BACTERIOLOGY OF FRESH WATER

I. DISTRIBUTION OF BACTERIA IN ENGLISH LAKES

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(With 1 map and 5 Figures in the Text)

CONTENTS

	PAGE
Introduction	616
General character of the lakes under investigation	619
(*a*) Windermere	619
(*b*) Thirlmere	621
(*c*) Esthwaite Water	621
Sampling	622
Methods	622
Thermal stratification in lakes	623
Horizontal distribution of bacteria in Windermere	627
Comparison of the two basins of Windermere	627
Vertical distribution of bacteria	628
Anaerobic bacteria	630
Fluctuations in numbers of bacteria	631
Action of sunlight on numbers of bacteria	634
Discussion	634
Summary	636
References	640

Introduction

Few branches of research can have undergone such a development of specialization as the bacteriology of water. Towards the end of last century Frankland, Houston, Jordon, Miguel, and many other bacteriologists undertook the pioneer work underlying the present-day knowledge of the role of bacteria in water. This work, instead of continuing along the lines laid down by those pioneers, has largely developed into a study of bacteria which are not indigenous to water. The public health interest in water has become so strong that the study of pathogenic and coliform bacteria in water has by far outgrown the study of the more fundamental questions of the distribution, growth, and physiological activities of indigenous bacteria. The relations of bacteria to the activities of the phytoplankton and zooplankton, and to the general chemical and biotic conditions occurring in fresh water, are still to be accurately determined.

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Results obtained by previous workers, mainly in Europe and America, on the distribution of bacteria in lakes have not been entirely in agreement. Some of the discrepancies have been due to the fact that very few regular and systematic series of bacterial counts have been made over a long period; differences in types of lakes and methods of technique have been contributory causes. One of the earliest studies was made by Kleiber (1894) who studied the fate of bacteria washed into Lake Zürich by inflowing rivers. He concluded that the zone, rich in bacteria, did not extend into the lake much farther than 20 m. from the mouth of the rivers. Pfenniger (1902) found that the distribution of bacteria in Lake Zürich was generally uniform except for certain periodic fluctuations. Maximum bacterial numbers occurred during the winter and minimum numbers in summer, corresponding to the periods of circulation and stratification of the water. Pfenniger's work was to some extent confirmed by Minder (1920) who also found higher numbers of bacteria in winter than in summer. Minder, however, obtained much lower numbers in samples taken from the immediate surface of the lake, during the summer, than in samples taken at depths only a few metres lower. In general the number of bacteria increased with depth. He claimed that the amount and intensity of sunlight were directly responsible for the fluctuations in numbers of bacteria, such fluctuations being a result of multiplication of the autochthonous types and not of the washing in of soil bacteria. The amount and variation (if any) of nutritive substances in the water had no direct effect on the bacterial population. In a later paper (1927), however, averages of bacterial counts in Lake Zürich over a period of 12 years are given which show decreasing numbers in samples from different depths in the following order: 15, 5, 0, 30 and 50 m.

Fred, Wilson & Davenport (1924) made a comprehensive study of Lake Mendota, Wisconsin, and found that increases in the numbers of bacteria occurred in spring and autumn; the numbers were, however, comparatively small and the bacteria appeared to be uniformly distributed at all depths except when the dissolved oxygen was exhausted in the lower layers. Averages taken over a period of several years, however, showed that bacteria were less numerous at the bottom of the lake than in the layers above. The authors concluded that increases in numbers were largely a result of the washing-in and multiplication of soil bacteria, although insufficient evidence was obtained to support that conclusion. Temperature had little or no effect on the activities of the bacteria and there was no indication that sunlight inhibited growth. Incubation experiments showed that the food supply in the water of Lake Mendota was capable of supporting a far greater bacterial population than was ever recorded. The factors causing limitation in numbers of bacteria under natural conditions were not determined. Ruttner (1932), in a few isolated samplings of Lake Lunz, found that in August and September numbers of bacteria decreased slightly with depth, but in January the bacteria were fairly evenly distributed at all depths except very near the surface where much higher counts were obtained. Numbers were, in general, lower in summer than in winter. Graham & Young (1934) made bacterial counts in the water from different depths in Flathead Lake, Montana, and found wide variations; a lower count was obtained at a depth of 5 ft. than in the surface layer. Maximum numbers were found in spring and autumn; these maxima were thought to be caused by the melting of the snows in spring and by autumn rains which washed in material from the drainage basin. Stark & McCoy (1938) made bacterial counts on samples taken from different depths of many relatively shallow lakes of Wisconsin, on a few occasions during summer months. Bacteria were comparatively scarce in samples taken from three of the deeper lakes and no conclusions can be drawn concerning vertical distribution.

Henrici (1938) made counts on samples of water taken at a depth of 0·5 m. from Lake Alexander, Minnesota, at weekly intervals from May to October. During this period all the inflowing streams were dry and as the season was one of low rainfall the washing in of bacteria was presumed to be negligible. Counts of bacteria were made both by the plate method and by suspended slides. Numbers of bacteria, as estimated by both methods, followed closely the curve for total plankton, with a lag which was greater in the case of the plate counts. Henrici concluded that the production of organic matter by planktonic organisms was an important factor in determining the number of bacteria. Düggeli (1939) made counts of bacteria in water from different depths in Lake Wäggital from 1925 to 1927, but only one set of samples was taken each year, usually in the same month.

In recent years several investigators (Cholodny, 1929; Kusnetzow & Karzinkin, 1931; Jerusalimski, 1932; Rasumow, 1932 and Bere, 1933) have employed methods for the direct counting of the total number of bacteria in water. Such methods have necessitated the concentration of the bacteria in the sample by evaporation under reduced pressure, or by flocculation, or by filtration. In the hands of various workers, total count methods yielded figures which were between ten times and ten thousand times the counts obtained by the plating method. In view of the technical difficulties in these methods of direct counting, caution must be used in interpreting the results.

Although investigations into the distribution of bacteria have largely been concerned with vertical distribution, several workers have also made studies of horizontal distribution. Fred *et al.* (1924), Graham & Young (1934), and Stark & McCoy (1938) all found that bacteria were much more prevalent in shallow bays than in deep open water. Stark & McCoy concluded that factors such as temperature, concentration of dissolved oxygen, and contamination from the shore were not responsible for the greater bacterial activity in shallow bays, but rather that the concentration of organic matter in the water was the important factor.

The fate of bacteria washed into lakes has also been studied. As previously reported, Kleiber (1894) noted that the zone rich in bacteria created by the inflowing river water did not extend out into Lake Zürich much beyond 20 m. Fred *et al.* made bacterial counts in the water of Lake Mendota at various distances from the mouth of a storm-water sewer at times of melting snow, and after unusually heavy rainfall. The bacterial count in samples taken at the mouth of the outfall was 400–600 times as great as the count in samples of water taken at a distance of 100 m. offshore; no increase in the bacterial count was noted at a point 3000 m. from shore as a result of the inflow of drainage water. The authors conclude that although drainage water is a continual source of contamination in the lake, contaminating bacteria do not remain long in large numbers, and except under unusual conditions they do not spread out any great distance from the shore.

The above review of previous work shows some discrepancies and inconsistencies. Therefore, when bacteriological research was started in the laboratories of the Freshwater Biological Association it was decided to study some of the fundamental aspects of fresh-water bacteriology, in particular the numerical distribution of bacteria in several lakes throughout the year. Accordingly a comprehensive study was begun; this has been made jointly with the Association's chemist and botanist.

