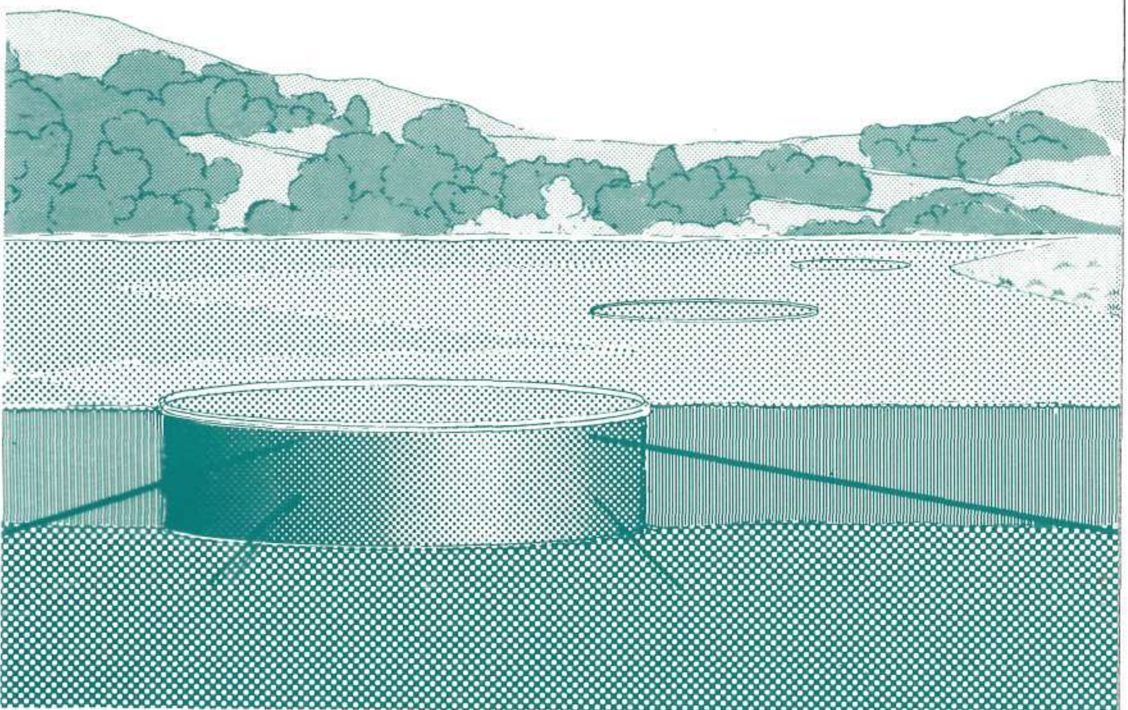
INVESTIGATIONS ON PHYTOPLANKTON with special reference to water usage

J W G LUND



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INVESTIGATIONS ON PHYTOPLANKTON WITH SPECIAL REFERENCE TO WATER USAGE

by

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PREFACE

The use of very large experimental enclosures as a means of studying natural phytoplankton populations was initiated by Dr J.W.G. Lund in 1970, when two cylindrical enclosures of butyl rubber, 46 m in diameter, 11 m in depth, each containing 18 000 m^3 of lake water, were installed in Blelham Tarn. With the inception of the 'customer - contractor' principle, enshrined in the Rothschild Report, some FBA research became commissioned and for a four-year period (1974-78) the Department of Environment partially funded the Lund tube algological studies under a contract (DGR/480/31) entitled "Experimental Lake and Laboratory Bioassay". The objectives of this contract were "to determine some of the major factors controlling the quality and quantity of phytoplankton and to study the causes and progress of eutrophication in lakes, as exemplified by those of the Windermere drainage basin". In 1978 Dr Lund retired but the Lund tube studies have continued to be partially funded by the DoE, under the direction of Dr C.S. Reynolds, and a third tube has recently been installed. A three-year contract (DGR/480/310) ran from 1977-80 entitled "Processes Controlling Algae in Lakes and Reservoirs", the objectives of which were "to quantify the effects of sedimentation, predation, decomposition, and oxygen depletion in the ecology and control of nuisance algae". This contract has been further extended to 1984.

Recent economies have prevented the DoE from publishing the Final Report of the 1974-78 contract. However, it has been mutually agreed that the results of this contract should be widely disseminated. In this way, reservoir managers and other possible users of these results may most benefit. The FBA *Occasional Publication* series has permitted this to be accomplished.

The Ferry House
March 1981

E.D. Le Cren
Director

ABSTRACT

Experiments and observations on the phytoplankton of certain lakes in the English Lake District were made from early 1973 to the end of March, 1974. They included laboratory and lake bioassays and observations on the quantity and quality of the phytoplankton in six lakes. The introductory sections of the report are about algae, the ecology of phytoplankton and the scope of the contracted work.

Laboratory bioassays on water from one lake, Blelham Tarn, showed that phosphorus, silicon (for diatoms) and organic substances forming complexes with iron were the major substances limiting the growth of the algae tested. The growth of the test algae was limited to different degrees by those substances and, to some extent, to a greater or lesser degree at different times of year. It is suggested that a relatively simple form of bioassay could give valuable information to water undertakings.

Lake bioassays and other experiments were carried out by using large *in situ* tubular plastic enclosures. Two such investigations are described and reference is made to others, some of the results of which have been published. Evidence was obtained supporting the view that the size of the spring diatom population is controlled by the supply of silicon and that additional enrichment of the lake with phosphorus would increase the abundance of gas-vacuolate blue-green algae which can form waterblooms. Thermal stratification also affects the size of the spring diatom population by affecting the rate of loss of cells from the well-illuminated upper layers of the water. This feature is more pronounced in the deeper Windermere than in Blelham Tarn. An attempt at producing a more natural system of fertilization by weekly additions instead of adding nutrients in relatively large doses on one or two days was not wholly successful, though natural phytoplanktons were produced in each enclosure and there were interesting differences between their waters and that of the lake water outside.

The effects of a change in sewerage in two drainage basins on the phytoplankton of three lakes is described and some data given about changes since 1945 in three other lakes in the same overall drainage basin. These latter lakes have been affected too by changes in sewerage and by increasing inputs of domestic and agricultural wastes.

Throughout, the relevance of the work done to practical problems of water usage is kept in mind and discussed. In the last section special reference is made to the largely unpredictable results of water transfers. The report ends with a note on river phytoplankton.

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1. INTRODUCTION

1.1. ALGAE AND THE ECOLOGY OF THE PHYTOPLANKTON

Phytoplankton consists of a variety of photosynthetic organisms multiplying in the open waters of lakes, reservoirs, rivers and seas. Algae form the major part of the phytoplankton and include both eucaryotic and procaryotic organisms. The blue-green algae (Cyanophyta or Cyanophyceae) are procaryotes and are now classed by some people as bacteria (Cyanobacteria). Other algae are plants in the ordinary sense of the word, for example the single-celled *Chlorella* or *Chlamydomonas* so common in sewage ponds and so often used in physiological, biochemical and genetical researches. Others show affinities with animals in combining photosynthesis with the ingestion of other organisms, including other algae, and some are animals in that they depend on the latter (holozoic) method of nutrition. Yet others are saprophytes (heterotrophs). Among eucaryotic algae there are species showing all transitional stages between autotrophic ('plant'), heterotrophic and holozoic ('animal') modes of nutrition. There are even single-celled species capable of carrying out all three nutritional methods. Algae may be parasitized by viruses, bacteria, fungi or protozoans. In addition, other bacteria and protozoans often occur on algal cells or in the mucilage surrounding them. It is not known to what extent these non-parasitic micro-organisms affect the growth of algae.

So far as water supply in the British Isles is concerned, it is photosynthetic algae with which we are concerned, though they are not all strict autotrophs, that is they may need organic substances (e.g. vitamins) or have some capacity for heterotrophic nutrition. Other types of algae scarcely ever cause problems.

The word *growth* is used here to include any process which leads to an increase in mass. Thus a multiplication in numbers may be termed growth, provided that enlargement of the daughter cells follows after their liberation. The growth of algae in a waterbody is affected by many factors, the more important of which are light, temperature, water movements, losses by sinking, gains by inflow and losses by outflow, the supply of nutrients, grazing by invertebrates and parasitism.

The multiplicity of interacting factors varying in importance in time and space and the diversity of the organisms called algae make an understanding of their ecology difficult and so pose problems for the water engineer and chemist to which biologists often cannot supply a wholly satisfactory answer. If all concerned with water supply and environmental matters who are not biologists were better aware of these facts, they might be less disappointed by what they sometimes consider the unjustifiable impreciseness of their biological advisers. There is now, thanks to modern statistical procedures and computing power, the technical basis for producing models on the basis of which some of the variations in phytoplankton in space and time can be explained or, to some extent at least, forecast. Examples are models of the general relationship between phosphorus and eutrophication and the control of the growth of phytoplankton by light, notably by its own interception of incident radiation (self-shading). However, our modelling capability has far outstripped our ability to produce more than very limited predictive models in which confidence can be placed. Hence the obvious need for better knowledge of the ecology of the phytoplankton. It will not be attained quickly because of this complexity of the natural world and the large number of algae in most planktonic assemblages. For example, in the phytoplankton of Windermere there are about 100 species which reach or exceed 1000 cells per litre at some time each year. Most of them are able to double or more than double their numbers in 24 hours under optimal laboratory conditions. Population increases in nature, from spring to autumn, not uncommonly equal 100 % or more per week. It follows that the potential for trouble that the water engineer faces is considerable. On the other hand, the Water Research Centre's recent survey (1) shows that the number of algae commonly causing difficulties is not large compared to the number of species which must be present in the reservoirs listed. It is interesting to note the similarity between this list and that of Lund (2) over twenty years ago.

If we are to understand potential algal threats to water quality, it is obvious from what has been said that the more we know about their inherent characteristics the better.

The practical problem for the water industry relates to the factors controlling the growth of algae in waters which enclose a natural world, even if they are built by man or have been altered by him. A fundamental approach is the study of the growth of a given taxon* in pure (axenic) culture of a given species using one or more genetically uniform populations (clones) in a defined medium, that is distilled or deionized water plus known chemical substances in certain concentrations which may also be supplied at known rates under controlled light and temperature conditions in the continuous culture technique (3). In this way the needs and productive capacities of species can be determined. Such investigations provide us with basic data for ecological studies. However, there is the practical difficulty of applying the results from this laboratory world to nature, where many algae, bacteria, fungi, crustaceans etc. are living in a largely uncontrollable and only partially definable world. The natural world changes constantly, sometimes erratically, and every water body differs from all others to a greater or lesser extent. Bioassay is an attempt to produce a test which combines simplicity and rapidity with a measurement of growth which provides information about the potential productivity of a water which is of practical value.

