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FRESHWATER BIOLOGY AND WATER SUPPLY IN BRITAIN

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18 figures in the text

by

W. H. PEARSALL, D.Sc., F.R.S., A. C. GARDINER, M.A.,

and F. GREENSHIELDS, Ph.D.

CONTENTS.

FOREWORD.

This pamphlet is designed to give a general account of freshwater biology as it bears on waterworks practice. It does not attempt to enumerate or describe species of plants or animals likely to be found in British waters. Nor does it attempt to cover ground which is included in the standard works such as those mentioned below, while the freshwater biologist will find that large bodies of information about lakes are unmentioned.

The authors' names are employed in the order in which their main contributions are arranged viz., general limnology (W.H.P.), methods of freshwater biology in waterworks practice (A.C.G.) and biology in filtration and treatment (F.G.). It was originally planned to have these in separate sections, but this proved impracticable, not only because ideas and facts overlapped too much but also because of Mr. Gardiner's untimely death. The final form is due to the editor (W.H.P.), but it owes much to the friendly criticisms of Professor F. E. Fritsch, F.R.S., Lt. Col. E. F. W. Mackenzie, O.B.E., Mr. J. T. Saunders and Mr. R. W. S. Thompson (to whom we owe also figure 18). Grateful acknowledgements are made to these gentlemen and also to the Metropolitan Water Board, for the numerous facilities given to us.

REFERENCE BOOKS.

SUCKLING, E. V. (1944) *The Examination of Water and Water Supplies.* (5th Edition). London.

WARD, H. B. and WHIPPLE, G. C. (1918) *Freshwater Biology.* New York.

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1. *INTRODUCTION.*

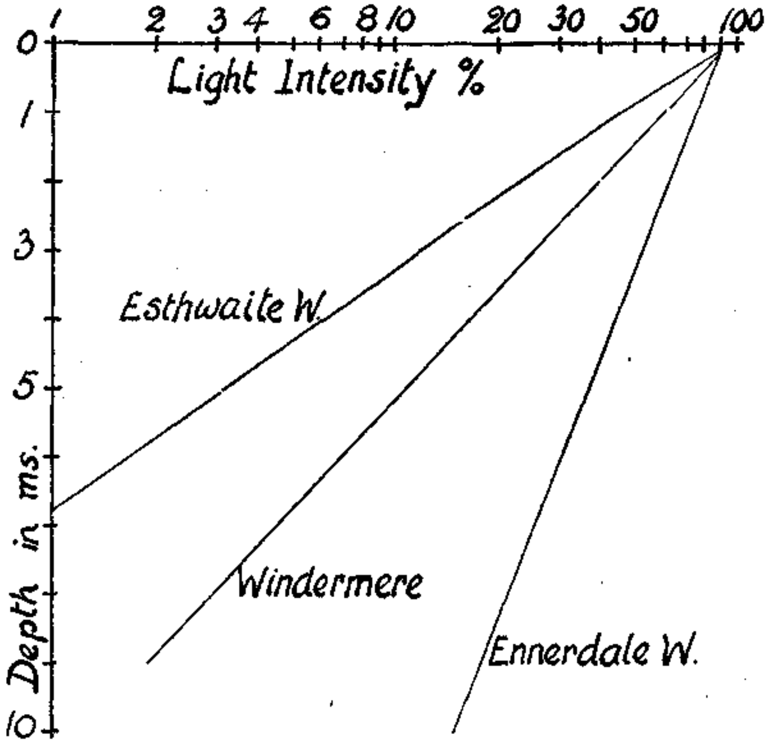
Water supply undertakings in this country are mainly concerned with providing water suitable for human consumption. The water as it reaches the consumer must be, therefore, of good physical quality and free from possibly deleterious substances, objectionable taste and smell. Still more important, it must be free from bacteria which might be injurious to public health. In order to ensure the requisite standard of purity, some control or treatment of natural waters is usually desirable before they are supplied to the consumers. The necessary treatment usually includes filtration and chlorination, with control increased by bacteriological, chemical and frequently biological examination.

Owing to the seasonal distribution of rainfall, water supply schemes, unless based on perennial wells, commonly involve some form of storage reservoir. The storage reservoir serves three other purposes in addition to that of maintaining a reserve of water. It allows any suspended matter in the water entering the reservoir to settle and it tends to reduce fluctuations in the quality of the water. At the same time it favours the removal by oxidation of organic matter dissolved in the water. Thirdly it leads to a reduction in the numbers of potentially harmful bacteria present in the water. Nevertheless, the reservoir introduces into the system a body of standing water which may be capable of supporting a large fauna and flora and it is known that undesirable tastes, smells and even bacterial elements may occur as a result of this freshwater life. Control of the reservoir in order to minimise these effects is often necessary, is frequently desirable and is commonly neglected. It may therefore be useful to discuss the principles which are involved in the development of freshwater life and the factors which favour its development. A reservoir is an artificial lake and the factors which affect its freshwater life are those which operate in other lakes. Many of the examples given below refer to natural lakes, but in fact many of the problems we shall deal with were first extensively studied in reservoirs. We shall therefore refer quite generally to lakes or reservoirs, although the two may at times present some differences.

2. BIOLOGICAL RELATIONS.

The total amount of life in any body of standing water depends mainly on the amount of plant life which the water can maintain. Many freshwater animals either feed directly on plants or else on other animals. Comparatively large animals like fish usually feed on smaller animals, although their " food chains " are usually complex, as their food requirements change as they grow older and larger. Again, several groups of small animals, crustacea such as water-fleas, the freshwater shrimp (*Gammarus*) and some insect larvæ, feed on vegetable detritus and these may be frequent in waters which contain much detritus carried in from outside, even though other forms of life are scanty. Generally speaking, however, the number of animals in a given body of fresh water is proportional to the amount of plant life. It is clear, then, that the starting point in the production of life is growth of plants, and it is accordingly important to consider the factors which favour this process.

The plants growing in water may live either entirely free-floating or else attached to the bottom. In the former case they make up part of the *plankton*, which also includes animals. The two parts are termed respectively *phytoplankton* and *zooplankton*. Attached plants also include two main groups, firstly those which are rooted such as the pondweeds, Canadian water weed and certain large algæ, the stoneworts (*Chara* and *Nitella*). Secondly, there are microscopic algæ which grow on stones, on the surface of mud and on larger plants. These algæ probably provide food for many aquatic insects, such as fly larvæ. The only species among these larger attached plants which often have marked direct effects on water quality are the stoneworts, which give rise to unpleasant odours. The larger attached plants, though less important in this respect, are more important in their effects on the bottom of a reservoir. Few of these larger plants can grow unless there is a layer of mud present. They are, therefore, most abundant in waters surrounded by fertile soils, which tend to be washed into a lake or reservoir, and to be deposited there as silt. When aquatic plants begin to grow on this silt, they gradually increase its organic content, and its reserves of plant foods such as nitrogen and phosphorus, so that it tends to become more fertile as time goes on, to support a larger and more continuous mass of vegetation, and also a far more abundant animal life. This stage of high fertility, once established, probably persists almost indefinitely in calcareous waters. In very soft or non-calcareous waters, however, the mud gradually becomes very organic, almost peaty, and it may then become poor in vegetation. The establishment of this last condition in a reservoir seems to be undesirable, however, because muds of this type liberate to the water compounds of iron and organic matter which facilitate the growth of iron-bacteria (see below, page 25). Generally speaking, the establishment of a large rooted flora in a reservoir is probably undesirable as it involves the accumulation of large reserves of nutrient materials in the mud. It can be shown that this may introduce undesirable effects (see page 26), and it certainly may tend to increase the fertility of the lake or reservoir as a whole. It is therefore important where such a possibility exists that the design of new reservoirs should allow for such a depth and such contours that only a relatively small area can support rooted plants.



1. Penetration of light into the water of different lakes. Plants grow to about 4 m. in Esthwaite Water and to about 10 m. in Ennerdale Water.

Effects of Light.

As plants require light in order to build up their food materials, the depth to which they can grow is determined by the extent to which sufficient light can penetrate into the water. In British waters this is rarely more than 30 feet (10m.), in clear waters, and it may be only 6 feet (2 m.), in turbid waters. Figure 1 gives the penetration of daylight into a clear water, that of Ennerdale Water, and into a turbid water, that of Esthwaite Water, with the limits of rooted vegetation in each case. Most British waters lie between these limits and perhaps in the South and East more commonly resemble the second example.

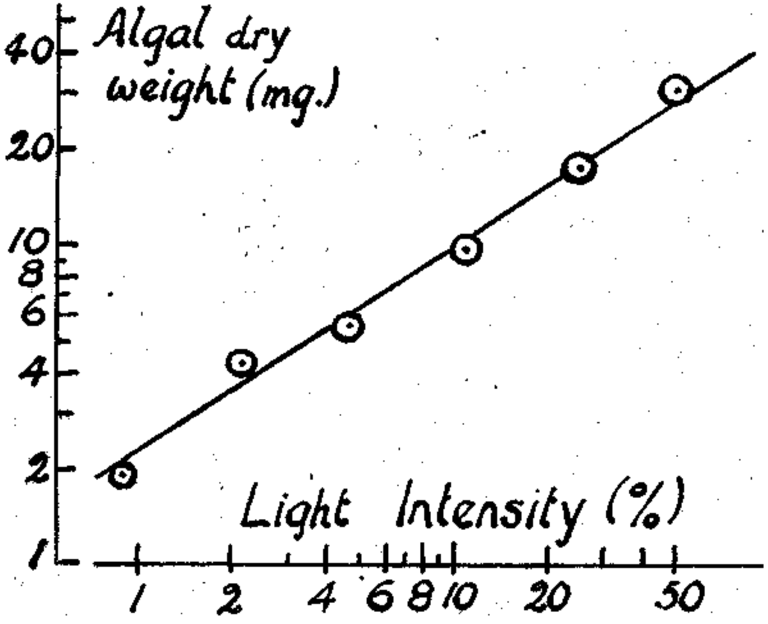
These figures, of course, refer to lakes in which the water level is more or less constant. In reservoirs, often with a very variable water level, light penetration below the average summer level is probably the important feature. The variable water level of many reservoirs does, however, limit the area available for aquatic plants to that part of the shores which is usually submerged, and probably, this, with the occasional exposure of the shore to still greater depths in dry summers, tends to increase the probability that species with resistant fruits (or other methods of propagation), such as stoneworts, will be most abundant. One feature of the varying water level of a reservoir which merits attention is that muds tend to be concentrated at and just below the usual summer level instead of being scattered over a wide area. Conditions favourable to the development of rooted vegetation are thus more quickly established than in similar waters with a more or less constant level. From the point of view of maintaining a reservoir in a condition of low fertility it would be useful to remove this mud when possible, and also to clear the banks of rooted vegetation.

The dependence of vegetation upon light is most clearly shown by methods in which glass slides, submerged to various depths, are left in the water for periods of a month or more. Algæ will usually colonise these slides and the weight of the algæ present is related to the light intensity at any depth as shown in figure 2. The data are for Windermere (one month, mid-July—August, 1938).

Depth in m.	Dry weight of algæ present, mg. per slide	Light intensity per cent of full daylight
1	30.8	57.5
3	17.7	25.1
5	9.8	10.9
7	5.5	4.8
9	4.8	2.1
11	1.9	0.9

These results also illustrate the fact that the algæ grow freely to much greater depths than larger attached plants, in Windermere, for example, to at least 10 m. as compared with about 5 m. for the rooted plants.

The algæ growing on these slides are mostly those which grow also on attached plants and on the rocks of the shore. Far more important from the point of view of waterworks operation



2. Relation between growth of algæ at different depths and light intensity.

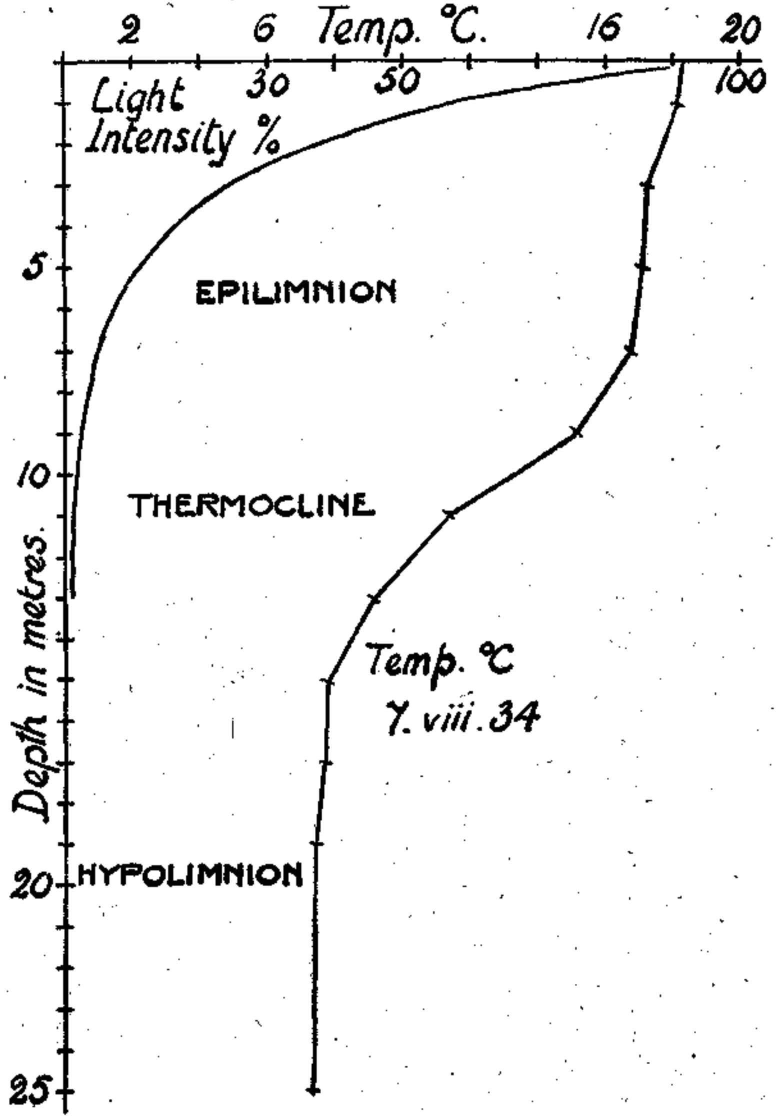
may be the *plankton* algæ, those drifting passively in the upper layers. These are also dependent on light and, although they may be scattered in an irregular fashion by water currents, they are generally most abundant at or near the water surface. Like all other plants they require for their nourishment certain elements which they obtain from the salts and gases dissolved in the water. In order of importance, these elements are, firstly, carbon, available to water plants as dissolved carbon dioxide or as bicarbonates; secondly, nitrogen, available mainly as dissolved nitrates, also, phosphorus as phosphate, silica as silicates and finally, other dissolved salts of sulphur, potash and magnesia which seem usually to be present in excess of requirements.

We recognise in practice that any water rich in dissolved bicarbonates, nitrates, phosphates and silica will be likely to support a large population of algæ, both planktonic and attached. This feature is illustrated in some detail at a later stage.

Another feature of the effect of light is of importance. When algæ assimilate carbon dioxide in the presence of light, oxygen is liberated. Algæ, therefore, serve as aerators of water as well as makers of organic matter. It is perhaps not generally realised how vigorous this aeration may be. A given dry weight of a green alga represents a similar weight of oxygen liberated in the water and about 1.66 times the weight of carbon dioxide absorbed. The production of oxygen in this way may affect many features of waterworks practice (pp. 58).

Stratification.

The fact that light and heat can penetrate only to a limited extent through water, not only affects the activities of plants, but also tends in other ways to cause stratification of any deep body of standing water. Water has its maximum density at 4°C, and in a typical lake in a temperate climate the bulk of the water acquires this temperature in winter. In spring the surface water tends to become warmer than this though lower temperatures persist at greater depths. This difference is accentuated by warm water coming in from inlet streams. As the warm water is less dense than that at lower temperature, it is maintained at the surface of the lake. Thus thermal stratification is set up which in deep lakes persists throughout the summer : the upper, warmer

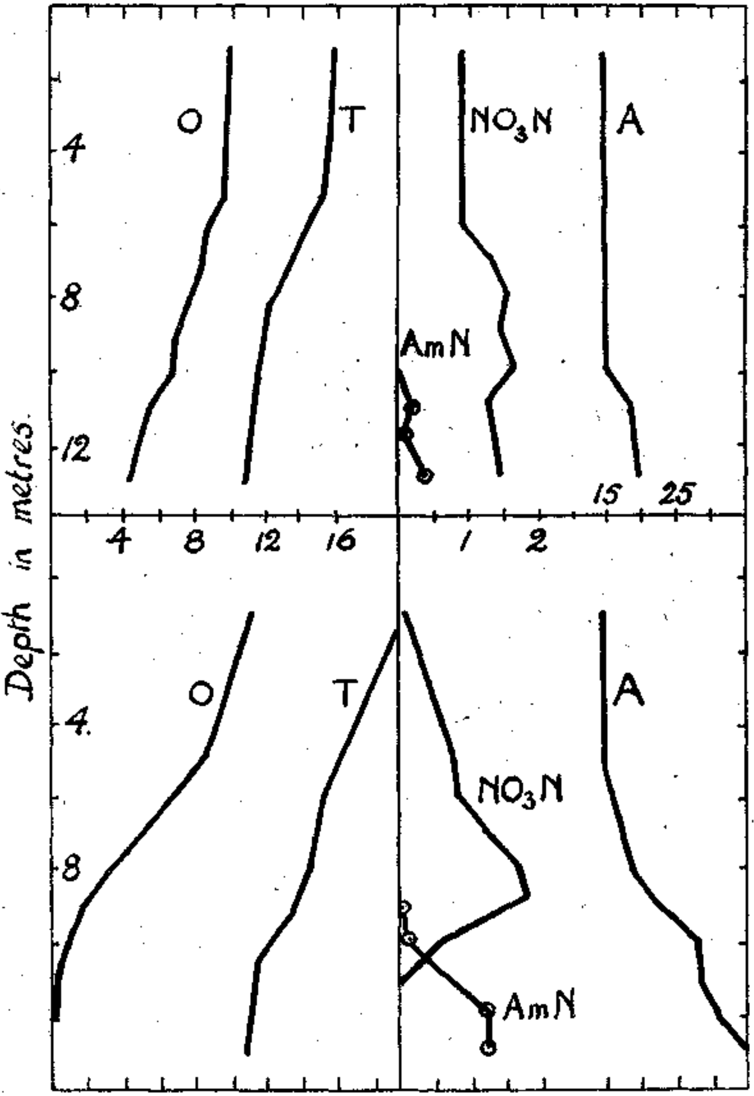


3. Diminution with depth of light intensity (as per cent of full daylight) and of temperature (°C) in Windermere during August, 1934.

and less dense water is circulated by wind but remains unmixed with and distinct from the lower, cooler and denser layers. As this thermal stratification has important physical and biological results, definite terms are applied to the two distinct bodies of water and to the junction layer, which latter is called the *thermocline*. The warmer water above it is called the *epilimnion* and the cooler water below is called the *hypolimnion*. The depth at which the thermocline is found in British waters is often about 30 feet, though it varies considerably with the shape of the lake and with its exposure to wind action. The temperature curves given in figure 3 show the temperature stratification in Windermere on August 7th, 1934. This is a large lake with considerable exposure and as a result the thermocline is comparatively deep. The lower layers here maintain a temperature of 7-8°C. throughout the summer.

Biologically, the importance of thermal stratification is that the two distinct bodies of water may acquire quite different chemical characters. The uppermost layer (*epilimnion*) is illuminated and is producing plant life and food materials. During their synthetic activities, plants give off oxygen and use up the carbon dioxide, available nitrogen and phosphates in the water (figure 4.). The upper layer may thus become depleted of nutrient materials but remains well aerated. When living plankton organisms die in this upper layer their remains fall into the *hypolimnion* and decay there. The processes of decay are brought about by bacteria, and while they are in progress, oxygen is used up, while carbon dioxide, soluble nitrogen compounds and phosphates are liberated. The water of the hypolimnion thus tends to become rich in organic matter and in available nutrient materials but much poorer in oxygen than the water of the epilimnion (see figure 4). The hypolimnion acts, in fact, as a reservoir of biologically important materials throughout the summer.

Many reservoirs do not exceed 30 feet (10 m.) in depth, and in this case the temperature stratification is often destroyed by strong winds, which cause mixing of the water layers. Even in this case, without marked variations in temperature, chemical stratification like that described above may develop during periods of calm weather if the bottom muds or the waters are rich in organic matter. The effects of the mud are shown in figure 5.