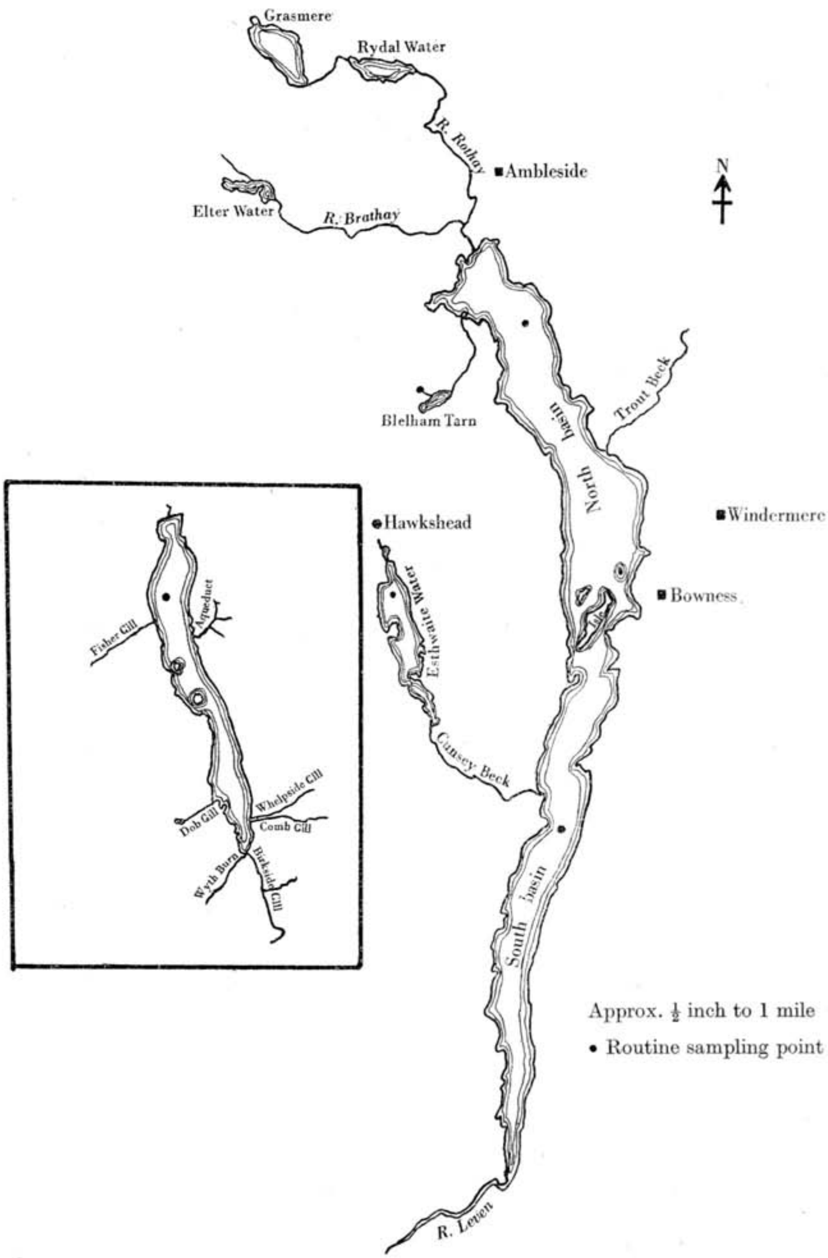
GENERAL CHARACTER OF THE LAKES UNDER INVESTIGATION

(a) *Windermere*

Windermere, situated on the boundary line between the counties of Lancashire and Westmorland, is the most southerly of a group of lakes of glacial origin which radiate from a central point rather like spokes of a wheel. Windermere is the largest and one of the deepest of the lakes; it is approximately 10 miles in length, and has an average width of about 0·4 mile and a maximum depth of 67 m. A series of islands almost divide the lake into two parts, the north and south basins, which are separated only by narrow channels. The main inflows are the River Brathay, which flows into the northern end of the north basin, and Trout Beck which enters the north basin midway down the eastern shore. The outflow, the River Leven, is at the southern end of the south basin. Mortimer (1938) has estimated that water entering the lake by the River Brathay would remain in the lake for at least 9 months before reaching the outflow, assuming uniform flow of all the water through the lake. The mean elevation of the surface of the lake is about 128 ft. above sea-level and the average rise and fall in level is only slightly over 1 m. The average annual rainfall on the drainage area is 86 in.

The south basin is narrower and shallower than the north basin; the water is considerably richer in dissolved chemicals, and supports a higher algal population. The north basin receives the sewage effluent from Ambleside (winter population about 2500) and the south basin receives the sewage effluents from Windermere town and Bowness (combined winter populations 6000).

Windermere and Tributaries
Thirlmere (inset)



Map 1.

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(b) Thirlmere

The original level of Thirlmere has been raised by means of a dam at the northern end, which was completed in 1879. Since that time the lake has been a water-supply reservoir for the city of Manchester. Thirlmere is approximately $3\frac{1}{2}$ miles in length with an average width of about one-third of a mile; the maximum depth is 42 m. when the water is flowing over the outflow sill. The level is subject to considerable variation and may fall as much as 8 m. during a dry summer. The lake is fed by many relatively small streams, which drain a very rocky drainage area receiving an average annual rainfall of 90 in.

As the water is used for human consumption great care has been taken to eradicate sources of pollution. Most human habitations have been removed from the drainage area and the sewage from those remaining is collected and taken away in tank waggons. The slopes of the mountains have been afforested. The only remaining source of pollution, apart from wild animals and birds, is that provided by a few cattle and numerous sheep which graze over most of the drainage area. This lake is an excellent experimental control for, in addition to freedom from pollution, the water is chemically purer than water from the other lakes under consideration and supports a relatively meagre algal population. The only disadvantages are the fluctuations in the level of the lake and the daily withdrawal of 45 million gallons of water from the lower depths for public supply and for compensation water.

(c) Esthwaite Water

Esthwaite Water is a comparatively shallow body of water lying in the Windermere drainage area, the outflow leading into the south basin of Windermere. The length of the lake is approximately two miles and the deepest parts do not exceed 16 metres. The lake is fed by small streams which drain a relatively large proportion of agricultural land. Pollution is appreciable and is mainly due to drainage from farmyards, sewage effluent, and crude sewage from the village of Hawkshead (population about 800). The water is richer in dissolved chemicals than the water of Windermere north basin or Thirlmere and supports a higher number of algae.

All three lakes are classified as oligotrophic but Esthwaite and Windermere have tendencies to eutrophism.

Table 1. *General features of the lakes under investigation*

Lake	Length miles	Area sq. miles	Max. depth ft.	Max. depth m.	Mean depth ft.	Mean depth m.	Av. annual rainfall over drainage area in.	Drainage area sq. miles	Nature of drainage area	Altitude ft. O.D.
ıdermere	10	5·70	219·8	67	75·5	23·0	86·8	95·0	Impermeable rock but much agricultural land	128
·lmere	3·5	1·29	137·8	42	67·0*	20·5*	90·3	15·8	Impermeable rock	587
ıwaite Water	2	0·39	52·5	16	23·0*	7·0*	74·1	6·7	Mainly agricultural	214

* Estimated.

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Sampling

Samples of water for studies on vertical distribution of bacteria have been taken from the different lakes as follows:

(*a*) North basin of Windermere; weekly samples from depths of 1 and 10 m. during the period July 1938 to October 1939; and weekly samples (fortnightly November 1938 to April 1939) from depths of 15, 30, and 50 m. from July 1938 (15 m. from October 1938) to July 1939.