1.2 THE WORK DONE UNDER CONTRACT

From 1 April 1974 to 31 March 1977, the Department of the Environment (DoE) supported research on phytoplankton at the Windermere Laboratory of the Freshwater Biological Association (FBA). The main emphasis was on experiments on lake water enclosed in large plastic containers within a small Lake District lake, and on laboratory experiments (bioassay) using the same lake water and certain test algae. The full details of this work are being and, in part, have been published elsewhere. Selected aspects of the experimental work form the main part

* A taxon (pl. taxa) is a taxonomic category; for example, the word might refer to a species, variety or even an unnamed alga which is referred to by code letters or numbers. It is desirable that any cultured alga used for experiments is both named, if this is possible, and given a coding - e.g. *Asterionella formosa* Hass. L 187. A sub-culture of such an alga, if the results of investigations on it are published, should be offered to the Culture Centre for Algae and Protozoa, 36 Storey's Way, Cambridge, CB3 ODT.

of this report with special reference to questions of water supply, eutrophication and protection of the environment.

DoE also supported on-going monitoring of the phytoplankton of certain lakes in the Windermere drainage basin. This work began in 1945 as a background to other investigations and later was continued as a separate programme because it was thought that weekly sampling over many years would provide a unique historical record and reveal gradual changes, especially those consequent on the possible or probable enrichment (eutrophication) of Windermere, Esthwaite Water and Blelham Tarn (fig. 1). Increasing general affluence, tourism and more productive farming would increase the output of domestic and agricultural wastes.

Less studied were Grasmere and Rydal Water (fig. 1) but there were sufficient data to compare the phytoplankton before and after a change, in 1971, from septic tanks to mains sewerage. Elterwater (fig. 1) had very rarely been sampled before the DoE contract started but it was known to be similar to the pre-1971 Grasmere and Rydal Water before a similar change in sewerage in 1973.

In relation to the monitoring studies of Windermere, Blelham Tarn and Esthwaite Water, there were water analyses for phosphate, nitrate and silicate and, for some years, total phosphorus, total iron, total manganese, ammonium nitrogen, alkalinity and pH. Chemical analyses were not carried out every year of sampling of Grasmere, Rydal Water and Elterwater. Temperature and dissolved oxygen were measured. The methods, with one exception, and equipment used, together with bathymetric and other details of the lakes can be found in the references cited. A method devised for estimating the length of *Oscillatoria* and other filamentous algae has not been published but does not differ significantly from method 5 of Olson (4).

Support for monitoring was given at first by the Development Commission, then by the Natural Environment Research Council (NERC) and DoE.

2. COMMENTS ON THE THREE MAIN TYPES OF INVESTIGATION.

2.1. LABORATORY BIOASSAY

2.1.1 *Description of batch assay*

This bioassay in its simplest form is a method of assessing or comparing the potential fertility of water bodies by growing algae in flasks containing treated or untreated water from them. Untreated samples from the waters concerned can be used so that the growth of a mixed population of algae is estimated. Alternatively, a single species, previously grown in unialgal (not bacteria free) or axenic (no other organisms present) clonal culture, can be grown in filtered samples of these waters.

A comparison can also be made between the growth potentials of the waters and the original concentration of nutrients, for example phosphate phosphorus or nitrate nitrogen, in those waters. In many oligotrophic waters a general but not necessarily exact relationship can be found between either the concentration of total phosphorus, phosphate phosphorus or both and the algal growth potential. However, such a simple relationship cannot be expected to hold generally or in a given water body all the time.

2.1.2. *Laboratory assays using an assemblage of algae*

The variations in the growth of different algae in relation to the 16 or so elements they need, to their ability to obtain them from different ionic species or via certain other sources (e.g. chelating agents for certain metals) and the need of some algae for organic compounds (e.g. vitamins) suggests that bioassay using a single test alga will be less satisfactory than one using a mixture of species. The most natural test for phytoplankton might seem to be to take samples from the waters concerned and incubate them in the laboratory at selected temperatures and conditions of illumination. Since counts of cells or plants of different size would not give comparable values for the amounts of algal matter produced, the results usually are given in terms of chlorophyll *a*, dry weight or optical density. The figures obtained commonly are referred to as measures of biomass, though only dry weight conforms strictly to the literal meaning of the word biomass. Wet weight cannot be estimated accurately, though it is sometimes used by assuming that the specific gravity of algae is equivalent, or closely so, to that of water, the algal numbers first being transformed into volumes. Since this involves an assumption about the specific gravity of algae which has not been substantiated, there seems little justification for this practice. Moreover, there can be considerable difficulty in estimating the volume of an alga accurately.

A bioassay using a mixed population is not without value but has certain disadvantages. Since the samples tested are small, one or more may differ significantly in the relative abundances of the algae, animals or parasites present. If a large enough number of replicate tests on each water are made, this problem may be overcome, though determining what is sufficiently large replication might be troublesome. For example, a crustacean such as *Daphnia* might well be present in such numbers that only a few (e.g. one in ten or more) 100 ml flasks would contain one or more specimens. A single *Daphnia* could have a very significant grazing effect. The same problems could arise, though probably to a less extent, with large algae containing many cells, for example *Microcystis* and other colonial or filamentous forms. Certain algae, for reasons which are not always understood, grow better than others in small flasks, for example small coccoid green algae and small diatoms belonging to the genera *Synedra*, *Navicula* and *Nitzschia*. These may not, indeed probably will not, be the major species in the sample when it was taken or of the phytoplankton at any time. Hence, though a measure of algal growth potential is obtained, it will tell those concerned with the ecology of the phytoplankton or those concerned with water supply little about the species which may be troublesome. In addition many, but not all, such small algae are more heavily grazed by any rotifers or crustaceans present than are the larger algae.

2.1.3. *Assays using single species*

For these reasons and others, in the majority of experiments using this now very popular type of test, a single alga is used, and the water sample is filtered before its addition. Using a single alga, the population added can be of a closely similar nature each time, since the alga can come from a clone cultured in a given medium and in a given part of its growth curve, normally the exponential phase of growth. Provided the same clone is used, comparison of waters even on a worldwide basis is possible. The most widely used species is *Selenastrum capricornutum* Printz* and a clone or clones isolated by Dr Skulberg of the Norwegian Water Research Association.

2.1.4. *A suggestion for water authorities and other bodies*

Other forms of bioassay are possible, for example, tests using continuous cultures (3). (This book can be consulted for a general understanding about the growth of phytoplankton.) All forms of bioassay can be criticised but, in my view, a commonsense approach for dealing with practical problems is to use as test algae species appropriate to the questions to be answered, the facilities possessed and specialist advice obtainable. Clearly these are likely to be species known to be or likely to be troublesome. A wide variety of algae can be cultured and, if necessary, a culture collection such as the Centre for the Culture of Algae and Protozoa at Cambridge can supply most of them. The FBA has carried out most of its work with the diatom *Asterionella formosa* Hass. because of its importance in the lakes studied; it is also a waterworks' pest (1, 2). The use of a planktonic green alga, a diatom and a blue-green alga will give a useful idea of the potential fertility of a water in relation both to water supply and environmental problems.

* This species has not been recorded in British waters, so that it might seem preferable to use another coccoid green alga for bioassay. There are many similar algae available, for example in the genus *Ankistrodesmus*, into which some taxonomists would place *Selenastrum*, or *Monoraphidium*, used in our bioassays, which virtually is *Ankistrodesmus* without its enveloping mucilage. It may well be that Printz's *S. capricornutum* is not a good species for it is not accepted by some specialists, though they are not all agreed as to what should be its correct name or taxonomic position. It is possible that *S. capricornutum*, under other names has, in fact, been found more frequently in Britain than the lack of records suggest, being listed under other names.

It is useful to know what is the potential maximal production in a water but in practice this is not as easy as might be thought. The final stages of growth may be slow and a similar period of incubation cannot be used for each water or the same water at different times of year. For example, in a water rich in nutrients the test alga may be able to produce such large and dense populations that the penetration of light into the flask will decrease markedly during the later stages of the growth of the population. Indeed, the maximal size of the population may be determined by light penetration and not by the availability of nutrients. In a water poor in nutrients the maximum may be reached much earlier and may not be detected before the population begins to die.

An alternative approach is to measure rates of growth in such a bioassay; this means determining the rate over the exponential phase of growth. It does not necessarily permit measurement of the maximal population. In practice, in virtually all batch bioassay procedures, there is a compromise in that the period of incubation may be long enough to include more than the exponential phase, but long enough to measure the maximal growth only in waters which are so poor that it is reached relatively soon. Arbitrary though this proceeding is, it permits a similar period to be set for all the tests (e.g. 7 days) and clearly distinguishes between oligotrophic and eutrophic waters, though it may fail to distinguish the degree of richness of the latter. Comparisons of such rich waters can be made if the samples are diluted with distilled or deionized water. Probably distilled is preferable to deionized water because of the possible importance of organic compounds.

The method can also be used to find out the effects of adding nutrients to the filtered waters. This may tell us the potential dangers of enrichment from various sources. It may also show that the growths of different algae used as test organisms are limited to different degrees by different nutrients, so producing evidence about quality as well as the quantity of algal production. Examples are given later.

In general, bioassay is a useful test but care must be taken in extrapolating the results of such tests into prognostications about water quality. The dangers of excessive confidence placed on laboratory bioassay will be much lessened if there is observational and analytical knowledge of the water-body concerned.

2.2. FIELD BIOASSAYS, WITH SPECIAL REFERENCE TO LARGE TUBULAR ENCLOSURES

The limitations imposed by the use of small containers can be overcome partially by using larger ones in the laboratory but only if their volumes are at least one or two orders of magnitude larger than usual. This poses spatial and manipulative problems when culture chambers have to be used. It can be easier, cheaper, can provide more material and permit whatever replication is wanted, if containers of polythene tubing are suspended in a water-body and this has often been done. Tubing of moderate diameter, for example one metre, is available commercially and usable, but large polythene tubes are likely to need protection and special support. Further they may have to be made in the laboratory instead of being prefabricated in a factory.

Tubes of moderate diameter are satisfactory for bioassays or field experiments lasting a week or more but usually not for experiments lasting months. The volume of enclosed water for a bioassay, using a selected test alga, is obviously limited by the practical problem of filtering it. The same problem arises when studying a planktonic assemblage as with laboratory flasks, namely that the phytoplankton is no longer a completely natural one, or is replaced by a benthic population. Grazing problems are likely to be reduced because the larger volumes of enclosed water will include more representative populations of invertebrates, though this is not to assume that grazing can be considered fully without reference to predation, including that by fish on invertebrates.