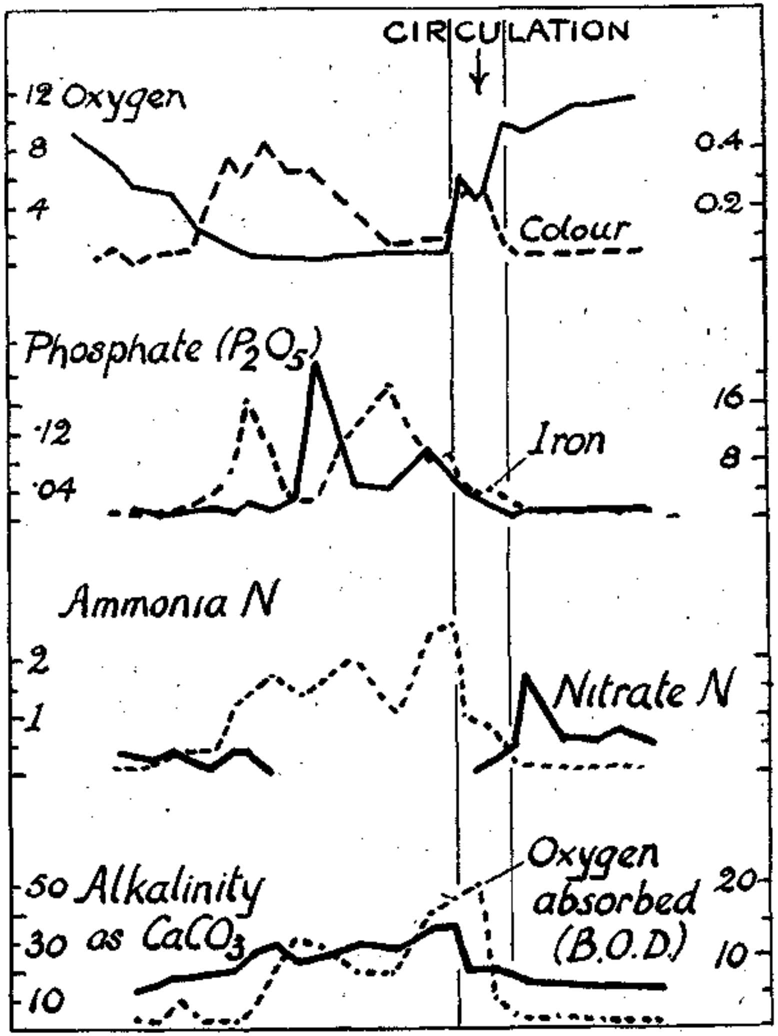
4. Esthwaite Water: Conditions at different depths at 4 weeks (22.vi) and 13 weeks (25.viii) after establishment of thermal stratification. O, oxygen (mg/1); T, temperature °C; AmN, ammonia N, mg/l.; NO_3N, nitrate N, mg/l.; A, alkalinity as $CaCO_3$ mg/l. Iron resembles AmN × 4 (After Mortimer). Upper graphs are for 22.vi.

Biologically, the next step in the sequence of events follows upon the cooling of the surface waters (*epilimnion*) in autumn. When their temperature as a whole approaches the temperature of the hypolimnion, the whole body of water becomes of equal density and it is then easily mixed by wind. The lake waters are then said to be in circulation. The result of this is that the plant nutrients in the hypolimnion are uniformly distributed throughout the lake water (see figure 5). At the same time organic matter which had accumulated in the hypolimnion rapidly decays owing to the higher concentration of oxygen available after mixing. For these reasons the surface waters of a lake tend to be richest in available nitrogen, phosphate and carbon dioxide in late winter and poorest in dissolved organic matter at that time (see figure 6).

There are other factors which contribute to these effects. Terrestrial vegetation dies down and leaves fall in autumn and hence the autumnal floods carry into surface waters large quantities of dissolved and suspended organic matter which decays in the lakes. Further, during summer, available nitrogen and phosphate, together with other nutrient salts, tend to accumulate in soils as there is little excess of rainfall to wash them away. Autumnal rains remove these substances and they are carried into the surface waters. Thus a seasonal cycle of organic matter and available plant nutrients is normally present in surface waters in this country (see figures 7 and 8) and in lakes it is augmented by the effects of circulation, which have already been discussed.

Seasonal Changes.

It is not, therefore, surprising to find that as soon as light and temperature increase in spring, there is a great outburst of plant growth. The algæ respond most quickly, particularly the plankton and littoral diatoms, and a considerable part of the available nutrient materials is usually absorbed and " fixed " in the form of organic foods. A maximum in the number of algæ is usually reached by the end of May (see figures 6 and 9), if not earlier. After this many of the algæ die, and the soluble organic materials they contain are released to the water while the insoluble residues sink into the hypolimnion and to the lake bottom. The maximum of plankton animals follows this burst of plant growth (figures 6 and 9). Their

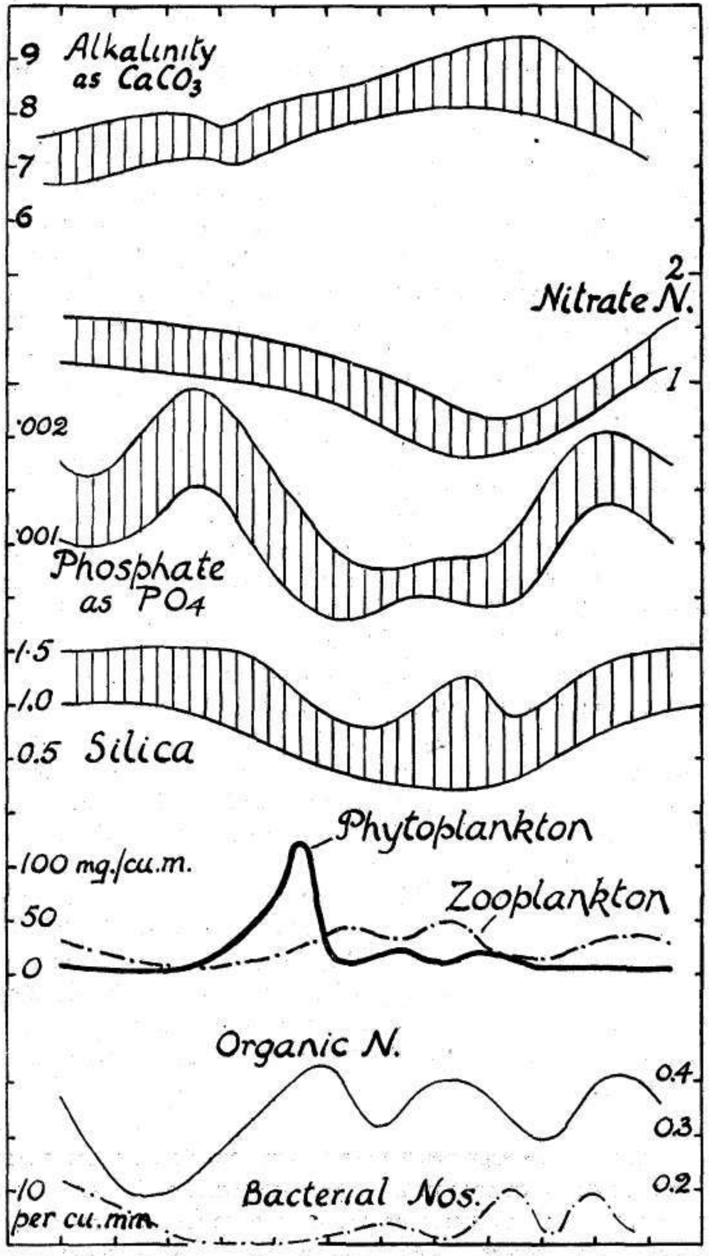


5. Esthwaite Water, 1940: Concentration of dissolved substances as mg. per litre just above the mud surface during the summer months. The vertical lines show the beginning and end of the period of circulation. (After Mortimer).

numbers often remain high during the summer and are augmented in the late summer by a maximum of larger carnivorous plankton animals such as *Leptodora*. During the summer there is usually some growth of algæ, generally of green or blue-green types, and the numbers of the latter are often closely correlated with the organic content of the water. Hence the presence of numerous blue-green algæ usually indicates either a large preceding maximum of diatoms or else some source of pollution by organic matter. It is not uncommon for example, to find a considerable increase in blue-green algæ due to organic substances resulting from the autumnal leaf-fall.

This outline of the sequence of events is illustrated particularly by figure 6 which shows a typical annual cycle in Windermere, made somewhat diagrammatic to eliminate disturbing effects such as those caused by floods or droughts. Windermere is a useful example, not only because we have a large body of information about it and because it represents a type common among British lakes, but also because the sequence of events we have described is usually readily distinguished as the season progresses.

The type of lake of which Windermere is an example, is that lying on poor soils in the mountainous parts of Britain. Widely different conditions exist in the south and east of this country, where the waters drain from fertile soils. The data given for water from the River Thames serve to contrast the characteristics of waters of this second type (see figures 7, 8 and 9). A comparison of the data given shows many features of interest and certain striking resemblances. It will be observed, for example, that both sets of data show considerable and rapid decreases of dissolved phosphate and silica in spring—these decreases being attributed largely to the effects of algæ, and particularly of diatoms, in removing these substances. Thus, for example, it can be shown that the amount of silica incorporated in the diatom crop suffices to account for the decreased concentration of silica in the water. Further, in some English lakes, *e.g.*, Ennerdale Water, diatoms do not grow in any abundance and in these waters there is no rapid decrease of silica in spring.

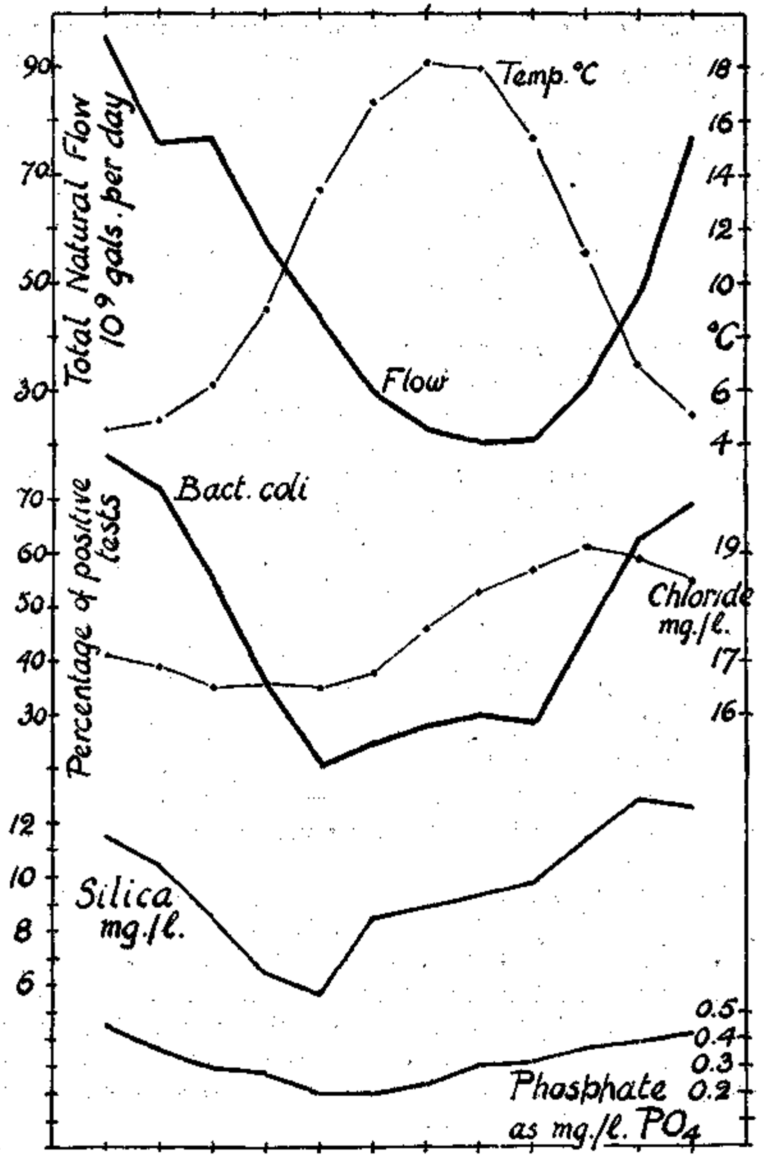


6. Monthly variation, starting in January of certain chemical and biological characters of Windermere water (chemical data as mg. per litre).

Algal Growths.

It will be evident from the figures that there are very great differences between the amounts of dissolved carbonates, nitrates, phosphates and silica occurring in Thames water and those occurring in Windermere. Hence the much greater abundance of algæ in the former water is readily understood. What it means in regard to waterworks operation may be perhaps best appreciated from an example. A reservoir supplied by Thames water and with a volume of 6.750 million gallons, might contain at times 110 tons of the diatom *Fragilaria crotonensis,* measured as dry weight. This involves a daily removal of a dry weight of a ton of the alga from the water filtered. In Windermere, on the other hand, the dry weight of the commonest diatom, *Asterionella,* in a similar volume of water would amount to only about one fortieth of this quantity. Similarly, a litre of Windermere water would rarely contain more than one million cells of *Asterionella,* while in the waters derived from the Thames, with much higher concentrations of nutrient salts, twenty million cells of *Asterionella* per litre would be not uncommon. These and other similar examples enable us to assume with some certainty that the chemical and physical environment ultimately controls the production of algæ in a given body of water. In agreement with this, we note that the *rate* of algal growth is far higher in waters of the Thames type (fig. 12) as we might expect from the greater concentrations of nutrients. In some cases, it seems certain that deficiency of certain nutrients *stops* algal development. Thus in the example given in figure 11, we should expect that the addition of more phosphate and silica to the water might allow growth to continue for a longer time. This type of experiment has been carried out successfully in some cases, especially in waters of the Windermere type. In other cases and, perhaps, particularly in the nutrient-rich waters of the Thames type, a burst of algal growth may sometimes cease before any serious depletion of the mineral nutrients in the water has apparently taken place, implying that other factors are important.

Although these arguments enable us to conclude that the chemical and physical environment largely determines the amount of algal production, the exact nature of the connection remains in some respects obscure. The most recent enquiries suggest that



7. Monthly means, starting in January for certain physical, chemical and biological characters of waters from the Thames. Figures for *Bact. coli* give the mean monthly percentages of samples positive for typical *B. coli* only in 0.1 ml. or less. (Silica and Phosphate, 1936-42; Temperature, 1919-37; others, 1908-37.)

there may be a very considerable delay between the time when the environment exerts its main effect and the time when a large algal growth results. We do not need to consider in detail here the factors causing *stoppage* of growth but only those leading to its initiation and encouragement. It seems probable that, in nature, density of algal population is largely determined early in the growth cycle, and, perhaps, even before a burst of growth starts. In order to explain this, it will be necessary to say something about the characteristics of algal growth.

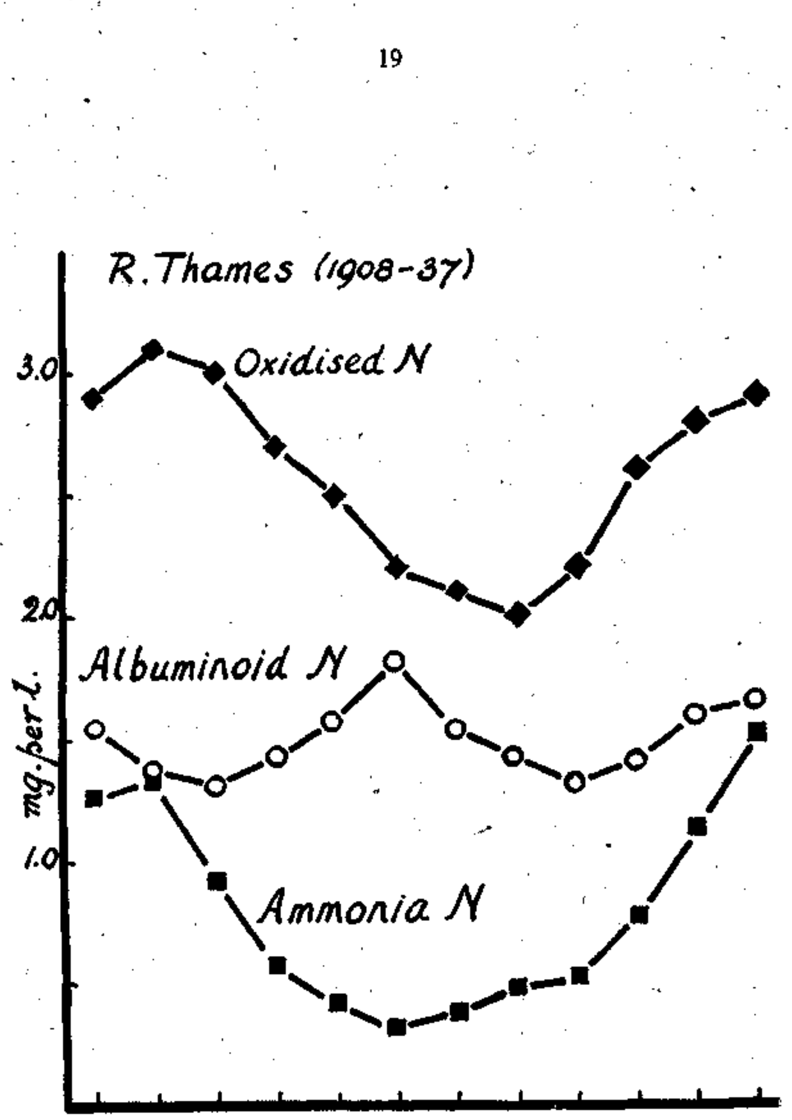
Under controlled conditions and so long as food supply suffices, the increase in numbers or in weight of an algal population is related to time in such a way that every unit increase in time results in a constant percentage increase in algal weight or number. This may be put in another way, for if every algal cell takes *g* days to divide, then in each successive period of *g* days the number or weight will double. Changes of this type are most conveniently expressed graphically by plotting the *logarithms* of the algal weights or numbers against time, as is done in figure 12, when a straight line will result. This characteristic of algal growth has of course been worked out under carefully controlled conditions. As long as there is no shortage of food and no other limiting factor, the number at any given time (N_t) will be related to the initial number (N_o) as follows :—

$$\log. N_t = \log. N_o + kt \qquad \text{or} \qquad N_t = N_o 10^{kt}.$$

where k is the logarithmic growth rate and t the time interval.

It is for this reason that, in trying to compare amounts of algal growth as in fig. 2, it is better to use the logarithm of the weight obtained for any particular condition. This will represent the comparative logarithmic growth rate. It may be noted that the absorption of light by water follows a similar rule; a constant percentage of the light entering each unit of depth is absorbed (cf. pp. 5-7). Hence the light intensity decreases logarithmically with increasing depth (cf. figs. 1 and 3).

Now it has been shown that standing crops of plankton algæ growing in lakes and reservoirs also appear to obey this logarithmic rule (see fig. 12). This is remarkable because instead of

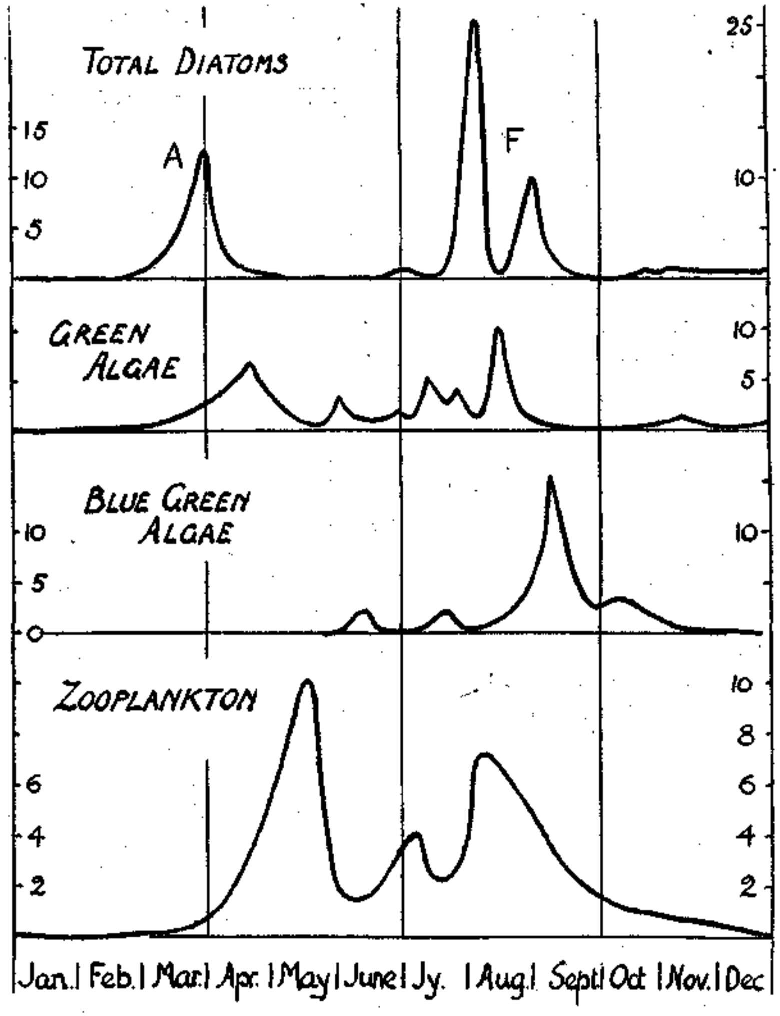


8. Mean monthly figures, starting in January, for different forms of soluble nitrogenous matter in Thames water.

these algæ growing under constant experimental conditions, they are in nature subject to considerable fluctuations of temperature, of light (intensity and duration) and often of diminishing supplies of foods. Nevertheless, as fig. 12 shows, the developing algæ continue to maintain a constant rate of increase all through the period of growth and without any apparent response to the frequent fluctuations in light and temperature. This suggests that the constant growth rate is rather an expression of the sum (or integration) of environmental factors operating in the earliest stages of growth and that it is not intimately related to the changing and immediate environment during development. From the point of view of waterworks practice, this conclusion would be a very important one if it were found to be generally true. It would mean that the average environment would tend to reproduce similar features of algal growth each year, and hence that we should have a rational basis on which to forecast the incidence and duration of algal growths. This subject is developed further on page 50. Here we need only point out, that as a basis for investigation or control, it would be necessary to remember (what is almost certainly true) that certain of the factors causing heavy algal growths operate at least from three to eight weeks before the heavy growths appear. It is, therefore, obvious that continuous and fairly frequent observations are required to detect them. If control is desired, it should come into operation at an early stage in the growth cycle.

