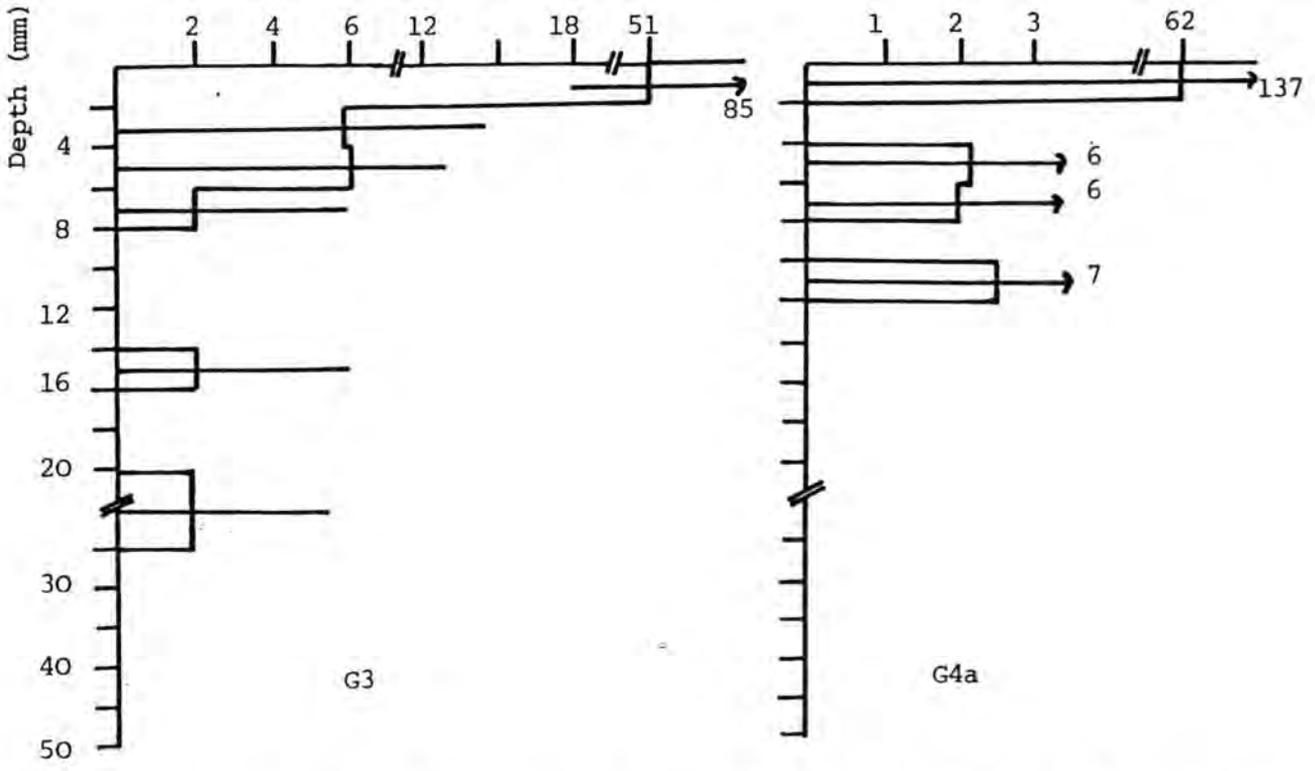
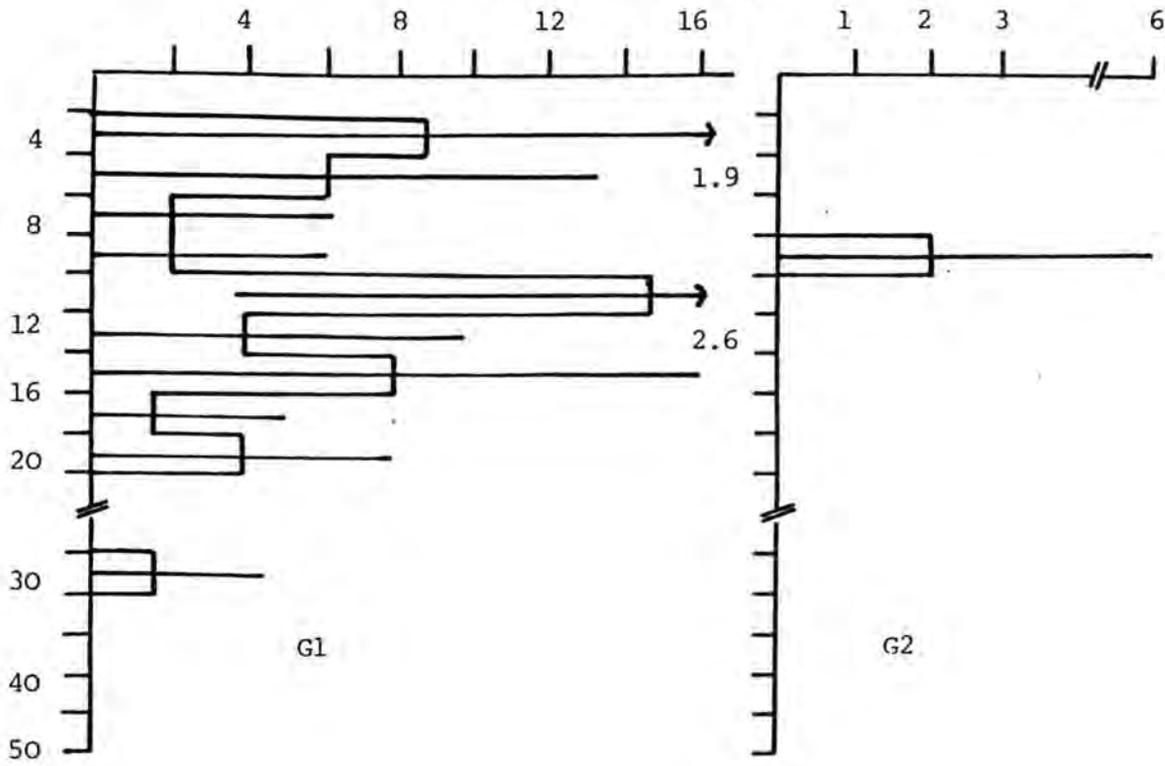


Fig. 73. The vertical distribution of filamentous bacteria in the profundal zone of Blelham Tarn. Conditions as Fig. 69. Note break in the ordinate and abscissa scales.

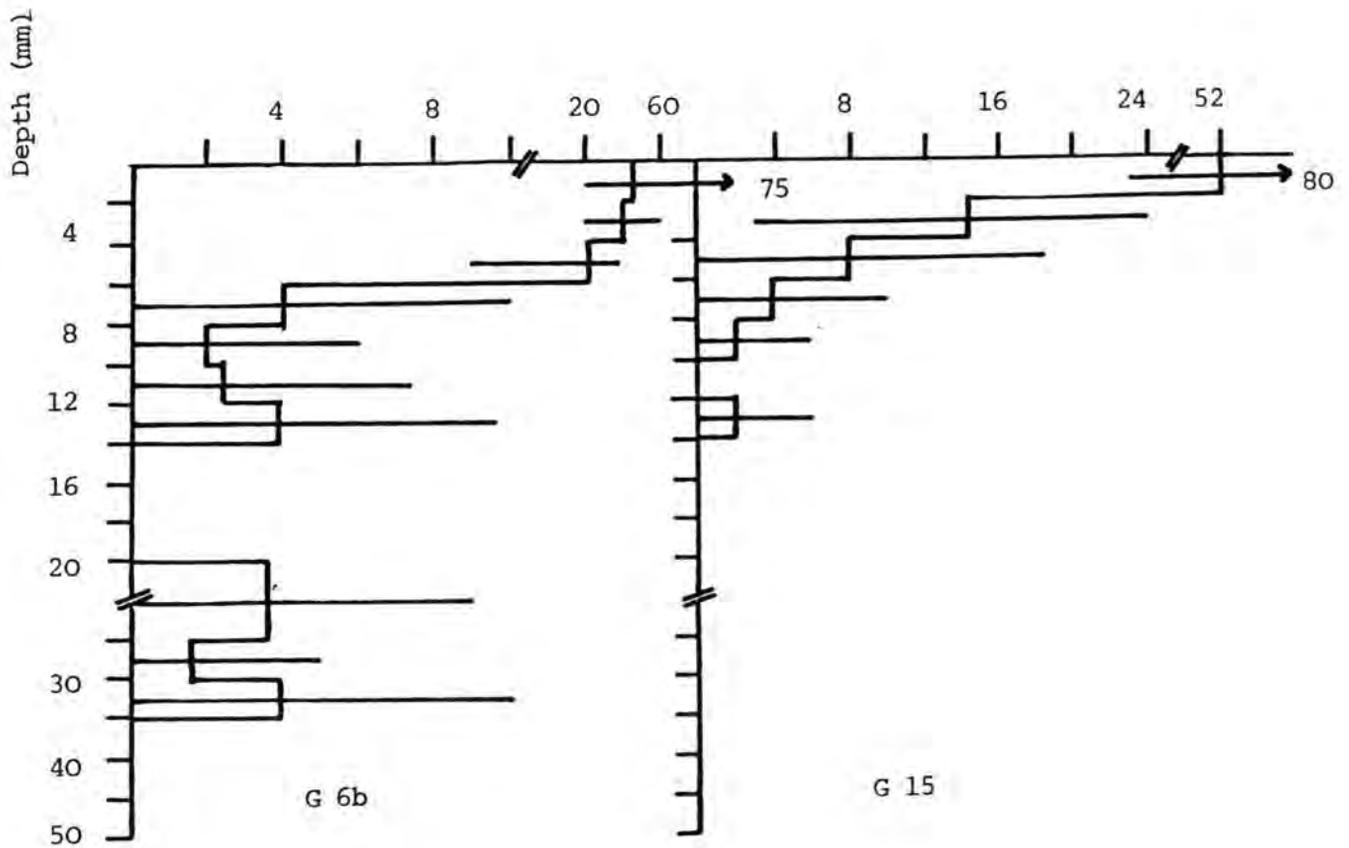
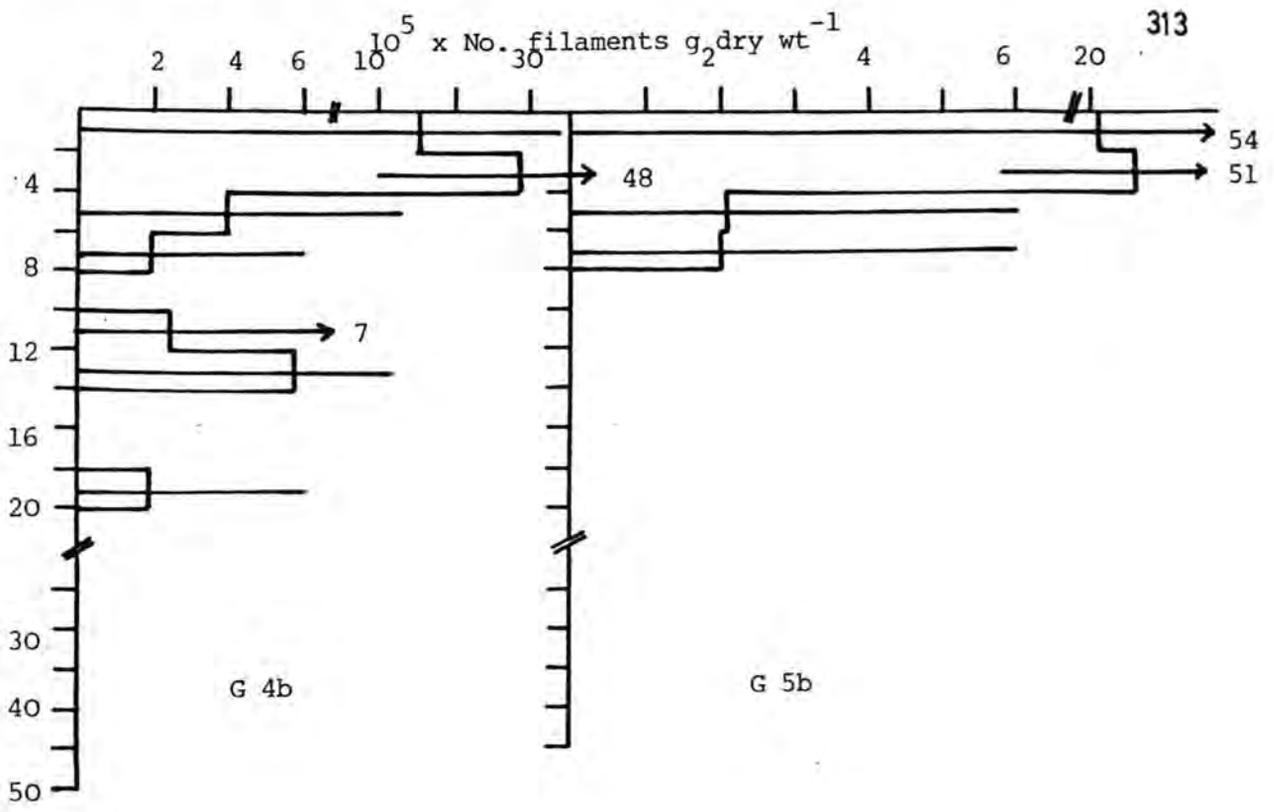


Fig. 73. Cont'd.

$10^5 \times \text{No. filaments g dry wt}^{-1}$

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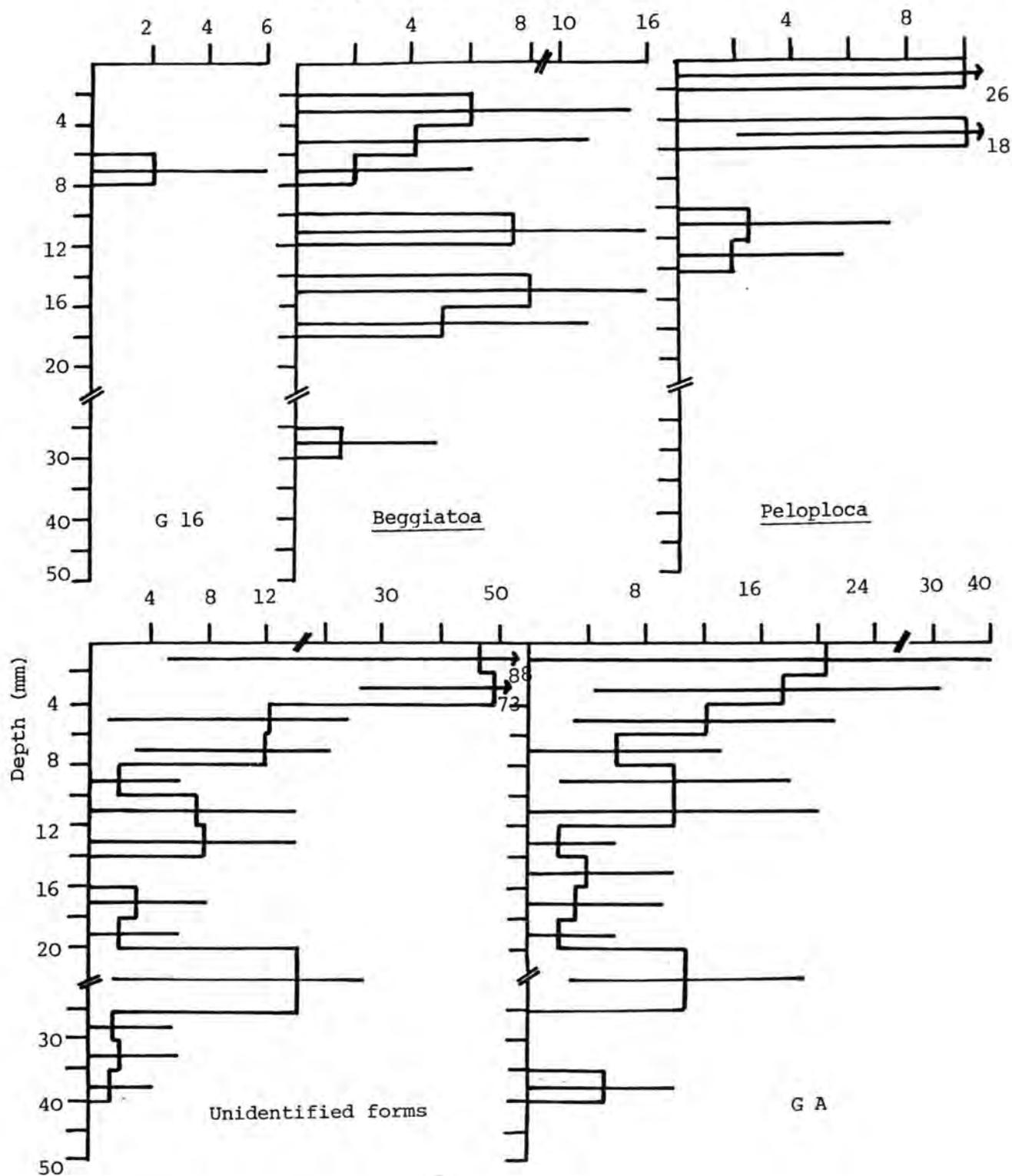


Fig. 73. Cont'd.

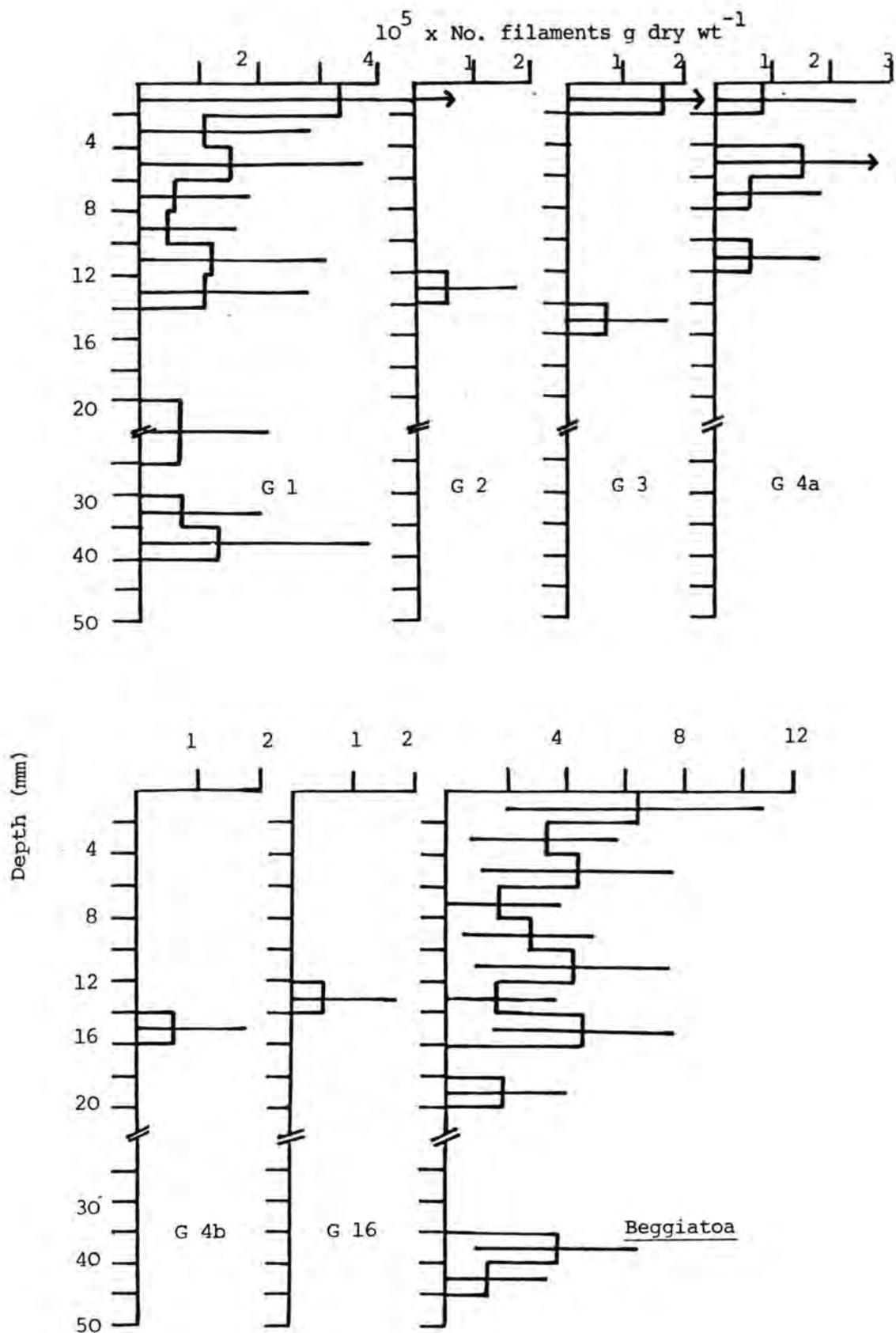


Fig. 74. The vertical distribution of filamentous bacteria in the littoral zone of Blelham Tarn. Conditions as Fig. 69. Note break in the ordinate scale.