The dominance of attached (benthic) or planktonic algae depends mainly on the ratio of wall-surface to enclosed volume. For example, the ratio of wall area to volume of a tube one metre in diameter and 10 m long, or, if fixed vertically in a lake, 10 m deep, is 3.0 m^{-1}; but for a tube 40 m in diameter and 10 m long or deep it is 0.1 m^{-1}. It was experience with small tubes such as the former, followed by tests with larger ones, that led to the use of very large tubes, 45.5 m diam., reaching the bottom of the lake concerned at between approximately 10.5 and 12.0 m depth and containing over 18 000 m^3 of water, and with a ratio of area of wall-surface to volume of 0.09 m^{-1}. The nature and functioning of these 'lakes within lakes', which are open at both ends, though the lower end is embedded in the mud, and the results of some observations and experiments, have been described (5, 6, 7, 8, 20).

It is common practice for industry when formulating a new process or plant to proceed from laboratory studies or small-scale tests to the erection of a pilot plant. The use of experimental basins, or the FBA's large *in situ* enclosures, or the circulating streams (9) are analogous in that they are methods for proceeding from experiments in laboratory containers and conditions to the environment of a lake, reservoir or river. Advantages and problems involved in using the FBA type of tube have been discussed in the papers mentioned. Here, the following features are mentioned.

1. The presence of natural populations, that is, populations consisting of algae and other organisms which are also present in the lake, including, if desired, fish.

2. A closely similar pattern of stratification to that of the lake water. Turbulence will not be the same inside and outside but the strong yet flexible nature of the plastic used, butyl rubber, is an advantage in transmitting externally-applied forces. Algae with life-cycles including growth in the open water, sinking, perennation on or in the superficial deposits and resuspension have the same phases within the tubes as in the lake, though sometimes with phase lags.

3. The freedom from gains or losses by inflow or outflow and so the removal of the most difficult parameter to estimate accurately in nutrient or algal budgets. Arrangements can be made to incorporate inflows and outflows, as has been done for the Water Research Centre's tubes and those in the Netherlands (10, 11).

4. The reduction of areal sampling problems, though this can also be considered as a weakness of the system. Vertical stratification remains but there is the possibility, not yet tested, of artificial mixing. Horizontal stratification can also be more important in natural water-bodies.

5. The ability to carry out experiments on a seasonal or longer term basis.

2.3. OBSERVATION AND ANALYSIS

Observation and analysis are the bases of our knowledge of the natural world because they tell us what is in it, how much and how it varies in time and space. This knowledge, in turn, is the basis for determining questions to which an answer may be obtained by experiment. If observation and analysis can be continued at suitable intervals for long periods there is the possibility of following gradual changes which might otherwise be missed and which may give warning of future events. This historical background offers a basis for considering or investigating what may be the causes of change or of stability and relating present and past production to chemical and biological traces preserved in the deposits.

Since, in most cases, water undertakings and similar bodies do not, as yet, have the time or staff to carry out much experimentation, some limited observation and analysis of their waters is very valuable and need not throw a heavy extra burden on them. In fact I believe that there is a large amount of unpublished and even unsorted information on phytoplankton in their archives. A record of such information kept at some suitable centre and available to biologists, with permission, could be of considerable value.

Permanent research institutes like the FBA have the continuity, opportunity and facilities to make relatively detailed observations over long periods and to relate these to known environmental changes, for example in land use and sewerage.

Ideally, observation, analysis and experimentation go hand-in-hand and if favourable conditions for research exist, as they do in the FBA, this can happen. However, in general, a balance has to be struck between the emphasis laid on and time allotted to each aspect. There is a danger of a monitoring programme enlarging, developing its own momentum and even breeding further such programmes without sufficient thought as to where it is going and for what reasons.

Monitoring also involves decisions about methodology and so standardisation. Standardisation without allowance for change is a recipe for fossilisation. More important is comparability, the ability to relate the results obtained by the use of one, maybe 'standard', method to another.

This discussion of methods of investigating phytoplankton only refers to those carried out under this contract and so is both partial and imperfect. Some results are now given.

3. EXPERIMENTS ON THE PHYTOPLANKTON OF BLELHAM TARN

3.1. LABORATORY ASSAYS ON WATER FROM BLELHAM TARN

This is a small lake (fig. 1), the phytoplankton of which has changed during the last 25 years from the effects of increased inputs of agricultural and domestic wastes (6, 7, 8, 12). Other biological changes are described in Macan (13). The bioassay method used is described in Lund et al. (14) and some results of work done in Lund et al. (15), using *Asterionella formosa* as the test alga. Assays have also been made with the green alga *Monoraphidium* sp. (previously called *Ankistrodesmus*) and less frequently with the diatoms *Fragilaria crotonensis* Kitton and *Tabellaria flocculosa* (Roth) Kütz. var. *asterionelloides* (Grun. in VH) Knuds. (*T. fenestrata* var. *asterionelloides* Grun. in VH). Dr J.D. Box carried out similar bioassays on *Microcystis aeruginosa* Kütz. emend. Elenkin in our laboratory, during the period of this contract but not supported by it (16). He also used water from the nearby lake, Esthwaite Water, which is similar to but more eutrophic than Blelham Tarn.

On the basis of the 33 years' observation of local lakes, growing the alga in culture and preliminary tests, the hypothesis was erected that the major elements limiting algal growth of *Asterionella* and perhaps other algae in Blelham Tarn would be phosphorus, silicon and iron. The results of four years' tests on *Asterionella* in filtered lake water have been published (15) and the results of less frequent bioassays since then have shown the same seasonal pattern. There is good growth from about November to March and, with a few exceptions, poor to very poor growth for the rest of the year (fig. 2). This pattern is similar to that in the lake, making allowance for the fact that physical conditions will restrict growth severely in nature in midwinter. This pattern of response is almost the exact opposite of that of *Microcystis aeruginosa* in filtered water from the same lake (fig. 2) and from Esthwaite Water (16). It grew better in summer and autumn than in winter and spring, even though it was always grown at 20 ± 2 °C and in continuous illumination. The results for *Monoraphidium* (fig. 3) parallel those for *Asterionella*, though it did not grow as well in the unenriched filtered water despite the fact that its potential growth-rate is similar to that of *Asterionella*. In 20 comparisons there were only two occasions when the growth of *Monoraphidium* was significantly ($p = 0.95$) better than that of *Asterionella*. If phosphate was added to the filtered water, both algae grew better on most occasions but their growth relationships to each other were reversed. In 19 tests *Asterionella* grew significantly ($p = 0.95$) better than *Monoraphidium* on only two occasions.

The inflows to Blelham Tarn also were investigated using *Asterionella* as the test alga. Fig. 4 shows the results for two major streams, Fishpond Beck and Ford Wood Beck. The former drains poor agricultural land and mixed woodland; the latter poor agricultural land and some relatively good, fertilized grassland. In addition Ford Wood Beck receives the outflow from a trickling filter treating the sewage from about 50 to 100 people depending on the season. Analyses show that Fishpond Beck has less phosphate and nitrate than Ford Wood Beck, but about the same amount of silicate, or at times more (see 6, 7). Filtered water from Fishpond Beck always supported less growth of *Asterionella* than that from Ford Wood Beck.

It seems that the FBA bioassay system can, like the widely used Skulberg and Algal Assay Procedures (17, 18, 19), be used to determine the relative potential fertilities of waters. However, bioassay is of little value if all it does is to produce results supporting suppositions based on those chemical analyses which it is routine practice to make. Nevertheless, support for predictions made from the results of such analyses may be necessary and it is not solely the concentration of a nutrient present at a given moment which is crucial but also the rate at which that nutrient is supplied and the efficiency of an alga in obtaining and utilizing it. Further, routine analyses are not always determined to low enough levels of concentration, though the position is improving. For example, it used to be common not to estimate PO_4P below 10 $\mu g\ l^{-1}$ which potentially would support 160 million *Asterionella* cells l^{-1} and, in turn, would cause very severe troubles in virtually any filtration system in the U.K. In fact, for a variety of reasons, notably reduced light penetration, lack of silicon, losses by sedimentation and the presence of other algae, such a large population has not been and is unlikely to be recorded. Nevertheless, winter phosphate maxima of 10 or less $\mu g\ PO_4P\ l^{-1}$ have been followed by diatom populations of 15 - 20 million cells per litre in our Lake District lakes, that is to a level likely to cause filtration difficulties.

The bioassays on water from Blelham Tarn (15), Esthwaite Water and Windermere (unpublished results) have indicated that iron can be a limiting factor. Standard analyses for iron give virtually no indication of the amount available to algae because the main part is in highly insoluble ferric hydrates. All modern experience in culturing algae points to iron and certain other metals (trace elements) being obtained via organic complexes, ethylenediamine tetraacetic acid (EDTA) being the complexing agent commonly supplied in the tests. In the investigation referred to, good to very good growth of *Asterionella* was obtained in filtered lake water when silicate, phosphate and the ferric complex of EDTA was added. This is an example of the fact that bioassay may supply information about the fertility of water not obtainable by routine analytical procedures.

The work of Box (16) on *Microcystis* shows that its growth in Blelham Tarn water is governed by a different nutrient factor or factors from *Asterionella formosa*. What the cause is we do not know, though Box suggests that it may be that the spring algal maximum, and so possibly that of diatoms, in some way alters the water so that it becomes more favourable to *Microcystis* than it was in spring, despite the fact that the concentration of phosphates (and nitrates) has decreased. He also found that EDTA increased its growth irrespective of whether it was supplied uncomplexed with iron. The effectiveness of EDTA alone may be because it complexes iron present in the lake water. On the other hand, the addition of EDTA alone to *Asterionella* in our tests sometimes produced good growth and at other times did not, whereas the ferric complex of EDTA always supported good growth, provided, of course, that other essential nutrients, such as phosphorus and silicon, were not limiting growth. The addition of nitrate, provided EDTA and phosphate were also added, increased the growth of *Microcystis*. The only time when adding nitrogen has been found to increase the growth of *Asterionella* is when its concentration has fallen to very low levels in a dry summer period (e.g. 1976). Bioassay also showed that *Monoraphidium* and *Asterionella* were affected to different degrees by added phosphate. Though it is necessary to be cautious in extrapolating results obtained from laboratory bioassay to the complex world of nature, it does indicate both quantitative and qualitative possibilities and that water may be more favourable for one alga than another at different times of year.