Effects of Organic Matter.

(a) *Organic matter and algae.* Although the algæ are the main producers of organic matter in water, they are themselves not unaffected by the presence of preformed organic matter. In pure waters it has long been recognised that any form of contamination by organic matter of animal origin, e.g. sewage, is invariably associated with higher algal production. It appears that materials of vegetable origin are less effective. Similarly, it is observed that blue-green algæ are often abundant in summer when mineral salts are least plentiful. Their ability to grow abundantly at this time may be, as has been shown recently, because they can utilise gaseous nitrogen from the air. But it is also observed that the appearance of blue-green algæ is often correlated with an abundance of dissolved organic matter. An example of this correlation is shown



9. Typical sequence of plankton algæ and animals in a reservoir fed mainly by water from the Thames.

Algæ vertical scales: for diatoms and green algæ, millions of cells per litre; blue-green algæ, millions of colonies per litre; zooplankton, *hundreds* per litre.

A = *Asterionella formosa.* F = *Fragilaria crotonensis.*

in the following table where the amount of albuminoid ammonia is taken as an estimate of the amount of easily oxidisable organic matter.

Lake	Albuminoid NH_3 in June (p.p. mill.)	Per cent of blue-green algæ in July*
Bassenthwaite L.	0.010	0
Wastwater	0.026	0
Derwentwater	0.036	3
Ennerdale W.	0.038	7
Ullswater	0.045	2
Crummock W.	0.057	19
Windermere (N)	0.061	26
Esthwaite W.	0.070	27
Windermere (S)	0.075	46
Bassenthwaite L.	0.080 (July)	51 (Aug.)
Lowes Water	0.082	61

*As per cent of total algæ.

The Bassenthwaite data in this Table represent the fact that an unusually large maximum of diatoms in June was followed by an unusually high figure for albuminoid ammonia and also by the appearance of numerous blue-green algæ (in August), an event which had not previously been recorded for this lake. The same suggestion of a connection between a large diatom maximum and a succeeding abundance of dissolved organic matter and of blue-green algæ has been frequently noted in Windermere. In this lake, the yellow-green alga, *Uroglenopsis americana*, also appears to show a similar connection with high organic content and with a preceding abundance of other algæ.

We do not know how the dissolved organic matter affects the algæ. It may be directly absorbed, of course, or it may be that there, are present special organic substances which are necessary for the growth of some algæ but not for others. It is probable that one factor involved is the breakdown of the organic matter to give available nitrogen, phosphates and carbon-dioxide which serve to replenish the stocks of these substances present in the waters. In waters poor in these nutrients, a high rate of replenishment might be very important in maintaining a high rate of algal production.

(b) *Organic matter and bacterial action.* If this is so, two factors are probably important. The breakdown of organic matter in water, as in soil, is probably brought about by bacteria and if so, a high rate of breakdown would depend, other things being equal, on the numbers of the effective bacteria and on the amounts and nature of the organic materials present. In regard to the latter subject, peaty vegetable materials as a whole tend to be relatively poor in nitrogen and also to be more slowly broken down in nature. On the other hand, organic materials from the plankton or from animal sources are commonly both rich in nitrogen and rapidly broken down under natural conditions. Hence we should expect these classes of organic matter to lead to higher algal production, as apparently they do.

Few details are available as to the exact rôles of the bacteria commonly found in water so that only certain very limited aspects of their bearing on waterworks problems need be mentioned. Three groups of bacteria are commonly present in natural waters. The first of these includes those organisms which are indicative of possibly dangerous types of pollution. Most important of these in practice is *Bact. coli,* which is a sign of contamination by fæcal matter. Bacteria of this type are likely to be most abundant during flood periods (see figure 7) when they may be washed in. There are well developed bacteriological methods for their detection and control. These lie outside the scope of this pamphlet.

The second and largest group of water-borne bacteria is probably derived in part from soil. We do not know much about the rôle of these forms in water and they require further investigation, but their gross numbers apparently tend to be highest when the amounts of organic matter in the water are high (see figure 6). Apparently they grow upon these organic materials, and they are certainly concerned with the breakdown of organic materials in soil and in water. They thus have an important rôle in the production of plant nutrients.

The third group includes organisms which may be responsible for unpleasant conditions (taste, smell or colour) developing in the supply mains or elsewhere. Chief among these are the *iron* and *sulphur* bacteria. Their activity depends on the presence of considerable organic matter in the water, but they only become abundant under conditions of oxygen scarcity.

The bacteria as a whole are extremely important in their effects on biological problems. They grow on organic matter and hence their numbers are usually correlated with the amount of organic matter present. They oxidise the organic matter to carbon dioxide, nitrates and sulphates and hence provide foods for green plants. In carrying out this process oxygen is used up. As a result, abundant dissolved organic matter usually means abundant bacteria and the rapid consumption of oxygen. Decay of this type is said to be *aerobic*.

Usually, decay goes on rapidly in the hypolimnion and in the bottom mud, both of which receive a continual supply of organic matter from planktonic and littoral organisms. In both cases, however, access to supplies of oxygen is limited. The result is that in water rich in organic matter, oxygen may rapidly be exhausted in the hypolimnion and so, during the summer, special types of decomposition without oxygen may be set up, which are said to be *anaerobic*. Such processes normally go on below the surface of lake mud. Their interest lies partly in the nature of the substances they produce and partly on the way they affect the exchange of materials from mud to water. In addition however, similar changes may occur in a waterworks wherever oxygen deficiency develops, a not uncommon event in dealing with water rich in organic matter.

The normal products of *aerobic* decay mentioned above always include substances rich in oxygen, while iron is present in the *ferric* state and hence, under most natural conditions is nearly insoluble. Under *anaerobic* conditions, substances *poor* in oxygen are formed, marsh gas (methane, CH_4), ammonia and sulphuretted hydrogen (all with unpleasant smells or other objectionable properties), while iron is transformed into the *ferrous* condition in which, under natural conditions, it is very soluble. Another important practical result of the development of anærobic conditions is that a great liberation of soluble nutrient substances from mud to water takes place (fig. 5). This has been studied in some detail in English lakes and it appears that as long as the water contains appreciable amounts of oxygen, there is little or no release of nutrients from the mud-surface to the water. In short, the presence of an oxidising layer at the mud-surface acts as a seal preventing leaching from the mud, in which anærobic conditions prevail at still greater depths. It will be seen from this that anærobic conditions in the hypolimnion tend to restore to the water much of the nutrient matter which has been carried down in the form of dead plankton. When circulation takes place in winter, these materials once more become available for algal growth. It would seem, therefore, that the continuous maintenance of ærobic conditions in the lower layers of water is likely to be an advantage in limiting algal growth, a point of considerable practical importance in reservoir design.

The third group of effects resulting from the onset of oxygen deficiency concerns the growth of the anærobic bacteria and fungi. Iron and sulphur bacteria are perhaps the commonest of these in waterworks, though other bacteria and also fungi, which may also give rise to unpleasant tastes or odours, are found at times.

A frequent effect is that the service mains become infected with iron-bacteria. This is primarily because organic compounds facilitate the solution of iron (mainly as *ferrous* compounds, as noted above), whereas otherwise, iron is in the *ferric* condition, and is almost insoluble in neutral or alkaline waters. Adequate aeration converts all the *ferrous* iron to the *ferric* state, in which form it is precipitated and may be removed by filtration.

The presence of organic matter in the water produces this result because it always tends to reduce the amount of oxygen, owing to the decomposition which takes place and, in dead-ends or when the service water is moving slowly, an almost complete absence of oxygen may be produced. This is particularly likely to happen below slow sand filters with a heavy algal or bacterial mat, and, as we have seen, it may occur in the water of the hypolimnion before filtration. Under such conditions large amounts of ferrous iron are likely to be present. The iron-bacteria use these as food, converting them to insoluble *ferric* compounds, which are precipitated among the slimy bacterial growths. These growths gradually reduce the diameter of the mains and so destroy their utility. At the same time the water may acquire an objectionable odour and a chalybeate taste. At times it shows a reddish-brown turbidity, and such water usually leaves reddish-brown marks where it drips on to white earthenware furnishings, and may be the cause of considerable trouble through staining textile fabrics. The factors that favour this sort of growth are a high organic content of the water and scarcity of oxygen.

A parallel case is given by the effects of sulphur-bacteria. Here again the detrimental effects are produced when waters of high organic content develop low oxygen concentrations, as they are liable to do in enclosed mains or on standing. If the water contains much sulphate this may be reduced to *sulphuretted hydrogen*, which has a characteristic smell of rotten eggs, or to other sulphides. Both sulphuretted hydrogen and sulphides are likely to be liberated from mud in the hypolimnion in waters of high fertility. In such an event, the greater part should be converted to sulphates in the normal processes of filtration, unless these are too rapid. Trouble is likely to arise, however, if the water retains a high organic content in the lower layers of slow sand filters and in enclosed mains after filtration, where deficiency of oxygen may develop. Extreme difficulty has been experienced in tropical waterworks (*e.g.*, at Madras) from these effects, and they are not unknown in Europe and North America.

It will be seen that these cases of bacterial growth depend largely on the organic content of the water and on the fact that this continues to decompose, using up oxygen in doing so. Many innocuous bacteria may help in these decompositions. Resulting difficulties in waterworks are liable to become most marked when the organic content of the water is highest, sometimes after leaf fall, but particularly in summer when plants and animals are commonly most abundant in the reservoir water. The higher temperatures in summer greatly accelerate the rates of organic decomposition and of oxygen consumption, as well as of bacterial growth, and hence, are particularly liable to cause trouble with iron- and sulphur-bacteria. At such times water is often drawn from the hypolimnion of a reservoir to avoid heavy growths of algæ and animals in the surface waters. This may increase the difficulties in other directions, as this water is liable to be deficient in oxygen

already and to be more highly charged with iron, sulphides, etc. From whatever angle we may consider these problems, however, it is necessary to emphasise the general principle involved, that a high content of organic matter in a surface water lies at the base of this varied series of biological changes. It decomposes to give nutrient materials for green plants; it is itself a nutrient for many sorts of bacteria; by using up oxygen, it tends to introduce conditions of low aeration, and hence to increase the solubility of certain products of anærobic decomposition, which are themselves important sources of nutrition for certain specialised organisms. From the biological point of view, therefore, many of the problems of waterworks practice centre in this general principle.

3. TYPES OF WATER.

Water supply is always controlled to a large extent by the nature of the water available. It may therefore be useful to consider somewhat in outline the biological types of water in this country. Excluding water from shallow wells, which is often dangerously contaminated by sewage or manure, there are four main types of water available for supply purposes in Britain. These are extremes connected by an indefinite series of intermediates. They are:—

1. Type from deep wells and boreholes.
2. Calcareous type of surface water.
3. Non-calcareous type of surface water.
4. Pennine type of surface water.

1. The water from deep wells and boreholes is commonly of good quality provided it is not stored in light. Bacterial numbers are normally low. This is because it has been filtered already through soil and rock and in the process most of the organic matter has been decomposed and bacteria removed. It is therefore usually rich in plant nutrients and will give heavy growths of plants if exposed to light. The chief disadvantage of this type of water is that it is usually ‘ hard,’ that is, rich in lime. In some cases, however, it may be nearly devoid of oxygen, and may contain ferrous iron or sulphuretted hydrogen in solution, usually also with some iron- or sulphur-bacteria. Treatment giving complete aeration is then

required. This usually stops the trouble, as, owing to scarcity of organic matter, oxygen deficiency is not likely to develop later.

2. In many parts of southern and eastern Britain surface waters are calcareous. Most waters drawn from deep or fertile soils are of this type and so also are the waters from most of the larger rivers. Because in most cases, they include drainage from fertile soils, such waters are almost invariably highly fertile. At the same time they are usually contaminated by organic matter of animal or manurial origin, and contain a large bacterial flora. Waterworks using waters of this type can hardly avoid difficulties with algal growths in storage reservoirs and on filter beds, and usually also require the most rigid bacteriological control. Storage reservoirs receiving river water develop a high fertility almost at once, as algæ are introduced with the river water.

3. Non-calcareous waters of the best type come from hard slatey or granite rocks, and as such areas have usually little soil and no agriculture, waters of this type are usually of high initial purity. Well known examples are the waters of such lakes as Loch Katrine, Lake Vyrnwy and Ennerdale Water. Algal production is normally low and danger of bacterial contamination slight, especially if grazing animals are kept out of the catchment area. In the best cases, little or no treatment is necessary. Reservoirs constructed for this type of water, however, though they usually start from thin soil and vegetation cover, may slowly build up fertility. Cases have been observed in which this reached an undesirable level after 50 years. Waters of this kind may be contaminated with peat and then they merge into those of the next class.

4. The extremely peaty type of water is most characteristic of the extensive gathering grounds of the Pennines. The waters are non-calcareous and they contain considerable amounts of dissolved and suspended vegetable matter, but few bacteria. In the Pennines they are often extremely acid, pH values as low as 4 having been recorded. This is usually due, not to the presence of peat, but to sulphuric acid. The presence of the acid is due partly to smoke contamination. In other areas, however, the peaty matter does not greatly affect the pH value, which is more usually 6 or higher. In either case, the content of plant nutrients is usually low and the decay of the dissolved organic matter slow. Plant life in the reservoirs is usually sparse therefore, but the plankton animals may be numerous, and are usually detritus feeding crustacea. One of the chief disadvantages of these waters is that they tend to dissolve lead pipes and to promote the growth of iron-bacteria. Only about one third to one half of the organic matter is removed by modern rapid filtration methods. Small amounts of lime are usually added to neutralise the acids and the neutralisation tends to accelerate the decomposition of the remaining organic matter. Hence growth of iron-bacteria in the service mains is frequent.

4. BIOLOGICAL CONTROL OF WATER SUPPLIES.

It will be observed from what has been said about these different types of water, that both general control and the most suitable treatment are likely to vary in different cases. In so far as the required treatment depends upon the numbers of algæ present we may say straight away that only two sorts of waters need to be considered. In the fertile waters commonest in southern and eastern England, high algal productivity is to be expected at certain times of the year and attention must necessarily be focussed on the day-to-day problems associated with the efficiency of the filters, with questions such as whether to use algicides or not and with attempts to forecast the periods of high algal production. In such areas, the water supply commonly includes drainage from fertile agricultural areas and it is clearly quite impracticable to attempt to reduce its initial fertility. This probably applies also to all waters which at any time are subject to contamination by organic matter of fæcal origin, and those who have to deal with such waters may pass at once to the later sections of this work in which we deal with the methods found useful in dealing with waters of high algal productivity, and with problems of filtration.

In the northern and western parts of the country, however, many water sources which have very low algal productivity are used. They normally come from areas with hard non-calcareous rocks and from soils of low or negligible agricultural value. Clearly in this case, some thought should be given to the steps necessary to ensure that the reservoir system does not become productive or at least to delay such a development as long as may be possible. There are good reasons for believing that reservoirs on these poor soils are always likely to become fertile, although possibly the process may take long to reach a level embarrassing to the waterworks engineer. Although most of our existing reservoirs are comparatively young, we know, in some cases, that undesirable algal productivity has been reached in forty or fifty years. The main reason for this increase in fertility is that any upland stream is carrying down to the lowlands soil (as silt), organic matter, lime and nutrient salts, washed away from the uplands. If we construct a reservoir, we are in effect putting a trap in the stream so that the materials which would normally be carried to the sea will be accumulated in the reservoir system, and lead to the growth of the plants there.

It is observed in many cases that a lake gains in certain materials during the summer so that the effluent is poorer than the inflow. This is illustrated by Dr. C. H. Mortimer's data for the total nitrogen carried into and leaving certain English lakes.

Average total nitrogen during summer (p.p. mill.) in waters of typical lakes:—

	Inflow water	Outflow water.
Thirlmere	0.51	0.53
Windermere*	0.71	0.55
Esthwaite Water*	1.16	0.74
Lowes Water	0.62	0.35

*contaminated by sewage.

It was estimated that during the year Windermere gained about 8 tons of nitrogen which, if it were converted to plankton would yield about 100 tons of dry matter. This gain in nitrogen, however, represents about only 3 per cent of the amount annually passing through the lake. Far more might be gained if such a lake became a reservoir, because during the winter, monthly amounts of 30-50 tons of nitrogen are carried away by the outflowing river, and much of this would be retained in the lake if the water were impounded. In other words a reservoir differs from a lake in that the escape of the waters when they are rich in nutrients is prevented. Hence anything which can reduce the amount of nutrient material accumulated in this way will be of advantage in waters of low fertility. With this end in view the biological control of water supplies involves :—

(*a*) Control of the catchment area and of the water supply before it enters the reservoir.

(*b*) Control of the reservoir system and its developing biological character.

(*c*) The treatment of the available stored water and its control in the service mains.

All of these have the same object, namely, reduction of the amount of soluble organic matter and of plant nutrients in the water (as the ultimate source of most types of unpleasant taste, harmful substance or bacterial growth). The two former mainly require long term planning, the last involves also a recognition of the seasonal biological changes in the water and an organisation which can recognise and meet the day to day changes which may occur. This aspect of the subject is discussed at some length later (p. 48), and we emphasize again that in waters of high fertility (*e.g.*, river waters), attention must necessarily be focussed on this feature and on the day to day control, because it is impracticable to alter materially the basal conditions.

a. *Control of the catchment area* should involve, first and foremost the prevention of contamination with animal or fæcal matter. It will involve also the reduction of soil erosion, and of the transport of organic remains (*e.g.*, vegetable detritus) and soluble matter into the reservoir system. When possible, agriculture should be eliminated because it usually involves manuring and tilling the land, and both operations result in greater fertility of the surface waters. Hence afforestation is usually the best method of maintaining the requisite control of the catchment basin, not only because it eliminates the undesirable effects of cultivation but also because it tends to prevent soil erosion and probably also to lengthen the time taken for rain water to run off into the streams. Hence it reduces flood effects, and maintains a more constant average flow. It should be noted that afforestation is beneficial in other respects. There is reason to believe that evaporation is less from a woodland than from a grassland, and hence that a greater proportion of the rainfall may reach the reservoir system.

One other question involved in the control of many catchment areas in Northern Britain is that of peat erosion. In many areas, hundreds of tons of peat may be washed into the reservoir system as the result of a single storm. To a large extent, peat erosion is the result of the methods now employed, of draining and of burning peat-covered areas, for both treatments contribute to the oxidation of the peat and hence increase the amount of soluble organic matter. Burning, in particular, not only causes the destruction of the vegetation cover and thus facilitates the removal of the surface peat, but also partly destroys the peaty stratum and leads to guttering and erosion which, once started, rapidly extends. Further, much of the plant ash left after burning is soluble and may act as a plant food in the water. Again, there is much to be said for leaving peat moors in catchment areas undrained, as they then act as sponges and tend to make the flow of surface water more uniform. Where peat erosion is already pronounced, the question of stabilising the peat by afforestation or by some other method of planting, *e.g.*, of grasses—is worth serious consideration.

b. *Control of reservoir system.* It will be obvious that one useful method of eliminating the effects of silts from soil or peat erosion is to have a small settling reservoir as an introduction to the series of storage reservoirs. This will permit the removal of accumulated materials at intervals, and is the first of the methods useful in the control of the reservoir system itself and its biological character. Silts will also form in each reservoir as a result of wave action on the shores and it is always advisable when these are exposed during periods of low water level, to consider the removal when possible of any accumulated mud containing organic remains

A second generally useful method of reducing the organic accumulations in reservoir systems is to use some method of cropping. In practice, the process of filtration is in effect one of removing parts of the crops of plankton algæ, although a very large proportion must remain in the waters of the reservoir. The catastrophic decrease of a population of diatoms must also mean that a large part of its material stays in the reservoir. The second method of cropping which merits more attention than it has hitherto received, is that of removing organic matter in the form of fish. Where productivity is small it ought to be possible to remove by this method annually a large part, if not all, of any organic matter *accumulated* in the reservoir during the year.

A third method which was suggested to us by Mr. R. W. S. Thompson of the Derwent Valley Water Board, is to use as compensation water that stratum of the reservoir water which is likely to be richest in organic matter (or plant nutrients), either as algæ or dissolved in the water. Where there is well developed thermal and chemical stratification in summer, for example, it will often be advantageous to withdraw compensation water from below the hypolimnion.