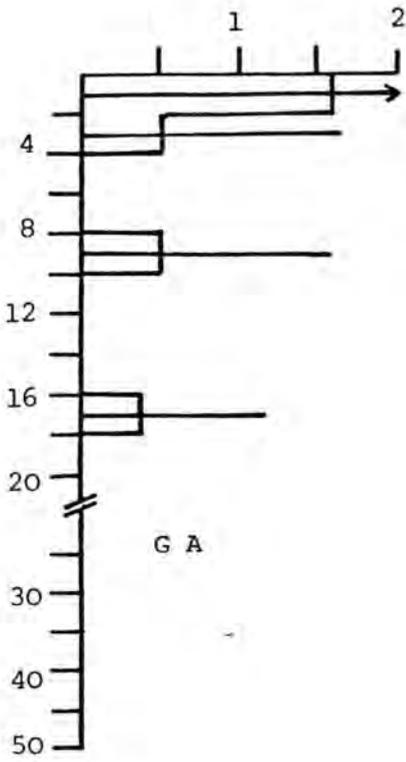
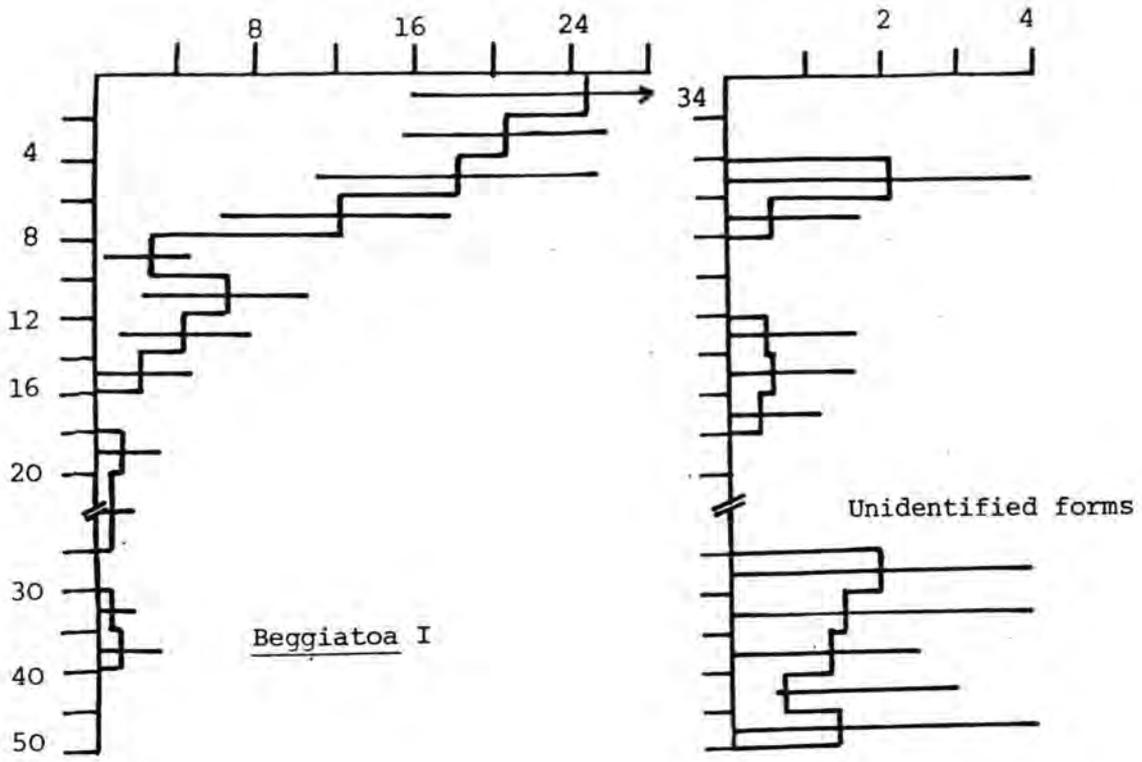


Fig. 74. Cont'd.

encountered but generally speaking counts were higher in the more eutrophic water. The results may be summarized according to the zone that exhibited higher number of filaments and in terms of the relation depths of the peaks and the differences between littoral and profundal counts (Table 21).

In Blelham Tarn the counts for all groups observed were higher at the profundal zone. In Windermere South Basin the counts were higher at the profundal site in most cases, exceptions being Beggiatoa, Pseudanabaena and Group 5b (Flexibacter?). The species diversity obtained is shown in Table 22. Blelham Tarn showed the greatest value with 12 and 8 different types of filaments in the profundal and littoral zones respectively. Six different groups were found in each of the profundal and littoral samples of Windermere South Basin while in Wastwater one type was observed in the profundal zone and two types in the littoral zone. In the three lakes sampled, several filaments observed did not fit in the groups described and they were grouped together as unidentified filaments. These consisted mainly of filaments attached to sediment particles or to diatom remains, non motile forms and on few occasions of slow gliders. Group 10 was observed only in Windermere South Basin. Groups 6b, 15, 16, Peloploca and Group A were observed only in Blelham Tarn. The organism designated Beggiatoa I observed in Blelham Tarn, was 3 μm wide without clear septation with a constriction towards the end of the filament and terminating in a rounded protuberance. This organism was tentatively identified as Beggiatoa arachnoidea. The group A also observed in Blelham Tarn was 2 μm wide and 281 μm long with cell constrictions and sometimes exhibiting sulphur inclusions.

3.532 Counts of filamentous bacteria using uncoated and agar coated slides.

The counts were made above and below the sediment-water interface. The counts below the interface showed that, once again, the filamentous

Table 21 Comparison of the vertical distribution of filamentous bacteria in the profundal and littoral zones of three lakes with wet mount preparation under phase-contrast microscopy.

Group No	Genus	Numbers higher in the Profundal (P) or Littoral (L) zones			Those which peak at greater depth in the littoral			Those which peak at or near the sediment surface		
		Wastwater	Windermere South Basin	Blelham Tarn	Wastwater	Windermere South Basin	Blelham Tarn	Wastwater	Windermere South Basin	Blelham Tarn
1	<u>Vitrioscilla</u>	-	P	P	-	+	-	-	+	+
2	<u>Leptothrix</u>	P	P	P	-	-	+	+	+	-
3	<u>Flexibacter</u>	-	P	P	-	+	-	-	+	+
4a	<u>Beggiatoa</u>	-	L*	P	-	-	+	-	+	+
4b	<u>Achroonema</u>	-	P*	P	-	-	+	-	-	+
5b	<u>Flexibacter</u> (?)	-	L*	P*	-	-	-	-	-	+
6b	<u>Cytophaga/Flexibacter</u>	-	-	P	-	-	-	-	-	+
10	-	-	P	-	-	-	-	-	+	-
15	<u>Pelonema</u>	-	-	P*	-	-	-	-	-	+
16	<u>Bactoscilla</u> (?)	-	-	P	-	-	+	-	-	-
	<u>Peloploca</u>	-	-	P*	-	-	-	-	-	+
	<u>Beggiatoa</u>	-	P*	P	-	-	-	-	-	+
	<u>Pseudanabaena</u>	L	L*	-	-	-	-	+	+	-
Unidentified forms	-	P	P	P	+	+	+	+	+	+
Empty sheaths	-	P	-	-	-	-	-	+	-	-

* Indicates that these organisms were found only at these sites.

Table 22. Observed distribution of different groups of filamentous bacteria using wet mount preparation under phase-contrast microscopy.

	Profundal													u.f.	A		
	1	2	3	4a	4b	5b	6b	10	15	16	<u>Peloploca</u>	<u>Beggiatoa</u>	<u>Pseudanabaena</u>				
Wastwater		+														+	
Windermere South Basin	+	+	+		+			+					+			+	
Blelham Tarn	+	+	+	+	+	+	+		+	+		+	+			+	+
	Littoral																
Wastwater		+												+		+	
Windermere South Basin	+	+	+	+		+								+		+	
Blelham Tarn	+	+	+	+	+					+			+			+	+

u.f. = unidentified forms

bacteria, in most cases, decreased with increasing depth. The counts on that part of the slide exposed to the water showed that the maximum number were, in the majority of the cases, between 0 mm and 4 mm above the sediment-water interface and the numbers decreased with increasing distance from the sediment surface. The results are shown in Figs. 75 to 86. Table 23 summarizes the differences obtained between profundal and littoral zones and also between the counts obtained above and below the sediment surface. The groups of organisms which peaked at or near the sediment surface is also shown. The Groups 5b and 3, in most cases were more numerous at the littoral site. Tables 24 and 25 show the diversity of species obtained. Pseudanabaena was observed only at the littoral site. Group 10 was observed only in Windermere South Basin and Groups 4b and 15 only in Blelham Tarn. Group 2c, observed mainly in Windermere South Basin, was 0.9 μm wide and 35-119 μm long. It was black under phase-contrast microscopy and characteristically the filament was not straight giving the impression that it was broken. The filaments were attached with one free end. The filaments were in certain cases impregnated with iron, (Fig. 87 and Plate 90).

Considering the different techniques used (wet mount, agar coated and uncoated slides) it was noted that one or more filaments were not in any case detected in one of the three lakes examined. They were as follows:

Wastwater: Groups 4a, 4b, 10, 15, Peloploca, Beggiatoa, A and 2c.

Windermere South Basin: Groups 6b, 15, Peloploca.

Blelham Tarn: Group 10.

Table 26 gives the number of groups of filamentous bacteria obtained with the three different techniques used.

The 95% confidence limits obtained with wet mount preparations were larger than the ones obtained with slides incubated in the sediment and the agar coated slides gave higher counts than the uncoated slides.

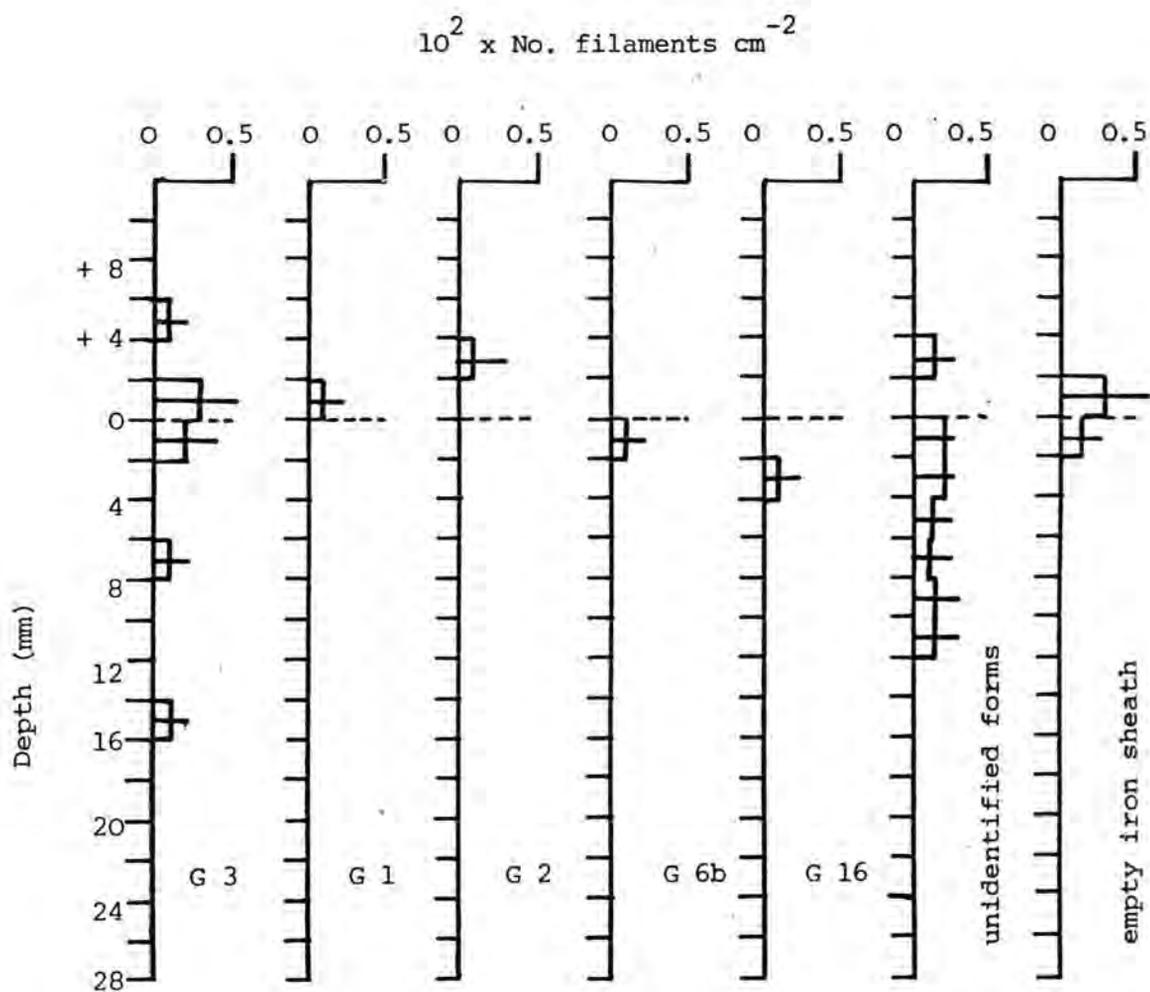


Fig. 75. The vertical distribution of filamentous bacteria in the profundal zone of Wastwater using uncoated glass slides. The counts were made above and below sediment-water interface and the slides incubated for a week at 8°C.

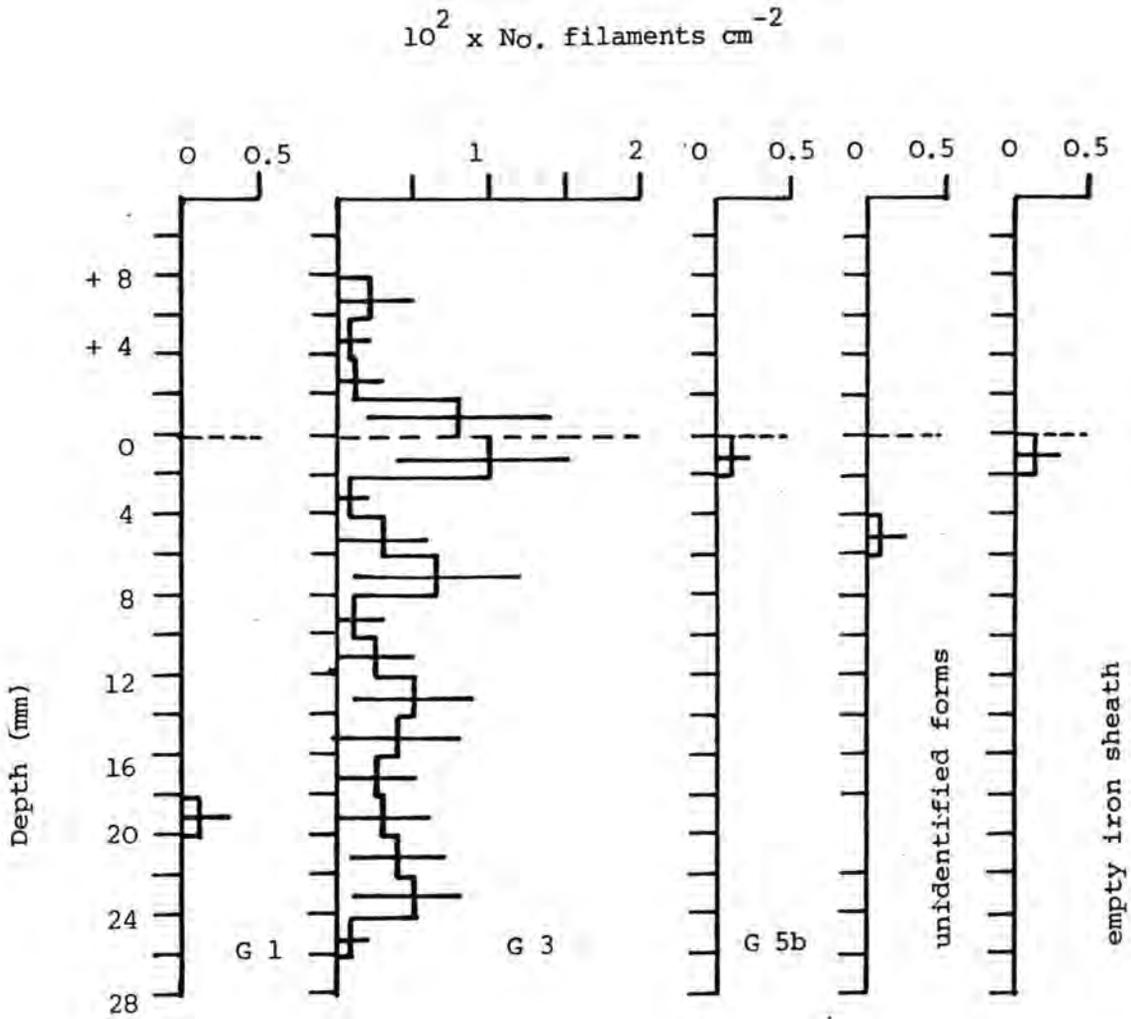


Fig. 77. The vertical distribution of filamentous bacteria in the profundal zone of Wastwater using agar coated slides. Conditions as Fig. 75.

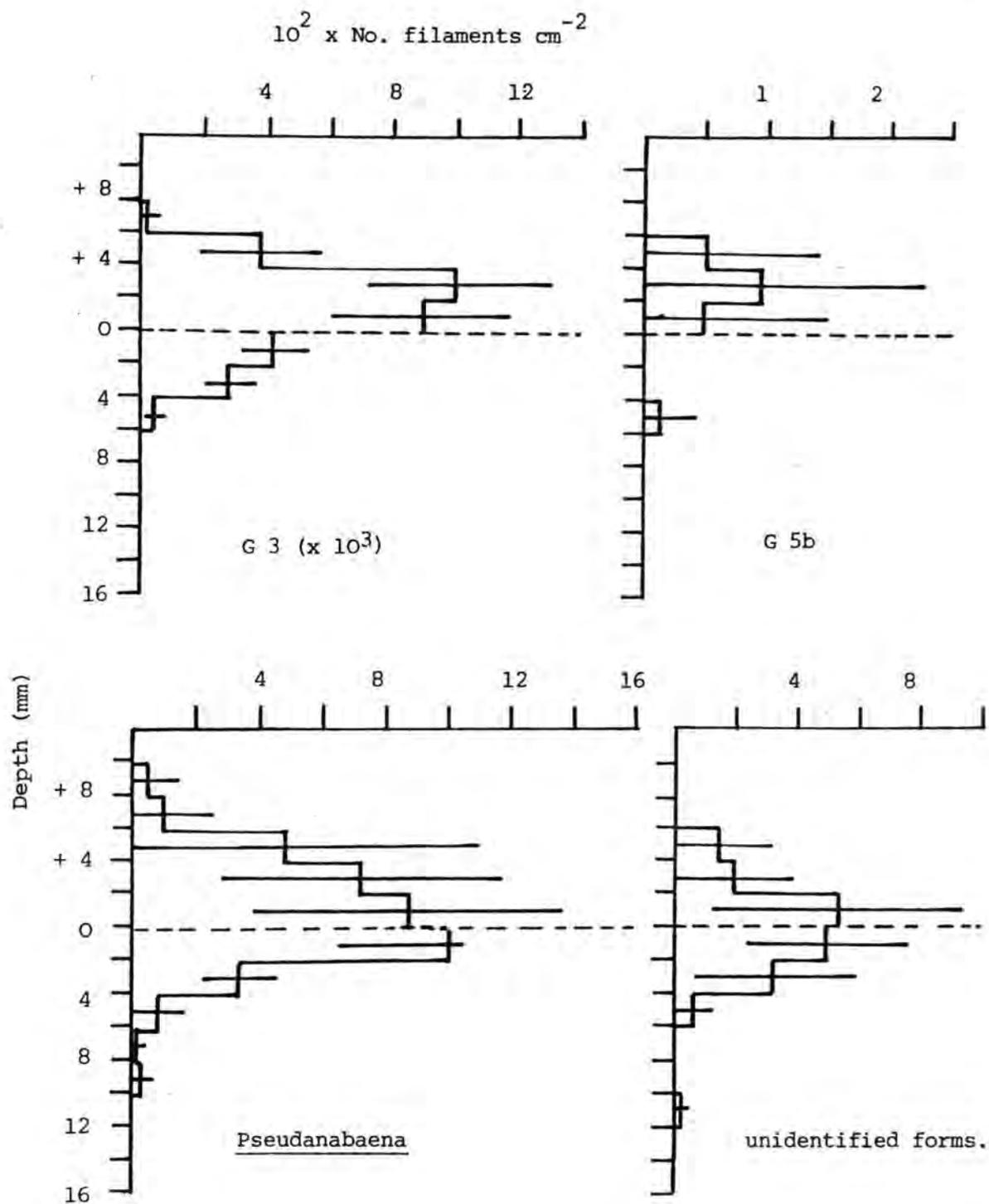


Fig. 78. The vertical distribution of filamentous bacteria in the littoral zone of Wastewater using agar coated slides. Conditions as Fig. 75.