3.2. EXPERIMENTS IN LARGE ENCLOSURES (TUBES) IN BLELHAM TARN

3.2.1. *General background*

During the period of the contract there were two enclosures in the tarn (fig. 1); in 1978 a third one was placed in the lake. These tubes are described in (8) and only some general features have been mentioned again in 2.2.

It was shown (5, 20) that when water was enclosed in the tubes and so unaffected by inflowing nutrients, it rapidly became oligotrophic and remained so for over a year. Part of the nutrients which enter a water body is incorporated into organisms living in it, part is lost by the outflow which includes organisms suspended in it, and part, largely in the form of organismal remains, is incorporated into the deposits. The nutrients regenerated from the decomposition of the organic remains in the deposits are, in part again, returned to the water column when thermal stratification breaks down in autumn or, in our climate, occasionally after a prolonged period of ice-cover and inverse stratification. Clearly, such decomposition and recycling of nutrients will be the more, the greater is the input of nutrients from the drainage area and so growth of organisms or, as the word is commonly used to-day, the greater is the eutrophication. In time, enrichment of the deposits may become so great that if the external source of this eutrophication is removed or substantially reduced, the water will remain rich in phytoplankton for years because of the recycling of the rich store of nutrients in the deposits. The small return of nutrients from the deposits in the tubes was evidence that the tarn had not reached a highly eutrophic state (20). Since that first experiment, the tube waters have been fertilized so often in experiments that it is now uncertain whether a similar experiment to the first would lead to a rapid return of the water in the tube to an oligotrophic state.

An important factor determining the size and nature of phytoplankton populations is retention time. So long as there is no thermal stratification, a knowledge of the rate of outflow may be used to make an approximate estimate of the loss of cells in unit time. When the lake is stratified this is not possible because of the difficulty of determining the distribution of the inflows in the water column and because algal numbers are also vertically stratified. If the inflows pass into the epilimnion of a lake such as Blelham Tarn they also pass into the zone where most of the algae are likely to be situated for most of the time. Hence, dilution and subsequent loss may be roughly proportional to the ratio of inflow to epilimnetic volume. If the water passes into the hypolimnion, the loss of cells will be nearer to the number in an equivalent volume of epilimnetic water, lifted up as it were and passing into the outflow. An accurate estimation of the loss of algae can only be made by direct counts in the outflow and measurement of its flow over the period concerned. The average retention time of Blelham Tarn is short, about 7 weeks. It may fall to about ten days during floods or become virtually infinite during a long drought. If retention time is short, losses of algae by outflow can reduce the population or its rate of increase. On the other hand, the basic source of nutrients is the drainage basin, so that a long retention time can increase the rate and degree of nutrient depletion. This aspect of the lake's ecology is illustrated by tube experiments described in 5 and 6. In relation to nutrients controlling the growth of the tarn's phytoplankton, the following experiment was carried out.

3.2.2. *Silicon and phosphorus and the spring diatom maximum*

Lund (21) found that the spring increase in the population of *Asterionella* in Windermere ended when the concentration of soluble reactive silicon (SRS)* fell to about 200 $\mu g\ l^{-1}$ and continued to fall during the maximum and decline of the population to about 100 $\mu g\ l^{-1}$, and suggested that lack of silicon was the cause of this decline. Improved analytical techniques (e.g. 22) and experiments (23) have shown that *Asterionella*, as well as other diatoms, can continue growing at lower concentrations of SRS. In Blelham Tarn the spring growth of diatoms can reduce the SRS from about 1000 to 5 $\mu g\ l^{-1}$†, representing the utilization of over 99% of the SRS originally present.

* In this and other papers I have used the 'chemically unrealistic convention' (22) of expressing SRS in terms of SiO_2 because the walls of diatoms consist of opaline silica. SRS is chiefly silicic acid.

† The minimum value may be less than this.

It seems that, apart from the less sensitive analytical method used, relatively high SRS concentrations then found at the time of the *Asterionella* maximum in Windermere were caused by the increasing rate of loss by sedimentation of cells from the productive zone during the latter part of the 'bloom', since, unlike Blelham Tarn at the present time, thermal stratification is well established in Windermere before the spring growth ends. Evidence for this loss by sinking can be found in 24 (fig. 9, see also 25, 26, 27). The experiment now to be described also illustrates the importance of losses by sinking.

The concentration of phosphate also had fallen to very low levels (1 µg PO_4P or less per litre) at the time of the diatom maximum. Since a cell of *Asterionella* consists of more than a thousand times as much silicon as phosphorus, the question arises as to which is the major limiting element, if indeed it is one of these two and not some other element for which there are no data.

In 1973 an experiment was carried out in the tubes to try and answer this question. The tubes, which had been open to the lake water, were closed when the SRS concentration in the lake water had fallen to 20 $\mu g\,l^{-1}$. At this concentration, taking an average value for the silicon content of *Asterionella*, there was sufficient silicon in the water to produce about 3×10^5 cells l^{-1}. Since the tubes were closed and little silicon comes from the deposits under aerobic (mixed) conditions, the rapid decrease typical of the end of the spring growth of *Asterionella* might be expected to follow. In addition, as there was incipient thermal stratification, the rate of loss, even of live colonies, by sinking might be expected to increase. Thus, after fertilization by silicon or phosphorus, even the prolongation of the period when large numbers were present would suggest that one or other element was the main limiting nutrient. Further, if the concentration of added SRS fell substantially, this would show that production of diatoms had continued since no other cause of a loss of SRS is known, except that from incorporation into cells of diatoms or other siliceous algae such as certain Chrysophyceae. The latter were not detected and so, at the most, were present in insignificant numbers so far as silicon uptake is concerned. Apart from *Asterionella*, a small

Stephanodiscus sp.* was an important part of the diatom assemblage. Before closure of the tubes there were 8700 *Asterionella* and 2000 *Stephanodiscus* cells per ml in the 0 - 5 m water column in the lake.

On 27 March, after samples had been taken for analysis and estimation of algal numbers, sufficient KH_2PO_4 was added to tube B (fig. 5) to increase the concentration in a completely mixed water column to 50 µg PO_4P l^{-1}, which is four or five times greater than the natural concentration before the vernal increase in diatoms begins. On 28 and 30 March and 2 April silicon as $Na_2SiO_3.5H_2O$ was added to give both tubes, in a mixed water column, an increase of 1120 µg l^{-1} SRS, approximately the winter maximum concentration in the lake. Fig. 5 illustrates results of these fertilizations.

In tube A the total number of diatoms varied between 8000 and 9000 cells per ml in the 0 - 5 m water column for the first two weeks. *Asterionella* maintained a population of about 8000 cells ml^{-1} but *Stephanodiscus* declined to 600 cells ml^{-1}. By 17 April, total diatoms, now almost wholly *Asterionella*, reached 11 500 cells ml^{-1}. From then until the end of May the population fluctuated between 7000 and 2500 cells ml^{-1}.

The changes in the SRS concentration in the 0 - 5 m water column were large. The early maximum of about 1400 µg l^{-1} shows that the added silicon had not mixed throughout the whole water column, which is over 11 m deep. The rise on 25 April was a consequence of increased mixing and so a temporary increase in the depth of the epilimnion and enrichment with silicon from the hypolimnion. By the third week in May the whole of the added silicon had gone from the 0 - 5 m water column, representing an overall diatom uptake in *Asterionella* units of some 20 000 cells ml^{-1}.

* I am grateful to Dr E.Y. Haworth for informing me that this species and that in Esthwaite Water belong to a complex of small species whose taxonomic limits are as yet uncertain. They are usually called *S. hantzschii* or *S. astraea* var. *minutula*. These are important plankton algae, especially in eutrophic waters, and the present uncertainties and disagreements about their taxonomy also illustrate the importance of taxonomic research because until this taxonomic tangle is resolved, ecological studies will be imperfect.

In the lake the diatom population fell from its maximum of 11 000 cells ml^{-1} to less than 1000 in the first week in May, rose again, with wet weather and so increased entry of nutrients, to 3000 two weeks later and fell again by the end of the month.

On 1 and 4 June more silicate was added to tube A to bring the concentration of SRS in the 0 - 5 m water column of the now-well-stratified lake back to 1120 μg l^{-1}. Again a higher value than would be expected was found at first, showing incomplete mixing. The diatom population, still dominated by *Asterionella*, showed no significant increase remaining at about 1000 cells ml^{-1} for about three weeks and then declined to less than 100 cells ml^{-1} a fortnight later. The SRS concentration fell to less than half by 5 June (490 μg l^{-1}) and thereafter fluctuated between 500 and 700 μg l^{-1}.

Of the phosphate added to tube B on 27 March to give 50 μg l^{-1} in a fully mixed column, only 5 μg l^{-1} was found on the next day in the 0 - 5 m water column, pointing to very rapid uptake by cells low in phosphorus. The addition of this phosphate plus the same amount of silicon as that added to tube A maintained a diatom population in the 0 - 5 m water column between 11 000 - 14 000 ml^{-1} for three weeks during which time SRS fell by 1000 μg l^{-1}. By contrast with tube A, *Stephanodiscus* increased during this period. The total diatom population decreased to less than 1 cell ml^{-1} and the SRS to below 50 μg l^{-1} by 5 June, this tube not receiving a second fertilization with silicate. Thereafter few or no diatoms were found in the phytoplankton counts of tube B's 0 - 5 m water column and the SRS fluctuated between 20 and 70 μg l^{-1}.

A striking difference between the tubes A and B was the much greater growth of the blue-green alga *Oscillatoria agardhii* Gom. var. *isothrix* Skuja in the latter, reaching a maximum during the last stages of the decline of the diatom population. It was also more numerous than in the lake.

The result of this experiment points to silicon being the major nutrient limiting the size of the spring diatom maximum and the length of the period when diatoms are numerous. Reynolds (28) carried out observations, analyses and bioassay experiments which pointed to phosphorus, not silicon, being the major limiting nutrient in the spring of 1977. The previous winter was abnormal in that the phosphate phosphorus was very low, indeed similar to that 20 or more years earlier, that is before sewage and improved farming led to the present enrichment of the lake. It may be that, as suggested by Lund (6, 7), the low winter phosphate, which arose because of the uptake of phosphorus by unusually large autumnal and early-winter algal populations, is a sign of increasing eutrophication which is beginning to disturb the previously typical seasonal succession of the phytoplankton. Alternatively, it may be a 'chance' fluctuation without long-term significance. Only continued monitoring and experimentation will enable us to decide which hypothesis is correct.