(*b*) South basin of Windermere; fortnightly samples at depths of 1, 10, 15, and 30 m. from September 1938 to April 1939 and then weekly until October 1939.

(*c*) Esthwaite Water; occasional monthly samples at depths of 1, 5, 10, and 13 m. from September 1938 to March 1939 and then weekly from April to October 1939.

(*d*) Thirlmere; weekly samples from depths of 1, 10, and 34 m. from February 1939 to January 1940.

For studies on the horizontal distribution of bacteria, work has been confined to Windermere north basin where samples have been taken at various distances from the mouth of the inflowing River Brathay.

Weather during sampling period

The months of June, July, October, and November 1938 were unusually wet with an aggregate rainfall of 50 in. and included two periods (29–30 July and 2–3 October) when over 5 in. of rain fell in 48 hr., producing small floods. The spring and summer months of 1939, however, were, with the exception of July, relatively dry and included a period of official drought (May–June). None of the lakes under examination was frozen over during the winter of 1938–9.

Methods

Some difficulty was encountered at first in finding a suitable device for taking samples at the different depths, without contamination from upper layers during the raising of the sample. Previous workers have, in general, used evacuated vials which were lowered to the required position; the neck of the vial was then broken by means of a messenger falling down the cable. As the making of the numerous vials required considerable time and the amount of water obtained was too small, a new device was invented by Dr C. H. Mortimer. Briefly, the apparatus consists of a sterile 1 l. Winchester bottle clamped into a stand and so arranged that a falling messenger operates a trip which releases a spring and causes a special fitting on the mouth of the bottle to be removed to allow the entry of water. Tests proved that mixing with surface water during raising did not take place and the apparatus worked successfully at all the depths at which it was employed. Details of the apparatus are given in the next paper.

The numbers of the bacteria have been determined in all samples of water taken, and the samples have been passed on for chemical analysis and for determinations of the number of algae. At the same time that samples were taken for bacteriological analyses, temperatures were recorded with a reversing thermometer. Additional samples of water were obtained with a closing water-bottle at similar depths, and often at additional depths, for the determination of dissolved oxygen.

The choice of a suitable medium for making bacterial counts was of great importance because with the plating technique the count obtained on any one medium represents only a small portion of the total bacterial population. It was important, therefore, to find a medium that would give the highest possible fraction of the total. Seven media were compared by "plating out" several samples of water on all of them. These were: (1) sodium caseinate agar, (2) evaporated lake-water agar, (3) synthetic medium together with yeast extract, (4) mud extract agar, (5) a synthetic agar medium containing asparagine and mannitol, (6) beef peptone agar and (7) nutrient gelatin. In all cases sodium caseinate agar gave far higher numbers of bacteria than any of the other six. This medium is almost identical with that used by Stark & McCoy (1938); it has the advantage that its inorganic ingredients are present in such small quantities that the effects due to the variation in composition of different batches of those ingredients are likely to be small. The composition of the medium was peptone (B.D.H.) 0·5 g., sodium caseinate 0·5 g., soluble starch 0·5 g., glycerol 1·0 ml., K_2HPO_4 0·2 g., $MgSO_4$ 0·05 g., $FeCl_3$ trace, agar 15·0 g., distilled water 1000 ml.

The optimum temperature of incubation was determined by incubating samples of water with sodium caseinate agar in replicate sets of Petri dishes at room temperature (16° C. approx.), 20 and 28° C. An incubation temperature of 20° C. proved far superior to room temperature and slightly better than 28° C. Incubation for 15 days was found to be the most suitable period. Five replicate plates were "poured" at a suitable dilution for each sample of water immediately the samples reached the laboratory. The plates were incubated for 15 days at 20° C.; the colonies were then counted with the aid of a "Quebec" colony counter.

Thermal stratification in lakes

Before presenting the results on the distribution of bacteria it is important to consider the physical conditions which occur in lakes, in view of the probable effect of those conditions on the bacteria. During the late spring and early summer the temperature of the surface water of a lake rises owing to increased solar radiation and the resulting difference in density of the water gives rise to the formation of two distinct layers of water, the upper layer or "epilimnion" and the lower layer or "hypolimnion". Under some conditions the temperature is approximately the same at all depths in the epilimnion; below this stratum, in the transitional zone known as the "thermocline", the temperature falls rapidly with increasing depth, and in the hypolimnion the temperature, which is considerably lower than that of the epilimnion, does not change appreciably with increasing depth. Under some conditions the temperature in the upper layer falls rapidly with increasing depth from the surface to the thermocline. Fig. 1 shows the temperatures at different depths in the north basin of Windermere over a period of a year, and in Fig. 2 the development and extent of stratification are shown. It is apparent from Fig. 2 that, following the period at which the temperature is uniform throughout the lake, the effect of warmer external conditions is to warm the water at all depths but decreasingly so at increasing depths. As circulation of the water gradually ceases, a point is reached at which the temperature at the bottom remains constant to within a fraction of a degree and stratification is then established. The nature of the thermocline and the depth at which it occurs vary greatly but in general the depth tends to increase as the summer advances and the ratio of volume of epilimnion to volume of hypolimnion therefore increases. During the winter months the stratification of the water in Windermere is destroyed by the cooling of the upper layer or by the circulatory motion set up by the action of strong winds blowing across the surface of the lake. In Windermere this mixing may take some weeks, but in the more shallow lakes, of which Esthwaite is an example, the "overturn" of the waters may take place during the first autumnal gale.

When stratification has begun, the amount of dissolved oxygen becomes gradually less in the hypolimnion owing to the stagnation of the water, but continues in normal amounts in the circulating waters of the epilimnion. The concentration of dissolved oxygen in the lakes under investigation appears to be related to depth, shallow lakes being depleted to a greater extent than deep ones. The nearer the bottom, the greater is the depletion.

Table 2 shows the minimum amount of dissolved oxygen (as percentage of the saturation value) observed in the bottom layers of the different lakes. Fig. 3 shows in detail the development and extent of the depletion of dissolved oxygen in Esthwaite Water, a relatively shallow lake. At the commencement of stratification in May 1939, depletion of oxygen began in the lower layers and two months later no measurable amount of oxygen was found below a depth of 12 m. Shortly afterwards there was none at 11 m. Two large fluctuations in dissolved oxygen content, which occurred during the summer at depths of 9 and 10 m., were attributed to the mixing of the waters with the more oxygenated surface layers, as a result of wind action. At depths of 1 and 5 m., the epilimnion remained approximately saturated with oxygen until mixing began. Week-to-week fluctuations which took place during the sampling period did not appear to be correlated with any variables such as temperature or rainfall. It can be seen that reoxygenation took place steadily downwards towards the end of September 1939 at the expense of the oxygen of the surface waters. In this particular instance the destruction of stratification did not occur suddenly as a result of a strong gale, but the surface water was cooled during a period of cold and relatively still weather.