It is necessary to emphasize again two points. First, these methods are not likely to be of practical value in reservoirs receiving river water of lowland type or in waters subject to contamination, even if remote, by organic matter of animal or fæcal origin. Secondly, they are not designed to remove *all* the annual harvest of organic material but simply to prevent *accumulation*, that is, to remove a fraction of the organic matter which will balance the *average annual gain* from the inflowing waters.

c. *Treatment of available stored water and its control in service mains.* The objects of treatment are the production of a safe and physically acceptable water, that is to say, one free from dangerous bacteria, clear and sparkling in appearance, having neither an objectionable taste nor odour, and no corrosive action. Of the many processes through which a raw water may pass before it is fit for the domestic tap, the most important are unquestionably those designed to remove undesirable bacteria, for failure in this places the lives of the consumers in jeopardy. The efficiency of filtration, of terminal chlorination and of adequate laboratory control, are, therefore, of paramount importance.

Treatment, strictly speaking, begins as soon as water enters a storage reservoir, because storage in a reservoir leads to the death of harmful bacteria, and also to the destruction by oxidation of dissolved organic matter. Both changes are brought about by natural processes which can be continued on slow sand filters if so desired. In either case, on the filter or in the reservoir, the destruction of organic matter will be speeded up by anything which favours the absorption, production or circulation of oxygen or the action of those bacteria which decompose organic matter. The kinds of treatment necessary to produce a safe and palatable water supply depends, moreover, upon the nature of the raw water and no two undertakings will be faced with identical problems. With the exception of waters from deep wells, which, after chlorination, are pumped directly into supply, nearly every type of water will require filtration and it will be generally accepted that a large part of the troubles that beset the water engineer are connected with the smooth operation of filters. The types of filtration most commonly employed are either " rapid " filtration (by gravity or pressure) or " slow " filtration through sand beds.

The method usually employed in rapid filtration consists in adding a coagulant such as alumina, and filtering through sand by gravity or under pressure. During filtration the sand grains rapidly become coated with a layer of reddish brown precipitate, and the surface of the sand covered by a skin of similar material and of detritus. When light is absent the difficulties due to growth of algæ in the filters do not arise, though, of course large numbers of plankton animals or plants shorten the life of the filter. The precipitate formed after treatment with alumina usually consists of a complex of partly decomposed organic matter (humus) with iron, manganese, aluminium and silica. Peaty waters and those drawn from an oxygen-poor hypolimnion already contain materials of this type and hence they usually give a copious precipitate and a shortened filter life, as well as usually requiring greater amounts of alumina. The chief disadvantage of the method from the biological point of view is that when abundant sources of raw organic matter are present (*e.g.*, plankton or the dissolved salts of hypolimnion water) dissolved organic matter tends to pass through the filter and it may induce the growth of bacteria or other organisms in the water supply after filtration (see page 25). Additional treatment, such as chlorination will therefore usually be required. Slow sand filters on the other hand, owe much of their value to biological agencies. They are normally exposed to light and the filtering of water through them is largely due to a surface skin of algæ and of bacteria, which removes organisms or detritus from the feed water. The effects produced by this skin are in many respects superior to the mechanical straining employed in methods of rapid filtration and are therefore discussed at some length in a later section.

The biological problems arising during the treatment of water are of three main types. These are, *firstly*, the problems connected with planning the work of treatment and filtration when heavy algal growths occur. These mainly involve a knowledge of methods of estimating numbers of algæ and of forecasting periods of great algal abundance, and they are discussed respectively in Sections 5 and 6. *Secondly*, there are the biological problems of slow sand filters, and their bearing on methods of operation, given in Section 7. *Thirdly*, and lastly, there are the problems involved in the use of algicides and bactericides, treated in Section 8.

5. METHODS OF PLANKTON ESTIMATION.

The proper biological control of a water supply implies a knowledge of the normal biological cycles of organisms to be met with in the unfiltered water and on the filters. In practice, this means mainly that it is necessary to be able to estimate the amount of planktonic growth carried to the filters and to know something of its normal seasonal variation in the water under examination. To obtain the latter knowledge it will be necessary to have records of the quantities of plankton over a considerable period. Given this information, it is normally possible to plan the filtration programme and to forecast the appearance of excessive or troublesome growths. In this section we shall therefore deal with the methods to be used in estimating plankton.

It may simplify matters if we note the following points. In trying to estimate the *plankton* or living matter in the water, we have first to make sure that we are working with a representative sample of water. We have usually also to distinguish the living matter from any detritus or silt present in the water. It will be convenient to use the term *seston*, which Kolkwitz coined, to cover all particulate matter in water, living and non-living, and to remember that the plankton individuals differ greatly in size.

The animals (*zooplankton*) are usually large, the plants (*phytoplankton*) usually smaller, while some plants, small protozoa and bacteria, are very small (less than 1/100 mm.) and usually distinguished as *nannoplankton*.

The Choice of a Method.

The ideal method would estimate the amounts of living algæ and of detritus, as well as the concentrations of the different species of algæ in the plankton. There is unfortunately no single method that reaches this ideal and the best that can be done is to select the one which seems most suited to the particular problem under investigation. In general, it will be sound policy to choose the simplest and quickest method, for the reason that observations at short intervals of time are likely to prove of greater value than those, which, because of greater complexity, can only be undertaken at longer intervals.

Broadly speaking, it is the seston content of a water that impedes filtration, from which it follows that it is not always necessary to distinguish between the amounts of the living and the suspended matter. The relative efficiency of different grades of sand, the optimum speed of filtration, the choice of the most satisfactory coagulant and the determination of the correct dose, the selection of one or another source of raw water, the decision to draw surface or bottom water or to restrict pumping into storage basins, are some questions to which a knowledge of the seston content will usually provide a sufficient answer.

On the other hand, methods which do not distinguish between the living algæ and the organic or inorganic detritus will not always serve. When we require to know the cause of an unpleasant taste or odour and the point at which to dose with 'activated' carbon to remove it, or whether to apply an algicide and the efficacy of the treatment adopted, we must know the ratio of organism to detritus. So too, measurements of the amounts of seston alone do not tell us when the seasonal maxima of algæ are to be expected or the probable duration of a period of growth. Hence diverse methods are at times needed and of these four will be mentioned, two of which will be described in detail. As a good deal depends on accuracy of sampling, some reference to the collection of samples is also desirable.

Collection of Samples.

The position of sampling points and the method of collection depend upon the purpose for which the sample is required. So long as the reservoir is in use, good samples can be drawn from the outlet main. Such samples, however, will be representative only of the level from which water is being drawn and the chemical and biological qualities of the water at this level may be very different from those either above or below it. This will certainly be so when a thermocline (see page 10) has become established and, in consequence, it may be of considerable practical importance to compare the conditions in the epilimnion with those in the hypolimnion. It is usual to provide facilities for the withdrawal of water from more than one depth, and it is certainly sound practice to incorporate such a provision in all new reservoirs. It is also sound policy to fit sampling taps to outlet mains close to the reservoir bank so that samples, which may prove to be of poor quality, can be drawn without running any considerable volume of such water to the filters.

As a general rule, samples dipped from the surface layers are best avoided. During spells of warm, calm weather, blue-green algæ rise to the surface as " water bloom " and samples dipped from the top few inches are unrepresentative. There may be, too, differences between the surface water on the lee and windward sides of a reservoir. Much may depend upon a water sample and the provision of adequate sampling facilities will repay additional expenditure. When a new reservoir is under construction the provision of a special sampling platform at some little distance from the reservoir bank and connected to it by a pier or bridge is worthy of consideration.

The collection of water samples taken from mains or drawn by means of a semi-rotary pump calls for no special sampling apparatus. Where samples cannot be drawn in these ways and collection from a boat is the only possibility, some form of sampler is essential. A very useful and simple piece of apparatus is the Friedinger water bottle (see figure 18 in Appendix). The general principle of this sampler is to lower what is virtually a wide tube open at each end, and then by sliding a weighted messenger down the wire to release two flaps, or lids, one at the top the other at the bottom of the tube. During lowering, the flaps are held open by springs. The collection of samples for both chemical and bacteriological examination may also be effected by a simple form of apparatus designed by Dr. C. H. Mortimer of the Freshwater Biological Association see, figure 17. (An apparatus for obtaining water from different depths for bacteriological examination. *Journ. of Hygiene*, vol. XL., No. 6, pp. 641-646. 1940). Heavily weighted bottles which are lowered with a stopper in the mouth, the stopper being jerked out when the bottle is at the desired depth are, in general, to be avoided.

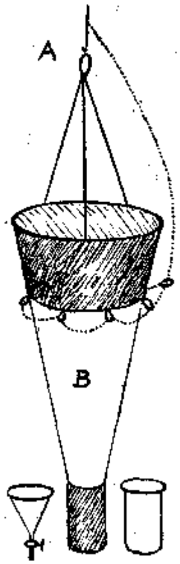
Net Sampling: Where other facilities are lacking or where information from the reservoir itself is required it may be necessary to take plankton samples by means of nets. Plankton nets are conical, with a circular mouth and are composed of bolting silk, 60 meshes to the inch for *zooplankton* and 180 for *phytoplankton*. Linen about 6 inches deep is used round the mouth and at the apex of the narrow end and the net is closed by a bucket or bottle which exactly fits it (and is usually tied in), or by a metal funnel with tap, in which the catch is collected. Metal rings are usually sewn on to the base of the linen mouthpiece and a cord through these will serve to close the net when desired (see fig. 10).

The nets are used in sampling either as tow-nets for qualitative surface samples, or, more usually, in a quantitative manner for vertical hauls. In this latter case, the net is weighted and sunk to the required depth, commonly either 5 m. for phytoplankton which is mostly near the surface, or 10 m. or more for zooplankton which is usually found at greater depths. The net is then drawn up at a convenient slow and constant speed, not exceeding 30 yards a minute, which should be standardised. (If it moves faster it pushes water before it without filtering it, especially when the net approaches the surface.) Any organisms caught in it can then be washed down into the bucket by splashing water from the outside.

When samples are required from intermediate depths (say 10-5 m.) the net can be closed at the required depth, 5 m. as follows: The ring at the top of the net is held in a quick-release catch (see figure 17). A cord running through the rings at the base of the linen mouth is securely fastened to the non-moveable part of the quick-release (D in fig. 17), and hence to the cable used for lowering the net. When the net has been drawn to the upper limit of depth required (say 5 m.), a weight is allowed to slide down the cable on to the quick-release catch (E), and the ring is then released at A, the mouth of the net falls, and the net is closed at the junction of linen and silk by the tightening of the cord.

Determination of Quantity of Seston.

Since the particulate matter in the water (seston) affects the clarity of the water, its colour and its filterability, measurements of these enable the amount of seston present at any time to be estimated. The following paragraphs give brief descriptions of how such measurements may be made and also of methods for the direct estimation of the quantity of plankton (as distinct from seston), which may be present in a sample.



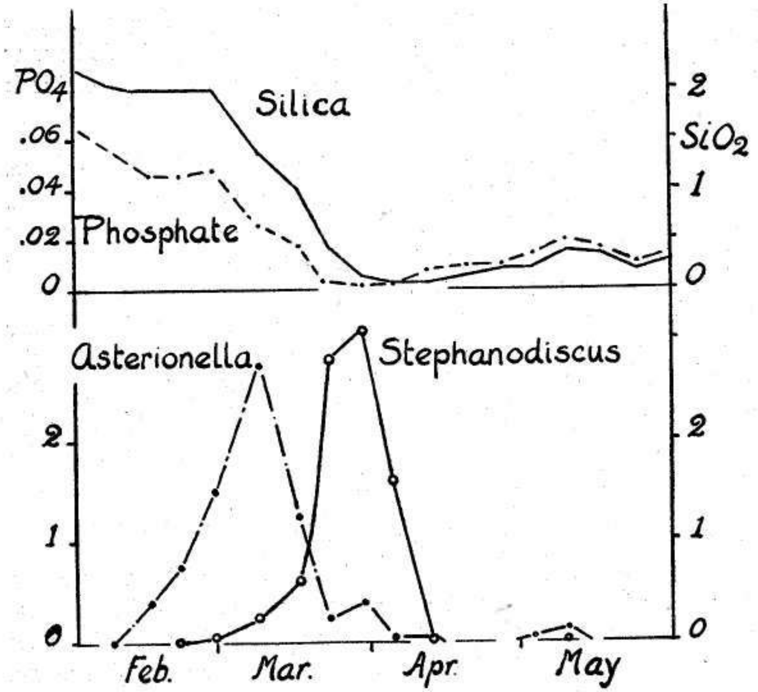
10. A typical plankton net with funnel and bucket, B being bolting silk and A a quick-release catch (see fig. 17) as used when the net is to be closed below the surface.

a. *Clarity*: Clarity is measured by lowering a white tile or plate into the water horizontally until it disappears from view, values being expressed in terms of the depth to which the tile was lowered. Intensity of illumination and the amount of ripple introduce errors. Moreover, only the top few feet of a body of water can be investigated. An alternative method of some interest has been described by Paterson. In essentials, the apparatus consists of a photo-electric cell (of the rectifier type) mounted at a fixed distance from a constant light source. The amount of light absorbed by passage through the column of water is a measure of the turbidity and colour. The instrument is enclosed in a light-tight casing and can be lowered to any reasonable depth.

b. *Turbidity*: There is a variety of ways by which turbidity can be measured and a number of standard instruments can be bought for the purpose. The Paterson turbidity meter is a proprietary instrument which gives good results. Instruments embodying a photo-electric cell also reduce the personal error and are easy to construct. If the apparatus be first calibrated in terms of a known concentration of finely divided silica, the amount by which light from a constant source is reduced by its passage through a column of natural water of fixed length is a measure of the turbidity of the sample in terms of the standard suspension. The colour of the natural water exerts a considerable influence upon the spectral composition of the light falling on the cell and a colour correction should be applied.

c. *Filterability*: Houston's original method (*Metropolitan Water Board*, 18*th Ann. Rep.*, 1924), was to deposit the seston contained in a known volume of sample upon a filter composed of four thicknesses of linen fabric and then to measure the volume of tap water which passed the filter in unit time. Houston's method has since been modified because, firstly, no two linen filters will allow the same volume of tap water to pass in unit time even although a constant head be maintained and the linen be cut from the same piece. Secondly, the four thicknesses of linen allow many of the smaller algæ, much of the finer silt and virtually all the nanno-plankton to escape. The first defect has been remedied by a somewhat different design, in which the filterability of each sample is expressed as a percentage of the volume of tap water passed by the

particular linen pad. The second has been overcome by inserting a strip of surgical lint between the second and third layers of linen. The new method is described in detail in the appendix (page 80).



11. Removal of dissolved silica and phosphate (as parts per 100,000) by the growth of the diatoms *Asterionella formosa* and *Stephanodiscus astraea* (as thousands per ml.). The data are for 1939 from a small reservoir belonging to the Metropolitan Water Board in which the water was not being used or renewed.

d. *Colour of deposit on a layer of magnesium carbonate*: The object of this method is to obtain a measure of the suspended matter in a sample, no distinction being made between living things and silt or detritus. The depth of colour of the deposit is used as the measure of the seston. The method is held to be an improvement on the simpler one described by Whipple (1927,) but is the same in essence. By itself the new method provides no better estimate than that given by turbidity measurements, but, used in conjunction with the pigment-extraction method (see page 46) it does enable the relative amounts of plankton and of inorganic detritus to be assessed. At those seasons when algæ are abundant the suspended colour will be high, but so also will be the extracted pigment value. When algæ are scarce but silt present in large amounts the suspended colour will again be high, but the pigment value low. Some knowledge of the relative amounts of living algæ and of silt may have practical value when addition of chlorine or of chloramine is under consideration.

The method depends on depositing upon a perfectly white, smooth surface (formed by a deposit of magnesium carbonate), a layer of seston, the amount of which is estimated by means of the tintometer in terms of standard colour units. Since in the pigment extraction method the depth of colour extracted by acetone from the algæ present in a sample is also measured in terms of tintometer units, it is possible to compare the amounts of algæ and silt in the same units. The data are not, however, directly comparable because the acetone colours are measured by transmitted light, whilst the suspended matter colour is viewed by reflected light. (For further details, see Appendix.)

Determination of the Quantity of Plankton.

The object of the waterworks biologist in estimating the amount of plankton in a water or of actually counting the number of algal cells is not quite the same as that of the limnologist. To the latter it may be of considerable scientific interest to study seasonal changes in the abundance of rare as well as of common algæ. As a general rule, the numbers of an alga which is only scantily represented in a plankton are of little practical importance in waterworks biology, though they may have value as indicators of changing conditions. Known exceptions to this rule are provided by *Synura* and *Uroglena,* colonial yellow-green algæ. These forms impart a strong odour to a water even when present in quite low concentrations. The odour has been described as cucumber-like, grassy or fishy and is surprisingly strong. In general, however, algæ will cause no trouble until their numbers have risen to a fairly high level. In these circumstances, then, there is usually no need to select methods in which algæ are concentrated before counting. If there are less than 100 algal cells per ml. in a natural water, there is every reason to believe that few filtration troubles will be experienced. This belief entitles us to omit descriprions of the better-known methods of the centrifuge and of Whipple's modification of the Sedgwick-Rafter technique, both of which will be found in Whipple's book (1927). The methods described in the appendix to the present pamphlet are sufficiently accurate for all practical purposes and are rapid—although the counting method scarcely deserves this description for there is no known way of making counts really quickly. The counting method has enabled the rate of increase of standing crops of dominant algæ to be followed from week to week and even from day to day. As has been pointed out (pp. 20), it is well worth while to do this for the commoner algæ, because a knowledge of these rates is of considerable service in forecasting.

a. *Direct counts by the " drop " method*: This method was devised in the Laboratories of the Metropolitan Water Board and has been the standard method there for some years. Its selection was determined by the desire to be able to make tolerably accurate counts with the least expenditure of time. On this account the volume of sample placed on the counting slide was made quite small (0.025 ml.), for it was found that most of the time consumed in making counts is taken up in moving the slide back and forth below the objective of the microscope. A drop of 0.025 ml. can be covered by a cover-slip only 19 mm. square and if the nannoplankton be ignored one such drop can be traversed in 10 to 15 minutes. For ordinary purposes it suffices to count the algæ in three such drops and to take a mean. The method cannot be used when the algal concentration is below 25 cells per ml., but, as has been explained above, such low concentrations are unlikely to give trouble on filtration stations.

b. *Utermöhl sedimentation method*: In the sea, as in many fresh waters the algæ may be so scanty that a drop as large as 1/10 ml. will contain none. If counts are to be made, therefore, the algæ must first be concentrated and one way in which this is frequently done is by means of a centrifuge. Alternatively, plankton nets with bags of fine silk may be used, although it is now recognised that a large part of a plankton is not retained even by the finest silk procurable. The centrifuge technique is subject to a number of errors from which the Utermöhl sedimentation method is freer, and the latter method is now widely used.

The algæ are concentrated merely by allowing them, after they have been killed, to settle by gravity to the bottom of a small tube of special construction. The tube is then placed upon the stage of an inverted microscope, in which the objectives are below the stage. The algæ are viewed through the floor of the tube, which is made from a large cover-slip so that objectives of high magnification can be used. Further details will be found in the appendix.

It was claimed for this method that virtually all algæ in a sample will fall to the bottom of the tube and that the distribution of organisms over the floor of the tube is so uniform that it is necessary only to enumerate the algæ in a few fields of the microscope. Recent work, whilst substantiating the first claim, has shown however, that the algæ tend to be deposited more densely in the area immediately adjacent to the walls of the tube than in the central area. On this account it is necessary to include a number of fields lying close to the margin of the sedimentation cells, the number of such marginal fields standing in the same ratio to non-marginal fields as the area of the cell floor lying about 1 millimetre from the walls does to the remainder of the floor. The simplest way to do this is to count the algæ in a number of unselected fields of the microscope along two diameters at right angles to one another. The correct proportion of marginal to non-marginal fields must first be determined from the dimensions of the tubes used.

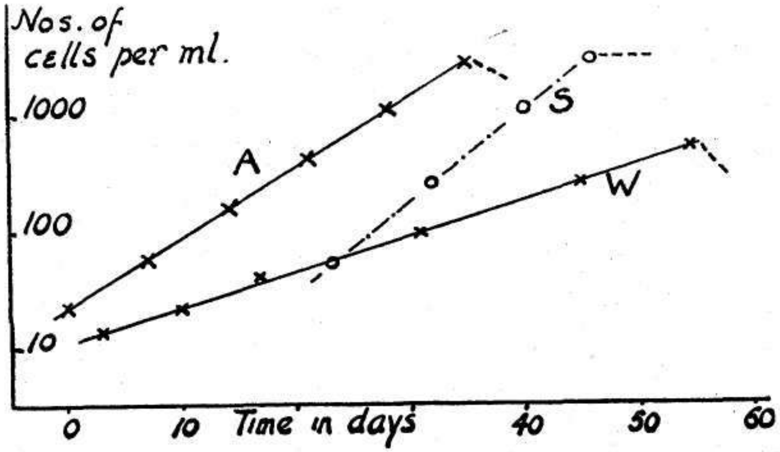
Although for most practical purposes it is unnecessary to concentrate algæ prior to counting, it is desirable to be able to do so simply and quickly in order that special investigations can be made. These would include the examination of the sparse winter plankton (see page 54), and the determination of the changes in the number of cells in a colony of *Asterionella*. It will be seen, also, that the use of sedimentation cells provides a simple way in which to enumerate Crustacea and Rotifera.

c. *A photographic method*: This method has undoubtedly certain points which must commend it to those who have little time to devote to a specialised study of algæ. It provides a pictorial record of what have been the dominant organisms in a water, a record which is permanent and which, within certain limits, enables

both the qualitative and quantitative changes in the plankton to be followed from week to week or, what is perhaps of equal importance, from year to year. Houston's original method was to throw down, by the use of a centrifuge, the suspended matter, including, of course the algæ, in a known volume of water to transfer the sediments to a small cell mounted on a microscope slide and to photograph one field magnified 50 times.