Even though all the details are not given here, for example the algal numbers and nutrient concentrations at all depths during the period of stratification, they illustrate the complexity of events in what is a natural experiment compared to laboratory bioassay. Four features are emphasized here. First, the growth of the diatom *Stephanodiscus* sp. was stimulated by the addition of phosphorus and silicon to tube B but not by silicon alone to tube A. It is possible that, unlike *Asterionella*, phosphorus was the major limiting factor for this diatom. It is of interest that this *Stephanodiscus* is a recent arrival in Blelham Tarn and, even more recently, it or another similar species has appeared in Esthwaite Water. The differences between *Asterionella* and *Stephanodiscus* are among the many differences between major planktonic diatoms in the lakes of the Windermere drainage basin which could be cited in order to underline the danger of making sweeping statements about the ecological needs of whole groups of algae such as diatoms, blue-green or green algae, which indeed do have marked differences from one another in cellular construction and some aspects of basic biochemistry.

Second, the extremely rapid uptake of the phosphorus added to tube B, moreover added to the surface to give a concentration far higher than ever occurs naturally in Blelham Tarn, shows that the cells were phosphorus-starved. Phosphorus was a limiting factor in that it was not present in optimum amount, even if it was not more important than silicon. If the addition of silicon had been larger, then phosphorus could have been the major limiting factor and this may have happened in tube A after the second addition of silicon when only half that added was utilized. Third, the addition of phosphorus to tube B resulted in a bloom of *Oscillatoria*. Other experiments described in Lund (6, 7, 29) produced similar but larger growths of *Oscillatoria* after the addition of phosphorus and in the presence of lesser numbers of diatoms. These and other unpublished results of experiments in the tubes support the view that if the input of phosphorus to the lake continues to increase, blue-green and other algae may be expected to become more frequent, as indeed they have done during the enrichment of the lake since 1945. Neither sewage effluent nor agricultural wastes will add significant amounts of silicon compared to phosphorus, as has been shown by analyses of the inflows. Fourth, there is clear evidence for the view that the development of large populations of diatoms is much affected by thermal stratification because of their relatively rapid rate of sinking and so of loss from the upper well-illuminated layers of a water-body (25, 26, 27). Blue-green algae containing gas-vacuoles, that is those species producing waterblooms (e.g. *Oscillatoria agardhii** and species of such genera as *Anabaena*, *Aphanizomenon* and *Microcystis*), can rise in the water rather than sink. Probably a failure to realize the implications of relative buoyancies has been one reason for the statement often made that diatoms are favoured by low temperature and light. They may grow better than some of their competitors under such conditions but these physical conditions also occur at a time when turbulence is more effective in keeping them in suspension because of the lack of thermal stratification, provided prolonged ice-cover is absent.

3.2.3. *Weekly fertilization. A more natural experiment?*

Adding a relatively large quantity of one or more nutrients in a single dose or a series of doses over two to three days, as in the case just described, is not a natural experiment, though it should not be forgotten that a replacement time of about 10 days in major floods can produce a large addition if nutrients were at low concentrations beforehand. However such large floods are most frequent in autumn or winter when concentrations of available phosphorus, nitrogen or silicon are likely to be at or near their maximum and the gain of nutrients from the inflows is largely balanced by loss down the outflow.

* This species belongs to a group which commonly produce maxima in or near the metalimnion. However, particularly when very abundant, these algae can also produce waterblooms.

In the hope of reducing this artificiality, nutrients were added weekly to the tubes from April to November 1975 in amounts related to analyses of inflow water and the known weekly input of water into the lake. However, the experiment still had several unnatural features. Even a weekly addition of nutrients differs considerably from the continuous additions in nature. None of the nutrients added to the water or utilized by the algae were lost by outflow as is the case in the lake. With one exception (see later), only phosphate, nitrate, ammonium nitrogen and silicate were added. They were added in amounts determined from estimates of inflow waters made weekly in 1974, not from continuous records giving an exact measure of weekly input. Nutrients were not added when the computer programme, based on continuous records of lake level, showed that in the previous week in 1974 the loss of a nutrient from the tarn exceeded its input.

Answers to two questions in particular were desired. Would these nutrient additions produce within a tube a phytoplankton similar to that of the lake water outside during the period concerned in 1974 or 1975? What would the effect be of adding twice as much phosphate to one of the tubes? The compounds added weekly were $Na_2SiO_3.5H_2O$, KH_2PO_4, $NaNO_3$ and NH_4Cl (Table 2). In addition to 11 September, in view of its unexpectedly low phytoplankton population relative to that in the lake, tube B was fertilized with 13 kg of $FeCl_3.6H_2O$ and 10 kg of disodium EDTA as it was thought that iron might be a major limiting nutrient, judging from laboratory bioassays using *Asterionella* as the test alga.

The phytoplanktons of 0 – 5 m water column in the tubes were markedly different from those of the lake in 1974 or 1975 (Table 1). The phytoplankton of tube B, which received the double dose of phosphorus, was richer than that of tube A. It was also richer in blue-green algae, notably *Oscillatoria redekei* Van Goor and, especially after the addition of iron and EDTA, *Microcystis aeruginosa*, both species often abundant in highly eutrophic lakes. However, it had less *Anabaena* spp. than tube A. The major algae in the lake were *Ceratium hirundinella* O.F.M. in both years, though less common in 1974 than in 1975, and *Mallomonas caudata* Iwanoff in 1975. The large numbers of certain algae, such as *Chrysochromulina parva* Lackey in the lake and *Chlorella* spp. in tube B in 1975, do not represent large biomasses as they have small cells.

Tube A's plankton was poor until mid-July when *Anabaena* became abundant and then poor again till November when there was a short-lived diatom maximum.

This experiment did not answer all the questions posed. Though there were marked differences from tube to tube and between the tubes and the lake, only one or two inferences from the differences observed can be made. It seems that what was considered to be a more artificial type of experiment, the adding of nutrients in single and perhaps relatively massive amounts as in the previous experiment can be as useful, especially when a single more sharply defined question is posed.

The low levels of chlorophyll *a* in tube A for most of the period (Table 3, fig. 6), despite weekly fertilizations, are reminiscent of the result when water was enclosed in a tube and no nutrients were added (20), but it is not known why this result was produced, though the greater algal growth in tube B suggests that lower supplies of phosphorus and chelated iron were at least partially responsible. Up to July allowance must also be made for the fact that nutrient loadings were low, corresponding to the dry period in 1974. Nevertheless the chlorophyll *a* values for tube A were markedly less than in the tarn in 1974 (and 1975) during this period and tube A's phytoplankton was about 6 weeks later than that of the lake in rising again after the early summer minimum which succeeded the spring maximum. Even then the increase in August was short lived. It may well be that the greater mean depth of the tube compared to that of the lake, including the absence of any shallow water, was also an important factor.

The addition of twice the computed 1974 input to the lake of phosphorus to tube B did not produce, on the average, more phytoplankton than in the lake. However it did produce twice the amount in tube A. These comparisons are based on chlorophyll *a* concentrations which involves the presumption, which may not be correct, that the various algae concerned all had the same amount of chlorophyll *a* per unit mass.

As in the previous experiment and others (6, 7, 29), increasing the phosphorus input produced waterblooms. From the point of view of water supply, production *per se* is not important; what is important is how much of the production accumulates, that is the excess of production over loss in time. Mass, volume and quality of the population produced are all of vital practical importance. In view of the many objections to waterblooms, the *Microcystis* waterbloom in tube B was more objectionable than the equally large or larger, on a chlorophyll *a* basis, populations of *Ceratium* and *Mallomonas* in the lake in 1974 and 1975.

4. OBSERVATIONS ON LAKES IN THE WINDERMERE DRAINAGE BASIN

4.1. GRASMERE AND RYDAL WATER

Before 1971, sewerage in the drainage basin of Grasmere (fig. 1) was by septic tanks. By June 1971, mains drainage having been constructed, effluent from an activated sludge treatemnt plant close to the entry of the main inflow was passing into the lake. Hall et al. (30) give a description of the lake and changes in certain chemical variables with special reference to inorganic nitrogen transformations.

The phytoplankton of Grasmere has not been so regularly investigated as that of Windermere, Esthwaite Water and Blelham Tarn. However, sufficient estimations have been made to detect any marked changes in its plankton before and after the change in sewerage and sewage treatment. In this comparison the year 1969 is treated separately, for reasons which are explained later.

Grasmere flows into Windermere via Rydal Water (fig. 1). The phytoplankton of the latter lake is closely similar to that of Grasmere except during droughts, because it is a smaller lake and all but a small proportion of its water comes from the Grasmere drainage basin. The similarity of the phytoplanktons of the two lakes is illustrated in fig. 7, in one year when *Asterionella* was never very abundant in Grasmere and another when it was abundant for an unusually long period. Table 4 also shows that when counts were made of *Asterionella* over periods covering most or all the year, the average and maximal abundances were similar in the two lakes. Therefore one can also compare changes in the plankton of Rydal Water before and after the change in sewerage in the Grasmere area as evidence of its effect on the phytoplankton of Grasmere.

There have been changes. The main differences since 1970 have been an increase in total phytoplankton and certain alterations in its quality. In both lakes diatoms, blue-green algae and certain minute algae have become more abundant and *Dinobryon* spp. and *Uroglena americana* Calkins less abundant.

This change is illustrated in Table 4 by the growth of the major diatom, *Asterionella formosa*. The data for 1950 and 1970 for Grasmere and for 1950, 1963 and 1975 for Rydal Water are excluded in the following comparison because estimations were made only for a short period of these years, though it may be noticed that *Asterionella* was very abundant in Rydal during mid-winter 1973 and spring of 1975. In the remaining years, even when relatively few estimations were made, they covered all or nearly all months of the year. The average yearly figure for live cells in the 0 - 5 m water column in the 8 years (1969 excluded) before the sewage treatment plant began is 6 cells ml^{-1} and in 7 years afterwards 1053 cells ml^{-1}. The highest average abundance before 1971, excluding 1969, was 20 cells ml^{-1} which is lower than the lowest average after 1970, 77 cells ml^{-1}. The highest averages after 1970 were in 1975 and 1976 when in both lakes they exceeded 1000 cells ml^{-1} and include the highest average abundances ever recorded in an English Lake District lake. The phytoplanktons of all the lakes in the district have been estimated over a period of two years or more from time to time since 1945.