$10^2 \times \text{No. filaments cm}^{-2}$

325

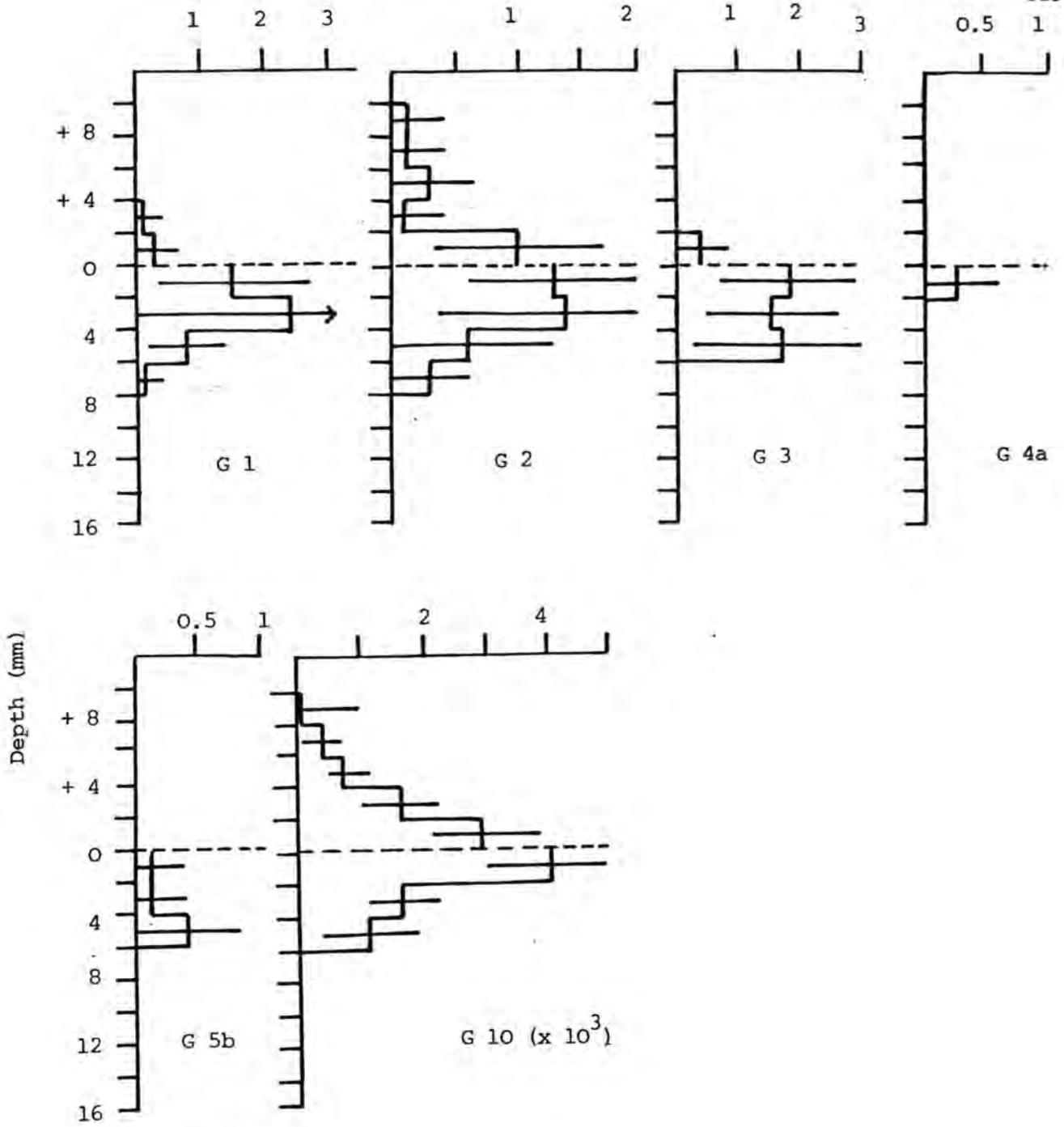


Fig. 79. Vertical distribution of filamentous bacteria in the profundal zone of Windermere South Basin using uncoated glass slides. Conditions as Fig. 75.

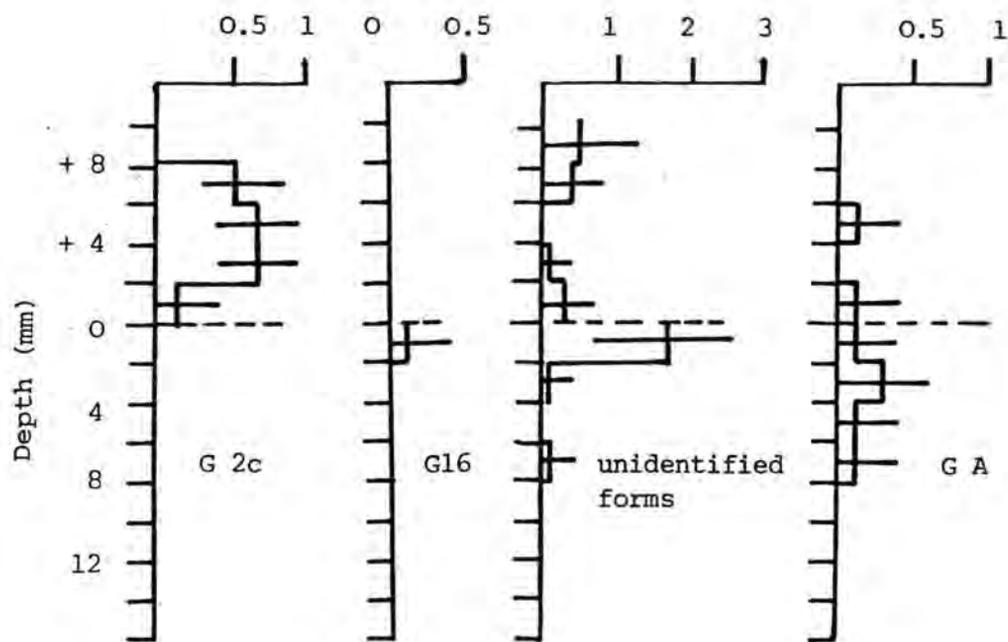


Fig. 79. Cont'd.

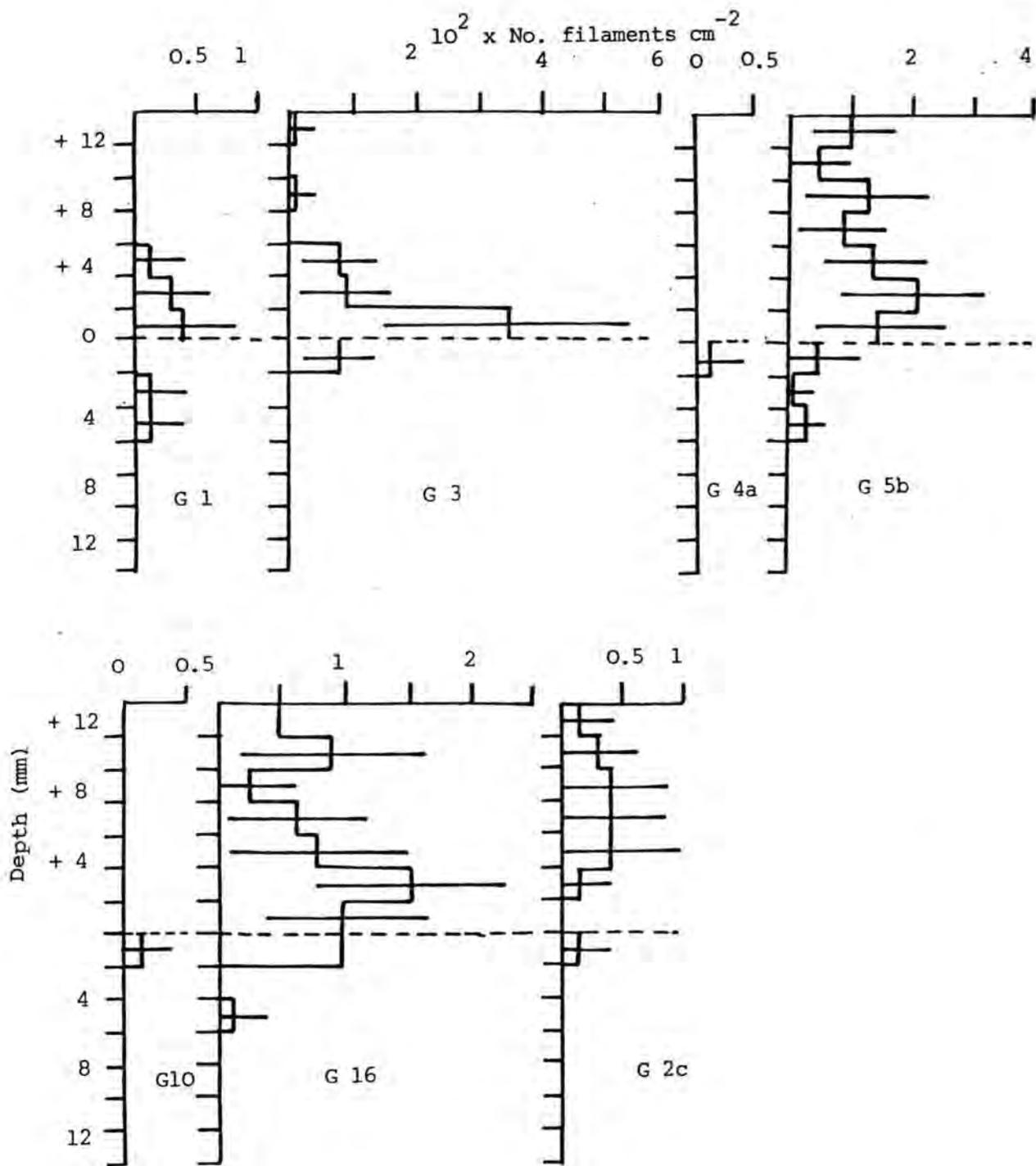


Fig. 80. The vertical distribution of filamentous bacteria in the littoral zone of Windermere South Basin using uncoated glass slides. Conditions as Fig. 75.

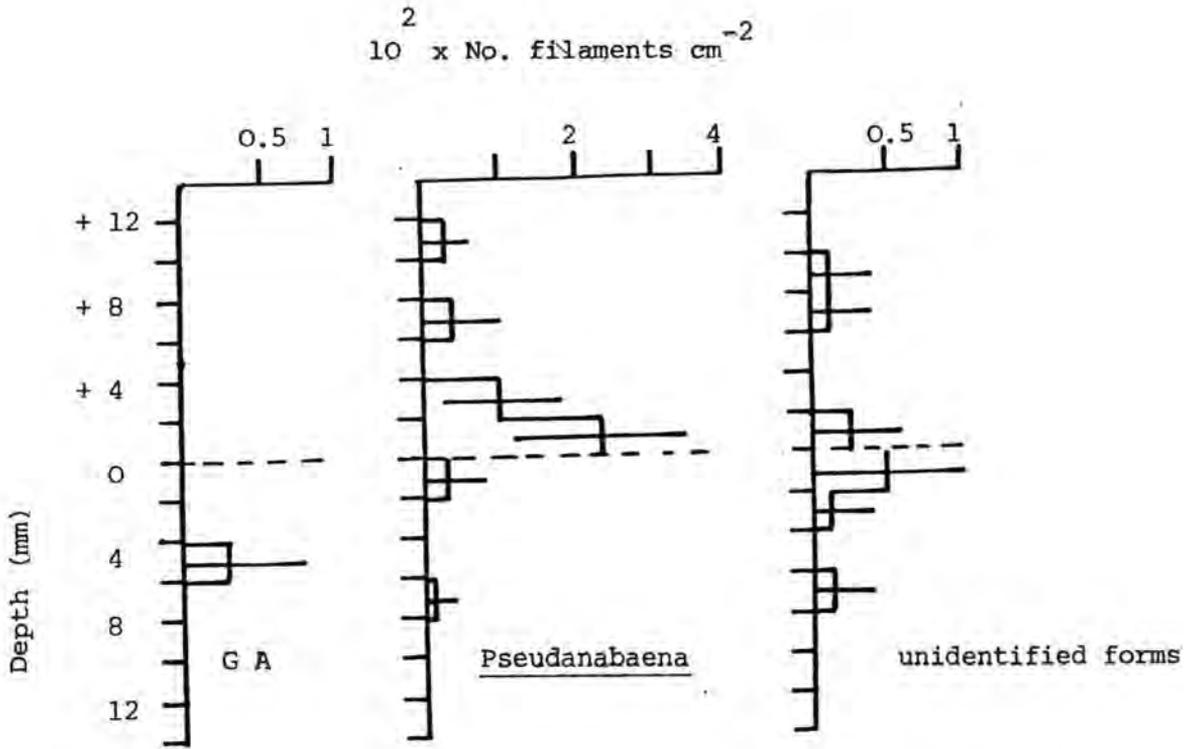


Fig. 80. Cont'd.

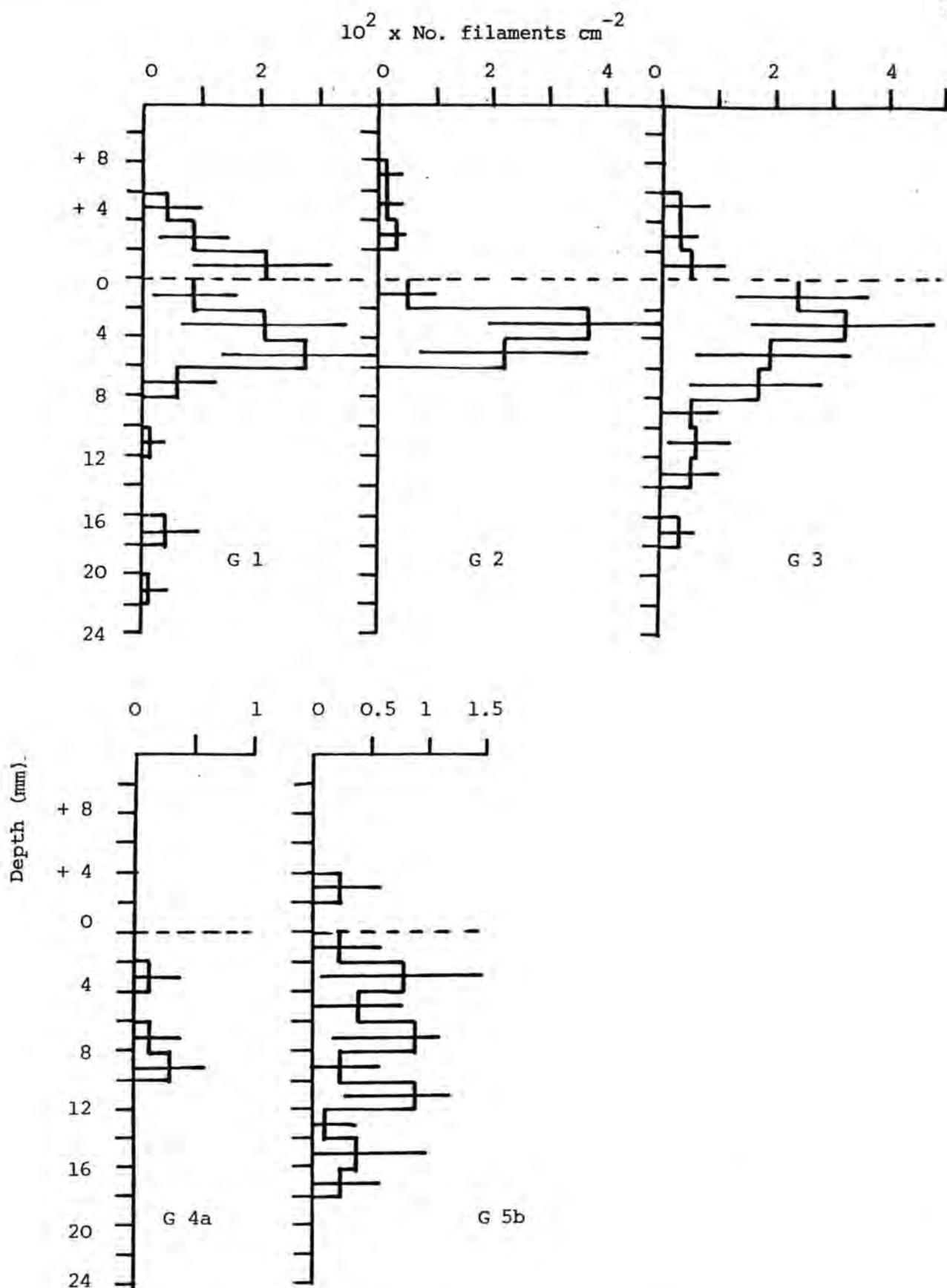


Fig. 81. The vertical distribution of filamentous bacteria in the profundal zone of Windermere South Basin using agar coated slides. Conditions as Fig. 75.

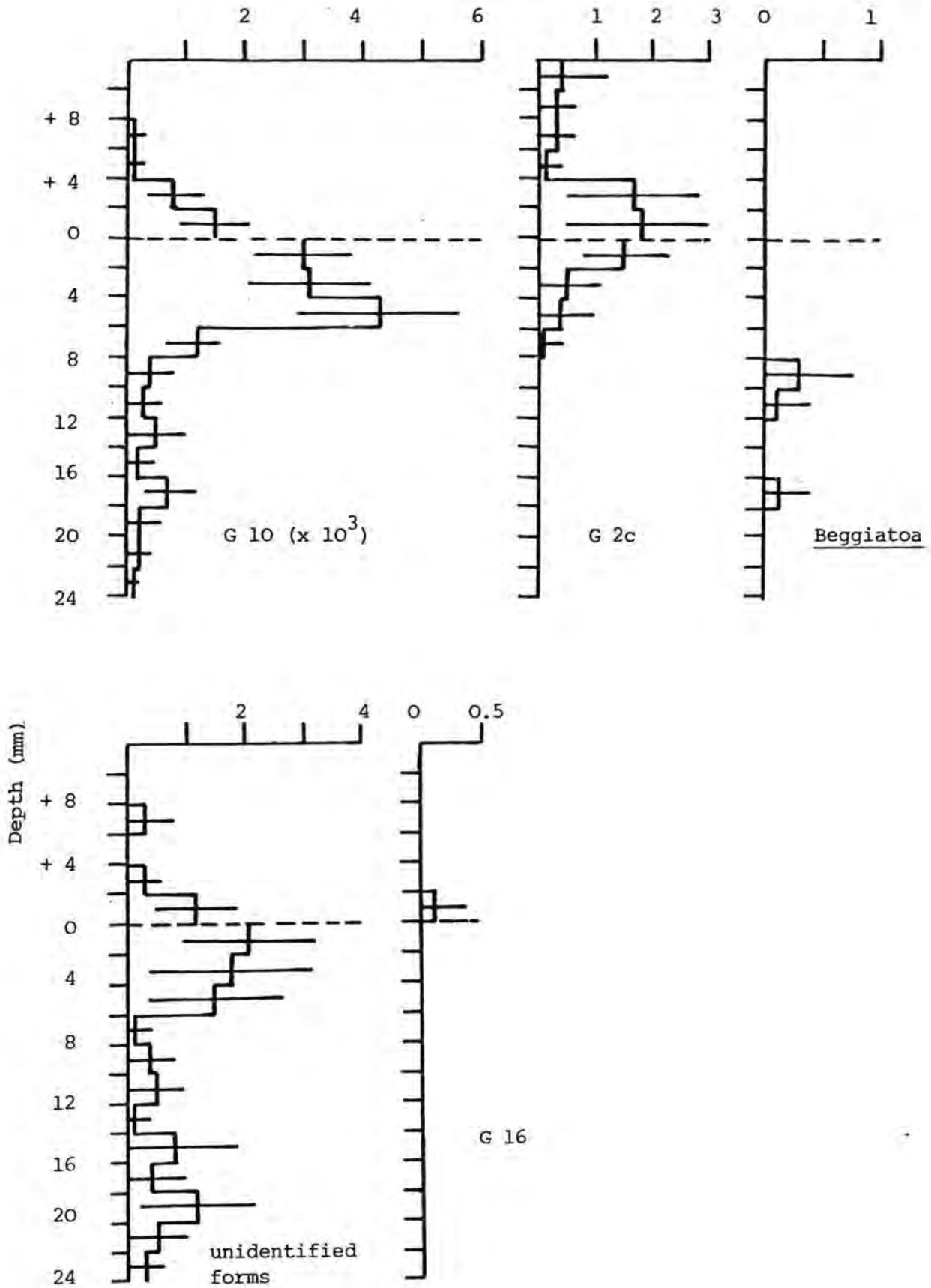


Fig. 81. Cont'd.