There is little doubt that the value of such photomicrographic records of seasonal change in algal abundance would be greater if the Utermöhl sedimentation technique were substituted for that of Houston. It is very difficult to distribute the sediments thrown down by the centrifuge so evenly over the floor of the cell that a photograph of a single field is truly representative of the suspended matter in the original sample. This criticism does not apply with the same force where the Utermöhl sedimentation technique is employed.

A disadvantage of the proposed modification is that unless the concentration of algæ is fairly high there will be so few organisms on the floor of the sedimentation cell that a field selected at



12. Numbers of algæ in standing crops plotted on a logarithmic scale against time. *Asterionella formosa* in a London reservoir (A) and in Windermere (W) and *Stephanodiscus astraea* (S) from fig. 11. The figures refer to the period of the vernal maximum.

random may contain only one or two individuals. This drawback can be overcome, however, by a system of two-fold concentration. If, for instance, an initial volume of 20ml. be first concentrated by sedimentation in a tube of 2 cm. diameter and about two-thirds of the clear liquid carefully drawn off by a fine pipette, the sediments can then be brought back into suspension in the volume of liquid remaining in the tube and the lot transferred to a sedimentation cell of considerably smaller diameter.

The value of photographic records is greatly enhanced if the prints are mounted on cards of such size that there is space in which to write down a list of the dominant algæ, recognised if necessary by special examination under the microscope, for it is difficult and often impossible to distinguish small algæ from detritus in a photomicrograph taken under a small magnification.

Records that are good enough for most purposes are obtained from photographs made directly upon contrasting bromide paper, that is to say without the use of plates or films. The picture obtained will, of course, be a negative and not a positive, but it is quite easy to recognise algæ from such negatives and the substitution of this for the more usual method saves money and time.

d. *The pigment-extraction method*: This method, devised for marine plankton by Harvey (*Journ. Mar. Biol. Assoc.*, XIX, p. 761), consists of extracting the pigments present in the algal cell by means of acetone and comparing the depth of colour imparted to the solvent with a standard. In marine work this method has been shown to work well, but with freshwater plankton certain modifications are necessary, chiefly for the reason that the colour composition of extracts from different classes of algæ, the diatoms, the green and the blue-green algæ, is not the same. Acetone extracts from green algæ, for instance, are often much more coloured than those from diatoms, whilst those from blue-green algæ may be reddish. Hence the " Harvey standard " cannot be used. One modification which has been suggested is to separate the green pigments (*chlorophylls*) from the associated pigments and to compare the amounts of chlorophyll present in different samples with a standard prepared from pure chlorophyll. This introduces considerable complications and is probably unnecessary when the method is used by waterworks biologists to obtain a measure of the seasonal changes in abundance of algæ. The following modification is suggested as being adequate for most purposes. It is, however, subject to errors due for the most part to the fact that whereas the pigments present in diatoms are all soluble in acetone, some of those present in other types of algæ are not soluble. Hence, it is not possible to compare by this pigment-extraction method the absolute amounts of diatoms present in a spring plankton with those of other types of algæ in a summer or autumn plankton. None the less, the method provides information of high practical value, it is simple and rapid and is of particular service in assessing either the amounts of algæ at different depths of a reservoir or in estimating the improvement due to primary filtration. There is some evidence that methyl alcohol is usually superior to acetone in its solvent action upon the pigments present in algæ other than diatoms, though for the latter acetone is superior to other solvents which have been tried.

e. *Estimation of the quantity of zooplankton*: It is difficult to obtain reliable estimates of zooplankton from samples drawn from a main or taken by pumps from tubing lowered to a predetermined depth. This is partly because the planktonic animals are commonly most frequent at a considerable depth and not at the surface, as plants usually are. It is partly, however, because they may show diurnal migrations, tending to go deeper during day-time. Finally, their powers of swimming often suffice to enable them to avoid a narrow orifice emitting suction. Consistent estimates of their numbers may usually best be obtained by vertical hauls with a coarse plankton net of 60 meshes to the inch. It is desirable to use the largest convenient opening and to have the net stained dark green or black. With such a net the animals caught in a vertical haul between known depths (*e.g.*, from 20 m. to the surface) are washed down into the bucket and preserved in a known volume of liquid. Counting is done by shaking the sample and withdrawing a known fraction (*e.g.*, 1-10 ml.) with a wide tube. This is placed in a suitable counting cell and examined under the microscope with a two-thirds inch or one inch objective, the organisms present being counted. The count should be repeated on different fractions (5 to 10) to obtain a fair average and the number in the total volume of the catch can then be computed.

The estimates made in this way are comparative rather than absolute, as the net does not remove all the animals from the volume of water it passes through. But each net used in a constant manner will tend to catch a constant proportion and if this proportion (the coefficient of the net) is determined it can be used to compute the approximately correct numbers in place of the comparative ones. The coefficient of the net is best obtained, when zooplankton is abundant, by direct counts on a series of water-bottle samples from different depths. (In each sample the animals in a considerable volume of water will have to be killed and allowed to settle for concentration into a smaller volume.) Numbers for the whole column of water may thus be obtained by summation and this may then be compared with the number estimated in the corresponding vertical net haul.

The sedimentation method may also be used for counting, either from net collections as above or from samples which have undergone previous concentration of the animals present. Larger tubes, containing 20-50 ml. of water are often necessary, but the size should be varied according to the numbers of animals present. It will be realised in any case that a direct count is generally more accurate than a net method, but it concerns only one depth, or a few, whereas a net method involves the whole column of water.

6. FORECASTING.

The object of carrying out estimations of algal and plankton numbers is primarily to accumulate sufficient information to allow the average seasonal condition (or normal periodicity) to be recognised. It is impossible to forecast the times when heavy algal growths are to be expected, unless this information is available. Satisfactory biological tests which would enable the necessary information to be accumulated, can be carried out in the course of a day and repeated periodically. They should include:—

(i) Records of seasonal periodicity of algal abundance.
(ii) Examination of the form and structure of cells and colonies in certain abundant algæ.
(iii) Examination of the winter plankton.
(iv) Records of seasonal periodicity of animals.
(v) Records of phosphorus, nitrogen and silica concentrations.

These observations should be made frequently enough to give a record of the life in the reservoir. The seasons of greatest production can be obtained fairly well from the filterability test (Method 1 above) or from measurements of the amounts of extractable plant pigments but the fact should be faced that some form of counting is highly desirable, if not essential for real progress.

Successful forecasting depends, however, not only on the existence of an adequate picture of what usually *has* taken place but also on the existence of certain constantly reproduced features in the algal growths themselves ; for it is necessary to be able to recognise whether the growth is beginning or ending before one can make a reasonable prediction as to whether the algal numbers will fall or will go on rising. Now it is known that periods of algal growth do show certain recognisable characteristics.

These recognisable features are of two sorts. (a) Those that deal with form or structure, and (b) those that are numerical or quantitative. As to the former, experience shows that populations of algæ that are not growing or that are approaching their numerical maximum tend to show certain changes in appearance (see page 53). When these changes can easily be recognised, they afford useful indications of the end of a period of algal growth. This is often extremely useful when the question arises of whether to apply treatment or not.

Reference has already been made (page 20) to the fact that algal growths also show certain numerical or quantitative characteristics. Indeed, present evidence suggests that standing crops of algæ tend to maintain a constant growth rate during their period of growth, and it is suggested that this represents an integration of the environmental factors which operate at an early stage in the growth cycle. As a basis for forecasting, this would mean that the average environment would tend to produce similar amounts of algal growth each year. Experience suggests that this is a *useful* working basis even although the underlying theory may prove to require modification. We should expect, on this basis, that each year we might get an algal growth that would tend to consist of a similar number of cell divisions in succession, to show a similar rate of growth and to yield a similar maximum number. It is clear that the possibility of forecasting depends largely upon this being *approximately* correct, as it appears to be. The possibility of *accurate* forecasting depends on a much more detailed knowledge than we as yet possess of the factors that cause deviations from the average condition, or that cause one reservoir to differ from another in biological character.

Analysis of data: The records of algal and other growths can be treated in the following ways, which indicate what should be looked for. No two bodies of water will behave in just the same way, although, in general, there are likely to be at least two well-defined peaks of algal production, the one in the spring and the other in the autumn. Both peaks may be due to diatoms, although the autumn is commonly the season of greatest production of blue-green algæ. In summer the most abundant planktonic algæ will, frequently, be green algæ. A characteristic seasonal succession is shown in figure 9. It is taken from records of a reservoir fed with water from the River Thames which is representative of many rivers and lowland lakes.

It will be of value to try to fix the dates of the most important periods of algal growth. Records of the spring maxima of diatoms in London reservoirs indicate, what is usually the case, that the maximum of *Fragilaria crotonensis* is to be expected later in the year than that of *Asterionella formosa.* They also suggest that, among the London reservoirs, the spring maximum of diatoms may be looked for earliest in Queen Mary Reservoir, slightly later in Island Barn and latest of all in Walton Reservoir. The collection of similar records from other bodies of water also tends to show that some degree of constancy in the dates of algal maxima is to be expected. But the date of the spring maximum often varies with differences in the dominant species, so that where two or more species of diatom are at times abundant it will be useful to find out as early as possible which is going to form the spring growth.

a. *The rate of increase of standing crops*: It has been observed that natural populations of the diatom *Asterionella formosa* and of other algæ increase exponentially, the rate of increase remaining constant over the whole period of growth in the cases most fully examined (see page 45, fig. 12). Two sorts of information can be obtained from estimates of the numbers of such an alga, most simply if they are plotted on a logarithmic scale in relation to time as in figure 12. The *rate* of increase will depend on both the particular alga counted and on the habitat. Thus *Asterionella* doubles its number in four or five days in a London reservoir, while in Windermere such a doubling takes about eight or ten days. Clearly, the latter is usually a much less favourable habitat for the rapid growth of this diatom. *Stephanodiscus astraea* grows even more rapidly in the London reservoir than does *Asterionella.*

Secondly, if we know the usual maximum numbers of the alga under examination in the particular reservoir, then we can, quite early in the growth period, estimate the time when the maximum number will be reached. For this purpose, it is helpful to know that the rate of growth of a particular alga, e.g., *Asterionella* in a particular body of water seems to remain nearly constant from year to year, as far as present evidence goes, so that once estimated, it is not likely to increase suddenly and so upset calculations.

b. *An approximately constant upper limit to production*: It is probably quite safe to assert that, although in a given body of water the concentration of one particular species of alga may vary in different years from say 10 to 16 millions of cells per litre, it is most unlikely to reach 100 millions per litre and unlikely even to rise to 32 millions per litre. There is, in fact, likely to be an upper limit to the concentration for the particular environmental conditions. Precisely what these conditions are is not known but, assuming them to include such factors as light intensity, turbidity, temperature, and concentration of dissolved nutrient salts, the area and depth of the reservoir and the slope of the banks, it will be realised that violent environmental fluctuations are not to be expected. The probability—for it is no more—that the concentration of one species of diatom is unlikely to exceed greatly the largest number recorded over a number of years sets an appreciable limit to the number of weeks that must elapse before a population increasing at a steady rate reaches this upper limit. The date for which the maximum was forecast may very well be too late for it is not to be expected that the upper limit will be reached each year, but it will be only in exceptional circumstances that the maximum will be reached earlier than the expected date. It may also be pointed out that when a population is increasing exponentially, and is, let us assume, doubling each week, an increase from 8 to 16 millions per litre will take place in the course of a week. An error of this magnitude in the estimated concentration may make all the difference between possible and impossible conditions of operating filters.

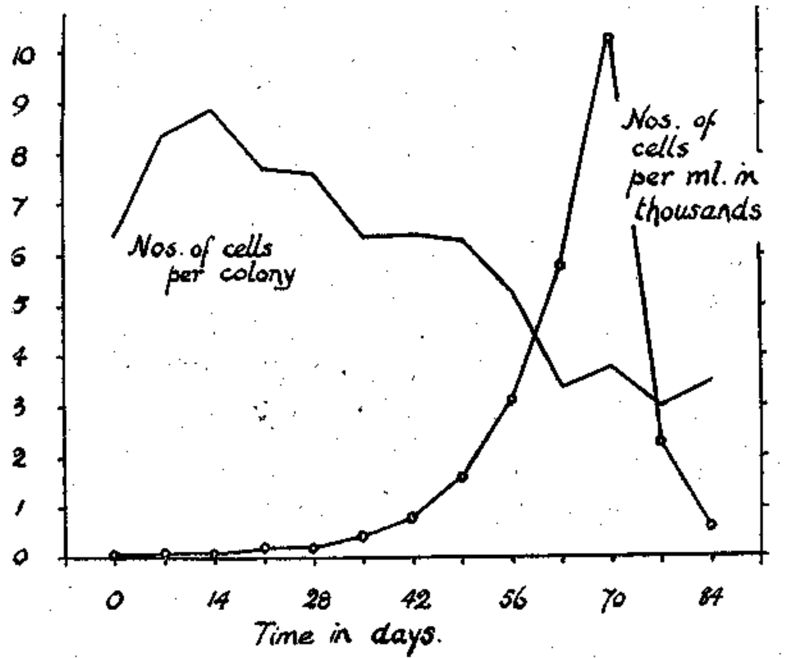
c. *An approximately constant duration of the period of growth of a particular species of alga*: As an alternative to the idea that there is likely to be a constant upper limit to production, recourse may be had to the possibility that for any one species growing in a particular reservoir, the duration of a period of growth at any given season of the year is approximately constant. This has been observed to be true, or approximately so, in the case of certain reservoirs, though it may not always be so. On present evidence, it is perhaps less likely to be generally useful than methods a and b, largely because it is often difficult to say when the algal growth started.

d. *Changes in appearance of algae*: It is often very useful to know whether an alga is going to continue growing as this may decide whether it is desirable to apply treatment or not. It is often the case that the cell walls of diatoms (and of other algæ) approaching the end of a period of rapid growth are abnormal. They may either appear to be encrusted or, sometimes, as when silica becomes deficient, may appear to be unnaturally thin. In other algæ, particularly those with gelatinous, and not siliceous, walls, the termination of a growth period may be marked by the growth of small epiphytic algæ or rotifers on the walls of the planktonic forms. These are generally certain indications that numerical increase has ceased in the infected alga.

In a healthy diatom the pigmented cell contents (the chloroplasts) occupy the greater part of the cell and are usually of a fairly deep brownish-green hue. On occasions it will be observed that the chloroplasts are shrunken and very pale. This condition usually denotes the rapid approach of the end of a period of growth. Its recognition is perhaps of small value in forecasting, for the reason that the occurrence of poorly developed chloroplasts often immediately precedes the disappearance of the species. In those places where chlorination prior to filtration is practised a study of

chloroplast colour must be made prior to chlorination since algæ which have been acted upon by chlorine are conspicuously paler than normal ones.

e. *Changes in the numbers of cells per colony of Asterionella*: In a great many of the fresh waters of the world one of the commonest diatoms is *Asterionella formosa* (or the very similar species *A. gracillima*). Frequently, though by no means always, it is one of the chief constituents of the spring plankton. It is on this account that some knowledge of the way in which the duration of a period of growth of *A. formosa* may be foretold has been included. Colonies of both species of *Asterionella* usually look like stars with 7 or 8 cells arranged like the spokes of a wheel. The colony is frequently found to be made up of this number of cells, but much



13. Decline in the average number of cells per colony during the growth of a population of *Asterionella formosa*. Data for Queen Mary Reservoir, starting January 20th, 1942.

larger ones with 16 to 32 cells are also encountered as well as smaller ones with 6, 4 or only 2 cells. On occasion the species may be represented in a plankton by a high proportion of single individuals. In the London reservoirs it has been found that during any one period of growth the population with the greatest mean number of cells per colony occurs at the start of the growth period and that on the whole the mean number of cells per colony falls as the population increases in numbers so that at the time of maximal concentration the mean number of cells per colony may have fallen to 3 or 4 (fig. 13). It is probable that similar changes in the make-up of colonies will occur elsewhere. Should this be so, a record of the shift in number of cells per colony would provide one basis for the issue of a forecast. It is not known whether other species of diatoms behave in the same way. Two species which might be investigated are *Diatoma elongatum* and *Tabellaria fenestrata* both of which are known to occur in star-shaped colonies. Periodic determinations of the make-up of say, 100 colonies using the sedimentation technique do not occupy more than 15-20 minutes each.

In the case of *Asterionella formosa*, growing in culture solution and in artificial light, Chu (*Journ. Ecol.* 21, p. 25) found that the average number of cells per colony decreased with decreasing nitrate concentration of the culture solution as shown below:—

Nitrate N. in parts per million	0.51	0.85	1.7	8.5	17.1
Average number of cells per colony	3.2	3.7	7.4	10.5	11.2

It is quite possible that the *internal* nitrogen of cells growing in a reservoir may decline as the population increases. On the other hand, in the example given in fig. 13, the nitrate-nitrogen content of the water changed hardly at all (3-2.7 p.p. mill.) during the period included in the figure. Hence it seems possible that other factors besides nitrogen supply may affect the form of the colonies in *Asterionella*.

f. *Examination of winter plankton.* It has been noted that the date of the spring maximum often varies with the dominant species. Hence it is clearly of importance to find out as early as possible which species is likely to form the greater part of the spring growth. There is some divergence of opinion as to where and how the plankton algæ spend the times separating two periods of growth. One possibility is that they lie dormant on the bottom, and that it is from these resting cells, particularly near the shores, that a new period of growth starts when conditions again become favourable. On the other hand, it is a fact that many species of planktonic algæ are never entirely absent from the free water of a lake or reservoir, although the numbers of such survivors may be very small. It is then possible that an increase in population comes about from the division of these survivors. The examination of the algal content of volumes of natural water somewhat greater than those normally used for routine counts should reveal the presence in late December and during January of occasional specimens of diatoms which may prove to be the forerunners of the spring populations. In this case, the appearance of the cells and colonies (e.g., *Asterionella*) will be important as it will show whether the cells are old ones or are just starting to grow.

The sedimentation technique (Method IV, Appendix) is, possibly, the simplest way to carry out the examination; filtration through linen (Method I, Appendix) can be used to detect the presence of diatoms in the early part of the year, but it must be recognised that there is a risk of damaging cells and of breaking colonies when the linen is squeezed on to the slide. It may often be of advantage to know in advance what species are likely to form the bulk of the spring growth, for the reason that one form may be found to give rise to much greater or much smaller filtration difficulties than another. Routine examination of the sparse winter plankton has allowed the dominant forms of the ensuing spring growth to be named accurately, on occasion, as early as January 21.

g. *Increase in the numbers of Crustacea and Rotifera.* It is commonly observed that the planktonic crustacea enjoy a period of great abundance in the late spring and again in the autumn. The spring increase usually does not coincide with the spring growth of diatoms, but may follow closely after it. The same applies to the rotifers of the plankton. The decrease in the numbers of diatoms is not believed to be due in any large part to the inroads made by the consumers—the zooplankton—upon the diatoms. The cause of the increase of zooplankton is not known, but may be due in part to the presence of an abundant food supply in the form of detritus, of bacteria or of larger numbers of small unicellular green algæ following the decrease in diatoms. An increase in abundance of such crustacea as *Daphnia*, *Cyclops*, *Diaptomus* and *Bosmina*, of the rotifers *Keratella cochlearis*, *K. quadrata* and of the relatively large *Asplanchna priodonta* may usually be regarded as an indication that the end of the spring growth of diatoms is drawing near. If there is no time to carry out regular investigations into the periodicity of numbers of the zooplankton a rough idea of their date of first increase can be gained from examination of the linens (Method i) or even from the inspection of the basins holding unfiltered water at primary filtration stations. Two examples of the periodicity of zooplankton and phytoplankton are given in figures 6 and 9.