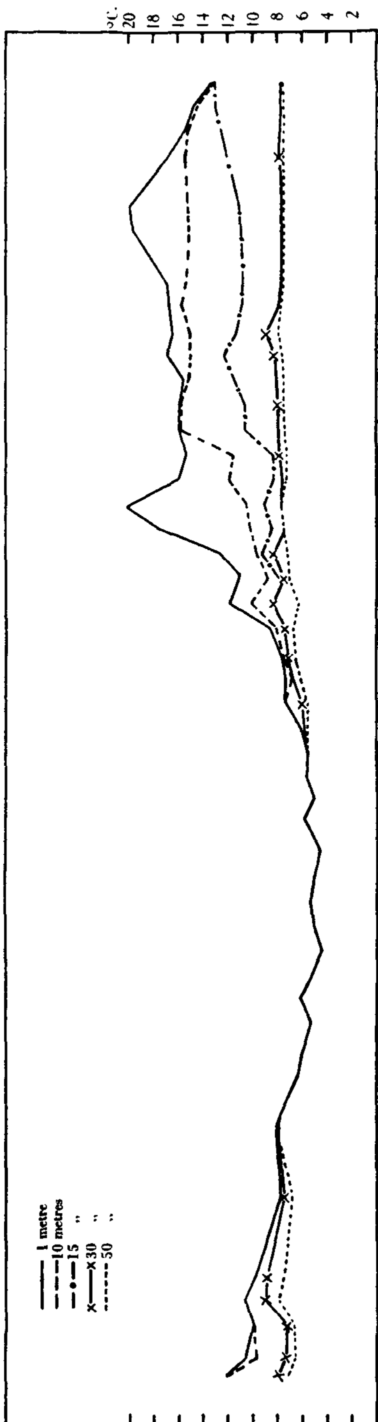

Fig. 1. Temperatures at different depths. Windermere, north basin, 1938–9.

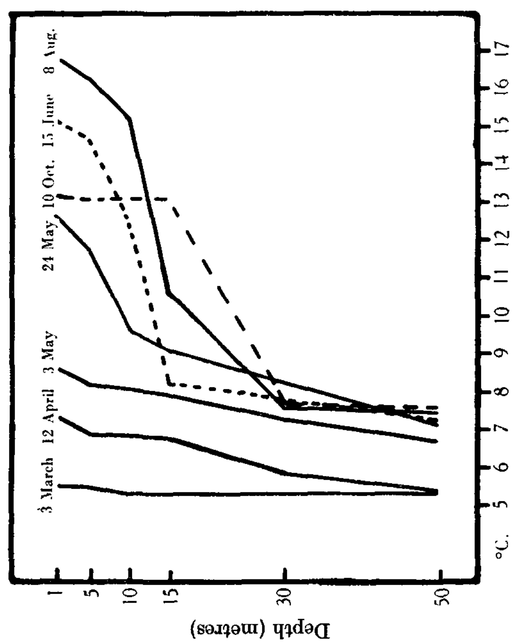

Fig. 2. Thermal stratification. Windermere, north basin, 1939.

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The changes described above are typical of lakes in temperate latitudes, and show that there are two definite conditions existing during the year: the winter period when the waters of a lake are circulating freely, and the summer

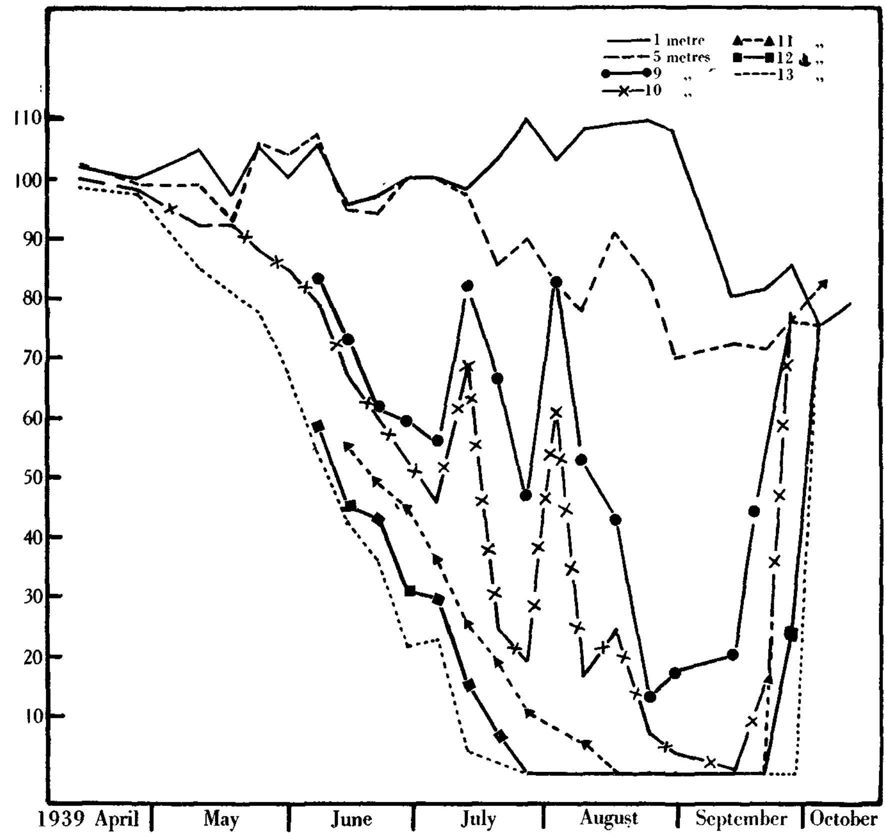

Fig. 3. Dissolved oxygen (percentage saturation) at different depths. Esthwaite Water, 1939.

Table 2. *Minimum amounts of dissolved oxygen present near the bottom of various lakes during the period of stratification*

Lake	Depth at which bottom samples were taken m.	Minimum content of dissolved oxygen % saturation value
Esthwaite Water	14	0
Windermere, south basin	30	52
Thirlmere	34	62
Windermere, north basin	50	72

period of stratification when circulation is confined to the epilimnion. In countries where lakes are frozen over for many weeks in winter, conditions are more involved and there is an additional period of stratification with a subsequent overturn after the ice melts in the spring. On the other hand, tropical lakes are usually subject to complete circulation throughout the year, except in the case of very deep water where a condition of permanent stagnation may exist.

Horizontal distribution of bacteria in Windermere

Three series of samples at a depth of 1 m. have been collected in Windermere, at various distances from the mouth of the inflowing River Brathay. Results of examination of these samples are shown in Table 3. From the observations made on 22 July 1938 and 2 November 1939, it is evident that there is a rapid and substantial decrease in the number of viable bacteria when the river meets the lake. On the first occasion there was no substantial decrease between the point of entry and a distance of 133 m., but numbers fell almost to one-third between 133 and 536 m. No significant decrease was obtained over a further 221 m.

On 2 November 1939, the bacterial count decreased to one-thirtieth at a point 191 m. from the point of entry. From this point to 4180 m. there was no significant change. The third set of samples was taken in order to find out whether any variation in numbers occurred at widely separated points in deep open water. Between points 2800 and 6000 m. from the River Brathay no significant decrease occurred.

Comparison of the two basins of Windermere

In view of the evidence that no large variation in numbers of bacteria occurs beyond about the first 200 m. from the mouth of the River Brathay it is of interest to compare the bacterial counts in the two basins of the lake. One of the selected positions of sampling in the work on the vertical distribution of bacteria in the north basin is 1·7 km. from the mouth of the River Brathay, and the other at a distance of 11·2 km. is in the more northerly end of the south basin (see map). The first point receives normally only the water from the River Brathay, whilst the second point receives all the water from the north basin, the sewage effluents from the towns of Windermere and Bowness, and water from Cunsey Beck, the outflow from Esthwaite Water.