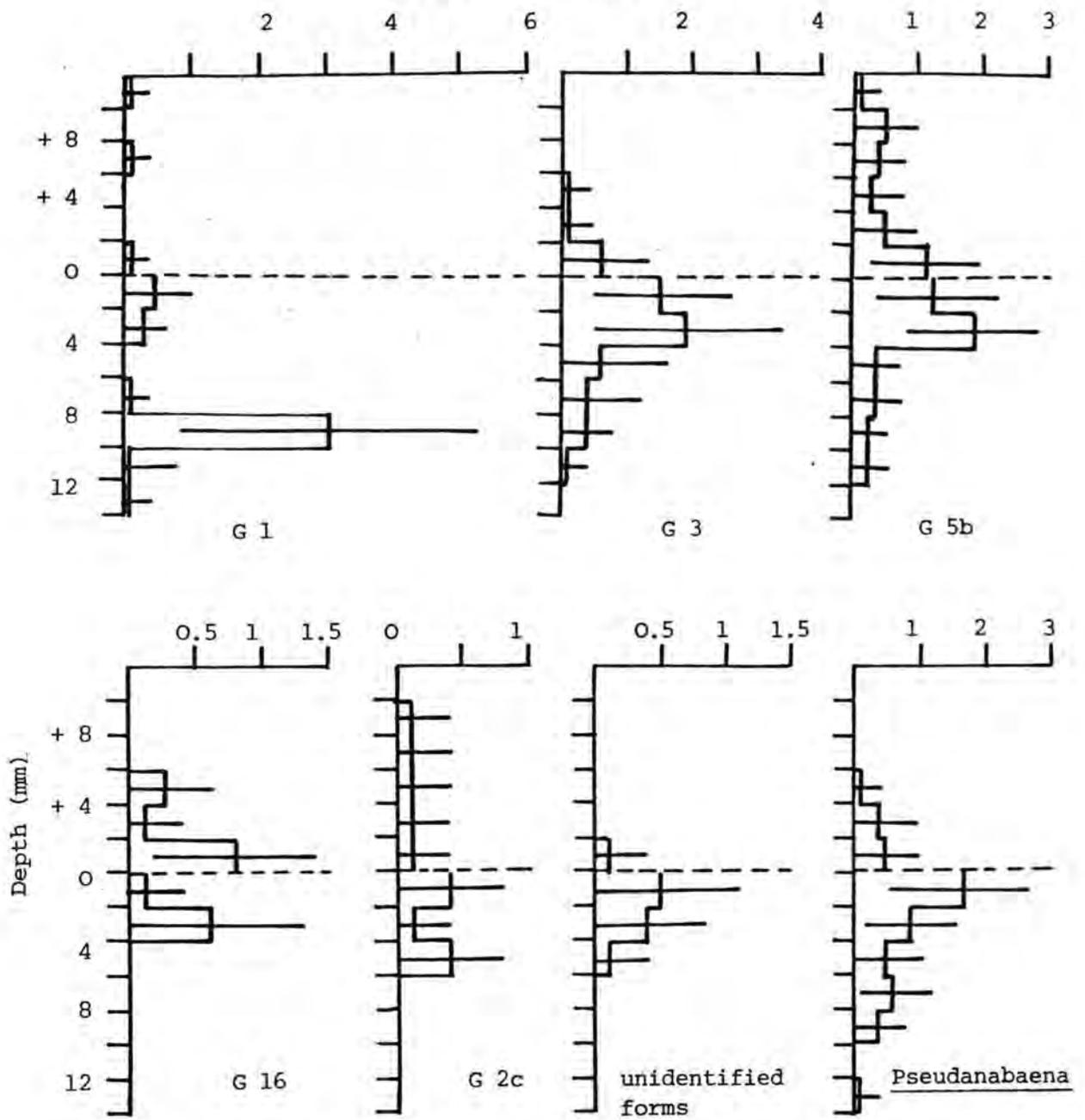


Fig. 82. The vertical distribution of filamentous bacteria in the littoral zone of Windermere South Basin using agar coated slides. Conditions as Fig. 75.

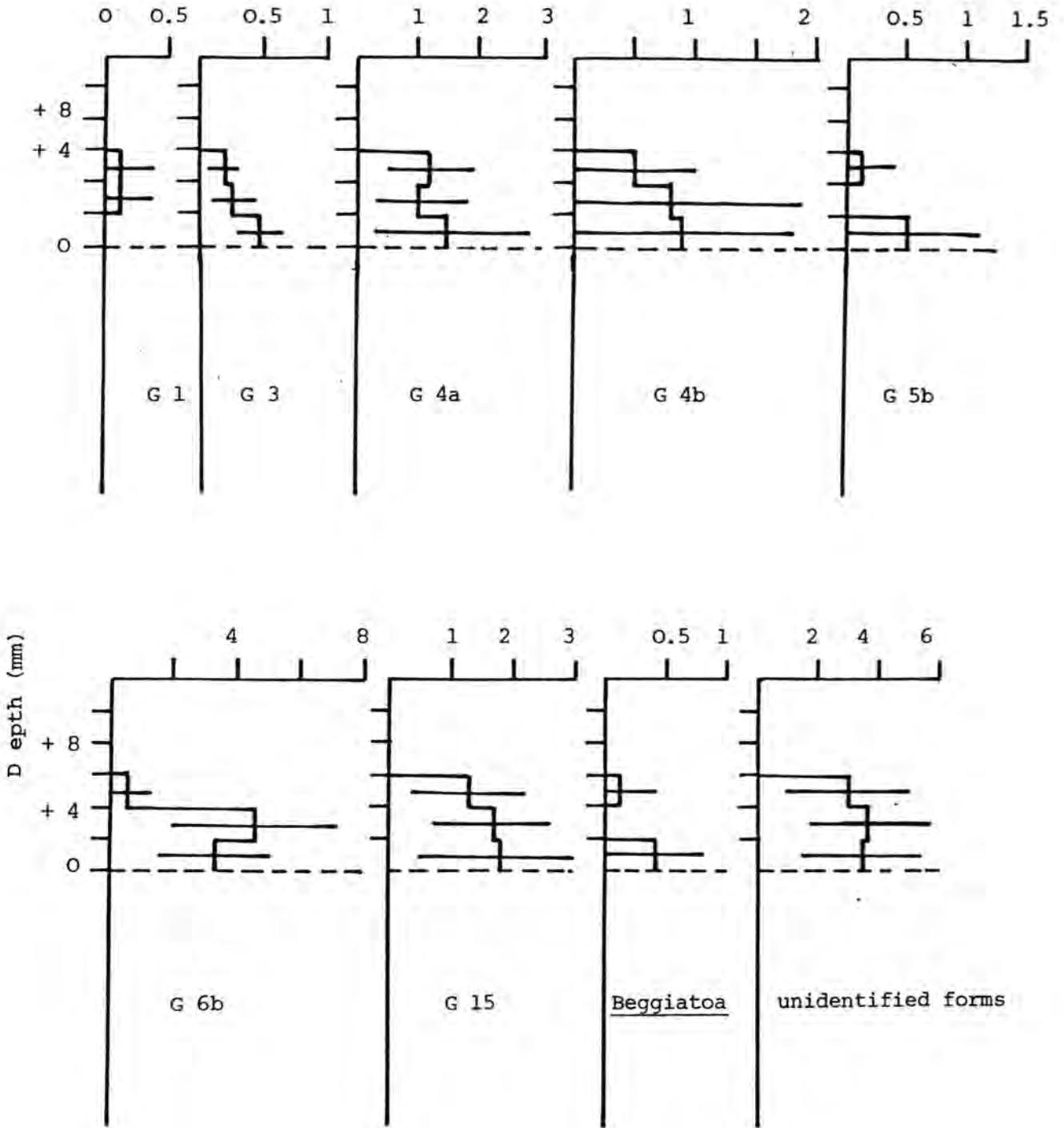


Fig. 83. The vertical distribution of filamentous bacteria in the profundal zone of Blelham Tarn using uncoated glass slides. The counts were made above sediment-water interface only, and the slides incubated for 4 days at 8°C

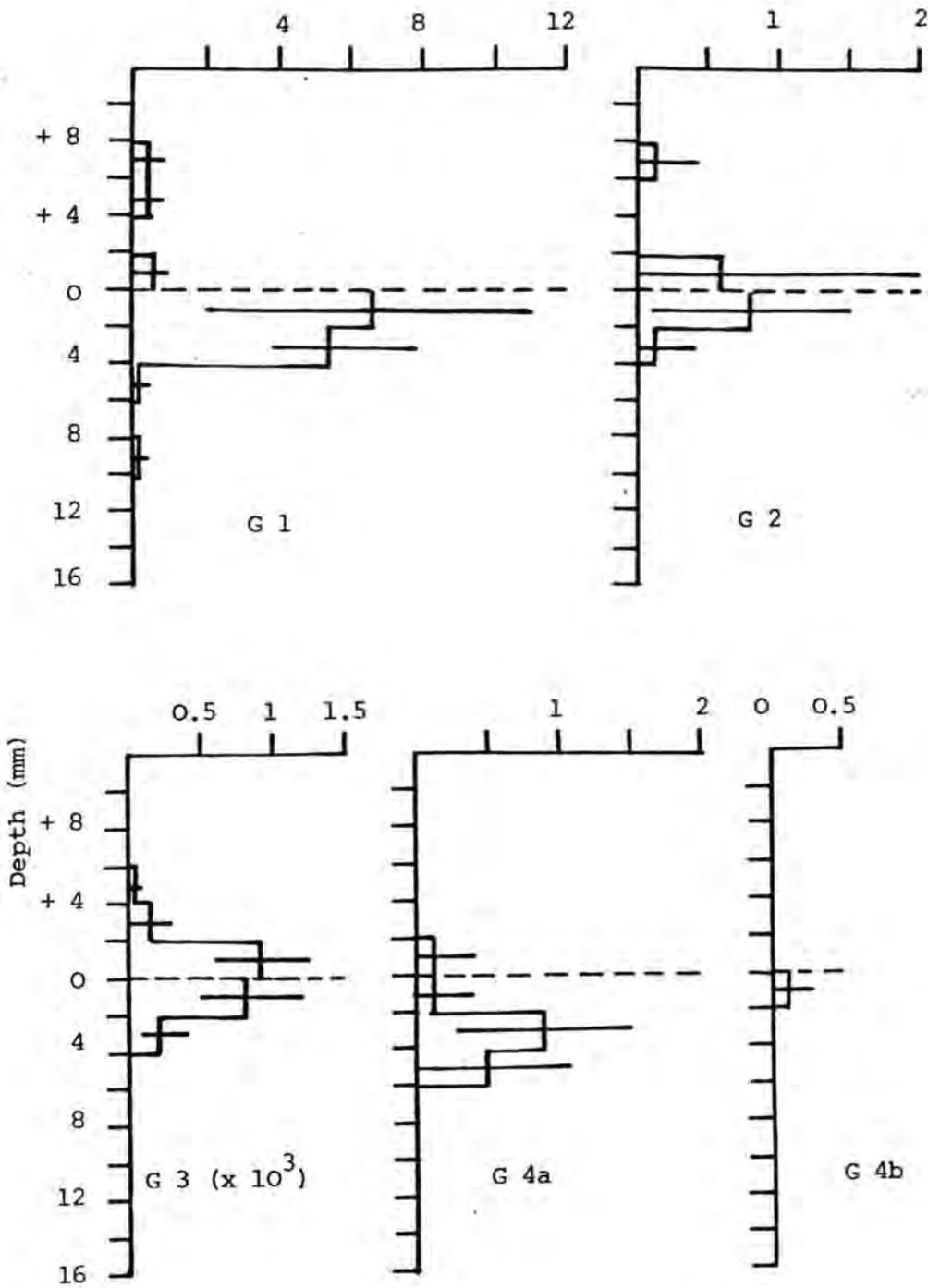


Fig. 84. The vertical distribution of filamentous bacteria in the littoral zone of Blelham Tarn using uncoated glass slides. The counts were made above and below sediment-water interface and the slides incubated for 4 days at 8°C .

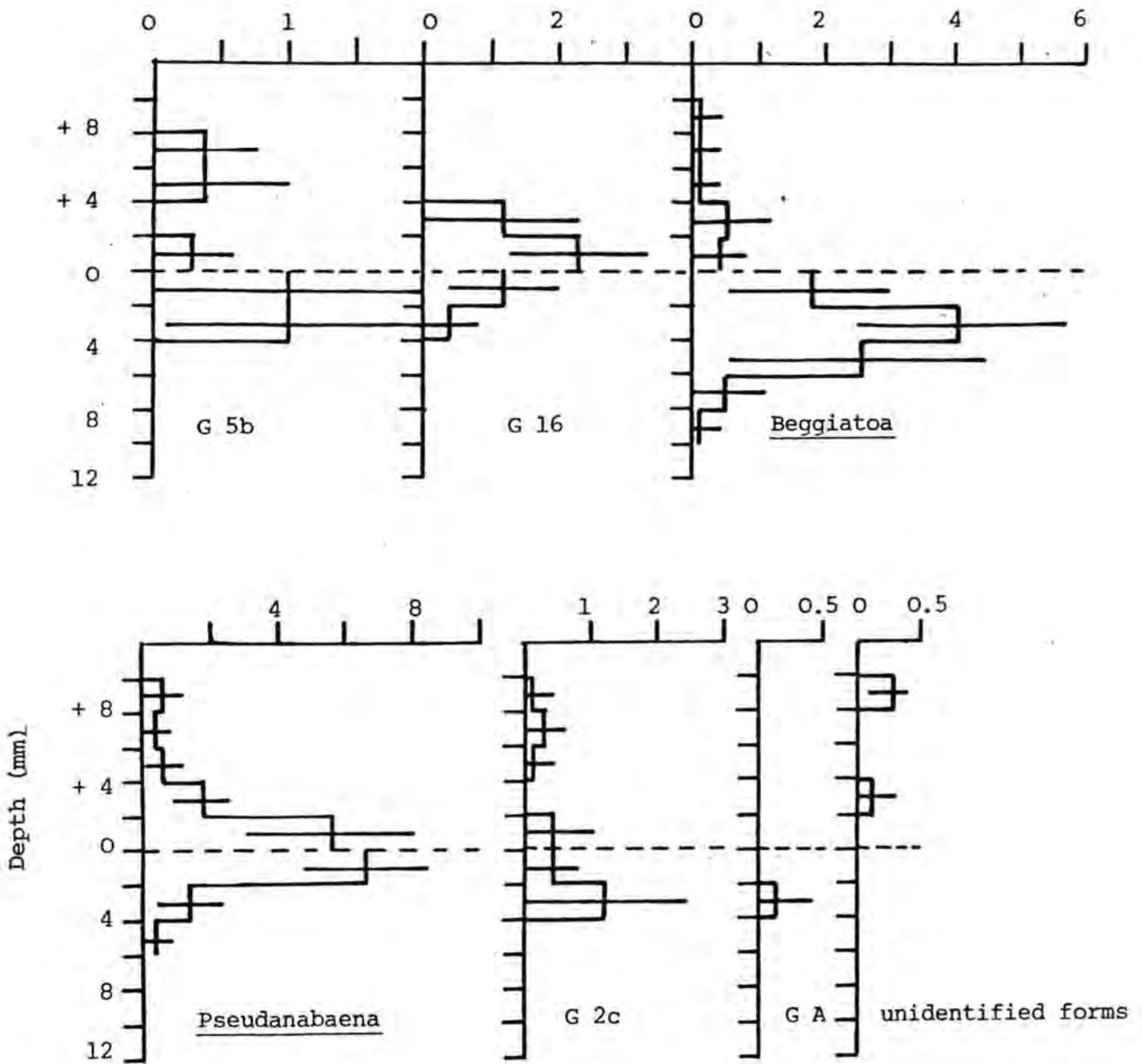


Fig. 84. Cont'd.

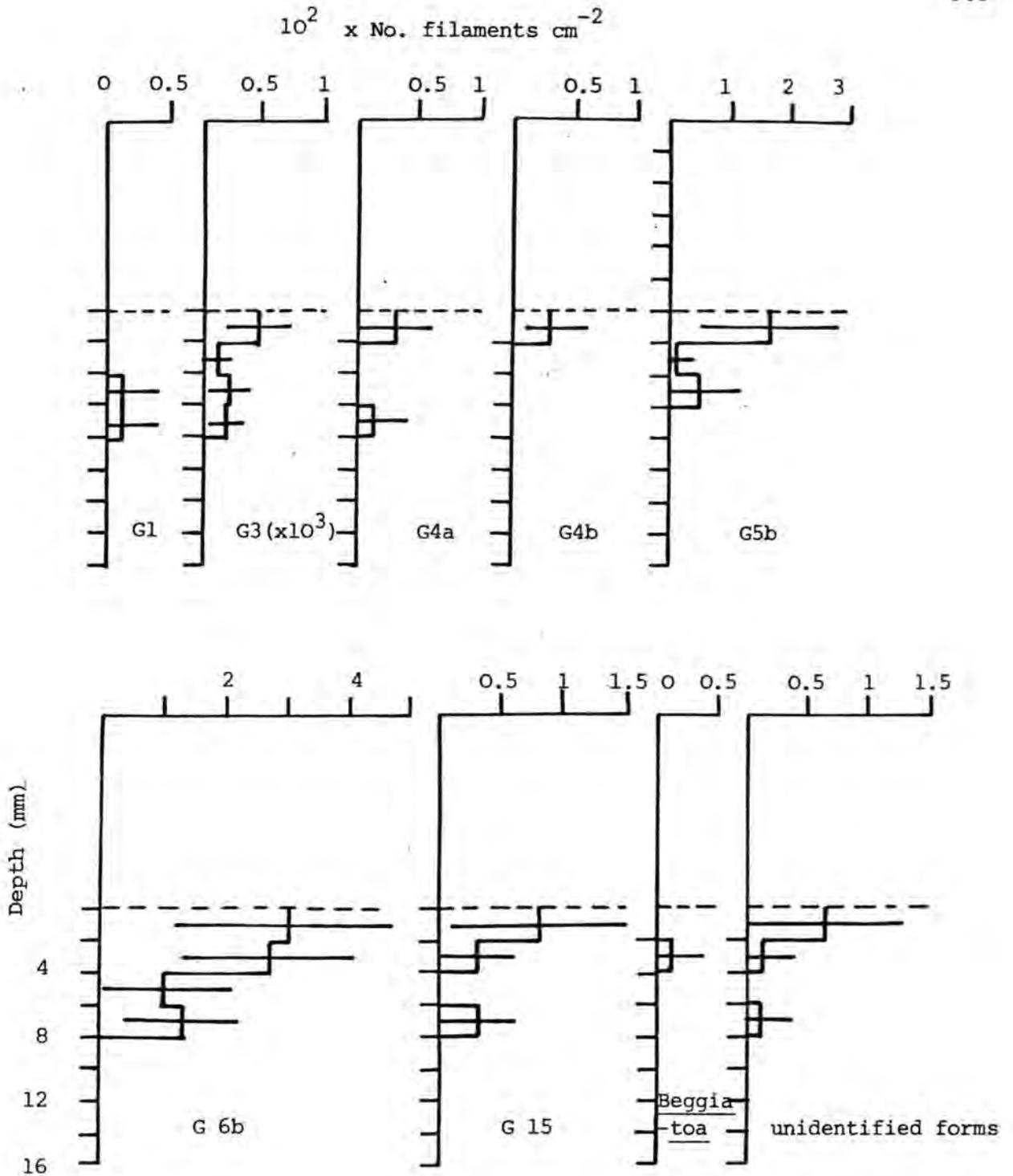


Fig. 85. The vertical distribution of filamentous bacteria in the profundal zone of Blelham Tarn using agar coated slides. The counts were made below the sediment-water interface only and the slides incubated for 4 days at 8°C .