A study of fluctuations in the abundance of planktonic animals at seasons other than the spring has not so far yielded information of much practical value in Southern England, but elsewhere the principal filtration difficulties may be associated with the autumnal maximum of zooplankton.

h. *Changes in dissolved substances*: Some information on the question of how long a growth of algæ is likely to persist can be gained from a study of the rates at which the concentrations of dissolved phosphorus, nitrogen and silica are falling. There is reason to believe that, in the spring at least, concentrations of phosphorus and silica are inversely related to those of diatoms, and that the concentrations of the former will usually have fallen to a low level just prior to the moment when the period of growth comes to an end. Similarly, it appears that no considerable growth of diatoms can be expected if silica falls below 0.5 mg./1. Unfortunately, it has also been found that the numbers of one species of diatom may decline from the maximum before the phosphorus and silica has fallen to the lowest level and that the period when the numbers of one species are decreasing rapidly may actually coincide with that during which the numbers of a second species of diatom are on the increase, see fig. 11. None the less, the probable duration of the spring period of growth of diatoms may often be forecast from a study of the rate of decrease in concentrations of phosphorus and silica. Such factors as the ratio of the volume of the reservoir to the daily rate of replenishment, the position of the inlet and outlet, with their influences upon circulation and short-circuiting, and the phosphorus and silica content of the incoming water must however be taken into consideration. At seasons other than the spring, inverse correlation between algal production and concentrations of dissolved phosphorus, nitrogen or silica may be less clearly shown. The reasons for this are not known, but the lack of correlation may be due in part to a greater rate of return to circulation of the chemical elements from the bottom deposits, or to the more rapid decomposition, with consequent liberation of salts, of certain classes of algæ or of organic matter, present in the late summer, particularly the blue-green algæ. Examples of this type of inverse relation between concentration of algæ and of phosphorus and silica are shown in fig. 12. The way in which one species of diatom may decrease from its maximum at the moment when a second diatom is on the increase and of the contemporaneous changes in the phosphorus and silica content of surface water is also shown in this figure.

By themselves, perhaps, none of these methods of forecasting is very exact, but none is intended to stand by itself. Used collectively, they have yielded a measure of success sufficient to engender a feeling of confidence that they embody the correct principles.

It should be observed that the principal difficulty in the way of working out a technique of forecasting has been the absence of numerical data which would enable general conclusions to be drawn. Though there are abundant records of seasonal chemical changes in natural waters, similar quantitative data of the algæ floating in this water are deplorably scanty and this omission must be made good before real progress can be made.

7. BIOLOGY OF SLOW SAND FILTRATION.

We have already (on page 34) dealt in outline with the main methods of filtration that are commonly employed. One method, that using " slow " sand filters, depends for its success on biological agencies and so merits detailed attention. In this case, the filtering of water as it passes through open sand beds is brought about largely by the community of algæ and of bacteria that develops on and below the surface of the sand.

This living filter is not homogeneous. It consists of animals and plants, including bacteria, in complex association and with much interdependence. German writers often distinguish between the association of the interstices and the general surface of the bed and that of the coating, mainly bacterial, that develops on the surfaces of the grains, the former being referred to as the " schmutzdecke " or dirt layer and the latter as the " zooglea." The distinction, necessarily rough, is useful and will be observed in what follows. The schmutzdecke is composed of organisms (and, strictly, silt and organic detritus) brought to the bed in the feed-water and of forms native to the superficial layers of the filter. Of the latter the commonest are sessile algæ, diatoms mostly, together with varying proportions of protozoa, a few larger animals such as the larvæ of midges (*Chironomus*) and a host of bacteria. With increasing depth this layer rapidly changes its character so that within a few inches of the surface the other forms are no longer present and the community is now almost wholly bacterial. Thus the schmutzdecke is rather a loose aggregation that lodges in the spaces between the grains and is readily removed by washing. The zooglea on the other hand is an envelope of jelly that surrounds the individual grains and requires for its removal when wet a pressure several times greater than that required to loosen the surface layers. The difficulty of its removal when wet is in marked contrast to the ease when dry. Where the water being filtered contains appreciable amounts of mineral substances, say as bicarbonates, the zooglea is a colloidal matrix of bacteria and precipitated mineral salts.

Certain of the algæ of the filter bed, particularly the *Eugleninae*, are facultative saprophytes and use organic matter as food. They therefore directly assist in purification by decomposing and digesting particulate organic matter as well as by using certain inorganic salts, such as nitrates and phosphates. But on the whole the greatest single effect of the algæ on the quality of the filtrate results from their synthesis of carbohydrates from light and carbon dioxide. Oxygen is the by-product of this photosynthesis and its liberation into the water assists in the oxidation of dissolved and suspended materials. The surface layer of the filter is thus likely to be well oxygenated, whereas, especially if organic matter is rapidly being decomposed by bacterial action, the lower layers of the filter may become deficient in oxygen and hence be working under *anaerobic* conditions. This is an undesirable condition (see page 24) normally prevented by rapid photosynthesis in the surface layers. In addition, increased supplies of oxygen encourage the growth of *aerobic* or oxygen-requiring bacteria, such as those oxidising ammonia to nitrites and nitrates ; they also ensure the production of insoluble forms of iron and manganese and in various ways greatly improve the palatability of the water.

The animal population of the filters seems to bear a direct relation to the amount of organic matter accumulated on them. If the raw water has already been partly purified by prolonged storage in reservoirs, the filters may accumulate little organic matter and they will then have few protozoa. On the other hand, at, and immediately following, periods of maximum planktonic abundance, especially of *Myxophyceae*, there may be a recognisable increase in the numbers of protozoa on the filter. Helminths, annelids, rotifers and the larvæ of aquatic insects may be found. These forms appear to accelerate the changes in the particulate organic matter deposited on a filter, for a rapid recovery of lost " head " has been repeatedly observed following an accession of matter of this kind. Working through and consuming large quantities of the grosser particles the larger animals so comminute them as to make a suitable medium for the protozoa and bacteria which, in their turn, continue the process of degradation. The insects, alone of this fauna, migrate from the filter, as winged imagines. The remainder either die where they were bred or are removed during bed-cleaning. Death may come as that of the prey of a larger form or it may be merely senescent decline. The result is the same, their tissues, like the organic substances that sustained them in life, are decomposed and mineralised.

Great as are the common changes wrought by the agencies mentioned they are not so important as those caused by the bacteria native to the filter-bed. These play the principal parts in the breakdown of organic matter and in its ultimate conversion into simple, inorganic salts. What would, but for them, remain objectionable or injurious matter is thus rendered harmless. Organic carbon, hydrogen and oxygen, as in the carbohydrates, fats and proteins, are ultimately, after a series of decompositions, passed into the filtrate as carbon dioxide and water, free or in inorganic combination. Organic nitrogen, as in proteins, is ultimately converted to ammonium compounds, or to nitrites and nitrates, and organic phosphorus, as in the phosphatides, goes to form inorganic phosphates. The great importance of *aerobic* conditions in these changes has already been emphasized (see also page 24).

Although different types of organic detritus differ in the ease with which they are decomposed, the numerical balance of the microflora of the filter continually tends towards a dynamic equilibrium with the character of the matter to be digested, with the result that the filtrate of slow sand beds tends to be uniform in character. The more insoluble substances such as the chitinous remains of arthropods and the constituents of humus, may be but little altered on the filter, most being removed at bed-cleaning.

The filter-bed performs its greatest service in rendering a polluted water pure, wholesome and palatable but other important benefits follow efficient practice. One of these has already been noted, namely the ability to recover from the hydraulic effects of a sudden accession of organic matter that is conferred on the filter by the activities of the micro-fauna and micro-flora. The value of this will be immediately evident. Efficiency in the breakdown of organic matter is also important because of the encouragement organic matter gives to the development of growths that cause blockage in the filtered-water mains (page 25). The removal of manganese, iron and other metals as precipitated salts on oxidation is another advantage of good filtration. The uniformity of the filtrate is a feature that should not be overlooked, for constancy of quality in any product is always an economic advantage. Uniformity of the filtrate makes frequent variations in the dose of post-filtration bactericides unnecessary and thus renders the process more amenable to automatic control.

The depth to which the zooglea extends varies with the kind, amount and state of aggregation of the suspended matter in the water being filtered; it also depends, though to a lesser degree, on the amount and kind of dissolved matter. It will also vary with the rate of filtration and with the "effective size" of the grains. Being thus dependent on factors in which filters differ considerably, substantial differences are observed in the depths at which the zooglea is just no longer detectable; but it is rare to find it much below 9 inches.

In its simplest condition the zooglea is a viscous gel, but this is usually much modified by the admixture of silt. Suspended or colloidal clay in a water is trapped in the jelly and imparts a fine granular texture to it, as do precipitates of inorganic salts.

Because algæ are dependent upon light for their continued existence, local algal growths do not develop on covered filters and as a result these filters are usually able to discharge more water between successive cleanings than do the open kind. It is, however, at the cost of a lower quality. Besides being more likely to allow the passage of objectionable bacteria the filtrate will be less palatable, because it is less oxygenated. The biological film will consist largely of bacteria, fungi and actinomycetes so that anærobic decomposition, with its frequent accompaniment of foul-smelling and foul-tasting products, may be reckoned among the common disadvantages of covering a slow sand filter. Such covered filters are not uncommon on the American and European continents. In the United States of America they are chiefly to be found in the northern districts, having been adopted mainly because of their greater immunity from the effects of frost. Berlin, on the other hand, provides an example of a preference resting on the greater protection from avian and aerial pollution that a cover affords. An obvious additional advantage is a saving in man-hours through freedom from interruptions of the work of cleaning the filters caused by rainstorms.

To infer from the emphasis laid on the value of the schmutzdecke and zooglea that for an open filter to be effective it need not be deeper than, say, a foot if composed of fine sand would not be correct, both for mechanical reasons and because purification continues, though in diminishing degree, throughout the depth of the filter. It should be said, however, that the question of an excessive amount of the aggregates being employed is one deserving attention. Recent decades, as readers know, have witnessed a great expansion in the use of rapid filters, with or without chemical aids to purification, in preference to the slow. Rising costs and technical developments within the service have favoured the incorporation of rapid filters, in most cases with chemical aids, in the design of new plants. Nevertheless there are many slow sand filters still in service, especially with concerns supplying old communities, and it is known that many of these are not essentially different from the originals of a century ago, when they were relied upon to purify the water without other aid, often indeed without the advantage of prior storage, and were so designed. In particular, depths and degrees of fineness of sand greater than are now known to be necessary were generally employed.

The rate at which the water passes through the sand has a considerable influence on the quality of the filtrate for two reasons. First, increased velocity tends to drive through the sand bacteria that would otherwise be entrapped in the schmutzdecke or zooglaea ; and secondly, it reduces the period of retention in the filter where oxidation of organic matter is taking place. It is possible, nevertheless, to filter certain types of water at high rates without causing an appreciable deterioration in their physical qualities: but where vertical velocities much in excess of 4 inches per hour are required it is usual to take other measures to ensure bacterial purity. Coarse, rapid (the so-called " primary ") filters, which may be easily cleaned by back-flushing or rotary screens of fine mesh wire are used to remove the coarser suspended matter from the water and lengthen the life of the slower (" secondary ") beds and bacterial purity is secured by chlorination or some equally efficient method of chemical sterilisation. It is thus evident that although a judicious combination of chemical processes and dual filtration can be relied upon to produce a water of the highest bacterial quality no single process can achieve this with the reliability and simplicity of slow sand filtration.

In the determination of a plan due weight must be given to the local circumstances. Thus, for example, where finely divided particles of clay giving a turbidity in excess of 50 parts per million or colloidal " colour " in excess of 30 parts per million, have to be removed this may best be done by chemical coagulation and sedimentation followed by rapid filtration ; yet some waters do not readily lend themselves to this treatment and for them slow sand filtration may remain the method of choice.

Algae of the Filter Surface.

Enough has been said to make it clear that it is wrong to regard the purification effected by a slow sand filter solely as the result of straining. Equally it would be wrong to suppose that all the algæ on the surface of an open slow sand filter have been strained from the feed-water. In general, a large part of the algal population taken from the bed in cleaning has never known another environment. The dominant forms are normally sessile, or sub-sessile, diatoms. Filamentous Chlorophyceæ, it is true also appear but they do not replace the diatoms. In spring, the first diatoms to appear are the small, sub-sessile Naviculoid forms and these hold the field alone until early summer is reached when filamentous Chlorophyceæ such as *Spirogyra* and *Cladophora*, become prominant, alongside the vigorous filamentous diatom, *Melosira varians*. These flourish throughout the summer, but with the coming of autumn the Chlorophyceæ begin to disappear, *Melosira varians* quickly follows them, leaving the field to the Naviculoideæ. With the onset of winter these also disappear, certainly at least as active habitants of the area. Although the successions of the algæ on the filter surface are by no means as distinct as those in the plankton, nevertheless at certain times, especially in the height of summer, luxuriant growths of algæ may develop on a filter. They may have apparently erratic effects on the flow and, for instance, similar densities of growth may be associated with different losses of " head " through the filter. Part, perhaps most, of the variation is attributable to fluctuations in the amount of solar energy striking the filter. The brighter the weather, within limits, the greater the rate of photosynthesis and the greater the production of oxygen by the algæ. The algæ being thus rendered more buoyant block the apertures less and thereby spare " head." The production of oxygen may be so vigorous as to raise large sheets of the growth and attached sand to the surface of the water—an event unreservedly welcomed by the operator of the filter in days when he was less conscious of the demands of quality than now. Indeed so valued were the mechanical results of the occurrence that it was often surreptitiously initiated by drawing a rake across the surface of the sand, " to give the bed new life." The floating masses of algæ that arise in this way commonly die and their decomposition may impart unpleasant tastes and odours to the filtered water. Even should they descend alive to the surface of the sand it is unlikely that they will settle upon the denuded areas—more likely is it that they will create areas having a skin of double thickness, thus impeding the flow where filtration is most effective and increasing it through those areas where the sand is bare. During prolonged spells of hot and sunny weather the decay of such floating masses of algæ may give to the waterworks something of the appearance and odour of a sewage works. They should, therefore, be removed from the water surface at regular intervals.

Effects of the Planktonic Algae.

The effects of planktonic algæ are, of course, very diverse. The most troublesome blockers of the filters are the diatoms, because of the persistence of their siliceous shells. Moderate growths of planktonic green algæ are readily broken down and digested on the filters so that, unless the number of them is abnormally great, lost "head" is rapidly recovered. Occasionally, complaints of " green water " are made when the beds are invaded by the reproductive " swarm-cells " of *Chlamydomonas* type. This is always an awkward event for the water engineer.

The blue-greens may afflict the purveyor of water by imparting objectionable tastes and odours to it. All algæ are capable of giving some taste or other if conditions are favourable to their doing so. They range from the " grassy " tastes caused by most green algæ through the " geranium-like " tastes imparted by certain diatoms to the " pig-pen " odours of the blue-greens. " Grassy " tastes are mainly attributable to the chlorophyllous cytoplasm of the cell ; the pungent " geranium-like " and similar aromatic taste are of essential oils, liberated by mechanical bruising of the cells or by their chemical disruption (as witness the effect of chlorine on water containing even minute quantities of *Synura*.) The tastes and odours associated with the blue-green algæ are largely the products of the decomposition of their comparatively large content of proteins. To prevent the occurrence of such visitations and, when this is not possible so to study the situation as it exists from time to time that reliable and timely prediction of the incidence of the growths can be passed to the engineer, are some of the most important parts the waterworks biologist has to play, as also is the devising of measures to cope with the trouble when it arises.

As has been said, heavy growths of the planktonic diatoms are responsible for excessive blocking of the filters. Thus it is that the biologically quiescent period of mid-winter should not be a period of hibernation for those responsible for the management of the plant. As soon as the worst of the winter floods have passed and the signs of the coming spring are unmistakable the beds should be cleaned in preparation for the vernal outburst of diatoms. Should the winter be mild there ought to be little intermission in bed-cleaning, for the set-back occasioned by early diatom growths may be such that the works may not completely recover until the following winter brings relief.

Weight for weight, and bulk for bulk, different species of planktonic diatoms cause widely different degrees of blockage in the filters. As an adaptation to their drifting habit of life, the structure of the cell, or colony of cells, is such as to favour flotation. Thus long, fine setæ (which may be roughly described as bristles) are characteristic structures of the shell and a radial disposition of individuals in a colony (as in *Asterionella*) or their union into long, narrow chains serves the same purpose. The setiferous forms are usually cylindrical cells, with varying proportions of breadth to height so that some may best be described as flat plates, often forming colonial chains. Such chains usually fragment on bruising, as do the setæ, and the single cells or short chains can thereupon pave the surface of the sand or fill the interstices and produce the greatest obstruction (examples of this are species of *Stephanodiscus*). It may be that the open, spatial arrangement of the cells in colonies of *Asterionella* and their comparatively great length cause the formation of a lattice on the bed and that this is why they block the apertures less than deposits of say *Stephanodiscus*.

It has already been stated that the appearance of " green " water in the consumer's supply may be caused by the passage of cells of *Chlamydomonas* through the filters. The complaints are commonest in the late spring and early summer and although many are justified, not a few are the result of the consumer storing his supply of water in containers exposed to light and allowing it to stagnate. When the waterworks is involved it will be found, as a rule, that the cells have passed through beds which have been cleaned and put into service a few days prior to the outbreak, or during it; whereas beds but a day or two old are more retentive.

Removal of Tastes and Smells.

Maxima of Myxophyceæ (or Cyanophyceæ) are particularly liable to give unpleasant tastes or smells in summer. Although these algæ are popularly referred to as the blue-greens the colours presented to the eye may be red, violet, brown, green or even grey. Grey species of *Oscillatoria* appear to the inexperienced so like *Beggiatoa alba*, a sewage bacterium, that the confusion frequently causes undue alarm.

The blue-greens often form "water-blooms" in summer and these generally coincide with the period of maximum demand and minimum supply. These three conditions are related through the common cause of an extended period of warm, bright and windless weather. To meet the demand for a palatable water a heavy expenditure has often to be incurred in attempts to remove the offensive odours and tastes caused by the decomposition of the algæ. Filtration through sand is generally ineffective in removing the taste on such occasions because the odour or taste of the noxious substances is so great that they are detectable in high dilution. The digestive powers of the bed are overwhelmed and the taste may persist until the bed is cleaned, even although the offending organisms may have disappeared from the feed-water. Treatment with ozone may be effective and recent American experience shows that with certain waters chlorination is effective if sufficient of the chemical be added to form tasteless compounds with the objectionable substances. Many tastes and odours have been successfully removed by administering powdered "activated" charcoal to the water and allowing it to settle in basins or on the surface of the sand filters, and this method is easily adapted to existing works. Doses of 1 to 5 parts per million have been found necessary. However effectual a method of this kind may be, it is to treat a symptom and leave the real disease untouched if concomitant steps are not taken to remove the underlying cause.

Complaints of "mustiness" and of ammoniacal odours in the water are likely to occur chiefly in winter when the weather is cold enough to cover the free water of slow sand filters with a layer of ice. By limiting the diffusion and solution of oxygen, this leads to the development of anærobic conditions, as indeed it may also do in the storage reservoirs. The deficiency of oxygen results in the diminution or suspension of the activities of the nitrogen-oxidising bacteria. Thus the bacterial degradation of organic matter continues, whereas the final oxidation of the products of this decomposition is in abeyance. In addition, winter floods bring to the filters more fine matter than at other times and this may smother the zone of aerobiosis, so that breakdown of organic matter goes on anaerobically in the depths of the filter.

The solution of this difficulty is not easy to find. Ozone would doubtless provide a remedy; powdered "activated" charcoal, dosed into the feed-water, has proved successful in removing similar tastes even although they originated in the schmutzdecke; and so-called "breakpoint" chlorination, that is chlorination in such proportions that the addition of more chlorine would result in the water acquiring the exact equivalent of free chlorine, is another possible method of destroying the taste in the filtrate.

Structure and Control of Filters.

It should be the aim of good management to encourage by every means the development of an adequate schmutzdecke and no practice should be countenanced that might tend to suppress this valuable agent. Nevertheless it would be wholly advantageous if some control could be exercised over the amount and type of algal growth on the surface of the sand.

The walls are of some importance in determining the character of the local flora of a filter. Sloping walls were formerly popular, but these have now been superseded by walls presenting a vertical surface to the free water and having a sill or foot projecting into the body of the sand. By this change local stagnation, initiated in the stiller and warmer water that lies above an inclined wall, has been greatly reduced. Algal growths on the walls contribute little to the process of purification yet may promote stagnation. In addition to keeping these growths down by attending to the smoothness of the walls and scraping with wire brushes at the time of bed-cleaning, it is usual before recharging the bed to apply to the walls a coating of lime and copper sulphate as inhibitory agents. In addition to its inhibitory action this coating provides a substratum no more attractive than the plain wall, yet easily removed, with its attached algæ, by light scraping. The texture of the wall of the filter bed also influences the growth of sessile algæ, and this should be as smooth and impermeable as possible.

Cleaning of filters is usually effected by draining the bed and removing as much of the top sand as is considered desirable, usually the surface inch. Because of the weight of traffic on the sand during these operations, consolidation of the bed occurs. This is at times a costly matter and not infrequently the blame for its effects is wrongly placed on algal growths. Those responsible for the operation of the filters should observe the " loss of head " at a standard flow, immediately the bed is put into service after cleaning, in order to avoid this error.

The " skimmings " from the filter surface are gathered into heaps which are then washed in hopper type washing machines above the sunken filters. In older works the washed sand is frequently delivered by the machines either into the bed from which it is being taken or into an adjacent one, which may be empty or in service. This practice is undesirable because the washed sand and the water that transports it are often heavily charged with bacteria, including organisms of the coliform group. An unnecessary burden is thus thrown upon the filter receiving this discharge. It is better practice to wash all sand centrally and to store it clean in bins against the time of re-sanding. If the storage is sufficiently prolonged all objectionable bacteria will perish.