Table 4 shows the numbers of bacteria in samples taken at depths of 1 m. during summer and winter periods. The average bacterial count was about 30% lower in the south basin than in the north basin, during both summer and winter. It is therefore apparent that the water flowing into the south basin from Trout Beck and the sewage effluent from the towns did not increase the bacterial population at the particular position of sampling. The water had in some way become stabilized, or in the language of the water engineer, "mature".

In general the results confirm the work of Kleiber (1894) and Fred *et al.* (1924), in that bacteria washed into a lake from an inflowing stream tend to disappear very rapidly. In Windermere the numbers of bacteria are reduced drastically in the first 200 m. from the point of entry of the river, after which a relatively insignificant decrease takes place at increasing distances.

Table 3. *Numbers of bacteria at various distances from the mouth of the River Brathay, Windermere, at a depth of* 1 *m.*

Distance from river mouth m.	Bacteria per ml.	Distance from river mouth m.	Bacteria per ml.
22 July 1938		2 November 1939	
0	14,300	River	20,200
67	15,260	0	18,500
133	12,120	191	620
536	4,720	351	1,060
757	4,400	542	960
30 November 1938		670	960
2,800	4,300	861	650
3,200	3,900	990	700
4,000	4,150	1,212	640
4,800	4,180	1,467	570
5,300	3,420	1,690	550
6,000	3,260	4,180	580

Table 4. *Numbers of bacteria in the north and south basins of Windermere*

Season 1938–9	North basin	South basin	Difference bacteria per ml.	Difference %
Winter:				
Bacteria per ml. (averaged over 10 weeks)	4060	2710	1350	33
Summer:				
Bacteria per ml. (averaged over 12 weeks)	1880	1290	590	31

Vertical distribution of bacteria

Results of the routine determination of numbers of bacteria at different depths in the north and south basins of Windermere and in Thirlmere are shown graphically in Figs. 4 and 5. Fig. 4 (north basin) shows that during the period July to November 1938 there were no great changes in the numbers of bacteria in the 50 m. (bottom) layer of the water; at depths of 1 and 10 m. the numbers were, in general, much higher and showed fluctuations from week to week. This period corresponded with the period of stratification. After the overturn which occurred at the end of November 1938, the numbers of bacteria at a depth of 50 m. increased enormously and were for some weeks greater than the corresponding counts at 1 and 10 m., but later there was no significant difference between the bacterial counts for the water at the three depths. This condition continued until May 1939. The period November 1938 to May 1939 represented the period of circulation during which the temperature and the concentrations of dissolved oxygen and dissolved solids were approximately the same at all depths at any one time.

Table 5 gives the mean bacterial counts during the periods of stratification and circulation in 1938 and 1939; it includes data for depths of 15 and 30 m. not shown in Fig. 4.

It can be seen from Table 5 that during the period of circulation bacteria were equally distributed at all depths; the small differences found do not exceed those found between replicate subsamples of the same sample. During the period of stratification, the numbers of bacteria tended to decrease with depth as far as 30 m., below which there was no further decrease. The fact that more bacteria were present in the bottom layer (50 m.) than at 30 m. was probably due to the proximity of the mud.

Table 5. *Numbers of bacteria (thousands per ml.) at different depths during periods of stratification and circulation. Windermere, north basin,* 1938–9

	Depth (m.)				
Period	1	10	15	30	50
Period of stratification July 1938–Nov. 1938	5·40	4·43	3·55	1·27	1·40
Period of circulation Dec. 1938–April 1939	3·33	3·21	2·98	3·06	3·17
Period of stratification May 1939–Oct. 1939	1·56	0·96	0·37*	0·25	0·40*

* May 1939–July 1939.

Sampling in the south basin of Windermere was begun in September 1938, by which time the overturn had taken place and, according to measurements of temperature and dissolved oxygen, the lake was in circulation. From Fig. 4 it would appear that there was little change in numbers of bacteria with depth except during the months of May, June, and July 1939. During this part of the stratification period numbers at the bottom (30 m.) remained at a low figure, usually lower than at depths of 1 and 10 m. When the bacterial counts are averaged (Table 6) it can be seen that the results obtained were similar to those for the north basin; numbers of bacteria during the period of circulation were approximately equal at all depths, but during stratification the numbers decreased with increasing depth down to 15 m. At a depth of 30 m. the number of bacteria was greater than at a depth of 15 m.

Table 6. *Numbers of bacteria (thousands per ml.) at different depths during periods of stratification and circulation. Windermere, south basin,* 1938–9

	Depth (m.)			
Period	1	10	15	30
Period of circulation Sept. 1938–April 1939	2·38	2·21	2·04	2·22
Period of stratification May 1939–Oct. 1939	1·22	0·74	0·53	0·81

An interesting point brought out in Fig. 4 is that although fluctuations in numbers of bacteria were less striking in the south basin than in the north basin of Windermere, the general behaviour of the bacterial population between October 1938 and April 1939 was similar in both.

Samples were collected in Thirlmere from February 1939 to January 1940 at depths of 1, 10, and 34 m. (bottom). The results plotted in Fig. 5 show that there was the same general sequence of events as in Windermere. During the periods of circulation, bacterial counts were approximately the same at the three depths on any one occasion and showed periodic fluctuations. During the period of stratification the counts were lower in the bottom layer and less subject to fluctuations. These findings are emphasized by the averages of the bacterial counts given in Table 7. As samples were taken at only three depths, information on vertical distribution of bacteria is limited. The findings confirm, however, those already described for Windermere: the bacteria are evenly distributed throughout the depth of the lake during the period of circulation. During the summer period of stratification the numbers are higher at depths of 1 and 10 m. than at the bottom.

Table 7. *Numbers of bacteria (thousands per ml.) at different depths during periods of stratification and circulation. Thirlmere,* 1939

	Depth (m.)		
Period	1	10	34
Period of circulation Feb. 1939–May 1939	0·79	0·74	0·74
Period of stratification May 1939–Oct. 1939	0·42	0·38	0·14

Bacterial counts in samples of water from Esthwaite Water were made on occasions during the period September 1938 to March 1939, and each week during the period April to October 1939. Averages of the numbers of bacteria for the period of circulation (Table 8) show that, during the winter, bacteria were fairly evenly distributed throughout the depth of the lake. During the summer months when the lake was stratified, the bacterial numbers decreased with increasing depth.

Table 8. *Numbers of bacteria (thousands per ml.) at different depths during periods of stratification and circulation. Esthwaite Water,* 1938–9

	Depth (m.)			
Period	1	5	10	13
Period of circulation Oct. 1938–April 1939	8·93	8·84	9·24	9·81
Period of stratification May 1939–Sept. 1939	13·00	10·92	7·11	4·33

Anaerobic bacteria

Since the lower layers of the lakes, particularly of the shallower lakes, become deficient in dissolved oxygen, it seemed possible that the conditions might encourage the growth of anaerobic bacteria. Such bacteria would not appear on Petri plates, incubated under aerobic conditions. It was decided therefore to make bacterial counts under strictly anaerobic conditions, employing McIntosh and Fildes jars. Determinations of numbers of anaerobes were made on samples of water from Windermere and from some shallower lakes. The numbers were of a similar order as for the counts of aerobic organisms; in almost every case there were more anaerobic bacteria in the highly oxygenated surface layers of the lakes than in the lower layers containing no dissolved oxygen. Table 9 shows the number of anaerobes and the amount of dissolved oxygen at different depths. When such bacteria were isolated and grown in pure culture it was found that the large majority were facultative anaerobes, many of which grew more luxuriantly under aerobic conditions. Strict anaerobes in the waters of these lakes must be relatively scarce.