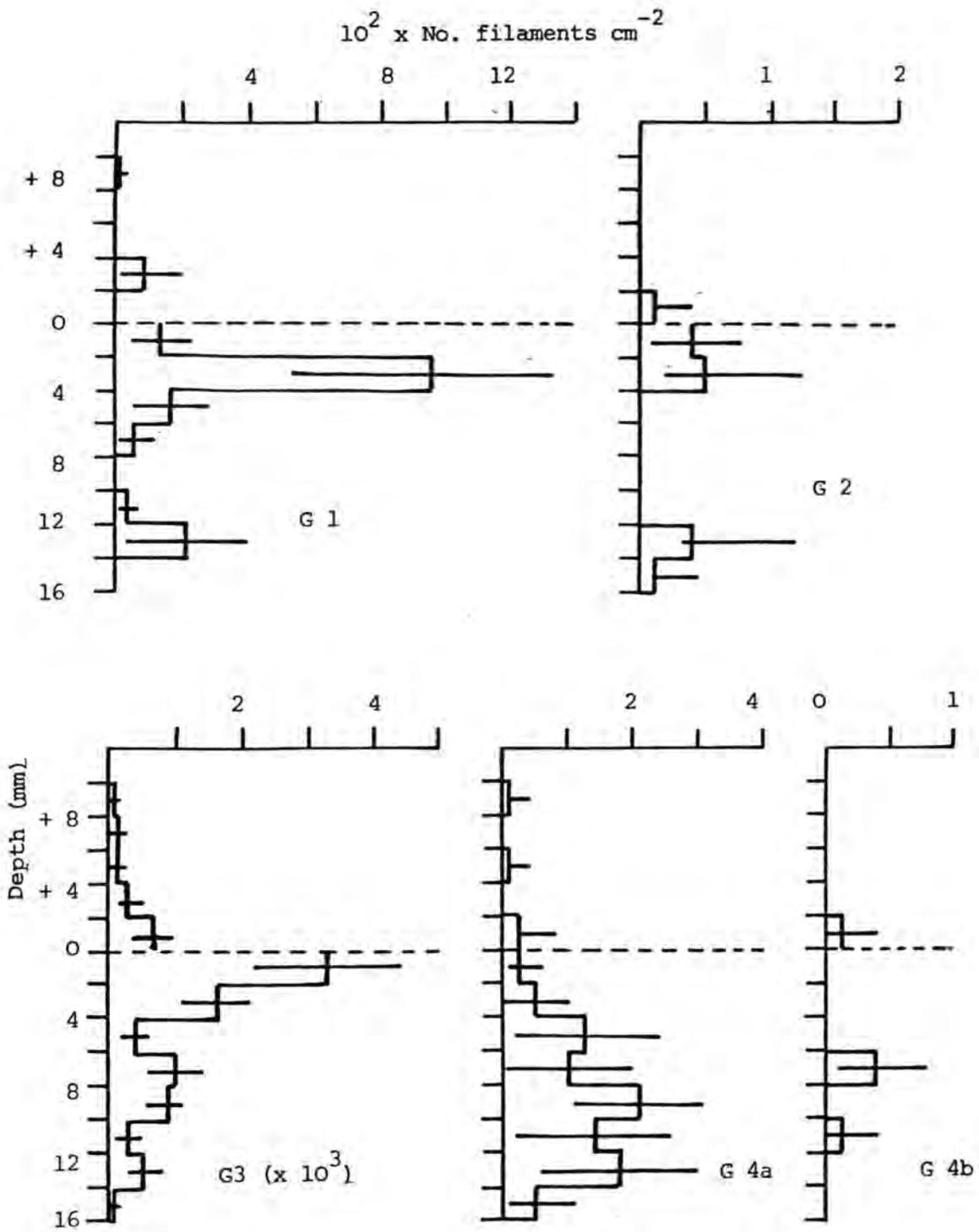


Fig. 86. The vertical distribution of filamentous bacteria in the littoral zone of Blelham Tarn using agar coated slides. Conditions as Fig. 84.

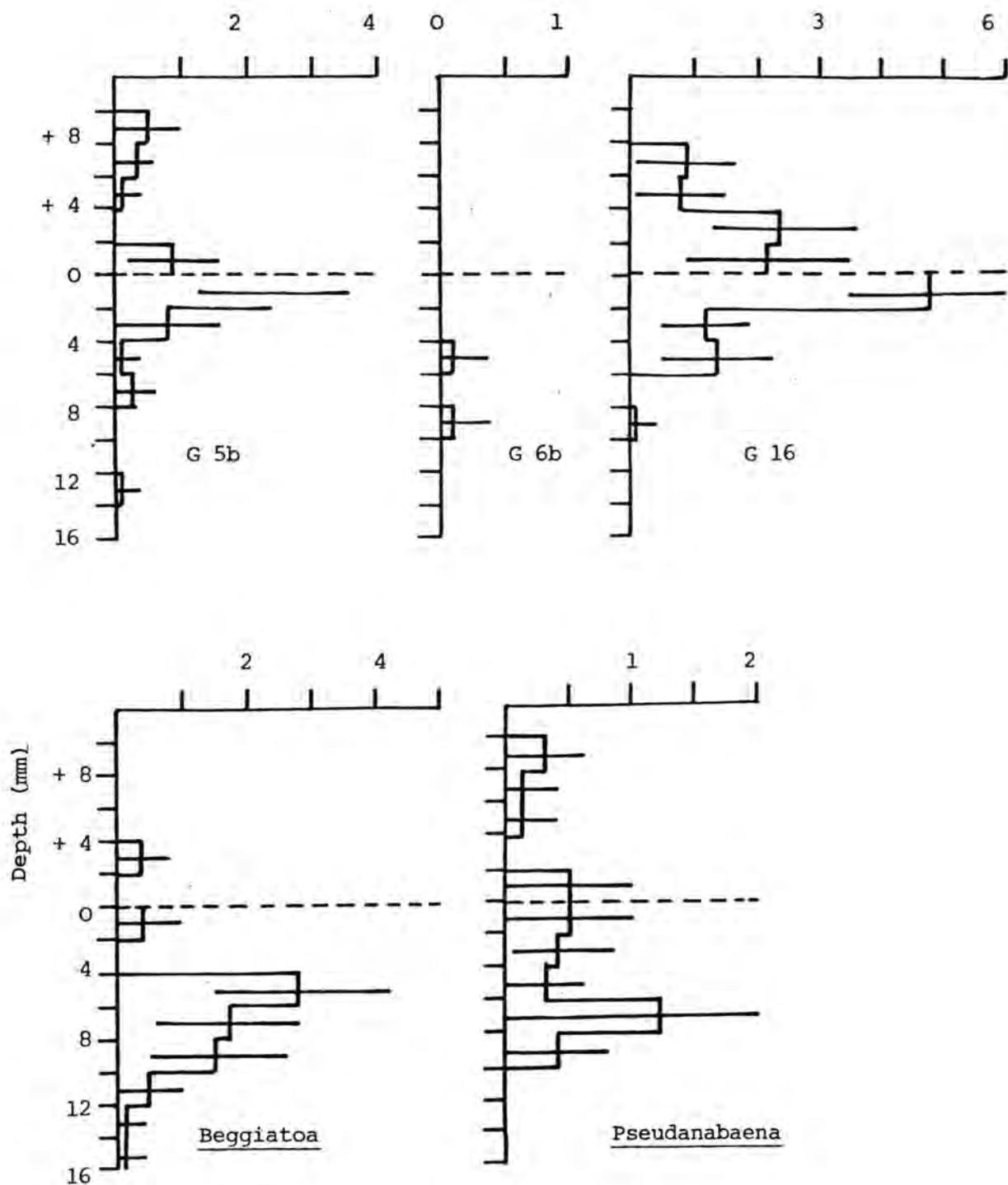


Fig. 86. Cont'd.

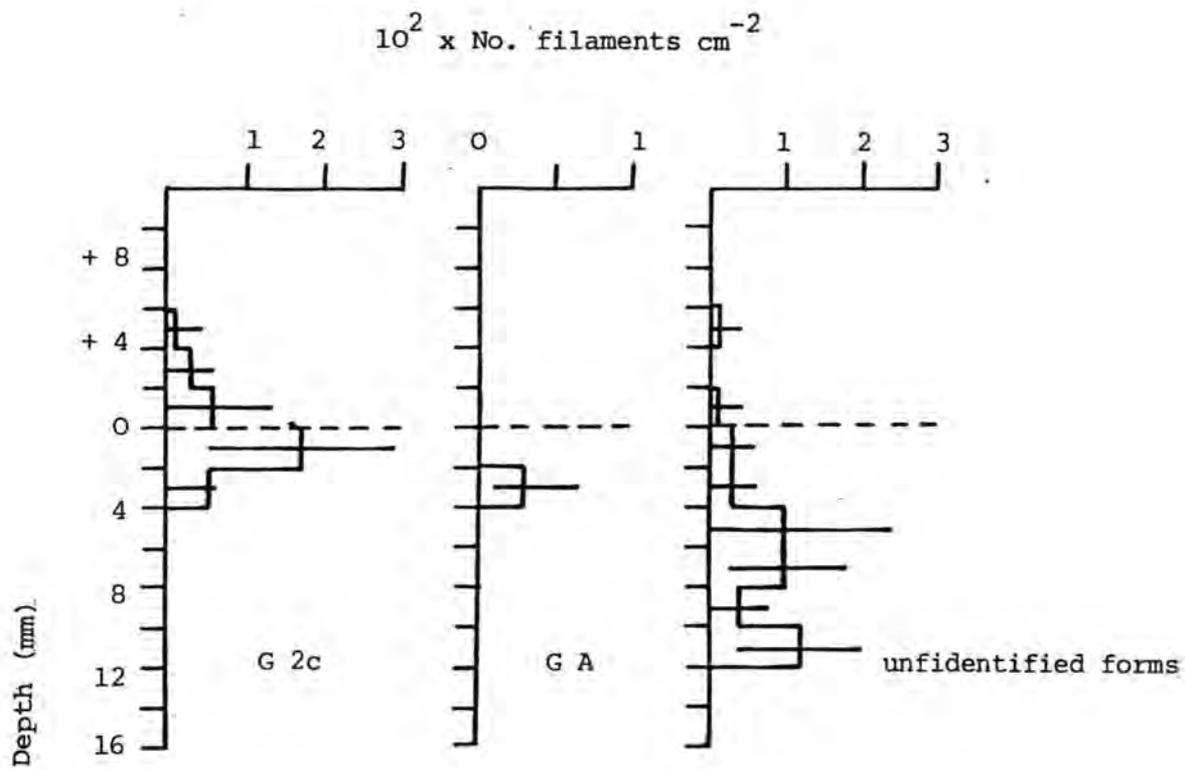


Fig. 86. Cont'd.

Table 23. Comparison of the vertical distribution of filamentous bacteria in the profundal and littoral zones of three lakes using agar coated and uncoated slides

Groups	Genus	Uncoated slides			Coated slides			Those which peak at or near the sediment surface					
		Counts higher above the interface (AP)-profundal (AL)-littoral			Counts higher above the interface: (AP)-profundal (AL)-littoral			Wastwater		Windermere		Blelham	
		Counts higher below the interface (BP)-profundal (BL)-littoral			Counts higher below the interface (BP)-profundal (BL)-littoral								
		Wastwater	Windermere South Basin	Blelham Tarn*	Wastwater	Windermere South Basin	Blelham Tarn**	U	C	U	C	U	C
1	<u>Vitreoscilla</u>	BL	BP	P	BP	BL	L	-	-	+	+	+	+
2	<u>Leptothrix</u>	AP	BP	L	-	BP	L	+	-	+	+	+	+
3	<u>Flexibacter</u>	BL	AL	L	AL	BP	L	+	+	+	+	+	+
4a	<u>Beggiatoa</u>	-	BP	P	-	BP	L	-	-	+	-	+	+
4b	<u>Achroonema</u>	-	-	P	-	-	L	-	-	-	-	+	+
5b	<u>Flexibacter(?)</u>	-	AL	P	AL	BL	L	-	+	+	+	+	+
6b	<u>Cytophaga/Flexi -bacter</u>	BL	-	P	-	-	P	+	-	-	-	+	+
10	<u>Lieskeella</u>	-	BP	-	-	BP	-	-	-	+	+	-	-
15	<u>Pelonema</u>	-	-	P	-	-	P	-	-	-	-	+	+
16	<u>Toxothrix</u>	BP	AL	L	-	AL	L	+	-	+	+	+	+
	<u>Beggiatoa</u>	-	-	L	-	BP	L	-	-	-	-	+	+
	<u>Peloploca</u>	-	-	-	-	-	-	-	-	-	-	-	-
	<u>Pseudanabaena</u>	AL	AL	L	AL	BL	L	+	+	+	+	+	-
Unidentified forms		BL	BP	P	AL	BP	L	+	+	+	+	+	+
Empty sheath		AP	-	-	BP	-	-	+	+	-	-	-	-

* In the slides incubated in the profundal zone the counts were made only above the sediment-water interface.

** In the slides incubated in the profundal zone the counts were made only below the sediment-water interface.

Table 25. Observed distribution of filamentous bacteria above and below the sediment-water interface using agar coated slides. Conditions as in Table 16.

	Groups of filamentous bacteria											u.f.	2c		
	1	2	3	4a	4b	5b	6b	10	15	16	<u>Beggiatoa</u>			<u>pseudanabaena</u>	
Profundal, above															
Wastwater			+												
Windermere South Basin	+	+	+			+		+		+				+	+
Profundal, below															
Wastwater	+		+			+								+	
Windermere South Basin.	+	+	+	+		+		+		+		+		+	+
Blelham Tarn	+		+	+	+	+	+		+			+		+	
Littoral, above															
Wastwater	+		+			+						+		+	
Windermere South Basin	+		+			+				+		+		+	+
Blelham Tarn	+	+	+	+	+	+				+		+			
Littoral, below															
Wastwater			+			+						+		+	
Windermere South Basin	+		+			+				+		+		+	+
Blelham Tarn	+	+	+	+	+	+	+			+		+			

u.f. = unidentified forms

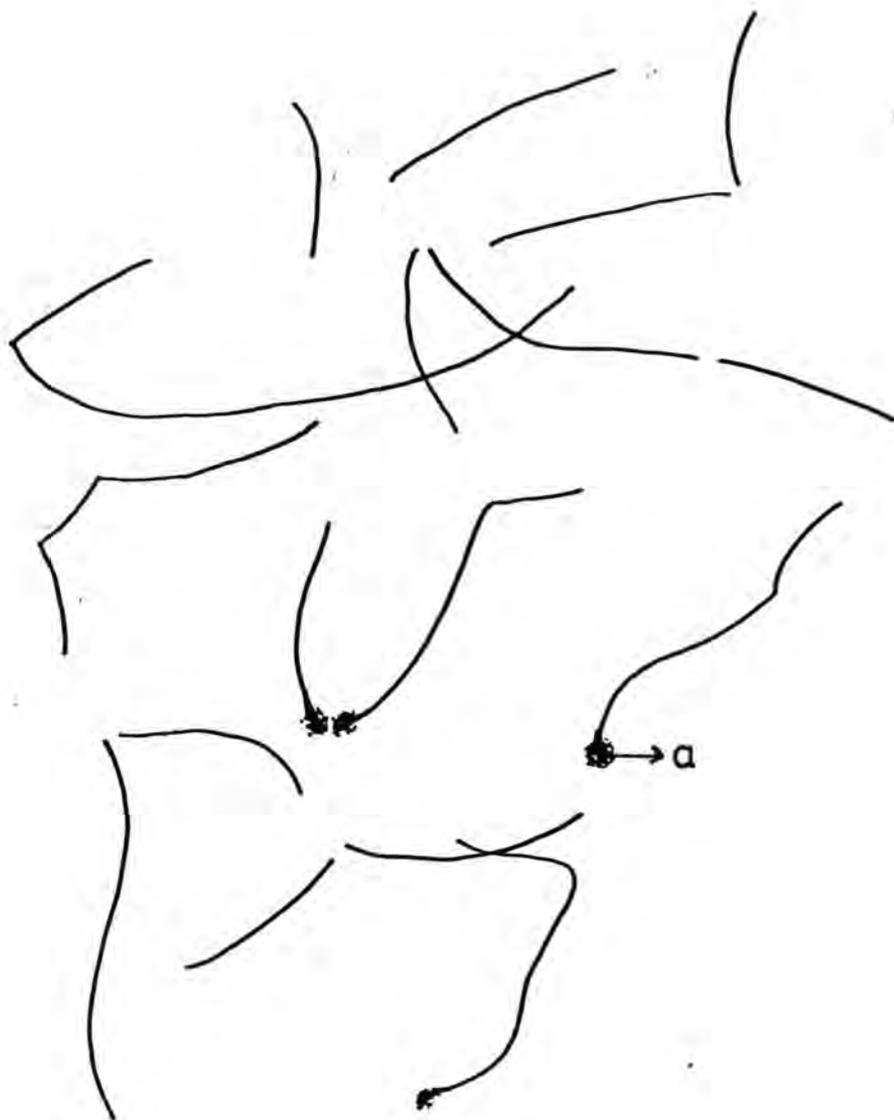


Fig. 87. Group 2c organisms observed in Windermere South Basin.
(a) attachment.

Plate 90 Group 2c organisms. (a), (b): the arrows show the attached region of the filament. (c) Filament with sheath impregnated with iron. Bar represents 20 μm .

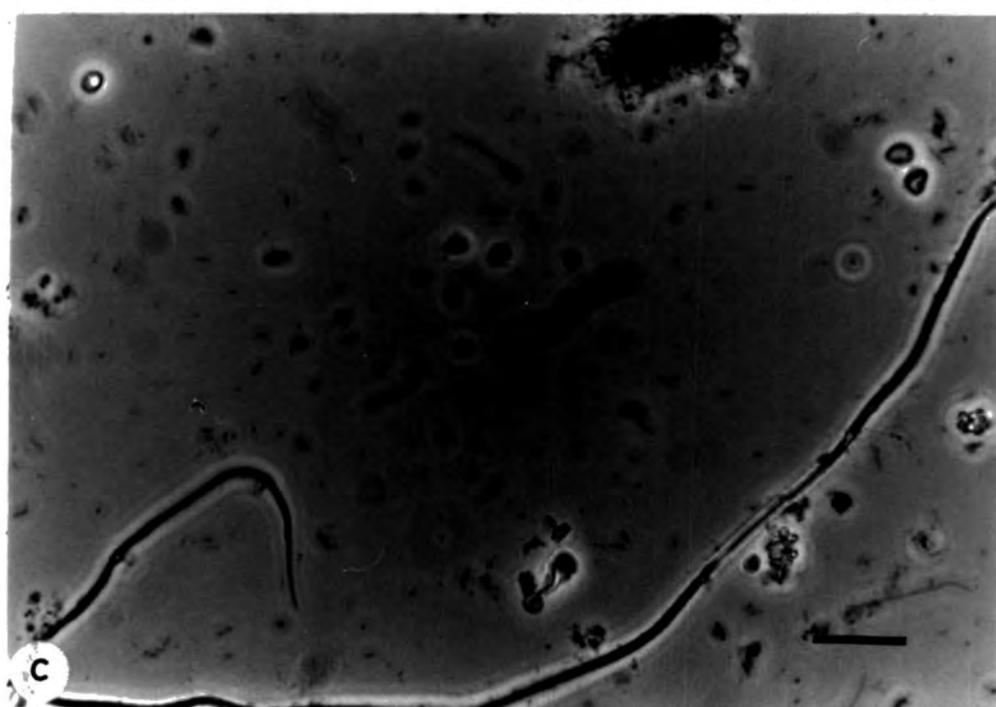
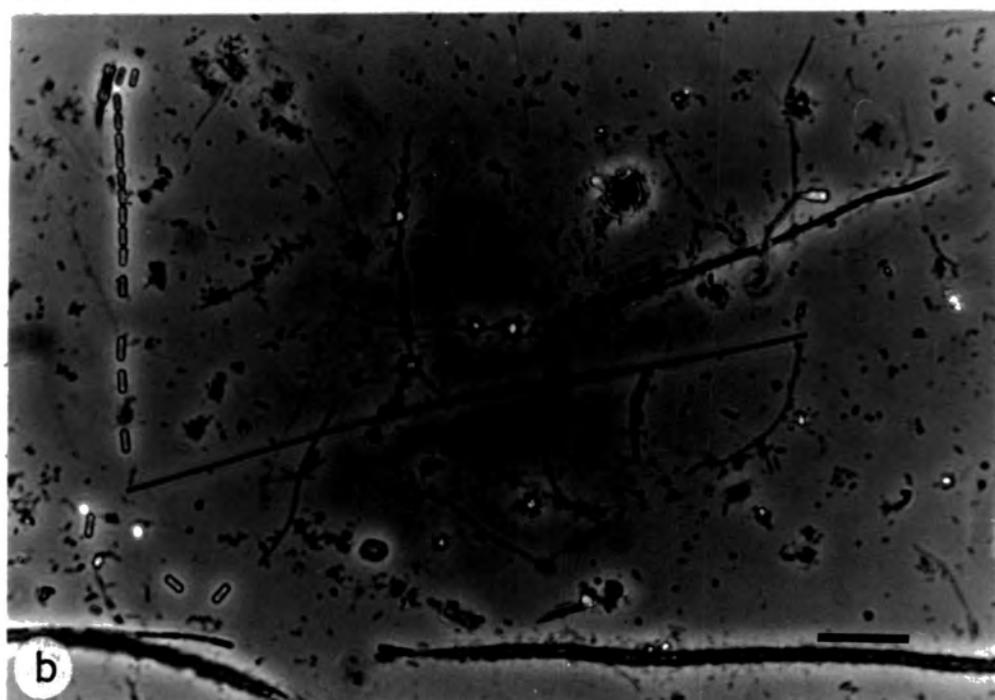
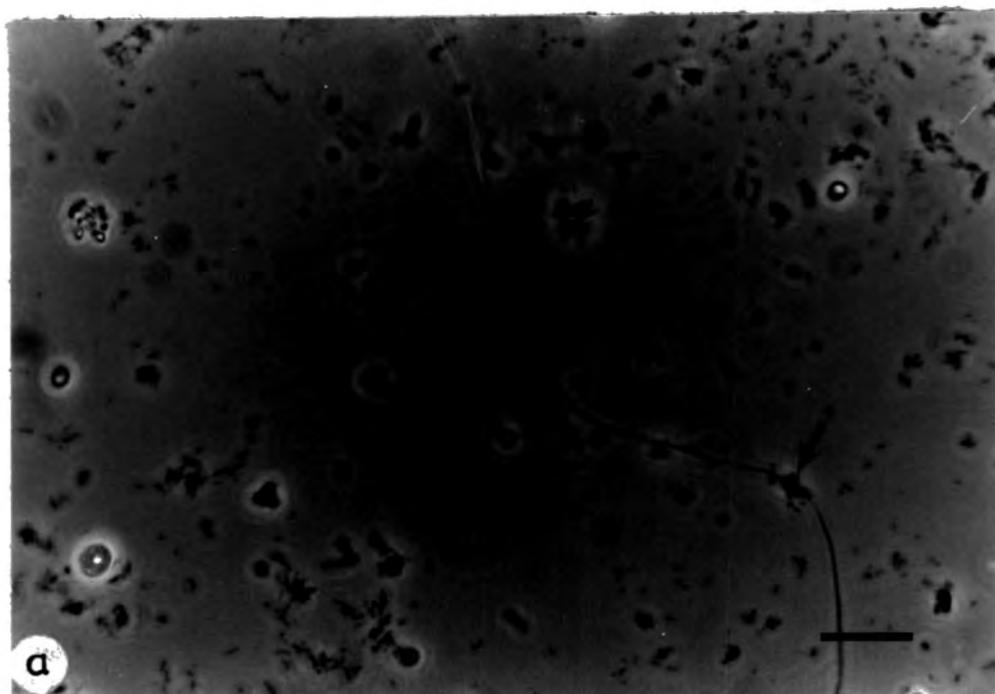


Table 26. The number of groups of filamentous bacteria observed using the three techniques.