It is not good practice to store unwashed sand for more than a few days, for if appreciable amounts of organic matter are left mixed with it in the heaps, anærobic decomposition will take place and it may not be possible to put the sand into the filter again unless special precautions are taken in the washing, because objectionable tastes, odours and coloration may be imparted to the filtrate by sulphides, ammoniacal compounds and other products of such decomposition. For this reason and for the effect on the bacteriological quality of the filtrate it should be made a rule, not lightly to be disregarded, when central washing is not practised, that washed sand may not be delivered into a filter that has been in service for less than a week since it was last cleaned. Washing sand back into a bed that is being cleaned is particularly objectionable. Attempts have been made to overcome this difficulty by washing the sand with water containing chlorine but the method has not been uniformly successful.

Should a filter of sound structure be efficiently managed the amount of organic matter entering the under-drains and other filtered-water channels will not be sufficient to support a considerable biological population there. Nevertheless it is not rare to find organisms in these places. The commonest forms inhabiting the under-drains and culverts are a few species of free-living nematodes, a rotifer of the genus *Colurella*, crustacea such as *Gammarus* sp., and *Eucrangonyx* sp., and the repulsive looking " water louse " or " water slater " *Asellus aquaticus*, the appearance of which in a consumer's drinking water usually evokes bitter complaint. Growths of sponges (*Spongilla*), " pipe mosses " (*Polyzoa*), and of the so-called iron-bacteria, e.g., *Crenothrix* and *Gallionella* may also occur and are usually most troublesome on account of the obstruction they offer to the flow of water and by the corrosion they cause, especially in iron mains. To reduce the volume of complaint wire screens are sometimes interposed at some suitable point in the system, but this should be regarded as a somewhat undesirable and temporary expedient and steps should be taken to improve filtration. The presence of any of these creatures, including even the iron bacteria, in a system delivering water to the drinking vessels of the public is an indication of defect. Heavy chlorination of the filtered-water channels is an effectual method of ridding them temporarily of most of these growths.

8. ALGICIDES.

There are always likely to be times when, alternative sources of supply having failed, it may be necessary to use water containing large numbers of algæ. If the load on the filtration plant is likely to be severe, it may be advisable to consider whether important advantages will result from the use of an algicide. The decision can usually be made logically either if some alleviation of filtration difficulties *must* be had at any cost, or if information is available as to whether algal growths are likely to go on increasing. There would be much less justification for such treatment if there were signs that maximum numbers had nearly been reached (see page 52). In general, if an adequate knowledge of the plankton or algal periodicity is available, it will usually be possible and preferable to kill the objectionable algæ before they reach numbers which are likely to give difficulty. Experience suggests that this not only avoids difficulty but is also usually cheaper.

There is an important point in this connection which requires further practical investigation. If it is right to suppose that both the *rate of growth* and the *duration of growth* in an algal population are determined at an early stage in the growth cycle, then reduction of the algal numbers at an early stage would also reduce the *final* numbers. This would mean that smaller quantities of algicides would be needed as the poison could be applied when few algæ were present.

We may illustrate the argument by a purely hypothetical example. We shall suppose that the algal numbers were being doubled each week over a period of, say, seven weeks. Thus the actual numbers would increase as shown below if no treatment were applied. On the other hand, if in the third week, an algicide were added which reduced the number of cells to one quarter, its effect would be to reduce the *final* number to one quarter, *if the duration of the growth period were fixed in the early stages of the growth-cycle, i.e., before week three.* The following numbers illustrate the types of change that would be expected in such a case.

Week		1	2	3	4	5	6	7	8
Nos. of algal cells, millions per litre	untreated	0.25	0.5	1.0	2	4	8	16	dying
	treated	—	—	0.25	0.5	1.0	2	4	dying

The effect of the treatment in such a case, would be to reduce a large algal maximum to manageable proportions. Further, there will be less accumulation of organic matter in the water and in the bottom mud and consequently less material which might give rise to heavy growths in a subsequent growing season.

The use of algicides depends on certain other principles which are, perhaps, not always fully appreciated. When an algicide is employed its effects are twofold. It kills or damages most of the algæ and bacteria in the water. The soluble substances in the living cells escape to the water, which therefore becomes enriched by the addition of sugars, amino-acids and other organic materials of high nutritive value. In addition of course, the remains of the dead algæ will in time decompose to yield nutrient materials. Sooner or later these materials are likely to accelerate the growth of algæ, fungi or bacteria and particular attention ought to be paid to the possibility of their removal.

From this point of view, copper sulphate is one of the best of the algicides, not only because it is effective in small amounts and cheap, but also because it forms insoluble compounds with the nitrogenous constituents of the living cells as well as with other materials therein. In normal circumstances these compounds sink to the bottom of the reservoir and they are not immediately attacked and decomposed by living organisms. They do increase the organic content of the hypolimnion and of the bottom mud and eventually their breakdown, though possibly slow, will accentuate anærobic conditions in these places (page 10). The re-utilisation of the organic materials in the dead algæ is, however, deferred for some time.

Success has also followed the application of potassium permanganate or of activated carbon, the latter floating on the surface and acting partly as a screen to the penetration of light. These substances have other virtues in addition to their action as algicides. Potassium permanganate increases the rate of oxidation of dissolved organic matter in the water. Activated carbon clears the water by absorption of dissolved and of fine suspended materials. Both substances are perhaps less effective in inactivating the algal material after death than is copper sulphate.

The use of a coagulant, and particularly of alum, has also proved a valuable method of combatting algal growths in some cases. The most important requisite for success is likely to be a specially constructed sinking chamber and sedimentation basin. Without these, attempts to throw down an algal population appear to meet with very variable success. It has been found for example, that a change in the direction of the wind may prevent sedimentation, with the results that both floc and a large proportion of the algal cells are carried over to the filter, which clogs with extreme rapidity. The formation of a satisfactory floc depends also on the pH of the water. Though this will *usually* be favourable in a water of high algal productivity, the choice of a suitable coagulant should always be made after preliminary experiment.

In using algicides like copper sulphate some consideration should always be given to the conditions of light and of temperature. Both strong light and high temperature greatly increase the rate of absorption of a salt like copper sulphate. Hence the tendency now is, if the conditions are favourable, to use lower concentrations of algicides than were formerly recommended. (A list will be found in Whipple's book. *The Microscopy of Drinking Water*). When temperature and light are high—this has been thoroughly justified.

A good example of modern practice is the case of Lake Monona, along the shores of which the city of Madison, Wisc, is situated. Here, copper sulphate has been applied regularly for some years. (Domogalla, *Symposium on Hydrobiology*, Madison, Wisc., U.S.A. 1941, p. 305). In this case experience has shown that it is cheaper to kill the objectionable growths when they start, before the organisms have developed enough to form a dense, foul-smelling mass. The old bag-dragging method has been discontinued, and all the chemicals are now applied with power spraying equipment. It is possible to keep the lake free from bad odours and excessive growths for at least a month after the surface has been sprayed. In June 1940, for instance, following treatment with copper sulphate at the rate of .05 parts per million, there was an immediate reduction in the numbers of certain troublesome algæ and the improvement continued for some 20 to 30 days. The following table taken from Domogalla's paper shows the results obtained.

Lake Monona plankton counts before and after spraying with copper sulphâte, June—July, 1940.

(Composite samples over surface of lake ; number of organisms per litre of water).

Organism	Just before treatment June 18th, 1940	Time after treatment (on June 22nd).			
		5 days	10 days	20 days	30 days
Anabæna ...	117,000	55,400	29,800	22,700	30,200
Microcystis ...	76,200	39,000	30,200	42,600	150,500
Pediastrum ...	61,160	29,600	30,800	18,000	45, 600
Lyngbya ...	35,300	22,700	19,800	16,900	29,800
Staurastrum ...	28,200	19,900	15,500	11,300	20,200
Fragilaris ...	15,500	11,300	8,900	3, 400	12,700
Hydrodiction ...	10,160	7,100	3,400	8,900	20,800
Pandorina ...	5,080	2,200	1,600	2,800	3,400

Domogalla points out that " many of our obnoxious algæ can be kept under control with our power spraying methods with far less chemical than is prescribed in Whipple's '*Microscopy of Drinking Water*.' " With these smaller doses the zooplankton was abundant all summer and throughout the fifteen years of chemical treatment. The dose applied by Domogalla, equivalent to only one-twentieth of a part per million, is only one-tenth of that normally used. According to Whipple, two of the blue-green algæ treated in Lake Monona, *Anabaena* and *Microcystis*, require for eradication respective doses of 1.0 and 1.7 lb. of copper sulphate per million U.S. gallons. These amounts are two to three times greater than that used by Domogalla. It is possible that the observed differences in lethal concentration may be due to the blue-green algæ living in the top few inches of surface water, in which the concentration of copper, delivered from the spray, would initially be greater than the calculated concentration. For the diatom *Fragilaria* on the other hand this explanation seems unlikely, for not only is the lethal concentration, as given by Whipple, much higher (2.1 lb./million U.S. galls.) but also the diatoms do not rise to the surface as do the blue-green algæ.

The use of copper sulphate to kill algal growths is attended by a certain degree of risk owing to the fact that there are species of algæ which are highly resistant to copper (see p. 78). Experience has shown that the destruction by copper of one species of alga may be followed by " after-growths " of some other species, usually the smaller members of the Chlorophyceæ (Green Algæ). Such algæ as *Scenedesmus*, *Ankistrodesmus*, *Pediastrum*, *Oocystis*, *Crucigenia*, *Tetraedron* and *Chlamydomonas* are particularly likely to occur and these will increase rapidly in the presence of concentrations of copper sulphate in excess of the dose of half a part per million (0.5 pt. $CuSO_4$, $5H_2O$/million). After-growths however, do not invariably follow treatment, but the trouble is that there is no way of predicting whether or not they will develop. Hence the decision to apply copper sulphate is a difficult one to make. The danger is that the algaæ which may develop as a secondary result of treatment are usually minute and may pass through sand filters into supply, giving rise to complaints of green water. Certain of the after-growths have been shown to be extremely resistant to copper, concentrations of four times the normal being quite ineffective.

In these circumstances, it is preferable, wherever possible, not to dose a main reservoir, but to run or pump the water into a smaller basin, applying the treatment to the intake of the latter. This method is usually successful provided that the length of time that the treated water takes to traverse the smaller basin is sufficient for the dead algæ to settle to the bottom. Where a second basin is not available, treatment may be restricted to the outlet area of the main reservoir, using a boat. Success will depend upon the positions of the inlet and outlet relative to the prevailing wind, upon the area and shape of the basin and upon the rate of abstraction. Treatment applied at a point close to filter beds is unlikely to be effective for the reason that dead algæ will block sand filters no less than living ones and may, in addition, give rise to tastes and smells.

It must always be remembered that heavy additions of algicides are likely to affect not only conditions in the surface water of the reservoir, but also the algal and bacterial film on the surface of slow sand filters. As the use of compounds containing chlorine has become a common feature of water treatment, it may be advisable to consider some of the biological effects of chlorine at this stage, though this is commonly used as a bactericide rather than as an algicide.

Pre-chlorination.

That chlorinous compounds could be used against epidemic diseases was recognised more than a hundred years ago, but their regular use for water purification dates from the early decades of the present century and spread rapidly after the war of 1914-1918. Treatment of the water prior to its filtration, so-called " pre-chlorination," the more certainly to provide against the passage of pathogenic bacteria into supply, then became a well-established part of British and American practice. Among the advantages attributed to the method was the fact demonstrated by Sir Alexander Houston that, by removing the necessity for retention of polluted river waters in reservoirs to promote natural purification a great saving in fuel for pumping into the reservoirs was effected.

Houston's findings have since been amply confirmed and it cannot be gainsaid that, where there is a reasonable chance of pathogenic bacteria reaching the filters, prior treatment with a bactericide has much to commend it. The important thing to observe is, that unless the filters are to be operated as mere strainers, the dose of chemical in the water passing to the filters should be so adjusted, or other means should be taken, to ensure that the natural flora of the filter is not destroyed or its function will be seriously impaired. In theory, if a water has been stored for 30 days (actual and not nominal storage) there is little necessity for the application of a bactericide, but large storage reservoirs are subject to pollution by birds, and, although a reduction in the bacterial indicators of dangerous pollution is achieved by storage alone, the water reaching the filters may contain such numbers of bacteria that slow sand filters do not produce a filtrate in keeping with present standards. This is particularly the case where the slow sand beds are used in conjunction with primary filters, for the increased rates of filtration then practicable render the removal of bacteria less complete. Hence, the use of pre-filtration chemical treatment must always be related to the prevailing conditions. It has been found that a continuous concentration of the order of 0.2 parts of chlorine per million of water on reaching the filters may radically change the biological association of the surface of the sand. The most striking changes are in the algal flora, though important alterations occur also in the bacterial. The blanket-like algal association normally present may be destroyed and with it some of the filter's efficiency. Experience suggests, however, that especially if the weather is bright, other more resistant algæ are likely to develop on the surface of the filters, so that after a time their efficiency is recovered. An example of the effects of pre-chlorination is given in detail below (p. 77).

Treatment of a water with chlorine is often attended by the production of unpleasant odours and tastes. Chlorinous tastes are generally the result of excessive dosage; while chlor-phenol tastes result from the interaction between chlorine and aromatic hydrocarbons of vital or industrial origin. The latter may yield to treatment of the water with more chlorine. When algæ are involved the cause is usually the liberation of essential oils from the cells on their death and the condition may be made worse by the chlorine. Often too, trouble is experienced when aqueducts and filters are first treated because of the dislodgment of sessile plants and algæ from the sides, and because of the death of fish and other animals that accumulate down-stream or on the filters to undergo decomposition. Freshwater mussels in culverts are readily killed by chlorine and dislodged, to decompose at some point where the concentration of the bactericide may be insufficient to inhibit the process. This has caused tastes in the filtered water following the initiation of treatment but these have readily yielded to the application of activated charcoal. Once cleared of obstructing growths in this way no further troubles have been experienced and increased freedom of water movement has accrued. Certain other advantages, both in the aqueducts and on the filters, are often very marked. Where algal growths are heavy, the summer condition of filters receiving chlorinated feed-water and of those without it must only be seen to win approbation for pre-chlorination. Unsightly floating masses of decomposing algæ, plagues of midges and obnoxious smells are often a feature of slow sand filters where "pre-treatment" is not practised and algal growths are heavy. Then, too, fish avoid chlorinated water and the point of administration of chlorine acts as a barrier to their passage downstream. Hence, apart from any pollutive effect the fish may have there is less to attract gulls to the beds—too often from a nearby sewage farm.

An Example of the Use of Algicides in Filtration.

The diverse types of problem which may be involved in treatment are so varied that it is always difficult to lay down general rules. The following example is therefore given as a good illustration of the operation of algicides during treatment and as a contrast to their use in the storage reservoir.

The particular case is that of a filtering plant receiving water from reservoirs in the lower Thames valley. The water is chlorinated before filtration and this destroys the normal algal growth on the slow sand filter beds. A new growth develops, however, in which the principal element is macroscopically a green jelly. In this the living green cells, in groups or singly according to the physiological age of the colony, are surrounded by a clear gelatinous envelope of many times the volume of the cellular contents. In a young, actively growing colony the separate envelopes are discrete but in the mature state the boundaries are lost so that the whole becomes a mass in which the green cells are uniformly dispersed. At times, the gel may be rapidly dissolved and the cells liberated as naked, free-swimming individuals apparently indistinguishable from *Chlamydomonas* sp. The gelatinous form of such an algæ is often called its "palmelloid" form, after the resemblance to the genus *Palmella*, a type now known to include stages in the life history of very diverse types of green algæ. When this palmelloid form first develops on the surface of a filter little increase in head is necessary to maintain the flow during daylight, because of the buoyancy imparted to the alga by the vigorous production of oxygen in photosynthesis. Commonly, the growth then resembles filamentous fronds of seaweed attached by their base to the sand and extending sinuously into the water above. Often, large masses are detached in the way described earlier. Although growth is most luxuriant during the warm "algal season" an established colony is able to withstand severe conditions of water temperature and may even persist in water bordering on freezing. The soft, yielding colonies are not detached by currents of 1 mile per hour.

The association of the growth with pre-chlorination is doubtless a sign of a greater resistance in this organism towards chlorine than that shown by its competitors and it is likely that the gelatinous envelope is its most important defence. There is no detailed knowledge of the concentration of chlorine beyond which the defence breaks down ; a continuous concentration of 1.0 part per million of water has certainly been tolerated.

The growth may not at first create any greater difficulty in filtration than does the " blanket weed " it displaces. But, later in the run of the filter bed it may suddenly collapse and form an impermeable mass upon the surface of the sand through which the water cannot pass. At other times the growth may remain reasonably buoyant until the water in the filter is drained down, when it settles on the surface of the sand and forms what is virtually a water-tight seal, so that the last few inches of water can be removed only with difficulty. The growth itself when fresh cannot readily be removed by shovelling in the usual manner and it is often necessary to use a squeegee in order to drive it towards drains or to accumulate it in quantities convenient to handle. Speaking generally, if the weather is likely to be warm and dry during the twenty-four or thirty-six hours following the completion of drainage it is cheapest to allow dehydration to proceed, so that flakes which are easily shovelled are formed. When, however, the atmosphere is moist and likely to remain so for more than a day it is usually best to remove the growth as a sludge.

Because of these difficulties, the attempt was made to reduce or modify the algal growth still further by treatment with copper sulphate. It was found to be greatly reduced by an application of 0.5 part of the hydrated salt per million of water. At first this was applied continuously during the algal season. It required a year of almost continuous application of the stated dose before copper was detected in the filtrate of a slow sand filter. The copper was removed by the algal material and retained in insoluble form on the filter.

The water handled by the works under consideration is one of from 210 to 290 parts per million of temporary hardness and of pH 8 so that it would also tend to precipitate the copper as the hydroxide or the basic carbonate. It must not be assumed, therefore, that copper would always be removed in such quantities from solution in waters of different character. In this case the growth on the filter removes some of the copper by absorption but the greater part is held as particles of insoluble salt in the sand. Under these conditions it is easy to avoid the appearance of copper in the filtrate by careful regulation of the dose and period of administration. The accumulation of insoluble copper compounds in the filter bed suggested that a considerable saving might result if the bed was treated intermittently—a moderate amount of copper sulphate being used when it was first put into service. This is now being tried.

It might reasonably be expected that on the addition of 0.5 part of copper sulphate per million to a water that has but a short time previously received the same dose of chlorine it would be impossible for algæ to survive in it, but this is not so. An algal population still flourishes on the filter beds. The growth that develops is much more friable than is the palmelloid type previously described and it dries more readily when exposed to the atmosphere so that much less trouble is experienced during bed-cleaning. A large proportion of the growth consists of new dominants, particularly small algal species of *Characium* and *Scenedesmus.* But there is also another unicellular alga of palmelloid type in which the cells are now surrounded by much less of the gel and are grouped into packets or morulae. Photosynthesis does not now cause large masses of the growth to leave the sand and float on the surface of the water.

The benefits of the treatment are not, however, unaccompanied by disadvantages, for it is reported by those in charge of sand-washing that the algal growth now travels through the battery of washing machines further than the algæ commonly encountered. Care must be exercised also in adjusting the dose of the copper sulphate for the substance is bactericidal in fairly high dilution and the biological properties of the filter might be weakened, with the extra danger that the filtering layer may recede from the surface into the depths of the filter. One of the objects of the intermittent treatment now adopted is to reduce the danger of undue modification of the bacterial films in the sand bed. Rigid and continuous chemical and bacterial control of the filtrate is, of course, necessary during such experiments. It would be false economy to save labour on the filters, while allowing an increased possibility of the development of organic growths or the accumulation of silt in either the lower parts of the filter or the mains and supply channels.

9. CONCLUSION.

The problems discussed in the preceding pages serve to illustrate some of the biological problems most widely met with in dealing with water supply. There are many others which it has not been possible to mention in this brief survey. The difficulty of including them in a general outline lies partly in the fact that each source of water is likely to have a different range of problems and a different set of causal conditions. Further, there are many biological problems of economic importance in waterworks, for the solution of which the data are quite inadequate. This is largely because of the scarcity of routine information. In dealing with periodic and recurring biological changes, continuous records are of the greatest possible value. Not only are they the first requisite of enquiry into a particular case, but they also serve at a later stage to examine hypotheses of a wider nature. Also because of the absence of general data, few examples of practice are included and those that are given should be regarded as suggestive only, subject to the special conditions obtaining in the particular case and requiring during their inauguration the usual bacteriological and chemical safeguards.

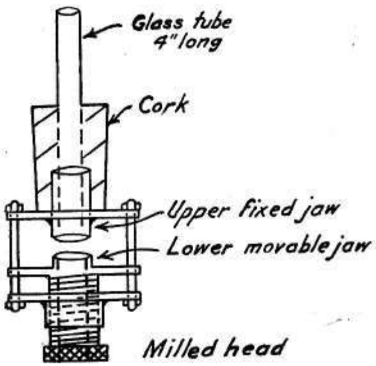
Many of the comparisons made in this pamphlet are good examples of the value of having parallel bodies of data from waters of types as different as those of the River Thames and Windermere. The pamphlet will have served its purpose if it makes possible the collection of a still more varied and wider range of data.