Table 9. *Numbers of anaerobes and concentration of dissolved oxygen at different depths in Windermere, Esthwaite Water, Rydal Water, and Blelham Tarn*

Depth m.	Dissolved oxygen % saturation	Anaerobes per ml.	Depth m.	Dissolved oxygen % saturation	Anaerobes per ml.
Windermere, north basin (averages of four weekly samples)			Esthwaite Water		
1	90	270	1	86	372
10	87	388	9	52	160
30	76	106	13·5	0	50
50	72	225			
Rydal Water			Blelham Tarn		
1	92	385	7	50	670
9	50	340	8	11	270
12	20	270	9	0	70
17·3	3	270	10·5	0	60

FLUCTUATIONS IN NUMBERS OF BACTERIA

One of the outstanding observations during the investigation of the distribution of bacteria in lakes was the fluctuation in numbers which occurred in samples of water taken from all depths. In summer the fluctuations were less marked than in winter and were mainly in the upper layers. From a comparison of Figs. 4 and 5, showing numbers of bacteria in the north and south basins of Windermere and in Thirlmere, it can be seen that with the exception of certain sharp fluctuations during the summer months the general trend of the bacterial population is similar. Fluctuations in the concentration of dissolved substances in the waters, which included ammonia, nitrate, silicate, phosphate, and oxygen, bore no relation to the fluctuations in bacterial numbers. It was found, however, that the bacterial count at a depth of 1 m. was closely related with the amount of rainfall falling on the drainage area for the seven days previous to sampling. The results for Windermere north basin have been published in part (Taylor, 1939; Water Pollution Research Board, *Annual Report*, 1939). The relationships between rainfall and numbers of bacteria at different depths in Windermere, Thirlmere, and Esthwaite Water have been determined statistically by calculating the correlation coefficients. As the significance of the calculated values of r depend upon the numbers of pairs of variables, the corresponding values of P have been taken from the Tables for the values of P at different levels of significance, given by Fisher (1936), and are shown in Table 10. Values for P of 0·05 or less are considered significant. The values of P show that there is a significant relationship between bacteria and rainfall in both basins of Windermere, and in Thirlmere, over the period under consideration.

In the north basin of Windermere the correlations are very significant at all depths during the winter period of circulation. Marked differences exist between the summer periods of 1938 and 1939. In 1938, when there was exceptionally heavy rainfall during the summer, significant values of r were obtained as far down as 30 m., whereas in the summer of more normal rainfall, 1939, the relationship held for the 1 m. layer only. The results for Windermere south basin are similar to those for the north basin except that no significant relationship was found at any depth during the summer of 1939. For Thirlmere, where work was begun in February 1939, the winter period has been divided into two parts, preceding and following summer stratification. For the period 14 February to 23 May the correlations between bacterial numbers and rainfall are just significant at depths of 1 and 34 m., but not at 10 m. During the post-stratification period the correlations are very significant at the three depths. During the summer the correlations are just significant for the 10 and 34 m. depths, but not for the 1 m. depth. Over the total sampling period of 11 months the relationships are significant for all three depths. Insufficient samples were taken from Esthwaite Water during winter months for purposes of statistical analysis but correlations for the summer period of stratification show no significant relationship between bacterial population and rainfall.

Table 10. *Total correlations between bacterial counts at different depths and rainfall over drainage area of lake during seven days before sampling*

Windermere (north basin)

Depth m.	Summer 1938 r	Summer 1938 P	Summer 1939 r	Summer 1939 P	Winter 1938–9 r	Winter 1938–9 P	Total period 1938–9 r	Total period 1938–9 P
1	0·56	0·02	0·63	<0·01	0·89	<0·01	0·63	<0·01
10	0·85	<0·01	0·39	(0·1)	0·88	<0·01	0·58	<0·01
15	—	—	0·35	(>0·1)	0·71	<0·01	0·75	<0·01
30	0·87	<0·01	0·11	(>0·1)	0·87	<0·01	0·47	<0·01
50	0·49	(0·1)	—	—	0·76	<0·01	0·43	<0·01

Windermere (south basin)

Depth m.	Winter 1938–9 r	Winter 1938–9 P	Summer 1939 r	Summer 1939 P	Year 1938–9 r	Year 1938–9 P
1	0·65	0·01	0·00	(>0·1)	0·31	0·05
10	0·72	<0·01	0·04	(>0·1)	0·49	<0·01
15	0·59	0·02	0·08	(>0·1)	0·41	<0·01
30	0·62	0·02	0·00	(>0·1)	0·34	0·05

Esthwaite Water

Depth m.	Summer 1939 r	Summer 1939 P
1	0·07	(>0·1)
5	0·31	(>0·1)
10	0·01	(>0·1)
14	0·30	(>0·1)

Thirlmere

Depth m.	Winter 14 Feb.–23 May 1939 r	Winter 14 Feb.–23 May 1939 P	Winter 3 Oct.–18 Dec. 1939 r	Winter 3 Oct.–18 Dec. 1939 P	Summer 1939 r	Summer 1939 P	Total period 1939 r	Total period 1939 P
1	0·55	0·05	0·76	<0·01	Neg.		0·61	<0·01
10	0·42	(>0·1)	0·82	<0·01	0·50	0·05	0·70	<0·01
34	0·52	0·05	0·73	<0·01	0·52	0·05	0·60	<0·01

Non-significant values are placed in parentheses.

The significant correlation coefficients that have been obtained leave no doubt that, at least during the winter months, the numbers of bacteria in Windermere and Thirlmere are directly related to the amount of rain which has fallen on the drainage area during a period of a few days before the samples are taken.

Although periods of high rainfall corresponded with high bacterial counts, there were, in the summer of 1939, certain exceptions when bacteria had been stimulated over a short period by some factor other than rainfall. These exceptions occurred in all the lakes under examination and although not on the same date they took place within a few weeks of each other. In Windermere south basin a significant increase in numbers of bacteria took place at depths of 1 and 10 m. on 3 May, in the north basin at a depth of 1 m. from 31 May to 3 June, in Thirlmere at 1 and 10 m. on 30 May, and in Esthwaite Water from 25 May to 8 June at depths of 1, 5, and 10 m. The increases in the population of bacteria were not found to be coincident with any marked fluctuations in the concentration of dissolved substances in the water.

Table 11. *Comparison of the dates of algal maxima with the dates of early summer fluctuations in numbers of bacteria unrelated to rainfall*

Lake	Depth m.	Date of maximum number of bacteria	Date of algal maximum
Windermere: South basin	1	3 May	12 April– 3 May
	10	3 May	12 April– 3 May
North basin	1	31 May and 5 June	26 April–31 May
Thirlmere	1	30 May	30 May
	10	30 May	—
Esthwaite Water	1	25 May to 8 June	11 May and 1 June
	5	1 June	11 May
	10	1 June	—

When these early summer fluctuations were compared with fluctuations in the total number of algae in the same samples of water, as determined by Dr Marie Rosenberg, it was noticed that some relation existed (Table 11). The increases in numbers of bacteria occurred either at the same time as, or a week or more later than, the early summer activity of the algae. No direct general relation was obvious between numbers of bacteria and numbers of algae. Increases in algal population which occurred at depths greater than those shown in Table 11 were not accompanied by increases in bacterial numbers, a fact which would tend to disprove any hypothesis that bacterial multiplication was due to the increase in numbers of algae by virtue of the increased area of surface to which bacteria could attach themselves. It would seem more probable that the theory of Henrici (1938) "that the production of organic matter by plankton organisms is an important factor in determining the number of bacteria in water", is applicable to these early summer fluctuations in numbers of bacteria.