	Wet mount	Agar coated slides	Uncoated slides
		Profundal	
Wastwater	1	3	5
Windermere South Basin	6	9	9
Blelham Tarn	12	8	8
		Littoral	
Wastwater	2	3	4
Windermere South Basin	6	6	9
Blelham Tarn	8	12	11

4. DISCUSSION

In the early stages of development of microbial ecology, sediments were often used as sites for the isolation and observation of bacteria including filamentous forms. Quantitative observation on population density and activity were not made at this stage. The study of bacterial ecology then moved into a semiquantitative state when bacteria were counted by viable count techniques e.g. plate count and MPN (Hayes & Anthony, 1959), although by this time the majority of the studies had been conducted on marine sediments (ZoBell, 1946). Although it is probably true to say that the emphasis in aquatic microbiology had been on bacteria in the water column, this was probably due to the interest in public health aspects but may also have reflected the difficulty in sampling the sedimentary environment. Work on sediments then progressed to an examination of the functions of the bacteria and measurement of the processes which they were involved e.g. respiration (Hargrave, 1969), nitrogen cycling (Keeney *et al.* 1971), sulphate reduction (Jørgensen, 1977; Cappenberg, 1974) and methanogenesis (Zeikus 1977; Winfrey & Zeikus, 1979; Cappenberg, 1976). This examination of processes has awakened an interest in the organism which may be performing these functions and a realization that perhaps the techniques used originally would not necessarily isolate all the relevant bacteria (Brock & Gustafson, 1976; Bryant *et al.* 1967). In this re-examination of bacterial groups, those responsible for general heterotrophic processes have to some degree been ignored. In particular the role of filamentous bacteria required further studies. Much of our recent knowledge on their identification has been derived not from their natural environment, but from the study of man made processes e.g. sewage sludge (Farquhar & Boyle, 1971; van Veen, 1973; Eikelboom, 1975).

Because of the absence of information on filamentous bacteria in lakes it was necessary to conduct a general survey to provide a base of

information from which sites might be selected for more detailed investigation. With the exception of the filamentous cyanobacteria and possibly of Leptothrix (Jones, 1975) filamentous bacteria had been almost exclusively observed in sediments on the lake bottom and sedimenting organic material caught in traps. The few filamentous species described by Clark & Walsby (1978) rarely occur in significant number in the water column of the Cumbrian Lakes. Seventeen lakes ranging from oligotrophic to eutrophic, and for which background chemical data were available at the Freshwater Biological Association, were selected for this survey. The results presented in the first part of this study tended to confirm previous observation (Pearsall, 1921; Mortimer, 1942) that the lakes formed a trophic series. This, however, was the first detailed survey of the benthos and considerably greater variability was observed. The ranking of the lakes based on their sediment characteristics was generally in reasonable agreement with that previously published, based on results from the water column (Jones et al. 1979), but in particular the four lakes at the eutrophic end of the spectrum might be considered as a separate group; this is discussed later. Of the results not directly concerned with bacterial population the following were considered to be of some interest. Increasing chloroplast counts and higher chlorophyll a concentrations in the more eutrophic water bodies could be due to higher productivity and sedimentation from the water column as well as production in the benthos itself, whereas sediment carbon and nitrogen values correlated positively with lake ranking, analysis by loss on ignition failed to do so. Because handling errors are more likely with the latter I concluded that the element analyses were likely to be the more accurate. Of the remaining physical-chemical factors measured the Eh profiles proved to be the most interesting. There was not enough time to collect sufficient data to produce detailed depth time diagrams illustrating seasonal changes in Eh within the

sediment. However the data presented in Figs 30 to 46 provided sufficient information to indicate the magnitude of vertical shifts in Eh during thermal stratification. These have been discussed in more detail for the Cumbrian Lakes by Gorham (1958). There is no other accepted convention for summarizing Eh data and therefore three methods of presentation have been used (Fig. 47). Although Mortimer (1942) considered that + 200 mV represented the boundary between oxidizing and reducing conditions, Hutchinson (1957) extended this argument to suggest that the migration of the + 200 mV layer into the water column represented a basic feature for the classification of lakes. The present results show that such a migration occurred in the four lakes at the eutrophic end of the spectrum. This migration not only reflected the oxygen consuming process in the sediment (Jones et al. 1979) but also affected, in turn, the chemistry of the overlaying water. These seasonal changes in the physical chemistry characteristics of the sediment undoubtedly affected the microbial population. Such an effect is usually seen as a greater seasonal variability in the more eutrophic lakes. Increased variability with trophic status has been reported previously (Jones, 1971; Jones et al. 1979).

It is not the purpose of this thesis to discuss the position of individual lakes within the trophic series but it was interesting to note that based on sediment characteristics both Bassenthwaite and Ullswater appeared to be misplaced. The relatively small microbial population in Bassenthwaite may be due to the continual disturbance of the sediment but there appeared to be no explanation for the relatively high values obtained in Ullswater except to say that these are in agreement with the findings of Pearsall (1921).

During this study several methods were used to estimate bacterial populations in the sediment. The approximate values obtained by each method are summarized in Table 27. The differences between viable and

Table 27. Orders of magnitude of the counts obtained when different techniques were used for enumerating filamentous bacteria expressed on a volume basis (ml^{-1}).

Lakes	AO counts with membrane	Marked slides	Wet mount (AO)	Wet mount (Phase-contrast)	Agar coated slides	MPN
Wastwater	10^7	10^6	10^6	10^4	10^3	10^3
Windermere South Basin	10^7	10^6	10^6	10^4	10^6	10^2
Blelham Tarn	10^8	10^7	10^5	10^5	10^5	10^4

direct counts (i.e. approximately 4 orders of magnitude) were higher than those observed previously in freshwater (Collins & Kipling, 1957) and marine (Jannasch, 1958) systems. These results help to explain the difficulties encountered in the attempts to isolate filamentous bacteria from the sediments. Of the three direct count procedures used the highest counts were obtained on black membranes after staining with acridine orange, the lowest with wet mounts. The results with the marked slide technique (Trolldenier, 1973) occupied an intermediate position. These observations are not surprising because one might expect fragmentation of the larger filaments during the homogenization which preceded the membrane counts. The low counts obtained with wet mounts may have been due to masking of some bacteria by particles of detritus. In addition to this, wet mounts were more likely to contain nutrients released from reducing sediments and these are known to quench fluorescence (Jones & Simon, 1975). Additional evidence for this suggestion was obtained when filaments which were readily observed under phase-contrast microscopy did not fluoresce. Identical filaments in oxidized sediments fluoresced readily. This phenomenon might also explain some of the lower counts obtained in profundal samples from Blelham Tarn.

The necessity for using so many techniques to count the filamentous bacteria might be questioned but the absence of reliable information on bacteria in sediments (particularly filamentous bacteria) meant that as many approaches as possible had to be tried. Some of the media tested had been used successfully to isolate filamentous bacteria from sewage sludge (van Veen, 1973; Eikelboom, 1975). The failure to obtain isolates in this study may not necessarily reflect the inadequacy of the media but more the relatively small inocula of filamentous bacteria in the sediments. The authors cited above were able to inoculate with flocs

largely composed of filamentous bacteria. If viable isolates of filamentous bacteria are to be obtained from sediment then some method must be devised which encourages their growth against that of unicells. Some measure of success in this may be claimed for the MPN method using cellophane strips in the heterotrophic medium. The numbers obtained were in reasonable agreement with the wet mount by phase-contrast microscopy, but it should be added that species composition appeared to be somewhat different. The cellophane technique was particularly good for primary enrichment of those filaments which resemble Flexibacter. Although many attempts were made to obtain pure cultures of the filamentous bacteria only two were obtained, one tentatively identified as Cytophaga and the other as Pseudanabaena. The agar sausage technique provided particularly good enrichments of Pelonema. In all cases further sub-culturing resulted in a profusion of unicells. Clearly the media used did not reflect the chemical conditions in the sediment and there is reason to believe that the oxygen concentration and/or Eh should be given more careful consideration; this will be discussed later.

Some of the more unexpected results with the direct counts were obtained with fluorescein di-acetate (FDA). This substrate has been used successfully to count viable eukaryotic cells (Paton & Jones, 1975) but the results with the prokaryotes have been variable (Jones & Simon, 1975). Satisfactory fluorescence has however been obtained at this laboratory with filamentous cyanobacteria and therefore the technique was used in this study. The FDA counts of filamentous and total bacteria were consistently higher in the intermediate lakes of the series. Given the statistical variability of counts obtained in sediments (Table 7) the significance of such a trend might be questioned. But two factors indicated that it was a real trend: in the first place between-core variability with FDA counts of filaments was consistently lower than other direct count procedures and, secondly, the trend was observed in several lakes and was not an isolated incident. The only

explanations available are that either there was a real trend in viability or that viability was significantly lower at the oligotrophic extreme and that FDA fluorescence was quenched to a greater extent than that of AO in the reducing sediments of the more eutrophic lakes. Unfortunately this trend was not observed with the MPN estimates of filamentous bacteria which showed a slight, but not significant upward trend. The same was true of CPS viable counts (Fig. 14). AO counts of total and filamentous bacteria also increased with increasing degree of enrichment of the lake, as did all estimates of variability in the filamentous bacterial population.

Although the filamentous bacteria represented a relatively small proportion of the total population they were considered worthy of further study for several reasons. In the first place they were always enumerated as whole filaments and not as cells within the filaments and therefore this underestimates their numerical importance. In addition their number may bear no relation to their biomass and activity. For example, Jørgensen (1977) has shown that relatively low numbers of Beggiatoa were much more important in terms of biomass and made a major contribution in to carbon turnover in off-shore sediments. The preliminary examination had also shown that filamentous bacteria responded positively to the degree of lake enrichment and that they might exhibit a degree of microstratification within the sediment. Bearing in mind the lack of information on these groups in natural environments they were considered worthy of further examination.

The problems encountered in attempting to obtain pure cultures of a significant proportion of the filamentous bacteria left me with two options for the remainder of the study period. The first was to concentrate on one particular group, to attempt to obtain isolates, and determine the details of its ecology. Alternatively a more general approach to the whole population could be considered which would include measurements of population activity and determination of population

distribution based on tentative identifications made under the microscope. The latter course was chosen.

Available information on filamentous bacteria was so limited that it was not possible to determine whether their metabolism in the sediment was essentially heterotrophic or autotrophic. The only available technique which might provide information of this kind was microautoradiography. Samples of sediment were incubated under a variety of conditions in the presence of ^{14}C -, ^3H -labelled organic substrates and ^{14}C bicarbonate. Uptake of these substrates into the organisms should have indicated the major source of cell carbon. None of the trials proved satisfactory in spite of the use of the most stringent dark room procedures (Brock & Brock, 1968). Examination of the samples showed a significant loss of filaments during handling and high background particle counts presumably due to unicells. This approach was therefore abandoned although subsequent discussion (M. Fletcher, personal communication) suggested that the use of Ilford photochemicals might have contributed, at least in part, to high background counts. A more general investigation of metabolic activity in the filamentous bacteria was then undertaken using tetrazolium dyes (TTC and INT). These were incorporated into the agar on slides to be inserted into the sediments, in the hope that metabolically active filaments would reduce the dyes to their highly coloured insoluble formazans. Dye reduction associated with individual filaments was not observed and further trials were discontinued. The failure of this technique was probably due largely to diffusion of the tetrazolium salts out of the agar and therefore the inability to maintain an adequate concentration in association with the filaments. The INT was also particularly unstable in the buffer system used, and bearing in mind the findings of Zimmermann *et al.* (1978) this approach should be re-examined using an unbuffered INT solution applied generally to the water above and within

the sediment core. Apart from the need for a more detailed investigation on the metabolic nature of these organisms concerning their ability to use carbon dioxide or organic carbon to synthesise their carbon compounds, consideration should also be given to their energy-yielding metabolism. Differences in species composition were observed between light and dark incubations and therefore an examination of possible photorganotrophic growth should be included in future studies.

Clearly the failure to obtain satisfactory results with the metabolic tests left only one course of action in the time available i.e. an attempt to describe the general population ecology of the filamentous groups in the three selected lakes. Such a description would have to include a tentative identification of the organisms based on their morphology and their response to cytochemical tests. The characteristics chosen were those used in Bergey's Manual (1974) and Eikelboom (1975) and most of the cytochemical tests were performed according to Farquhar & Boyle (1971) or minor modifications thereof. Preliminary trials immediately demonstrated that the available keys were inadequate for the characterization of filamentous bacteria in freshwater sediments to genus level. The major problems encountered, and discussed to some extent in the key were as follows:

- 1) Sheath formation: organisms developing on slides inserted into the sediment were frequently observed to migrate out of their sheaths and to exhibit gliding motility. Dubinina (1976) reported the migration of filaments of Leptothrix out of their sheath which had become heavily impregnated with iron impeding the permeability of nutrients to the cells. Conversely other gliding filaments which did not possess a sheath when motile were observed with such a structure when stationary. Lewin (1970) pointed out that the secretion of extracellular slime trails by gliding organisms could accumulate and form a sheath and this could, in some cases, impede motility. These properties could obviously lead to confusion in the identification of members of group 1 organisms

(Vitreoscilla) and group 2 (Leptothrix) as well as Achroonema and Herpetosiphon. Similar problems might be encountered with sheathless forms of Flexithrix which might be confused with Cytophaga or Flexibacter. Such observations clearly raise doubts as to whether sheath formation is a sufficiently stable characteristic (or the conditions under which sheaths are produced are sufficiently well understood) for it to be used for identification at this level.

2. Motility is one key feature that is not necessarily always observed since stationary phases may occur.
3. The size range of organisms in many cases either overlapped or is not given in the literature creating difficulties. Under such circumstances it would be possible to assemble Vitreoscilla, Herpetosiphon and sheathless Leptothrix. In the present study the differentiation between species of Vitreoscilla and Achroonema was made according to the presence of constrictions at the cross-walls in the former and its absence in the latter. This is not in agreement with Bergey's Manual (1974) and further investigation may show whether these two genera need be separated at all.
4. In certain filaments, cross-walls, if present, may be invisible by ordinary light microscopy presenting problems in determining whether the organism was multicellular. Observations made with organisms stained with acridine orange and examined under fluorescence microscopy showed that this might be a satisfactory technique to determine multicellularity.
5. It was observed that pyridine caused the dispersal of gas vacuoles in several filaments examined, and therefore this solvent should be applied only after the pressure test for gas vacuolation.
6. The presence of inclusions in the cells is variable since it depends on the nutrient status of the organism. With the exception of sulphur inclusions (where organisms with cells devoid of elemental sulphur may deposit it in the presence of added sodium sulphide) there is little that can be done to stabilise these features.

The major differences between the key presented in this thesis and

the one in Bergey's Manual (1974) are shown in Table 28. Bearing in mind the above reservations about identifying filamentous bacteria by direct observations an attempt was made to obtain some information about their population ecology in the three lakes: Wastwater (oligotrophic), Windermere South Basin (mesotrophic) and Blelham Tarn (eutrophic). The results were based on the use of two techniques, direct counts in wet mounts and enumeration on slides inserted into the sediment. The former was used to provide an estimate of the real distribution of the bacteria within the sediment and the latter partially as an enrichment technique, possibly to provide a microclimate to encourage the growth of a greater number and variety of filaments. Such enrichments are analogous to the use of microcapillaries by Perfil'ev & Gabe (1969). Microcapillaries were not used routinely because they selected for a very limited range of filamentous forms, very low numbers were obtained and recovery was extremely difficult. Several slide coatings were tested and compared with uncoated controls. These included agar, gelatin and Ullrich's adhesive. Although the results obtained were not absolutely clear cut, there was, generally, a greater variety and higher population density on the agar coated slides. These were therefore chosen and uncoated slides were used for cytochemical tests.

Before the distribution of individual genera in sediments can be discussed those of the total populations should be considered (Figs 51 to 53). These results were obtained at the onset of stratification and in late summer when all three lakes were stratified and the hypolimnion of Blelham Tarn was anoxic. Consistently higher counts were obtained in the profundal zone and this was more marked in early summer. There have been relatively few microbiological studies which compare these two zones within a lake. Willoughby (1974) demonstrated differences in the benthic fungi, members of Saprolegniales being more active in the marginal than the deeper water sediments (Willoughby, 1965; Dick, 1971). The only

Table 28. Differences between the key presented in this thesis and the one in Bergey's Manual, 1974

Bergey's Manual	Proposed key
<p><u>Toxothrix</u>: it is keyed out twice in Section H.</p> <p>1) organism with sheath which split longitudinally into fine, hair-like sections</p> <p>2) sheathless organism and excretes a mucoid substance which is deposited as a double track of twisted strings and may be impregnated with oxidized iron.</p>	<p>It is keyed out once as in the second alternative described in Section H of Bergey's Manual.</p>
<p><u>Thioploca</u>: it is keyed out once.</p>	<p>It is keyed out once and there is a note pointing out that filaments which leave the sheath are indistinguishable from <u>Beggiatoa</u>.</p>
<p><u>Streptothrix</u></p>	<p>It is included as <u>Haliscomenobacter hydrosis</u>.</p>
<p><u>Herpetisiphon</u>: it is keyed out once as a sheathed organism with un-sheathed forms motile.</p>	<p>It is keyed out once as a sheathed non-motile filament. As a motile organisms, sheathed or without sheath it is entered as a note.</p>

Table 28 cont'd.

Flexithrix: it is keyed out once as a sheathed, non-motile organism with free cells motile by gliding.

It is keyed out once as a sheathed non-motile organism. As a sheathless gliding organism, it is entered as a note since it is indistinguishable from Cytophaga and Flexibacter.

Achroonema: it is keyed out in Section F as trichomes not constricted at the cross-walls. In Section H is keyed out twice

It is keyed out twice as filaments not constricted at the cell walls

1) with sheath

2) without sheath

1) sheathed filaments which may be constricted at the cross-walls.

2) sheathless filaments with cells frequently short and barrel shaped. A note is added here pointing out that it is impossible to separate some species of Vitreoscilla and Achroonema.

Sphaerotilus: it is keyed out once as a sheathed organism.

It is keyed out once as a sheathed organism. Since sheathless form has been reported a note to that effect is added when sheathless, non-motile organisms are described.

Table 28 cont'd.