Hall et al. (30, Table 4) have pointed out that though it is not clear whether the decrease in nitrate in the epilimnion after the sewerage change is significant, the increase in phosphate by an order of magnitude is significant. However, this order of magnitude is from an extremely low concentration of about 0.1 $\mu g\ l^{-1}$ PO_4P (the exact value for the lowest estimations is analytically uncertain) to 1.0 $\mu g\ l^{-1}$. Lakes with such low PO_4P concentrations usually have less *Asterionella* and, indeed less total diatoms than Grasmere. Further, this is equally true of many lakes with higher PO_4P concentrations than Grasmere. The total phosphorus concentration in Grasmere also is low, the average values for the years when estimations were made being 14 - 22 $\mu g\ P\ l^{-1}$.

The change in sewerage has led to a marked increase in algae, especially *Asterionella* whose abundance since has from time to time been so great that if Grasmere was a reservoir there might well be filtration problems.

The results for 1969 illustrate the effect of the digging and pumping during the construction of mains sewerage. The entry of soil and dirty water into the lake was noticeable, particularly in the summer during the period of abundance of *Asterionella*. In early work using bioassay, Lund (31) attributed filtration difficulties caused by *Asterionella* in a reservoir in northwest England to erosion caused by forest planting. Entry of soil will add nutrients such as phosphorus and nitrogen and also may supply organic chelators which the bioassay on Blelham Tarn water suggests are another chemical factor controlling the abundance of *Asterionella* at certain times of year (part. 3.1).

4.2. ELTERWATER

In this small and also originally oligotrophic lake (fig. 1) a change from septic tank to mains sewerage produced a different result to that described for Grasmere. The reason for this difference is the location of the sewage treatment plant and the morphometry of the lake. It has three basins separated from one another by narrows and the sewage effluent passes into a small stream which enters the innermost basin. Apart from field drains, this is the only inflow. Because the inner basin is small, the sewage effluent has a large effect on its chemistry. Analyses during the first year of entry of sewage, made at a point a few metres away from its entry into the inner basin between April 1974 and May 1975, gave values for phosphate phosphorus, total phosphorus, nitrate and ammonium nitrogen of 187 - 7480; 45 - 8950; 269 - 3172 and undetectable to 33 600 $\mu g\ l^{-1}$. The basin containing the outlet to the lake (outer basin) receives over 99% of the total inflow into the lake. The middle basin is affected mainly by the outflow from the inner one, despite the entry of one of the two rivers feeding the lake near its junction with the outer basin. However, in wet weather, water from the outer basin may pass into it and in floods the aforementioned inflow may overflow into it. All the Ordnance Survey maps show the entries of the two major inflows more or less incorrectly. Since the drainage basin is mountainous with high rainfall, the outer basin is often more riverine than lacustrine. Table 5 shows the variations in PO_4P, total P, NO_3 and NH_4N in the three basins during 1974 and 1975. The effect of the sewage effluent on the chemistry of the inner basin is marked. The middle basin's chemistry is intermediate between those of the other two basins, and the oligotrophic nature of the outer basin is clear. The chlorophyll *a* values, without allowance for phaeophytin, are low in all three basins in winter and almost always in the outer basin (Table 6). At other times of year they can exceed 100 µg l^{-1} in the inner basin; in 1976 (fig. 8) they exceeded 300 µg l^{-1} on three occasions. As with nutrients, the middle basin had higher values than the outer but lower ones than the inner basin.

Though *Asterionella* was at times abundant in the inner basin, other algae often were dominant. The highest chlorophyll *a* values (over 300 µg l^{-1}) were attained during vast blooms of *Volvox globator* L. in 1976, when the water was bright green in colour. Small nanoplanktonic algae were often abundant and these included the diatom *Cyclotella pseudostelligera* Hust., coccoid green algae and cryptomonads. Sudden decreases in the numbers of these small algae commonly coincided with great abundance of rotifers, especially *Keratella* spp. In addition to grazing, parasitism by fungi and protozoa sometimes was very severe. In all these features, the inner basin's plankton was similar to that common in sewage oxidation ponds. A feature of such highly enriched waters is the general absence of waterblooms, produced by the gas-vacuolate blue-green algae which are so common in lakes enriched by sewage. A single such alga, *Anabaena solitaria* Kleb., was present at times but never produced a big waterbloom.

As a result of the location of the sewage effluent, the lack of other inflows to this basin and the narrows between the inner and middle, and middle and outer basins, the large algal populations in the inner basin only entered the outer basin in significant numbers in times of flood. Then they were so diluted in this basin by the rivers entering it that they passed out of the lake only in low concentrations into the river which flows into the north (upper) basin of Windermere. Consequently the effect of the Elterwater plankton on this basin of Windermere, which is used for water supply, was slight.

4.3. WINDERMERE, ESTHWAITE WATER AND BLELHAM TARN (FIG. 1)

These three lakes have been studied on a weekly basis from 1945 to date and a number of papers have been written about their phytoplankton during the 33 years to 1978, apart from what has been said here about Blelham. In addition many papers have been written about special aspects of these lakes and their inhabitants*, so that only one or two points

* A list of all papers published by workers at the laboratories of the FBA can be obtained from the librarian on request (*Occ. Publ.* No. 7, price £1.50).

relevant to the period of the contract and its aims are mentioned here.

Windermere has two basins, separated by islands and shallow water. The north (upper) basin is deeper and less rich in nutrients and organisms than the south (lower) basin. It is the north basin which forms part of the North West Water Authority's water supply system from the Lake District. The oligotrophic nature of this basin combined with the fact that the Authority takes water from the constantly aerobic hypolimnion assures its good quality for supply. Although enrichment of waters passing into this basin (e.g. Grasmere, Rydal Water, Elterwater and Blelham Tarn) has taken place and there has been an increase in sewage passing directly into it, no changes have as yet taken place to interfere with its use as a reservoir. The large volume of the hypolimnion, about 1×10^8 m^3 (32), is a protection against considerable future enrichment of the basin.

The south basin receives sewage from the main centre of population and tourism in the Windermere - Bowness urban area. Even allowing for differences in the exactitude, and so comparability, of analytical procedures in the last 34 years, it is clear that the winter maximum of PO_4P is an order of magnitude higher than it was at the beginning of the period of weekly analyses. This change has not been accompanied by an equally striking one in the abundance of phytoplankton. A much greater relative change has taken place in Grasmere with increasing PO_4P concentrations from lower levels (section 4.1). Qualitative changes in the phytoplankton have taken place, some algae becoming more or others less numerous. The overall result is an increase in summer but it is difficult to translate qualitative changes into quantitative data because of the lack of estimations of chlorophyll *a* in the earlier years. The hypolimnion is still aerobic in summer, probably because of its large volume. Nevertheless, from an environmental point of view, as well as a scientific one, continued monitoring of water quality is needed.

One influence on the phytoplankton of this basin is the outflow of Esthwaite Water, now, and probably since the last ice age, the richest lake in the English Lake District. In recent years the summer population, dominated by the dinoflagellate *Ceratium hirundinella* (one of the largest single-celled freshwater algae), has become more massive than it was when Lund's paper (33) was published. Chlorophyll *a* values in the epilimnion are as great as those in the inner basin of Elterwater, despite lower concentrations of phosphorus and nitrogen in Esthwaite's inflows.

The village of Hawkshead and outlying hamlets at the head of Esthwaite Water were served by septic tanks until late 1973, when a sewage treatment plant near the entry of the main inflow to the lake replaced them. The difference in the amount of sewage entering this inflow was not as large as might be expected from a change from septic tanks to mains sewerage. Before this change, a large septic tank system for most of Hawkshead became more and more overloaded, in considerable part from the great tourist popularity of the attractive village together with its association with William Wordsworth. The tanks near the inflow often overflowed into it and so bad was the pollution that sometimes sewage solids (e.g. excreta) entered the lake. The sewage treatment plant certainly produces more effluent than the septic tank system because it serves a bigger area and tourism has continued to grow. Nevertheless a very important biological change has been the replacement of imperfectly purified sewage by a high-quality effluent. Despite this change, the massive growths of *Ceratium* have continued to dominate the summer phytoplankton. There has been a recent change in the vernal phytoplankton with the appearance in 1976 of a small *Stephanodiscus*, of a similar type to that in Blelham Tarn (2.2), and large populations of *Chlorella* spp. so that the long dominance of *Asterionella* and *Melosira italica* var. *subarctica* O. Mull. is threatened by new competitors. The decrease in *Oscillatoria* in spring, described in (32), continues.

Near the head of Esthwaite Water is a pool called Priest's Pot. Small though this pool is, its seasonal cycles of stratification and of phytoplankton are similar to those of the lakes. It is the richest water in the English Lake District with chlorophyll *a* values of over 100 $\mu g\ l^{-1}$ for most of the year, winter excepted. An interesting feature is that its highly eutrophic condition is caused solely by agriculture, the domestic sewage entering the mains sewerage system of the Hawkshead district. As with the very eutrophic inner basin of Elterwater, water-blooms of gas-vacuolate blue-green algae are rare.

5. DISCUSSION

5.1. GENERALITIES

In this section some matters concerning algae and water usage are discussed with special reference to the work done under contract.

It is probably true to say that the technology exists to supply water of satisfactory quality for domestic purposes from reservoirs with very large algal populations over long periods. However, water undertakings neither necessarily have the most modern equipment nor sufficient treatment facilities to allow for all possibilities, which possibilities, moreover, have not been or at present cannot be envisaged. Eutrophication, which was the centre of much work and controversy in the sixties, is now sufficiently well understood in general terms, so far as British waters are concerned, that methods of control or treatment can be suggested. However, suggestion is one thing and the cost or practicality of carrying it out another. Further, undesirable qualitative changes can arise without major quantitative ones, even with a reduction in nutrient input, as the example of Loch Leven illustrates.

The phytoplankton of Loch Leven has changed markedly since the reduction of the phosphorus input from an industrial source (34, 35 and pers. comm. A.E. Bailey-Watts). This change has been from a lake very rich in algae which, because of their minute size, had a large capacity to penetrate into or pass through filtration systems (36) to one which in mass is, on the average, less but over limited periods, greater, and is dominated by large algae posing different filtration and treatment problems. If a similar chemical change had been part of a water transfer scheme, it might have been envisaged that the consequences of the transfer would be wholly beneficial. If less algae, large or small, are desired, then it seems that the phosphorus input must be further reduced. However, the major importance of this loch at present is for trout fishing and nature conservation, and if its use for water supply is also to be considered, then the effects of the phytoplankton in relation to all three interests have to be considered.

If it seems that too much emphasis has been laid on the unpredictable and unquantifiable aspects of changes in the phytoplankton in relation to known or partially known alterations in land management and sewage practice, it is because this is a 'fact of life' and because I have found so often that this seems difficult for the non-biologist to accept or appreciate. The more that is learnt about the ecology of algae, the less this area of uncertainty will become.