Action of sunlight on numbers of bacteria

Minder (1920) came to the conclusion that bacterial periodicity in Lake Zürich was a function of the duration and intensity of solar radiation, and that the summer increase in bacterial numbers from the surface to the bottom of the lake agreed approximately with the decrease in photochemically active rays. It is difficult to assess the effect of sunlight on numbers of bacteria in the present work since samples were always collected in the morning before the sun's rays were strong, and since there were very few days when breezes or winds did not effect some disturbance of the surface waters. However, from Tables 5–8 it can be seen that bacteria were as abundant at a depth of 1 m. as at a depth of 10 m.; usually the numbers of bacteria were greater near the surface.

To obtain clearer evidence of the effect of sunlight, samples were collected from the immediate surface and from a depth of 1 m. in the north basin of Windermere during a period of eleven weeks. In Table 12 the numbers of bacteria are compared with the hours of sunshine recorded on the day before sampling. Except on two occasions, 19 July and 20 September 1939, the differences between bacterial counts at the two depths are no greater than might be expected between two parallel samples from the same position. On the two dates mentioned the relatively small amounts of sunshine on the previous days do not suggest that any bacteriostatic effect of the sun's rays is responsible for the lower bacterial count in the surface water.

Table 12. *Numbers of bacteria at depths of* 1 *and* 10 *m. and duration of sunshine on day before sampling. Windermere, north basin*

Date 1939	Bacteria (thousands per ml.) Surface	1 m.	Sunshine (no. of hours at Ambleside on day before samples were taken)
19 July	0·29	1·03	3·8
25	0·88	0·97	9·0
2 Aug.	4·43	4·32	0·1
8	0·72	0·71	4·1
23	0·66	0·82	2·2
30	3·45	2·77	1·5
13 Sept.	1·46	2·62	9·3
20	0·11	0·72	2·5
27	0·43	0·44	1·6
4 Oct.	0·67	0·68	5·2
11	1·17	1·27	2·6
Average	1·36	1·57	

Discussion

The data obtained from samples of water taken at different positions or at different depths at the same position in the north and south basins of Windermere, and in Thirlmere and Esthwaite Water have in part confirmed some results of previous workers and cleared up some of the apparent differences existing in those results.

With regard to the fate of bacteria washed into lakes by rivers, results obtained in Windermere have confirmed the work of Kleiber (1894) and Fred *et al.* (1924) in so much as very rapid reduction in numbers takes place when the river water enters the lake. In Windermere washed-in bacteria were reduced to between one-third and one-thirtieth of their original number at a distance of approximately 200 m. from the river mouth, or ten times the distance found by Kleiber in Lake Zürich. From this point to points farther down the lake there is a tendency for further reductions in numbers to occur, but they are so small that their significance is doubtful. Thus it would appear that sedimentation, or dilution, or death due to unfavourable conditions, or a combination of these circumstances takes place very rapidly, and in normal circumstances water a few hundred metres from river mouth and shore is unaffected by washed-in bacteria. It should be pointed out that differences in physical and chemical conditions may influence the fate of washed-in bacteria in different lakes. A case in point is a lake which is fed by mountain streams. In such a lake, in summer, the water of the inflowing streams may be colder than that of the upper layers of water of the lake, with the result that the inflow water, instead of mixing with the lake water, sinks and forms a stratum below. In other cases torrential rain, if of higher temperature, may form a surface layer over the lake.

The vertical distribution of bacteria in the lakes investigated has been found to be governed largely by the physical conditions and in particular by the phases of circulation. During the winter, if the lake does not freeze over and is subjected to the usual seasonal winds, the water mixes from top to bottom, and Mortimer (1939) has shown that the chemical composition of the water in Windermere and other English lakes is similar at all depths on any one occasion during that period. Data in the various tables in this paper show that the bacteria are also equally distributed. Fred *et al.* (1924) did not obtain such a result because Lake Mendota on which they worked froze in winter, and therefore the water was stratified. In the late spring and summer the lakes examined during the present investigation all became stratified in various degrees. In general the concentration of dissolved oxygen in the lower layers of shallower lakes decreased to a greater extent than in the deeper lakes. Stratification involves the immobilization of the lower layers or hypolimnion with resulting stagnation and reduction in concentration of dissolved oxygen, and the normal process may be hindered or destroyed by high winds or cooler external conditions, which cause mixing.

The results of bacterial counts made at weekly intervals during the period of stratification in Windermere, north and south basin, Esthwaite Water, and Thirlmere, show that from the beginning of stratification the bacterial count in the lower layers remains low and relatively free from fluctuations. These lower layers are approximately equivalent to the hypolimnion and it is suggested that the immobility of the water is the basic cause of the bacterial count becoming more or less uniform at relatively low numbers. There seems no reason to believe that depletion of oxygen by itself is responsible for this condition because, whereas the bacteria are fairly evenly distributed in the hypolimnion (with the exception of the water a few metres immediately above the mud at the bottom), the concentration of dissolved oxygen progressively falls with increasing depth. Nor does there seem to be any evidence that there exists a threshold value of dissolved oxygen below which there is a high mortality of bacteria. The bacterial activity in the mud and the disturbance of the mud surface by water movement may be responsible for higher bacterial population in the lower layers of the hypolimnion than in the layers immediately above.

Reduction of the content of dissolved oxygen in lake water during stratification does not, by reason of more favourable conditions, lead to increases in the number of anaerobic bacteria. Counts of bacteria made under strictly anaerobic conditions have shown that anaerobic bacteria were few in number and were more abundant in the surface layers. Isolation and study of these bacteria proved that most of them were facultative anaerobes.

The cause of the periodic fluctuations in numbers of bacteria which took place at all depths in the lakes studied during the periods of circulation, and in the epilimnion during stratification, has been shown to be the rain previously falling on the drainage basin. There is no evidence to show that the increases are due to washed-in bacteria. The theory of Minder (1920) that the intensity of sunlight was the basic factor underlying fluctuations of bacteria is not true of the English Lakes. The effect of water temperature on numbers of bacteria has not been apparent and higher bacterial populations in winter than in summer can be ascribed to the greater rainfall during that period rather than to any temperature effect. Evidence has shown that fluctuations in numbers of bacteria during early summer may have been a result of previous algal activity but in the oligotrophic lakes such as Windermere and Thirlmere such fluctuations are insignificant in comparison with those found by Henrici (1938) in a eutrophic lake.

The variable results observed by different workers on the vertical distribution of bacteria in lakes appear to have been caused largely by the taking of isolated samples with insufficient information about the prevailing physical conditions of the lake. When regular series of samples have been taken, the periods of circulation and stratification have not been separated.

The specific cause of the stimulating effect of rain upon the multiplication of bacteria in lake water has not yet been determined; details of the relations will be the subject of a further publication.

Summary

1. The vertical distribution of bacteria has been determined in Windermere (north and south basins), Thirlmere, and Esthwaite Water; these lakes are oligotrophic bodies of water in the English Lake District. Studies on the

horizontal distribution of bacteria have been confined to the north basin of Windermere.

2. Bacterial counts at the mouth of the main river flowing into Windermere were about 200 times as great as counts in samples of water from the lake at a distance of 200 m. offshore from the point of entry of the river. In general there was a gradual decrease in numbers of bacteria with increasing distance from this 200 m. point but such decreases were relatively insignificant.