Leptothrix: it is keyed out in Section F as sheathed filaments spirally wound around themselves or algal filaments. In Section H it is entered as a sheathed filament.

Felonema: it is keyed out once as a sheathless organism.

Flexibacter: it is keyed out once as a unicellular organism.

It is keyed out once as a sheathed organism. A note is included pointing out that the filaments may migrate out of their sheath in which case they are indistinguishable from Vitreoscilla.

It is keyed out twice 1) sheathless organism 2) sheathed organism. Since impregnation with iron has been observed a note is added to that effect.

It is keyed out once as a unicellular organism and there is a note pointing out that in some strains the longer filaments are multicellular.

The following organisms are described here but not in Bergey's Manual:

Chloronema

Chloroflexus

Microthrix parvicella

Nostocoida limicola

Pseudanabaena

comparable study on bacteria (Willoughby, 1969) demonstrated that the distribution of actinomycetes was different than that of the lower fungi and that numbers were higher in the profundal zone. It is doubtful whether actinomycetes were a significant part of my direct counts and this was tested by introducing species known to be frequent isolates in these lakes into sterilized sediments and observing any morphological change which occurred.

The other interesting point with regard to the total count distribution of filamentous bacteria concerned the depth at which peak population densities were observed in the three lakes. These occurred at the surface in Blelham Tarn but at depths of 0.5, 1.5, 2.5 and 4.5 cm in Wastwater and Windermere South Basin. The Eh profiles in these three lakes suggest that filamentous bacteria in general may occur in zones where oxygen concentrations are extremely low. These observations however were based on samples taken at relatively large depth intervals. To obtain more information shallower sampling intervals and analysis of the population composition would be necessary. This was done during thermal stratification when Blelham Tarn was anoxic, thus emphasizing the differences in trophic status between the lakes. The results are shown in Fig. 69 et seq.

The general observations of higher numbers in the profundal zone and greater depth distribution in the more nutrient poor lakes was confirmed for many individual groups. In addition to this, depth distribution was often found to be more limited in the profundal zone than in the shallow water sediments, again reflecting Eh conditions. This may be seen as further evidence of microaerophily in many filamentous forms, and might explain why the isolation procedures and viable counts techniques used were so unsuccessful. I attempted to grow the organisms under either aerobic or anaerobic conditions but there is now an increasing body of evidence to suggest that considerable care in controlling oxygen concentration is necessary if microaerophiles are to be grown successfully

(Stouthamer et al. 1979).

Among the particular groups observed Pseudanabaena was found only in littoral samples presumably because of its requirement for light. Beggiatoa spp were found only in Windermere South Basin and Blelham Tarn and in Windermere South Basin the population density peaked at a greater depth in the profundal zones. This genus was not found in the littoral zone of Windermere South Basin. These observations might be explained by the greater organic input in the richer lakes thus creating a potential for sulphate reduction, generating the sulphide ion used by the Beggiatoa (all those observed on the slides contained large numbers of sulphur granules). Group 1 organisms (Vitreoscilla) were found mainly in the two richer lakes and again at a greater depth in the littoral sediment, suggesting that these organisms may also have a preference for low oxygen tensions. Group 2 organisms (Leptothrix), on the other hand, were present in larger numbers in Windermere South Basin and Wastwater possibly suggesting a definite requirement for oxygen.

Although it was not possible in the time available to study seasonal fluctuations in each group it was noted that filamentous bacteria depositing iron were more consistently observed in Windermere South Basin than in the other two lakes. This may reflect the intermediate status of Windermere South Basin where oxygen concentration is reduced (but not to zero) and the Eh discontinuity layer migrates upward in the profundal zone (Mortimer, 1942) to an extent sufficient to provide a source of iron. A similar population of iron depositors might be expected to develop in Blelham Tarn during the critical period when oxygen and iron concentrations were suitable. In such a eutrophic lake this period would be very short and could easily be missed with all but the most careful sampling programmes.

In addition to the presence of microaerophiles some bacteria peaked only at the surface of the sediment in wet mount, and above the sediment (i.e., in the water column) on slide cultures. These results suggest

that the organisms were aerobes. Although there was very little evidence to suggest that some bacteria were obligate anaerobes the fact that species diversity was lower on the slides incubated in Blelham Tarn cores (as opposed to the wet mounts) suggests that some organisms might have been inhibited by the inevitable introduction of small quantities of oxygen during the handling procedure.

The population densities and the species diversity of the filamentous bacteria increased with increasing nutrient content of the lakes. Although this might be expected from surveys of other biological groups encountered in the Cumbrian lakes (see references cited by Jones et al. 1979) there is no quantitative information on the populations of filamentous bacteria in other temperate lakes with which the present results may be compared. Difficulties in isolating pure cultures of groups which represented only a small proportion of the total population resulted in tentative identifications based on morphological features and cytochemical tests. There is need for more critical studies of the distribution and activity of these organisms, and it is hoped that the present work will provide a useful basis for such studies.

5. SUMMARY

- 1) The sediments of 17 lakes in the English Lake District were investigated, with estimates of bacterial population density and other variables. The total counts of bacteria with acridine orange ranged between $1.5 \times 10^{10} \text{ g}^{-1}$ dry weight and $16 \times 10^{10} \text{ g}^{-1}$ whereas the filamentous bacteria counts ranged between $0.03 \times 10^8 \text{ g}^{-1}$ and $12 \times 10^8 \text{ g}^{-1}$. Viable counts were also made using fluorescein di-acetate and a most probable number (MPN) technique. The total counts obtained with acridine orange were up to one order of magnitude greater than the counts obtained with fluorescein de-acetate and two to four orders higher than those obtained by the MPN method. Chloroplast counts and chlorophyll a concentration increased with the increasing degree of enrichment of the lakes. The carbon and nitrogen values correlated positively with the lakes' ranking but this was not with analysis of the sediments by loss on ignition. Measurements of Eh showed the migration of the + 200 mV layer into the water column in the sediments of the four most eutrophic lakes.
- 2) Three lakes representing the oligotrophic (Wastwater), mesotrophic (Windermere South Basin) and eutrophic (Blelham Tarn) states were selected for more detailed investigation of the filamentous bacteria. Comparison between profundal and littoral zones was made. The range of values obtained for the population of filamentous bacteria was $0.11 \times 10^7 \text{ g}^{-1}$ to $8 \times 10^7 \text{ g}^{-1}$ in the profundal and $0.01 \times 10^7 \text{ g}^{-1}$ to $2.1 \times 10^7 \text{ g}^{-1}$ in the littoral zones. The highest densities of filamentous bacteria were observed in Blelham Tarn and the lowest in Wastwater. The number of filamentous bacteria expressed as a percentage of the total counts were 0.026%, 0.021% and 0.055% in the profundal zones of Wastwater, Windermere South Basin and Blelham Tarn, respectively. In the littoral zone the values were 0.007%, 0.009% and 0.020% respectively.

3) An identification scheme was drawn up for filamentous bacteria based on microscopic observations on wet mount preparations and on agar coated and uncoated slides incubated in sediment cores. Thirteen groups were described and identified tentatively on morphological and cytochemical criteria. The vertical distribution of the different groups of filamentous bacteria was surveyed in the three selected lakes. Higher numbers in the profundal zone and distribution through a greater sediment depth in the more nutrient poor lakes was observed for many individual groups of filamentous bacteria.

A tentative key to filamentous bacteria was provided since the key available for these organisms did not prove satisfactory during the present study.

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A Microbiological Study of Sediments from the Cumbrian Lakes

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The microbiology of the benthos of 16 lakes in Cumbria was studied. The lakes formed a series ranging from oligotrophic to eutrophic, and the results were compared with other surveys of their chemistry and biology. The sediments of the more productive lakes contained more organic matter, E_h measurements indicated that they were more reduced, and the overlying water was deoxygenated to a greater degree. These results correlated with greater microbial activity, biomass and numbers in the sediments of the richer lakes, as measured by electron transport system activity, ATP and direct counts. The data obtained from the sediments were, however, more variable, and showed poorer agreement with the assumed ranking of the lakes than the results obtained from the water column in this and past surveys. The microbiology of the benthos suggested that the more productive lakes might be considered as a distinct group, but more detailed sampling and careful choice of indicator micro-organisms would be required to provide statistically significant evidence for this.

INTRODUCTION

Interest in lake classification has led to a number of comparative studies of lakes of differing trophic status. This status is related to the size and shape of the lake (morphometric eutrophication) and its nutrient input (cultural eutrophication) (Odum, 1971). Although nutrient enrichment has received particular attention in recent years both components may be of importance particularly in relation to deoxygenation of the hypolimnion and metabolism in the profundal zone. Lakes in other parts of the world have been classified on the basis of catchment area: volume ratio (Schindler, 1971), hypolimnetic oxygen concentration and benthic fauna (Kitagawa, 1978) and oxygen deficits and clarity (Stewart, 1976). Indices of lake trophic state derived from multivariate analysis have been used successfully (Shannon & Brezonik, 1972) to determine fertilized cropland and urban areas as major influences on Florida lakes. However, maturity indices, based on primary productivity and other variables associated with the euphotic zone, were apparently less useful in Colorado (Winner, 1972), possibly due to inadequate characterization. Measurements of microbial biomass and activity (chlorophyll *a* concentration, direct counts of bacteria, ATP concentration, and heterotrophic potential - the V_{max} for single substrate metabolism) have been used to rank New Zealand lakes (Gillespie, 1976; Spencer, 1978).

Pearsall (1921) considered the lakes of the English Lake District as a series, illustrating the stages in post-glacial development, although within this series classes were also recognized, characterized by the degree of cultivation of the drainage basin and the degree of silting within the lakes. Since Pearsall's original paper, several studies have been published which are relevant to a microbiological investigation of the series. Not every paper is concerned with all the major lakes in this area, but valuable comparative information on

the series may be obtained from them. Details of the morphometry and drainage basin area of the 15 major lakes are summarized by Ramsbottom (1976). Phytoplankton and benthic algae were investigated by Pearsall (1932), Lund (1957) and Round (1957). The relationship between algal standing crops and productivities, and sediment and water chemistry were reported by Round (1957) and Gorham *et al.* (1974), the latter authors drawing together much of the earlier work on the lakes series. The underwater light climate, and depth of penetration of light of different wavelengths, has been considered as another factor controlling the production of phytoplankton in 16 of the lakes (Talling, 1971). Mackereth (1957) discussed the chemical differences between the Lake District tarns and lakes and Mortimer (1942), the physico-chemical properties, particularly E_h , governing nutrient exchange between the sediment and the water column. Decomposition processes in the hypolimnion are driven to a considerable degree by the input of sedimenting material. Gorham *et al.* (1974) studied sediment between approximately 5 and 20 cm depth and concluded that much of the organic matter in the sediments of productive lakes was autochthonous in origin. Mackereth (1966) accounted for longer term changes in the chemistry of post-glacial sediments (the depth range for these lakes is 4 to 6 m) in terms of erosion rate from the drainage basin (i.e. allochthonous input). This apparent inconsistency may reflect, in part, differences in sampling method and depth but can also be resolved by considerations of catchment land use, particularly deforestation (Pennington, 1978). A more detailed discussion on sedimentation in five of the lakes is provided by Pennington (1974). In a study of the planktonic community, Jones (1972) compared algal biomass, bacterial plate counts and microbial phosphorus mineralization (phosphatase activity) with phosphorus chemistry and dissolved oxygen concentrations. Although there were minor differences in the ranking of the lakes in the above papers, these were confined to those in the middle order, and agreement was generally good at the oligotrophic and eutrophic extremes.

This paper examines the relationship between lake trophic status and microbial numbers, biomass and activity, with particular reference to the benthic communities. Analysis of the planktonic communities had shown that the lakes fell into a true series with a steady transition from oligotrophic to eutrophic. This study was intended to examine whether this was reflected in the benthos and whether general microbiological variables might be used as indicators of the degree of enrichment.

METHODS

Sampling sites and programme. Samples were taken over the deepest point of each lake, the positions and depths of which are given in Ramsbottom (1976). Random samples were taken during 1977, but three sampling trips were made to each lake during 1978. These ran from 26 April to 11 May, from 17 July to 28 July and from 24 August to 8 September. The dates were chosen so that comparable estimates of hypolimnetic oxygen depletion could be made.

Sampling methods. Water samples were taken with a 5 m tube in the epilimnion (Lund *et al.*, 1958) and 1 m above the sediment with a Friedinger water bottle. Sub-samples were taken into sterile glass bottles for microbiological analysis and acid-washed polythene bottles for chemical determinations. Sediment samples were taken with a Jenkin surface mud corer and retained in their cores until returned to the laboratory. The sediment was then extruded using the device described by Jones (1976) during which E_h readings were taken. Sediment to a depth of 1 cm was then removed for analysis. Dissolved oxygen and temperature were measured in the field with a combination thermistor/oxygen electrode (Mackereth, 1964).

E_h measurements. The sediment E_h (cell potential plus the potential of the reference electrode) was measured with a bright platinum electrode and a calomel reference electrode as described by Jones (1979).

Sediment carbon and nitrogen analysis. Samples were dried to constant weight at 80 °C and their C and N content was determined on a Hewlett-Packard F & M 185 CHN analyser.

Chlorophyll a. The sediment chlorophyll *a* concentration was determined by extracting 2 ml of sediment in 18 ml of A.R. grade cold acetone, except for the more productive lakes, when 0.5 ml was extracted and the volume made up with distilled water. The extract was mixed thoroughly and then stored overnight at 4 °C. The contents of the tube were then agitated vigorously and the extract was cleared by centrifugation.

The absorbance of the extract was determined at 665 and 750 nm before and 1 min after acidification with 0.1 vol. HCl.

Chloroplast counts. The number of viable algal cells in the sediment was estimated by counting chloroplasts under an epifluorescence microscope. A 10^{-3} dilution of the sediment in tap water was homogenized for 1 min in an Ultra-Turrax homogenizer (Sartorius Instruments, Belmont, Surrey). A sample (5 ml) of the homogenate was then filtered through a 0.45 μm pore-size black membrane filter and counted under an epifluorescence microscope, using a Calflex B1/K2 interference heat filter and two BG12 filters (total thickness 4.5 mm) for an excitation spectrum which ranged from 320 to 490 nm with a maximum at 400 nm.

Bacteria, direct counts. Planktonic and benthic bacteria were counted directly using epifluorescence microscopy and the procedure of Jones & Simon (1975). Benthic samples were diluted 10^{-3} with membrane-filtered water and homogenized as described for chloroplast counts to disperse clumps before staining with acridine orange at a final concentration of 10 mg l^{-1} .

Bacteria, viable counts. The Harris & Sommers (1968) most probable number (MPN) procedure was used to enumerate aerobic bacteria on casein/peptone/starch (CPS) medium (Collins & Willoughby, 1962) and anaerobic bacteria on CPS medium plus 0.1 g l^{-1} each of NaNO_3 , $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ and Na_2CO_3 . The replicates used were incubated aerobically at 15 °C for 14 d, and anaerobically at 20 °C for 10 d using the GasPak anaerobic system (BBL).

ATP. The ATP content of water and sediment was determined using a sensitive modification of the conventional firefly luciferase method as described by Jones & Simon (1977). Sediment was diluted 10^{-3} immediately before extraction in actively boiling Tris buffer at pH 7.8. Internal standards (bacterial cultures) were analysed with each sediment sample.

Electron transport system (ETS) activity. This was used as a measure of microbial energy-yielding metabolism in the sediments. The method used was that of Owens & King (1975) as modified by Jones & Simon (1979) to ensure optimum conditions for extraction of enzymes from freshwater sediments.

Data analysis. Areal oxygen deficits in the hypolimnia of the lakes were calculated using the procedures described by Jones (1976) and the bathymetric data in Ramsbottom (1976). Where necessary, non-parametric statistical tests were used for data analysis (Jones, 1973) because the parent distribution of many of the variables was not known, and also because most of the comparisons were for the purpose of ranking the results from the lakes.

RESULTS AND DISCUSSION

Some features of the 16 lakes studied are summarized in Table 1. The lakes are ranked in order of increasing degree of enrichment according to Jones (1972). Further tables of background information have been provided by Gorham *et al.* (1974), Jones (1972), Pennington (1978) and Ramsbottom (1976) and therefore only the information of greatest relevance to a microbiological survey is presented. The human population estimates were taken from a more recent census than that used by Macan (1970), but some difficulty was experienced in estimating the population distribution on the basis of lake catchments. This was particularly true of Esthwaite Water and Blelham Tarn and those areas where a large tourist influx occurred. Some estimate of the latter could be obtained from sewage discharge volumes. These were subject to several errors, particularly the absence of calculated inputs from camp sites and establishments not connected to piped sewerage schemes, and clearly underestimated the population in the Esthwaite Water drainage basin. All measures of microbial population density, biomass and activity increased with the degree of eutrophication of the water body. Most of the data in Table 1 were obtained from the epilimnion and therefore this survey concentrated on the profundal zone, particularly the benthos.

The E_h profile of each sediment core was determined, and the results are summarized in Fig. 1. There has been no consensus on how such results should be presented, short of providing details of each profile or, if sufficient data are available, a seasonal depth-time diagram for each lake. The degree of reduction of a sediment will depend on the carbon and energy input and oxygen consumption as well as the reducing power of the sediment itself. Figure 1 attempts to illustrate this in three ways. The mean depth of the E_h gradient, and the depths of the 0 mV and +200 mV readings were calculated. Mortimer (1942) considered that the +0.2 isovolt represented the boundary between oxidizing and reducing conditions. Hutchinson (1957) extended this to suggest that the presence of an oxidized

Table 1. *Some physical, chemical and biological features of 16 Cumbrian lakes*

Lake	Maximum depth ¹ (m)	Volume ¹ (10 ⁹ m ³)	Human population ² per 10 ⁶ m ³ lake vol.	Depth of 1% light penetration ³ (m)	Total cations ⁴ (μ eq. l ⁻¹)	Phosphorus ⁵		Nitrate nitrogen ⁷ (μ g l ⁻¹)
						Total (μ g l ⁻¹)	Soluble reactive (μ g l ⁻¹)	
1. Wastwater	76	115.6	1.32	38.4	329	2	0.5	256
2. Ennerdale	42	53.2	1.65	35.4	361	2	0.6	219
3. Buttermere	28.5	15.2	11.6	38.2	340	5	0.1	115
4. Crummock	44	66.4	4.8	24.2	340	3	0.4	154
5. Coniston	56	113.3	12.3 (18.5)	14.4	568	3	0.5	300
6. Thirlmere	46	52.5	3.1	14.4	354	3	0.6	179
7. Windermere (N)	64	201.8	21.95 (42.6)	14.4	500	13	0.7	309
8. Ullswater	62.5	223.0	6.1	13.0	507	7	0.2	146
9. Derwentwater	22	29.0	40.5	14.8	487	6	0.7	99
10. Bassenthwaite	19	27.9	273 (502)	6.1	609	21	0.9	194
11. Windermere (S)	42	112.7	77.4 (150)	13.0	545	20	1.5	294
12. Loweswater	16	5.4	12.96	8.5	509	7	1.3	404
13. Rydal Water	19	—	—	—	486	11	0.6	178
14. Grasmere	21.5	4.9	201 (612)	9.7	455	19	0.8	172 (14)
15. Esthwaite Water	15.5	6.4	162 (212)	9.7	749	31	1.2	355
16. Blelham Tarn	14.5	0.69	141	8.7	755	29 (5.6)	1.8 (0.5)	342 (140)

Lake	pH ^b	Sediment organic matter ⁴ (% loss on ignition)	Chlorophyll <i>a</i> ⁷ ($\mu\text{g l}^{-1}$)	Algal biomass ⁴ ($\mu\text{g dry wt l}^{-1}$)	Bacteria ml ⁻¹		Phosphatase activity ⁷ ($\mu\text{mol PO}_4$ $\text{l}^{-1} \text{d}^{-1}$)
					$10^{-3} \times$ Plate counts ⁷	$10^{-6} \times$ Direct counts ¹⁰	
1. Wastwater	6.85	14.4	1.4	23	1.48	0.71	0.11
2. Ennerdale	6.7	13.8	1.0	93	0.82	2.04	0.36
3. Buttermere	6.85	13.2	1.6	70	1.28	1.25	0.38
4. Crummock	6.7	12.0	2.8	110	1.72	1.11	0.37
5. Coniston	7.05	13.0	4.2	790	1.54	2.43	0.49
6. Thirlmere	6.6	19.5	2.6	55	0.56	1.54	0.42
7. Windermere (N)	7.4	19.0	8.7	1400	1.49	1.84	0.64
8. Ullswater	7.15	10.2	4.7	3800	0.37	1.82	0.83
9. Derwentwater	6.9	15.2	3.1	1800	11.9	1.60	0.91
10. Bassenthwaite	7.6	11.9	11.1	4200	23.1	1.84	0.65
11. Windermere (S)	7.9	17.4	13.3	2800	1.23	2.28	0.97
12. Loweswater	7.05	11.7	6.8	2100	5.05	1.70	1.02
13. Rydal Water	7.1	21.0	9.6	1000*	6.45	2.52	1.41
14. Grasmere	7.2	39.4 ^c	15.6	910*	4.38 (2.3) ⁸	1.87	1.01
15. Esthwaite Water	7.6	24.0	33.4	6800	3.61 ⁹	3.41 ⁹	2.45
16. Blelham Tarn	7.4	26.5	29.2 (5.6)	3600	6.56 (2.73)	4.32	1.83

Chemical and biological data in columns 6 to 9, 11, and 13 to 15 are arithmetic means of data obtained from the epilimnion between April and September.