It does not follow that nothing should or can be done when a problem arises, or that there is not a sufficiently high probability that a certain action will lead to the desired result. For example, there is good evidence about the major role played by phosphorus in eutrophication in many parts of the world and so about the value of reducing its input to over-enriched (eutrophicated) waters. Moreover, as over-enrichment with phosphorus generally arises from point sources, such as sewage plants, the problem often is potentially solvable. In relation to eutrophication it should not be forgotten, as Collingwood (37) points out, that the deleterious effects of algae may be neither solely nor mainly in a reservoir or treatment plant, but may arise secondarily in the distribution system.

5.2. MODELLING

Modelling has been mentioned in section 1. It is not considered here in any detail but only in relation to its present practical utilization.

Most models are used to explain past or present situations. What the water industry needs are models which can be used to predict future events and it is not clear to what extent present models suffice for the purpose. The increasing work on this subject can only lead to an improvement in this situation.

Models such as Talling's (38, 39) and Steele's (40, 41), which are based on the photosynthetic capacity of algae and so on underwater illumination, are applicable to temperate (39, 41) and tropical (42, 43, 44) waters alike. The report of Vollenweider (45) concerning the nutrient bases of eutrophication has been followed by much discussion (e.g. 46) and the description of improved models by Vollenweider himself (47, 48), Dillon and others (e.g. 49 - 53), which include the retention time among other factors as well as lake morphometry, and which have predictive value for the areas concerned. Even so, there seem to be problems in using these models for a variety of regions and climates. It is also not always as easy as it may seem from published work to make sufficiently accurate estimations of nutrient loadings without the installation of special measuring devices and the expenditure of much effort and money.

In the cases of Esthwaite Water and Grasmere (sections 4.3 and 4.1) this has not been done. We do not know what the nutrient loadings, notably of phosphorus, are. The position for Blelham Tarn (section 3.2.3) is better, but the nutrient loadings have been estimated on the basis of weekly samples of the inflow waters and the presumption that the volumes of their inputs vary according to the areas of their drainage basins.

From a water supply point of view, the treatment problems would be different in Esthwaite Water and Grasmere. In Esthwaite Water there are the massive growths of *Ceratium* in summer and early autumn, and in Grasmere the large populations of *Asterionella* from autumn to early summer. It is not clear that present models could have predicted both the quantitative and qualitative nature of these changes in the phytoplanktons of these two lakes.

It could be valuable for the water industry if more research were initiated or supported for the production of predictive models applicable to British waters. The reluctance to forecast coming events is understandable, since the results of such forecasts may mainly indicate the lack of knowledge of the forecaster. However, they can, step by step, be improved just as has been done in other areas of ecology. The work of the Metropolitan Water Services of the Thames Water Authority (35, 40, 41, 54) is an example of what can be done by water authorities in collaboration with other institutions. So much emphasis has been laid on nutrients in relation to eutrophication that the importance of grazing on the quantity and, especially, the quality of the phytoplankton has not received sufficient attention. A study of zooplankton in turn calls for better knowledge about predation by fish (cf. 54). Changes in quality can be just as troublesome, sometimes more so, as changes in quantity.

5.3. WATER TRANSFER

Knowledge of the effects of water transfer is of particular present importance in view of the projects in train or proposed. There is little information on which to forecast what will happen if two waters of markedly different kind are mixed, for example a hard and a soft water. It is even not sufficiently certain what will happen when waters are mixed which from chemical and biological observations may be believed to be similar. Bioassay (section 2.1) can be valuable in that it can indicate what the nutrient potential for algal growth is in relation both to variable volumetric additions of one water to another and in relation to seasonal changes in quality. It can only give limited information by itself on what way and to what extent this potential will be realized. Its value is much greater if there is good information about the basic biologies of the waters concerned.

Simplistic predictions of what will happen may be made because the breadth of environmental conditions which algae will tolerate, or within which they have the potential to produce large populations, is underestimated. For example there is a wide diversity of algae which can be grown in some culture solutions but it does not follow that they do so equally well, that is that they grow as well as possible, and the same situation must occur in nature; of the 100 or so species growing in Windermere, less than half become abundant. The fact that most freshwater algae are cosmopolitan supports this view, apart from their ability to get from place to place, incidentally by largely unknown means. A proviso must be added to this viewpoint and that is that we do not know to how great an extent this morphological identity in different parts of the world is matched by physiological identity. Many algae considered typical of oligotrophic waters can be found in eutrophic ones, and the main reason why they do not occur in abundance seems to be that other algae grow better in eutrophic waters.

The less these differences in growth potential are, the more important the abundance of such algae in transferred water will be to their chances of success in the recipient water, though it has to be added that there is a lack of observation and experimentation to support what seems to be a reasonable hypothesis. Fig. 9 shows the abundance of *Ceratium* in Esthwaite Water and the south basin of Windermere during the last 34 years. The figure for 1978, kindly provided by Dr S.I. Heaney, is an estimation in that it is based on the data for the first eleven months. However, it will not be significantly incorrect because this alga is never common after spores have been formed, usually in October; by December the physical conditions are so unfavourable for growth that the population remains low. The numbers of *Ceratium* in Windermere have increased since it became very abundant in Esthwaite Water. This change in Esthwaite Water began in 1963 with an average weekly abundance of 30 cells ml^{-1} compared with the highest previous value of 7 per week for a single year. From 1945 - 1963 inclusive the average weekly abundance in Esthwaite Water was 2.6 cells ml^{-1} (range 0.6 - 6.9) and over the succeeding 15 years it has been 44.4 cells ml^{-1} (range 0.5 - 118.7). For the same periods in the south basin of Windermere the figures are 0.37 cells ml^{-1} (range 0.02 - 0.90) and 4.3 cells ml^{-1} (range 0.18 - 23.0). An exact relationship from year to year and so over the whole period is not to be expected. The number of *Ceratium* reaching Windermere depends on variations in rainfall and hence the volume of Esthwaite water entering Windermere and the percentage of the *Ceratium* cells which arrive alive. Further, *Ceratium* can grow in Windermere and the population will be affected by the number of spores which overwinter there to germinate in the succeeding year. However, the correlation between the increases in *Ceratium* in the two lakes is such that it can scarcely be doubted that the marked increase in Windermere is the result of that in Esthwaite Water.

The increased populations of *Ceratium* in Windermere have led to the largest recorded total phytoplankton maxima, expressed as chlorophyll *a*. The death of such maximal populations must lead to increased oxygen depletion and, though no part of the hypolimnion of the south basin as yet has been deoxygenated, the increased enrichment from Esthwaite Water and the fact that the sewage from the largest centre of population and tourism passes into this basin are potential threats to its oxygen regime.

Returning to the tolerances of algae to environmental conditions, reference can be made to Round's (55) valuable paper on factors controlling algal growth and succession (periodicity) with one partial disagreement. He discusses the effects of major seasonal changes on the underwater environment which he calls shock periods, for example the period of overturn, though the changes here usually are so gradual that it can be argued that shock is too strong a word to apply to their undoubted effects on the phytoplankton. So far as temperature is concerned, changes will affect algal growth but the number of algae which will only grow over a narrow range of temperature (stenotherms) is small. Most algae are well adapted to the temperature range of the water in which they live; they would hardly succeed if this were not the case. Moreover they can withstand considerable temperature 'shocks', for example changes of 10 - 15 °C over a short period of time. That this is so is clear from the common and successful preservation of samples by keeping them in a cool or cold place in the laboratory when they cannot be examined soon after collection. For example, in summer this is to put the samples in a refrigerator which in the case of sub-surface horizontal net hauls means a rapid drop in temperature of 10 °C or more. It follows that unless a water transfer has a large effect on the temperature of the recipient water it is unlikely, for this reason alone, to have a large effect on the algae therein. It may have an important indirect effect by cooling and so deepening the epilimnion which could favour the growth of diatoms.

It seems, however, that quite small changes in nutrients caused by a water transfer could have considerable changes in the abundance of the phytoplankton. The data for Grasmere (section 4.1) show that a low, though increased, concentration of nutrients, in particular (judging from bioassays on other Lake District waters) that of phosphate, led to a massive increase in *Asterionella*. In a richer lake an increase of one milligram of phosphate phosphorus per litre would be considered to be insignificant.

Relatively small changes in the nutrient supply to basically oligotrophic lakes may have disproportionate effects to similar changes in eutrophic ones. Neither the phosphate nor total phosphorus concentrations in Esthwaite Water are high compared to those of many other eutrophic lakes, but its enrichment has led to quantitative and qualitative changes in its phytoplankton which would be highly objectionable if it was a reservoir. The massive *Ceratium* populations during the last 15 years have produced chlorophyll a values of over 100 $\mu g\ l^{-1}$ and even up to 500 $\mu g\ l^{-1}$ at certain depths (56, 57) in the epilimnion. Talling's (58) investigations show that such populations so deplete the inorganic carbon supply of this softwater lake that the pH may exceed 10, and his experiments with *Ceratium* and *Microcystis* show that photosynthesis can continue up to about pH 11. Collingwood (37) discusses the deleterious effects of high pH on the coagulation process using aluminium and iron. In the case he mentions, Farmoor Reservoir, serious difficulties arose at pH values below those now common in Esthwaite Water in summer. Further difficulties were produced by the extracellular products of *Microcystis*. In Esthwaite Water there would be no escape from supply difficulties by using water from the lower layers, so avoiding the pH problem, because these are deoxygenated.

It may be that transfers of water can be made at preselected times of year, so that the question arises as to which time would be best for preventing or reducing possible increases in algae. We have little knowledge on which to base an answer but it does seem that there is no easy answer. Obviously addition at a time when the water to be transferred has large algal populations would be likely to be avoided (but see also section 6). If the transfer water has few algae, as is commonly the case in winter, it is likely to be relatively high in nutrients since there are not sufficient algae to utilize them. Hence this is only delaying the period when large algal populations may arise. Two or more reservoirs in series offer the possibility of reducing algal populations passing into a treatment plant, if only the last one is used for supply and presuming that the first one receives all the significant inputs of nutrients. The first reservoir could then act as a 'trap' for the products of primary production. However, this plan presupposes that the algae will not pass down the system but die and be sedimented in the first reservoir. In times of high inflow or high demand for water this cannot be assured. Further, water must continue to pass down the series in winter and hence a substantial part of the inflowing nutrients may do so as well. The relationship between the outflow of Esthwaite Water and the south basin of Windermere is one illustration of the complex problems involved in such a proposal, particularly since Esthwaite Water also passes through a pool (Out Dubs) on its way to Windermere.