3. During the winter period of circulation of water in the lakes numbers of bacteria were approximately the same at all depths on any one occasion. When the water in the lakes was stratified, however, the numbers of bacteria decreased with increasing depth in the epilimnion, but below the thermocline the numbers were approximately the same at all depths, except in the layer of water immediately above the mud where the bacterial population was somewhat greater.

4. Progressive depletion of dissolved oxygen with depth in the hypolimnion during the summer months did not stimulate the growth of anaerobic bacteria, nor was the degree of depletion related to numbers of bacteria.

5. Periodic fluctuations in numbers of bacteria which took place at all depths were found to be directly correlated with the amount of rain which had fallen in the drainage area during the week before the samples were taken. The correlation is most significant during the winter months, possibly on account of the high rainfall during those months. During the summer the relation was most marked in the upper layers of Windermere north basin, but in the lower layers to which rain had no access there was no correlation. In the south basin of Windermere, where the bacterial population has become more stable, numbers were not affected by the relatively small summer rainfall. There are indications that short early summer fluctuations in numbers of bacteria unrelated to rainfall may be related to maxima in algal growth.

6. Changes in the temperature and concentration of inorganic substances in the lake waters have not shown any relation to fluctuations in the bacterial population, nor has any noticeable bacteriostatic effect of sunlight been apparent.

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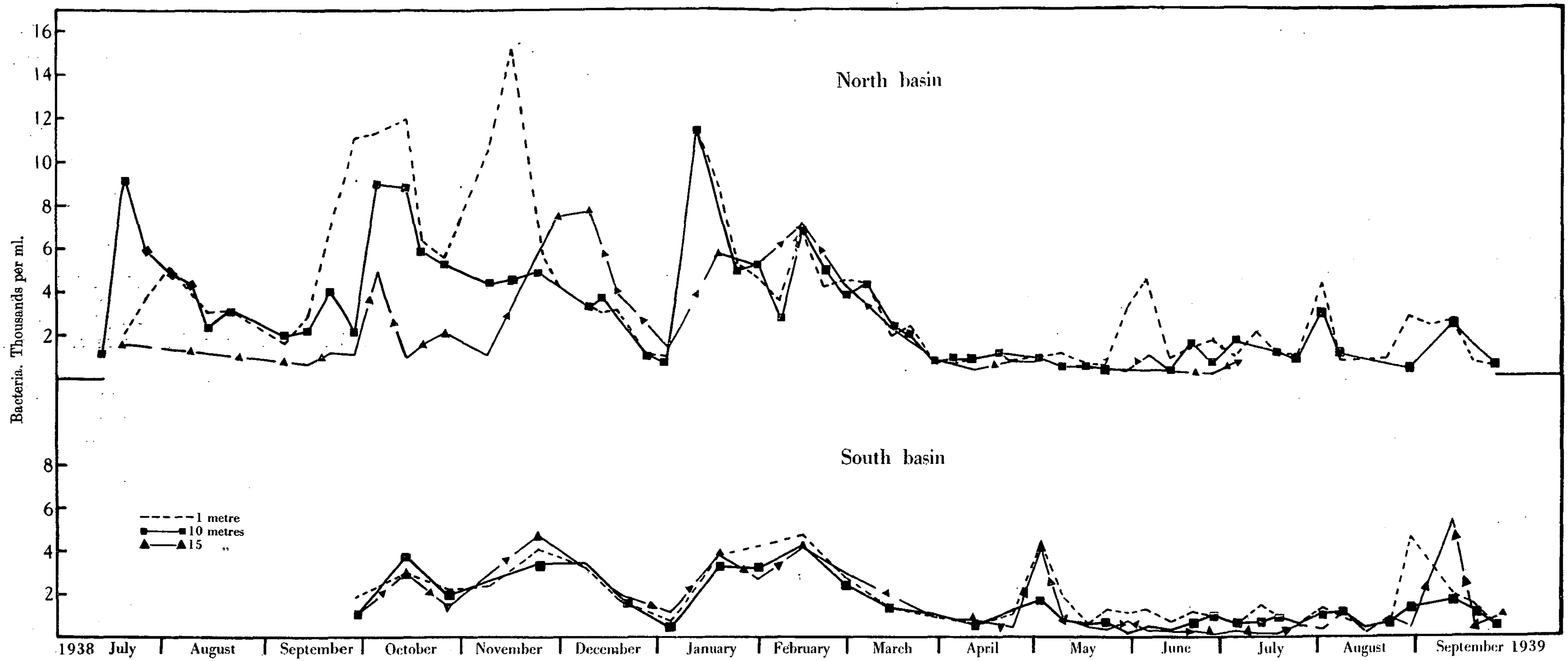

Fig. 4. Numbers of bacteria at different depths. Windermere, north and south basins, 1938–9.

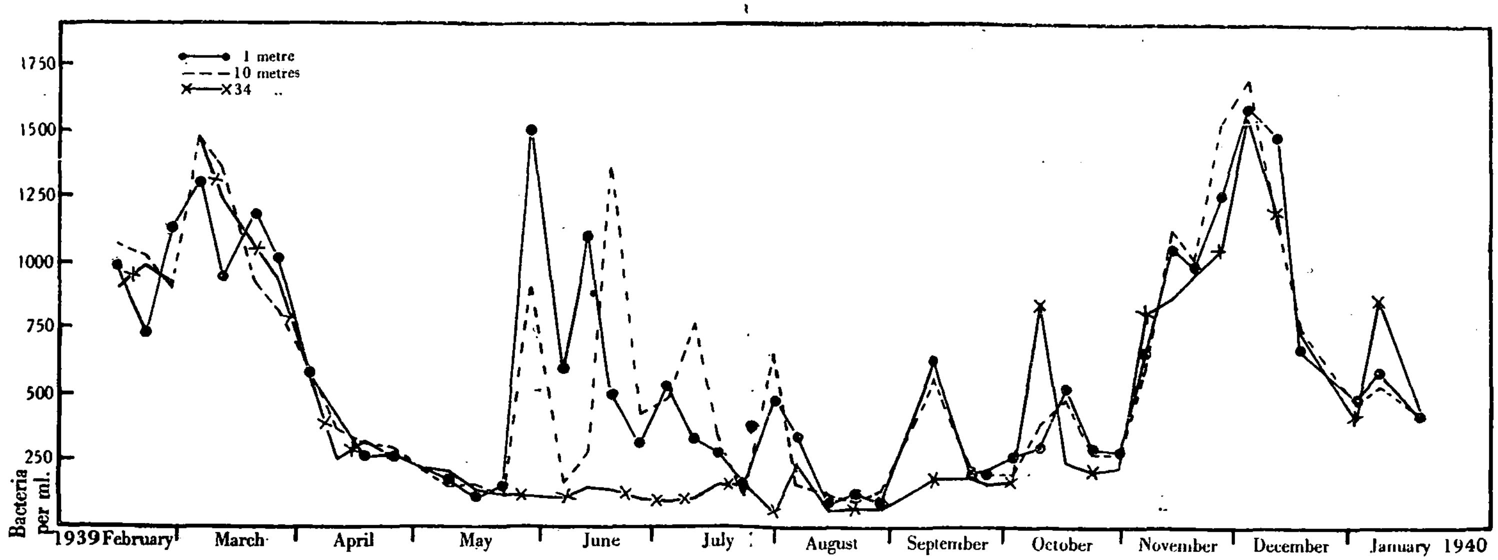

Fig. 5. Numbers of bacteria at different depths. Thirlmere, 1939–40.

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