Sources of data: ¹ from Ramsbottom (1976); ² from 1971 minor district census, () indicates increase due to tourist influx estimated from increased sewage effluent discharge; ³ from Talling (1971), values refer to penetration of green light; ⁴ largely from Gorham *et al.* (1974); ⁵ Freshwater Biological Association, unpublished data, () indicates standard deviation on seasonal mean; ⁶ from Round (1957); ⁷ largely from Jones (1972) and unpublished data, () indicates standard deviation on seasonal mean; ⁸ from Hall *et al.* (1978), () indicates standard deviation on seasonal mean; ⁹ from Jones (1977); ¹⁰ this survey.

* Underestimates because no large algae were counted.

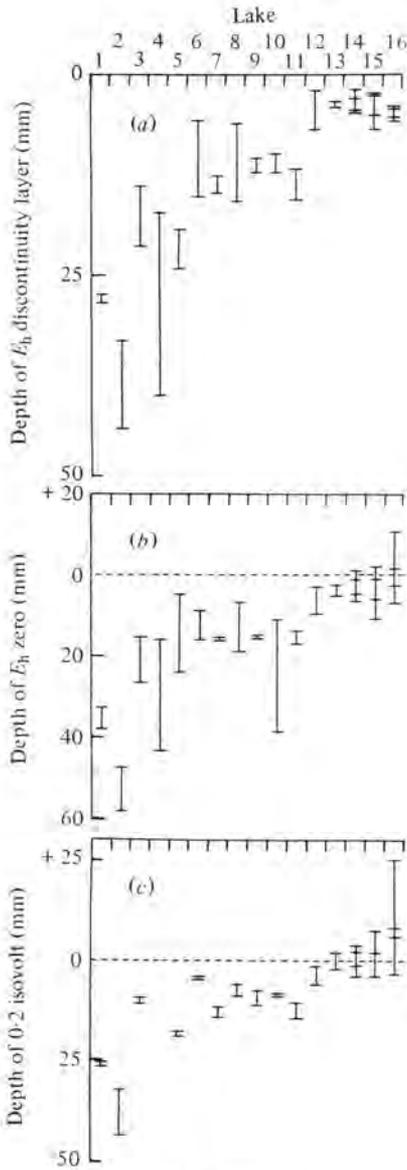


Fig. 1

Fig. 1. Analysis of E_h profiles in the sediments from the lakes described in Table 1 (lake numbering as in the Table). The ranges of readings obtained during the period of thermal stratification are shown (thermal stratification is defined, for this and subsequent figures, as the period between the onset of a 1°C temperature difference between surface water and bottom water, and the autumn overturn when isothermal conditions are re-established). The result of each profile is represented by a horizontal bar. (a) Depth of the E_h discontinuity layer (b) Depth of E_h zero. (c) Depth of the 0.2 isovolt.

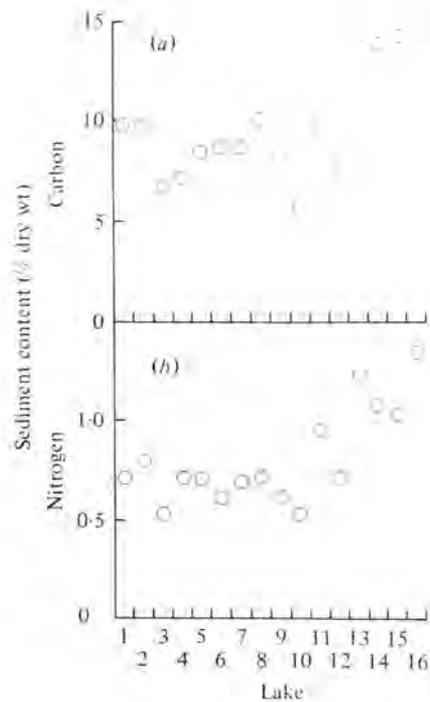


Fig. 2

Fig. 2. Carbon (a) and nitrogen (b) content of surface sediment from the lakes described in Table 1; samples were taken during thermal stratification in 1977.

microzone throughout the year (i.e. no migration of the $+200$ mV level into the overlying water occurred during summer) represented an important dichotomy in the chemical classification of lakes. All three representations of the data obtained on this survey illustrated the more reducing nature of the sediments from the more eutrophic lakes. The $+200$ mV value

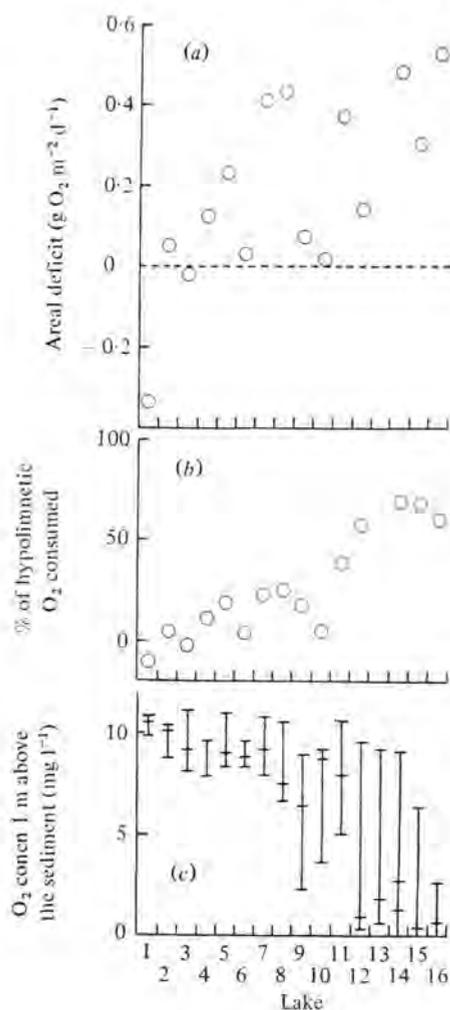


Fig. 3

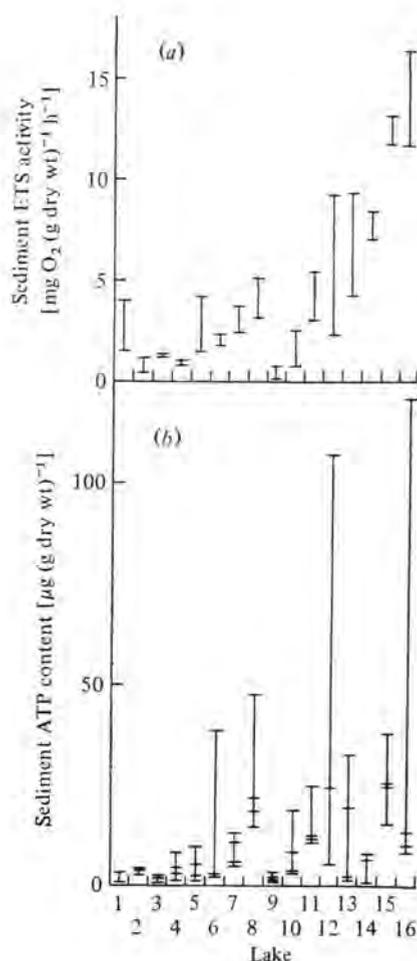


Fig. 4

Fig. 3. Hypolimnetic oxygen consumption in the lakes described in Table 1 between April and September 1978. (a) Calculated areal deficit. (b) Oxygen consumed, expressed as a percentage of the total oxygen content of the hypolimnion in April. (c) Range of oxygen concentrations observed 1 m above the sediment during the period of thermal stratification; each reading is represented by a horizontal bar.

Fig. 4. Electron transport system activity (a) and ATP content (b) of surface sediments from the lakes described in Table 1; each measurement is represented by a horizontal bar.

migrated into the overlying water in Rydal Water, Grasmere, Esthwaite Water and Blelham Tarn. These results correlated well with the carbon and nitrogen content of the sediments (Fig. 2) and the oxygen content of the overlying water (Fig. 3). The sediments of the eutrophic lakes contained more organic matter and the values obtained were in good agreement with those of Gorham *et al.* (1974), Pennington (1978) and Round (1957). Hypolimnetic oxygen consumption was expressed in terms of the areal deficit (Fig. 3a), the percentage of hypolimnetic oxygen consumed (Fig. 3b) and the range of oxygen concentrations observed 1 m above the sediment during the period of thermal stratification (Fig. 3c). All showed a trend of increased oxygen consumption with degree of enrichment, and the oxygen concentrations were similar to those reported by Jones (1972), with the possible exception of Grasmere, where increased oxygen consumption has been observed since the introduction

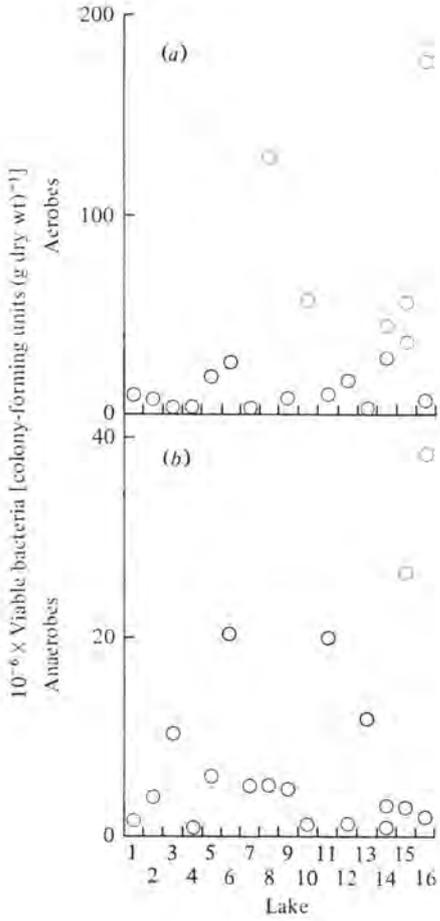


Fig. 5

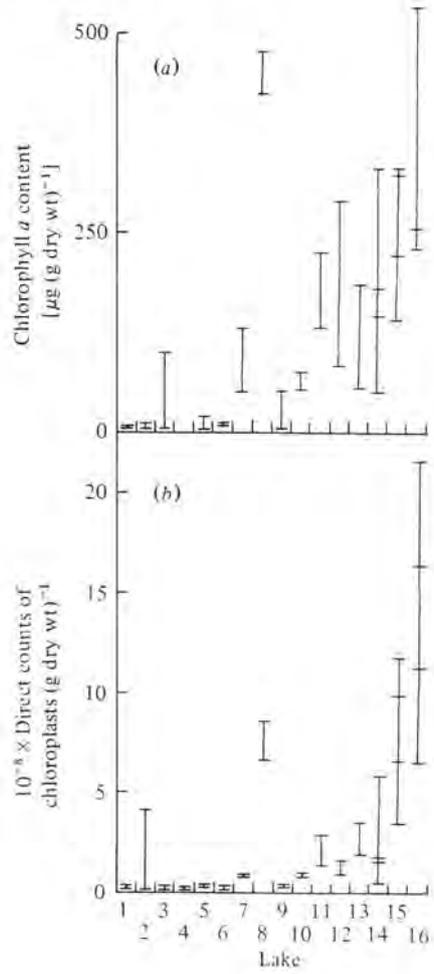


Fig. 7

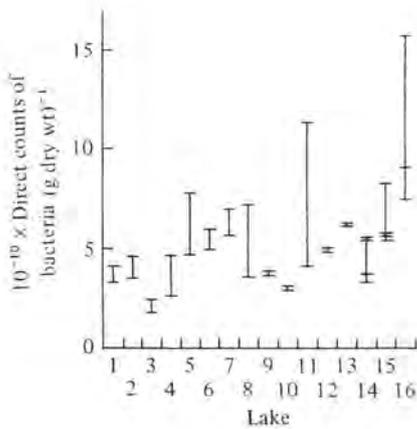


Fig. 6

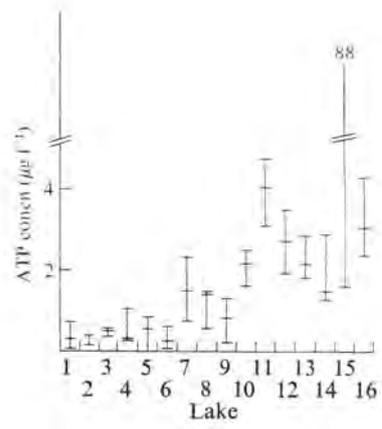


Fig. 8

Figs 5-8. For legends see facing page.

of sewage effluent to the lake in 1971 (Hall *et al.*, 1978). The areal deficits were somewhat variable, possibly reflecting the long time scale used, which might also account for the relatively low values obtained for the more eutrophic lakes (cf. Mortimer, 1942; Jones, 1976). Hutchinson (1957) related the rate of hypolimnetic oxygen loss to the type of lake and suggested 40 to 330 mg O₂ m⁻² d⁻¹ for oligotrophic and 500 to 1400 mg O₂ m⁻² d⁻¹ for eutrophic lakes, but also commented that Mortimer's values of < 250 and > 550 mg m⁻² d⁻¹ as the boundaries for oligotrophic and eutrophic waters might be more convenient. Although hypolimnetic areal oxygen deficits have been used successfully in comparative studies and in the estimation of decomposition processes (Edmondson, 1966; Jones, 1976), some doubts have been expressed concerning their general applicability (Stewart, 1976; Hall *et al.*, 1978). The values for the percentage of the hypolimnion volume deoxygenated (Fig. 3*b*), particularly if combined with a time factor, may provide a more useful guide to events in the lake.

The increased respiratory activity in the richer lakes suggested by the above results was confirmed by an examination of their microbiological characteristics. Energy metabolism, as measured by electron transport system (ETS) activity, was highest in Blelham Tarn and Esthwaite Water (Fig. 4*a*) and although an upward trend in ATP content was also observed (Fig. 4*b*) with increasing degree of enrichment, the range of values was much wider. This upward trend was also observed in viable counts of aerobic and anaerobic bacteria (Fig. 5), direct counts of bacteria (Fig. 6), chlorophyll *a* concentration (Fig. 7*a*) and direct counts of chloroplasts in the sediment (Fig. 7*b*). The viable counts did not agree particularly well with the ranking of the lakes, and the scatter of points with the viable counts and the range of the direct counts was greater than that observed for the plankton (Jones, 1972, and Table 1). The chlorophyll *a* estimates and chloroplast counts of this survey, and published estimates of benthic algal standing crops (Round, 1957), were not strictly comparable since the latter were made on samples taken from littoral sites. Analysis of the ATP content of the plankton (Fig. 8) showed that the general trends in microbial biomass observed by Jones (1972) still occurred.

The variables measured on this survey showed a high degree of inter-correlation as tested by calculating Kendall's rank correlation coefficient. The results from the benthos were, however, more variable, and showed poorer agreement with the assumed ranking of the lakes than had been obtained on analysis of data from the water column (Table 2). Data obtained from the plankton in this and previous surveys showed significant correlations ($P = 0.05$) between variables, and between the variates and assumed lake rank, in over 85% of cases. This was true of only 60% of the cases when results from the benthos were analysed. There was good agreement between results from lakes at either end of the scale, particularly from the more productive waters, and indeed the lakes might be conveniently placed in three groups, representing mean ranks of 0 to 5, 5 to 10 and 10 to 16.

The results from Bassenthwaite suggested that it might be incorrectly placed in the series, but whereas the plankton analysis suggested that it might be more eutrophic, the results from the benthos suggested the opposite. This may reflect the instability of thermal stratifi-

Fig. 5. Viable counts of aerobic (*a*) and anaerobic (*b*) bacteria from surface sediments of the lakes described in Table 1. Samples were taken during thermal stratification in 1977.

Fig. 6. Direct counts of bacteria in surface sediments of the lakes described in Table 1. Samples were taken in 1977 and 1978 during thermal stratification; each sample count is represented by a horizontal bar.

Fig. 7. Chlorophyll *a* content (*a*) and direct counts of chloroplasts (*b*) in surface sediments of the lakes described in Table 1. Samples were taken in 1977 during thermal stratification; each measurement is represented by a horizontal bar.

Fig. 8. ATP concentration in the surface water (to a depth of 5 m) of the lakes described in Table 1. Samples were taken in 1977 and 1978 during the period of thermal stratification; each analysis is represented by a horizontal bar.

Table 2. *Assessment of the ranking of Cumbrian lakes based on arithmetic mean values of planktonic and benthic variables**

Lake	Assumed rank	Plankton			Benthos		
		Mean rank	Rank CV† (%)	Rank displacement	Mean rank	Rank CV† (%)	Rank displacement
Wastwater	1	3.1	92	0	4.2	72	+2
Ennerdale	2	4.0	85	+2	5.7	60	+4
Buttermere	3	3.4	47	-1	3.5	87	-2
Crummock	4	3.9	54	-1	3.8	59	-2
Coniston	5	7.6	48	+3	7.6	32	+4
Thirlmere	6	4.0	47	-2	7.6	61	+2
Windermere (N)	7	9.2	24	+2	7.5	37	0
Ullswater	8	6.8	48	-2	11.7	22	+6
Derwentwater	9	7.2	49	-2	4.9	42	-5
Bassenthwaite	10	11.9	25	+4	5.2	78	-5
Windermere (S)	11	11.8	27	+2	10.8	21	0
Loweswater	12	11.2	29	0	9.4	38	-2
Rydal Water	13	10.2	31	-3	11.1	36	-1
Grasmere	14	10.4	25	-3	11.4	34	-1
Esthwaite Water	15	14.3	15	0	13.7	7	0
Blelham Tarn	16	14.6	9	0	15.4	7	0

* Based on eleven variables each for the plankton and the benthos, obtained from the results of this study, Table 1 and Jones (1972). For the purposes of this comparison, hypolimnetic oxygen depletion was considered as a benthic variable.

† CV, Coefficient of variation, expressed as a percentage of the mean.

cation in Bassenthwaite due to its shallow mean depth and exposure to winds. Benthic analyses indicated that Ullswater might be placed closer to the eutrophic end of the scale (the low coefficient of variation supported this). This would be in agreement with the findings of Pearsall (1921) and Gorham *et al.* (1974) but not with the general conclusions of the plankton analysis. The position of each lake in the series, and whether the lakes form a continuous series or fall into distinct groups could be discussed at length. The microbiological analyses of the plankton suggested the former whereas the results from the benthos indicated that the more eutrophic lakes might form a distinct group, comprising Rydal Water, Grasmere, Esthwaite Water and Blelham Tarn. This was the largest number of the more productive lakes which could be considered as a group which was significantly different ($P = 0.05$) from the remainder, on the basis of at least four of the variables measured. Such a distinction would correlate with the proposed autochthonous source of sediment organic matter in the more productive waters (Gorham *et al.*, 1974; Pennington, 1978). Multivariate analysis of the microbiological and other variables or construction of indices of eutrophication were not attempted because of insufficient data, which usually did not conform to the underlying assumptions of such analyses. Microbiological analyses of the benthos (Jones, 1976) and the plankton (Heaney, 1976; George & Heaney, 1978; Jones, 1977) have demonstrated the scale of spatial variability in individual lakes, and increasing seasonal variability with the degree of enrichment has also been observed (Jones, 1971). Clearly, if microbial indicators of eutrophication are to be used, effort would be more profitably spent in obtaining more reliable estimates of a few well chosen variables from larger numbers of samples than attempting sophisticated analyses on inadequate data. Careful consideration should also be given to the variables chosen. The development of anoxic conditions in at least four of the lakes would favour the use of anaerobes such as denitrifiers, sulphate-reducers and methanogens as indicators of eutrophic conditions, but there is insufficient information on their depth distribution and activity in the sediments of other lakes to determine whether such measurements would be of more general value.

Analyses of the plankton have shown a steady transition from oligotrophic to eutrophic conditions and therefore further study of this community might provide finer resolution of the state of a particular lake and its position in a series. The results from the benthos, on the other hand, were less reproducible and tended to be discontinuous, possibly placing the lakes in groups rather than on a series. The use of benthic animal populations as indicators of trophic status may be more successful (Brinkhurst, 1974) and microbiological studies might be more profitably applied to the plankton or certain taxonomic or metabolic groups in the benthos.

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