APPENDIX.

i. *Determination of Filterability*:

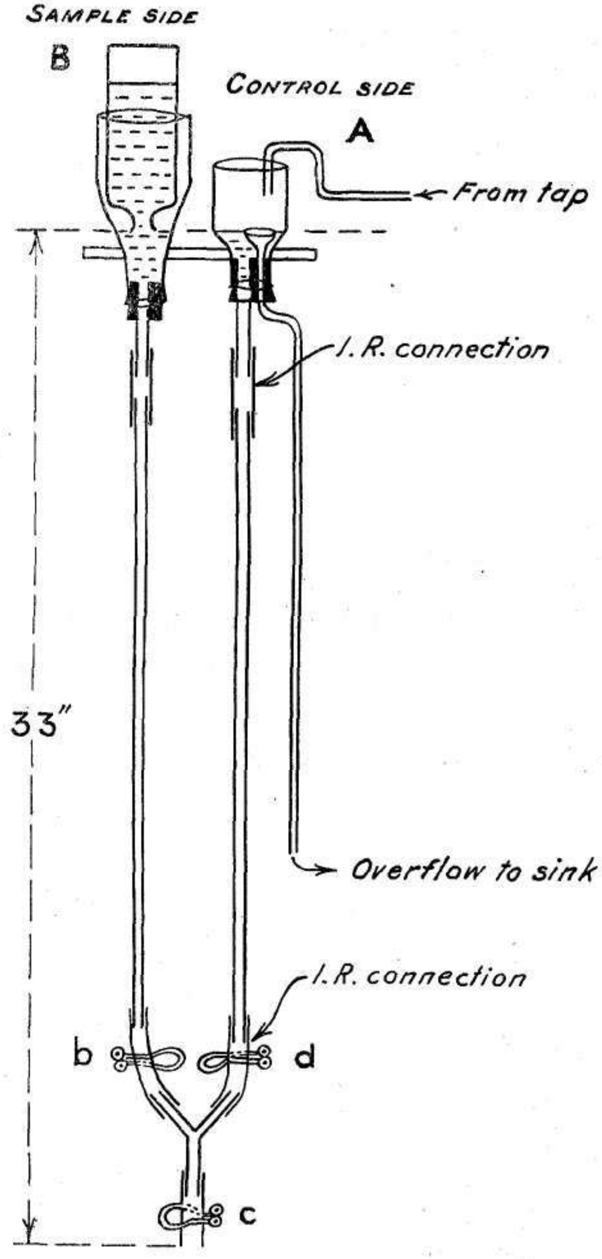
The object of the method is to obtain a measure of the degree to which a given stored water will clog sand filters. The general principle is to determine what volume of sample, delivered under constant head, will pass a small filter pad of linen and lint in unit time. The results are expressed as a percentage of the volume of tap (or distilled) water which has previously passed the filter pad in the same time and under the same pressure.

The apparatus is shown diagrammatically in Figure 15. It consists essentially of two open-ended containers mounted side-by-side at the same height above the bench. Each container is connected by lengths of glass tubing to a Y-piece, to the lower leg of which is affixed by means of a short length of rubber tubing the clamp, between the jaws of which the linen and lint filter pad is securely held. For details of the clamp see Figure 14.



14. Clamp for holding linen pad for filterability test. (Half size).

Container A holds tap (or distilled) water, container B the sample. A constant head is maintained in A by means of a wide-mouthed overflow, discharging to the sink. Tap water flows continuously into A. The same head is maintained in B by inverting a flat-sided sample bottle, of about 180 ml. capacity, into B. By opening and closing the pinch-cocks, water can be run from A or B as required. The volume of clear water from A, passing through the filter in one minute, is determined first and then the volume of the sample water (from B) which will pass through the *same* filter in a minute is estimated. The amount from B is then calculated as a percentage of A.



15. Apparatus used in determination of filterability.

The filter used consists of a strip of fine linen folded into four layers, between the second and third of which is inserted a piece of surgical lint. The lint serves to entrap algae that might pass through the linen. Even when these filters are made from the same larger sheets of material, their porosity is highly variable, so that each filter must be standardised for each sample by a separate run with the standard clear water, as described in the last paragraph.

The examination of the deposit upon the linen and lint is held to be an important part of the test. It provides a rough estimate of the proportion of silt or detritus to living algæ and enables seasonal changes in the qualitative composition of the plankton to be followed. The simplest way in which to make this examination is to separate the linen from the lint and to rinse the upper surface of the linen in a small drop of water placed upon a microscope slide. The lint may then be squeezed on to a second slide.

The great advantage of this method lies in its speed. The whole operation can be completed in about ten minutes, including the microscopic examination of the deposits on the linen. The actual filtration test only takes three minutes. On the other hand, there is no easily ascertained relation between the results of this test and the clogging of filters in the works. No doubt it would be possible to work out an empirical calibration that might be of service in strictly limited instances, but there are theoretical difficulties in the way, which depend not only on the fact that the linen filter is changing during the test as sediment accumulates, but also on the fact that different types of sediment affect the flow of water through the filter differently and in a manner not necessarily related to the effect produced on the work's filters. Lastly, of course, it is quite clear that while the mode of operation of this test may superficially resemble simple strainers such as some of the rapid filters used in waterworks, it has no resemblance either in size or in mode of action to the biological filtration carried out in a slow sand filter.

The numerical values obtained by the filterability method should not be used as estimates of algal abundance, firstly, because the method does not distinguish between living algæ and silt and, secondly, because equal numbers of different species of algæ do not clog the filter pads to the same extent. It has been found, for instance, that the diatoms *Asterionella* and *Melosira* and the blue-green alga *Aphanizomenon* in relatively high concentrations will give better filterability values than smaller numbers of a diatom like *Stephanodiscus*. The method, however, has proved of great practical value and for many years no small part of the day-to-day decisions taken in the Metropolitan Board to suspend the use of this or that reservoir have been based on this method.

ii. *Measurement of Suspended Colour* :

The same apparatus is used for this test as for the filtration of the algæ prior to extraction by acetone or methyl alcohol. (see iii). A circle of coarse filter paper, Postlip No. 633 Mill is a suitable grade, is held between the upper and lower containers in the manner described on page 86. Upon this paper is deposited a layer of a finely divided white powder and the sample is filtered under reduced pressure through this layer. An aqueous suspension of magnesuim carbonate, 8.0 g. per litre, is run into the upper container, no suction being used whilst the suspension is added. Suction is then applied and the water sucked through the paper, leaving a layer of magnesium carbonate evenly deposited upon the paper. As soon as the water is drawn off the magnesium carbonate, fill the upper container with a sample and keep full until all the sample has passed through. Then equalise the pressure, remove the paper with the deposited layer of magnesium carbonate plus suspended matter from the sample and lay face upwards upon a damp surface. The circle of paper is now transferred to a small square of wood or ebonite and held in position over the square aperture at the back of the Tintometer cabinet. The colour, composition and depth of tint of the deposit is now measured by reflected light. Full details of the use of the Tintometer instrument both for measurement of colours by transmitted and reflected light are given by the manufacturer of the apparatus.

A little practice is needed to avoid cracking the layer of magnesium carbonate before the first quantity of sample is added. If the layer is sucked too dry, the film may crack and if not dry enough a small amount of the magnesium carbonate may lift when the sample is first added. The same precautions must be taken at the end of the operation, for if, once more, the film is sucked too dry, it may crack. It is essential to run the magnesium carbonate suspension into the upper container without reducing the pressure. Clearly, if the pressure is reduced the water will pass through the paper so rapidly as to leave the magnesium carbonate deposited unevenly or in a heap.

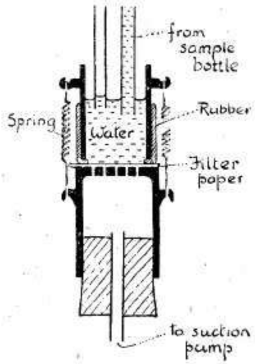
The volume of sample. As a rule 500 ml. of sample are sufficient. Volumes in excess of this may give colours too high to be easily read, but there is another reason why the volume should be kept low. The depth of tint of the coloured suspended matter increases linearly with added amounts of sample filtered until so much suspended matter has fallen upon the white surface that additional amounts merely pile up on those already deposited. As a result a curve relating depth of tint to volume of sample filtered ceases to be linear, but flattens. It will be wise, therefore, to make a number of preliminary measurements to determine the point at which the curve relating depth of tint to volume of sample departs from a straight line and to use a volume of sample of such size that the resulting colour of deposit falls within the part of the curve that is straight. A few determinations made at seasons of great algal abundance and at times when algæ are scarce, but silt present in large amounts, will indicate the volume of sample to be used in a particular water.

iii. *Description of the Pigment-extraction Method*:

Filter 1 or 2 litres of sample through a Whatman No. 42 paper, 5.5 cm. in diameter. Now immerse the circle of filter paper with the algæ deposited upon its surface in 5 to 10 ml. of acetone (or methyl alcohol), and leave in the dark for 12 hours. At the end of this period transfer the coloured extract to a 2.0 cm. cell and match the colour composition and depth of tint against the standard colours in the Tintometer. This instrument is manufactured by Tintometer Ltd., of Salisbury and has been found most suitable for making such comparisons.

Detailed description.

1. *Filtration of the sample.* This is done under reduced pressure. The circle of filter paper lies upon a flat-bottomed metal container, about 5 cm. in diameter and 7½ cm. in height. The flat bottom is perforated by a number of 1/33″ holes drilled through the bottom of the container. The lower end is plugged by a rubber bung through which a length of glass tube passes and this tube is connected to the vacuum pump.



16. Water filter used in pigment extraction method.

The circle of filter paper is held in position by a second metal container slightly smaller than the first, and open at each end. A rubber seating is made at one end by slipping over it a short length of the inner tube of a bicycle tyre. This rubber tube should extend a few millimetres beyond the metal tube, when the free end of the rubber tube will curl in towards the inside of the metal container, forming a water-tight seating. The upper and lower metal containers are held together by 3 rubber bands (or springs) stretched between lugs soldered to the walls of the two containers.

The water sample to be filtered is most conveniently contained in a narrow-necked flask, the open end of which is inverted into the upper container. Alternatively, a wide-mouthed bottle can be used, the mouth being closed by a rubber bung through which pass two short lengths of glass tubing, both of which dip into the upper container and regulate the flow of water from the bottle into the container on the principle of the "chicken feed." With bad samples filtration may take some time and an automatic filtration device of this kind saves time since it can be left unattended until the whole volume of the sample has been filtered.

2. *The method of extraction.* When all the sample has been filtered, separate the upper from the lower container and transfer the circle of filter paper with the deposited algæ to a small glass

tube, about 2½ cm. in diameter and 7½cm. long; add a known volume of solvent, usually 5 to 10 ml., cork and leave in the dark for 12 hours. The pigments present in the algal cells will have been more or less completely extracted during this time and the solvent will now be coloured. Pour the solvent into a small measuring cylinder and make up the volume to a known amount. There will usually have been small losses of solvent due to evaporation and the amount needed to make up the volume to 5.0 or 10.0 ml. can be used to rinse out the extraction tube.

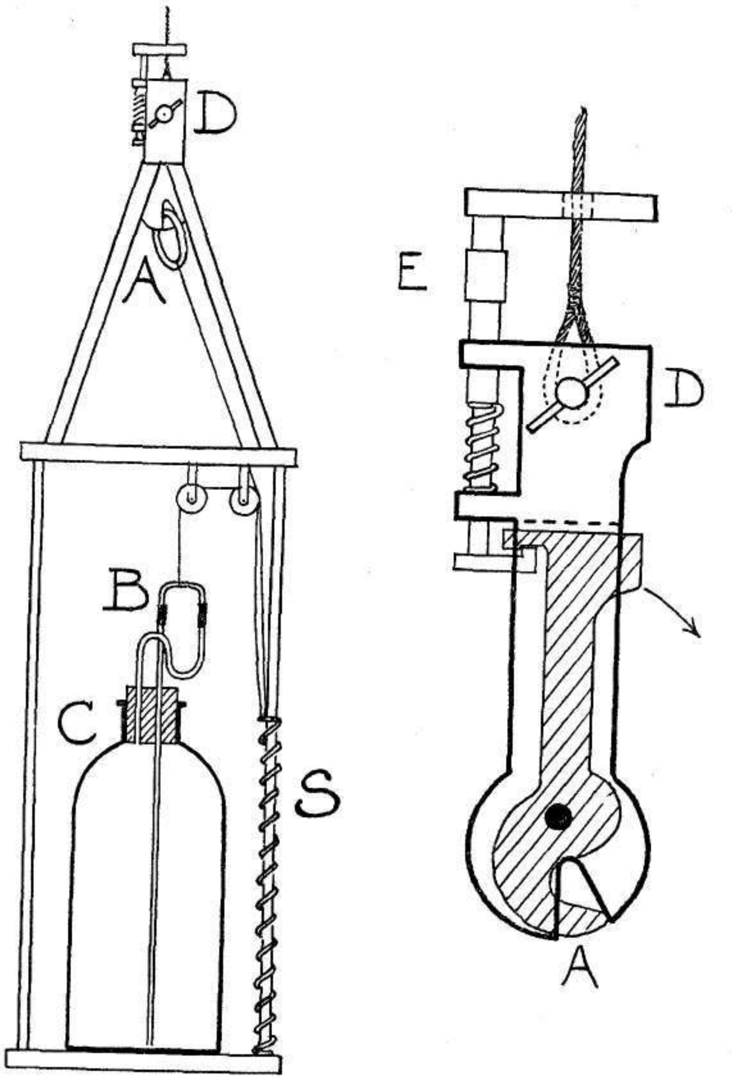
3. *The method of measuring the depth of tint.* Transfer the coloured solvent from the measuring cylinder to a 2.0 cm. Tintometer cell and measure the colour composition and depth of tint by transmitted light in the Tintometer.

4. *Expression of results.* The results are most conveniently expressed as Tintometer units per litre of sample filtered.

5. *Other information.* At times of very heavy algal growth, a volume of 1 litre may contain so many algæ as to give unduly deep colours, which are difficult to match against the Tintometer standards. In these circumstances use either a smaller volume of sample or dilute the extract to 10 ml. or more before reading. At times of very low algal production, on the other hand, it may be necessary to use a volume of sample in excess of 1 litre. At these seasons, however, it is unlikely that algæ in the reservoir will be of practical importance and the time spent in filtering larger volumes, which may have a high silt content and filter slowly, can probably be better employed.

iv. *Utermöhl Sedimentation Method:*

The principal requisites here are an inverted microscope and the sedimentation tubes. The latter are made from wide glass tubing, cut into appropriate lengths and ground level at one end. The ground end is then sealed with a microscope cover-glass. Canada Balsam, an adhesive of the Durofix type or one of the special glassware waxes of high melting point may be used to attach the cover-glass to the tube. The capacity of the tubes required depends on the richness of the algal flora. A tube of 1 cm. diameter and 4cm. length will be suitable for 1-2 ml. of water and one of double the



17. Apparatus for obtaining sterile water samples from known depths (left) and quick-release mechanism in detail (right).

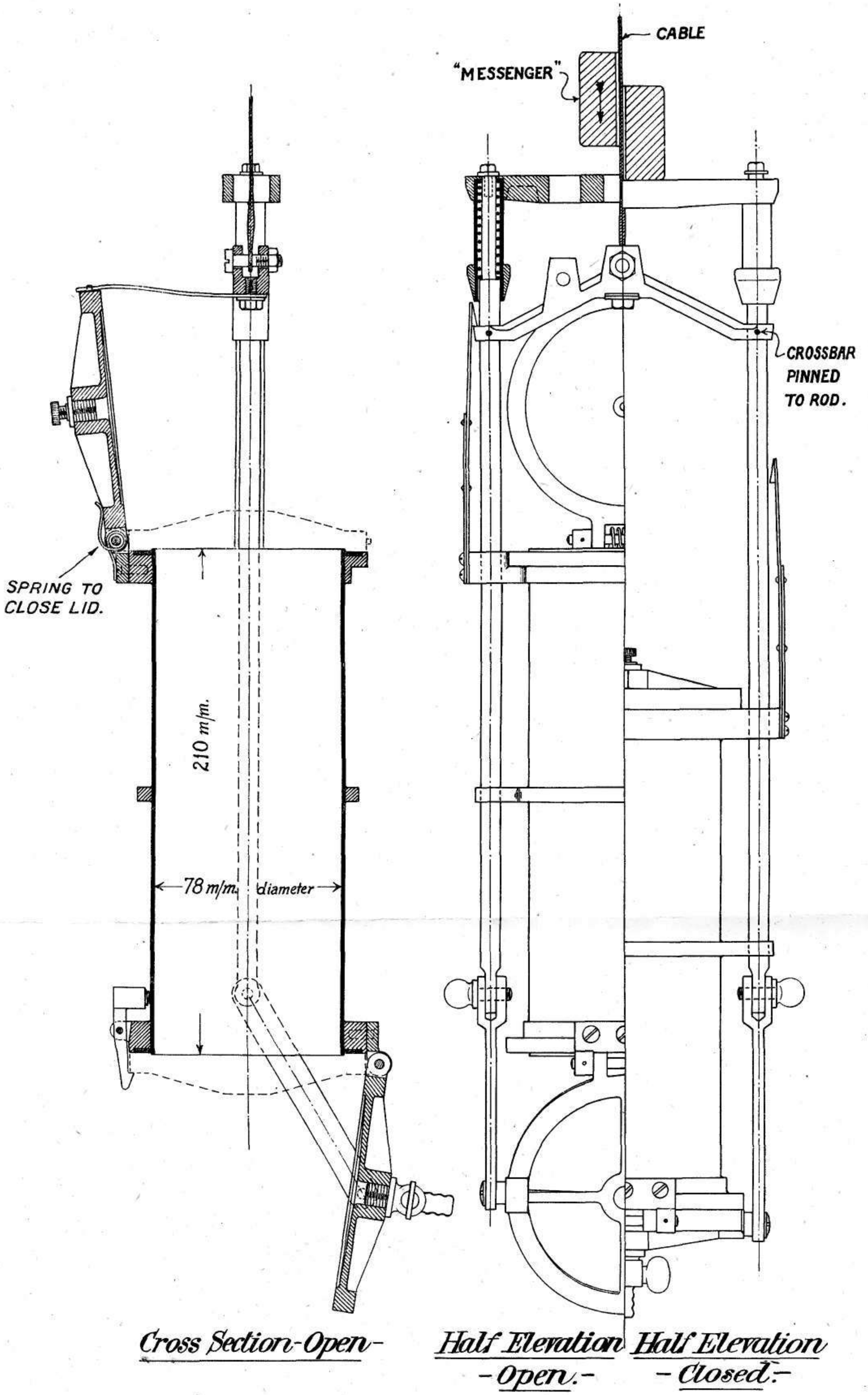
width and similar length will take 10 ml. These meet most requirements. The enumeration is carried out by first shaking the water sample thoroughly and then immediately withdrawing the required sample in a pipette, transferring it at once to a sedimentation tube. A drop of killing reagent is then added, a saturated solution of iodine in potassium iodide is suitable, and the tube is allowed to stand overnight. The numbers of algæ on known areas of the cover-glass are then estimated and the total number present is computed. It is useful to know the area of the field of view of the microscope used or to employ a micrometer eyepiece with squared units. Representative fields of view in all parts of the sedimentation cell should be included (see page 43) in counting.

v. *Water Sampling Bottles.*

The apparatus devised by Dr. C. H. Mortimer to take water samples from any convenient depth (see fig. 17), has the advantage for chemical and bacteriological work that the bottles used can be sterilised. It is simply a frame to which a bottle can be clamped (at C in the figure, where the clamps are not shown). The bottle is fitted with a stopper through which run two copper tubes, the shorter of which is bent as shown and the longer of which reaches to the bottom of the bottle. A small piece of glass tubing (B) makes a detachable connection by means of rubber joints. The spring (S) is extended by a cord terminating in a ring which is held in a quick-release catch at A. A cord of wire runs from the spring round the pulleys to B. When A is released, the spring contracts, jerking off the connection B. Water then enters the bottle, through the long tube, while air escapes through the short tube. When the bottle is full (in about one minute) an air bubble remains in the curve of the short tube and this prevents any further exchange of liquid between the inside and outside of the bottle.

The quick-release mechanism is based on one devised by Friedinger for use with closing plankton nets. It is shown separately in the figure, in which the metal base is drawn in heavy lines, the moveable parts in lighter lines. The actual catch of the quick-release is shaded, it pivots on a pin and is so balanced as to fall in the direction shown by the arrow, opening A. In the set position (as shown) it is held in place by a shallow lug on the arm

E. This arm can be moved up and down but is pushed upwards by the spring. A slight downward movement allows the catch to fall. Through an extension on the upper end of E runs the wire suspending the whole apparatus, which is lowered to the required depth from a measuring winch. The leaden messenger is then allowed to fall down the wire. It strikes E, forcing it down and so releases the catch, which falls, releasing anything held in A.



Cross Section-Open-

Half Elevation -Open.-

Half Elevation -Closed.-

18. Friedinger Water Sampling Bottle.

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