A better proposal, if it is also a practical one, might be to add the transfer water and then leave the mixture long enough for the nutrients to be utilised and the algae to decline before using the water. The evidence from the work on the Blelham tubes suggests that if the recipient water is deep enough and stratifies, much of the algal production would be lost to the deposits. This proposal would have a good chance of success if there were several basins rather than one relatively large reservoir.

In view of the weakness of our knowledge about water transfer, it is clearly essential that we should have information about the living world of the transfer and recipient waters, as well as their chemistry, with reference to the nature and capacity of the existing treatment plant. Equally obvious is the fact that if there is choice, a source which has been found to have the lowest potential fertility, for example by bioassay, should be chosen.

6. A NOTE ON RIVER PHYTOPLANKTON AND WATER TRANSFER

Though the study of rivers was not part of this contract, a few remarks may be made on river phytoplankton since rivers are so often the source for the transfer of water.

In Britain, rivers have virtually no true phytoplankton, or one dominated by small algae. In the great drought of 1976 there were exceptions to this statement because flow became so slight that conditions were lacustrine rather than riverine.

The major factor controlling the phytoplankton is time which in turn depends on the length of the river and the current speed. The many chemical analyses of our river waters show that the nutrients present are very often sufficient to produce large populations of phytoplankton. Hence, as would be expected from these facts, it is the large or slow-flowing British rivers in which such populations exist.

Nutrients, of course, are important because, until the cell demand reaches its maximum, increased supplies will increase the potential rate of growth.

Slow flow is likely to lead to loss of suspended matter, apart from algae, and so to improved conditions for photosynthesis. If a water transfer increases the flow in summer it will reduce the abundance of the phytoplankton unless the stimulation of the algal growth rate from added nutrients exceeds the rate of loss from reduced retention time. However, it should not be forgotten that less phytoplankton may improve light penetration and so produce more attached algae and that macrophytes are often a bigger problem than algae. In general, it is not clear whether planktonic or non-planktonic plants are more beneficial to fish, since their preferred food will vary with age. An abundant phytoplankton is likely to be accompanied by an abundance of crustacea or rotifers feeding on the small algae whereas mats or growths of larger plants will harbour many larger invertebrates.

It was pointed out in 5.2 that, if there was a choice of time for the transfer of water, periods when this water was rich in algae probably would be avoided. However, this is less important if the transfer is from a river to a reservoir rather than from reservoir to reservoir or reservoir to river, because most of the common algae of river plankton are not major components of reservoir plankton. Even reservoirs wholly dependent on water from rivers with abundant phytoplankton develop a lacustrine phytoplankton. The same applies to the transfer of reservoir water to a river, provided that the intake to supply is not close to the outlet from the reservoir. Lake phytoplankton usually soon decreases after entry into a river. It is necessary to say 'usually' because this may not be the case if the river is very slow flowing, for example during a drought. Britain does not have any large rivers comparable for example to the major rivers of the USSR in which typical lacustrine species may be abundant, though the effect of the large impoundments along the course of so many of them is a major influence on their phytoplankton.

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9. TABLES

TABLE 1

	L		A	B
Diatoms	1974	1975	1975	1975
Asterionella formosa	3200	900	500	
Cyclotella pseudostelligera				17000
Fragilaria crotonensis		300	3500	2300
Nitzschia bacata				900
Stephanodiscus sp.		700		
Tabellaria flocculosa var. *asterionelloides*	1400			
Blue-green algae				
Anabaena spp. (f)			400	
Aphanizomenon flos-aquae (f)	400	300		
Oscillatoria redekei (f)				12000
Microcystis aeruginosa (c)				100
Green algae				
Chlorella sp.			4300	58000
Colonial spp.	4200	700	3400	4500
Dinoflagellate				
Ceratium hirundinella	400	100		
Chrysophyte algae				
Chrysochromulina parva	5500	18300	1000	2200
Dinobryon divergens	1000	600		
Mallomonas caudata		600		

Table 1. Maxima of major (see text) algae in the 0 - 5 m water column from the beginning of May to the third week in November in Blelham Tarn (L) in 1974 and 1975 and in the tubes A and B in 1975. c, colonies; f, filaments; the rest cells; all per ml.

TABLE 2

	A	B
$Na_2SiO_3.5H_2O$	251	245
Si	47.5	46.4
KH_2PO_4	3.5	7.1
P	0.8	1.6
$NaNO_3$	79.4	79.4
N	13.1	13.1
NH_4Cl	1.15	1.15
	3.0	3.0

Table 2. Kilograms of nutrients added to the tubes A and B from April and 27 November, 1975.

TABLE 3

Year	Lake	A	B
		average	
1974			
Before 11 Sept.	24	-	-
After 11 Sept.	17	-	-
1975			
Before 11 Sept.	16	7	11
After 11 Sept.	11	8	23
		maximum	
1974			
Before 11 Sept.	69	-	-
After 11 Sept.	23	-	-
1975			
Before 11 Sept.	42	27	48
After 11 Sept.	24	24	35

Table 3. Average and maximum amounts of chlorophyll a, $\mu g\ l^{-1}$, without allowance for phaeophytin, in the 0 - 5 m water column of Blelham Tarn in 1974 and 1975 and in the tubes A & B in 1975 before and after 11 September, on which date in 1975 tube B was fertilised with ferric chloride and the sodium salt of EDTA.

TABLE 4

1	2	3	4	5	6
G	1949	0.3	5	13	
R	1949	0.2	3	14	
G	1950	0	0	2	1, 2
R	1950	0	0	2	1, 2
R	1962	0	0	3	4, 6, 9
R	1963	1	4	36	
R	1964	20	450	43	
G	1965	5	36	19	
R	1965	5	30	19	
G	1966	7	42	21	
R	1966	7	52	21	
G	1970	0	0	3	1, 4
G	1972	746	5200	34	
G	1973	1304	5700	52	
R	1973	10270	11210	4	11, 12
G	1974	439	4472	47	
R	1974	376	4404	45	
G	1975	1251	10090	42	
R	1975	2645	13532	11	3, 4, 5
G	1976	2037	11410	46	
R	1976	2196	12850	40	
G	1977	77	668	34	
G	1969	224	3073	31	
R	1969	578	1458	3	8

Table 4. The abundance of *Asterionella formosa* in Grasmere and Rydal Water before (above continuous horizontal line) and after the start of the Grasmere sewage treatment plant. Data for 1969 given separately (below broken horizontal line) for the reason given in the text. Columns: 1 – G, Grasmere, R, Rydal Water; 2 – year; 3 – average number of live cells ml^{-1} $week^{-1}$; 4 – maximum number of cells ml^{-1}; 5 – the number of weeks when counts were made; 6 – the months when counts were made in years in which the estimations only covered a small part of the year.

TABLE 5

	A	B	C	D
			I	
1974	4 - 123	32 - 206	ND - 666	10 - 300
1975	0.8 - 140	28 - 176	146 - 574	25 - 680
			M	
1974	0.8 - 17	19 - 94	34 - 286	2 - 285
1975	0.6 - 47	12 - 84	39 - 619	5 - 322
			O	
1974	0.2 - 21	7 - 21	104 - 350	5 - 44
1975	0.3 - 3.7	5 - 27	129 - 342	ND - 33

Table 5. Elterwater, 1974 and 1975. Ranges in the concentrations ($\mu g\ l^{-1}$) of phosphate phosphorus (A), total phosphorus (B), nitrate nitrogen (C) and ammonium nitrogen (D) in the inner (I), middle (M) and outer (O) basins. ND - not detected.

TABLE 6

	I		M		O	
	R	A	R	A	R	A
1974	1.7 - 113.5	30.5	1.0 - 28.9	9.4	0.1 - 10.7	2.9
1975	0.7 - 119.4	29.7	0.8 - 44.2	12.9	0.2 - 24.3	3.7
1976	2.7 - 359.0	65.3	2.4 - 93.8	20.9	0.6 - 19.8	3.7

Table 6. Elterwater 1974 - 1976. Ranges (R) and yearly average (A) concentrations of chlorophyll *a* ($\mu g\ l^{-1}$), without allowance for phaeophytin, in the 0 - 5 m water columns of the inner (I) and outer (O) basins.

10. FIGURES

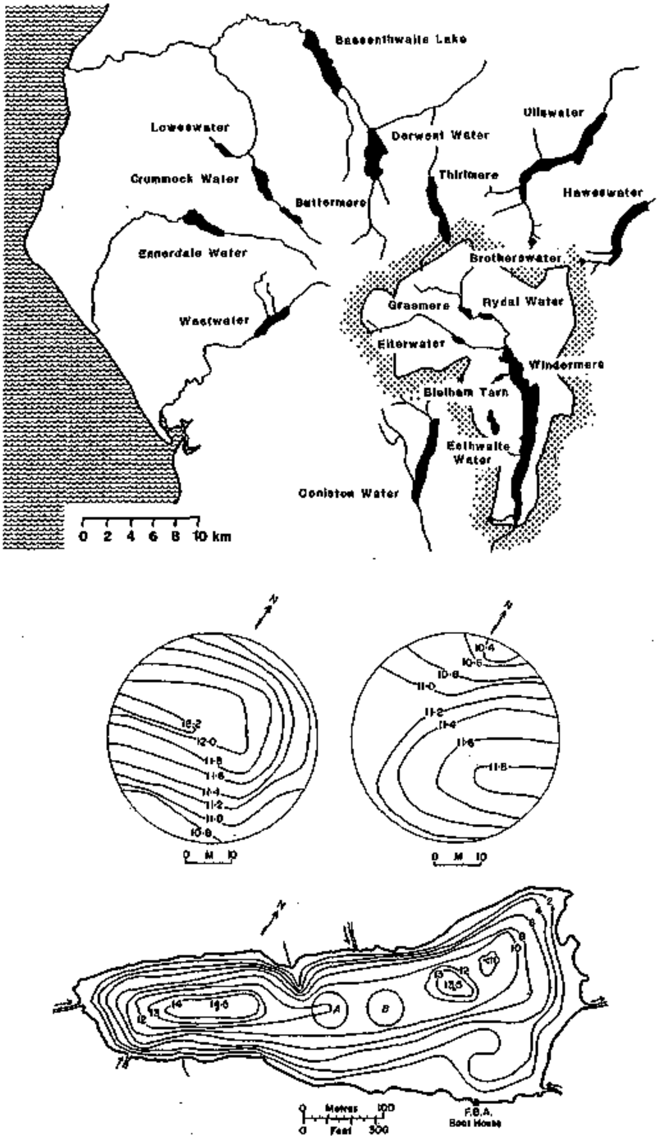

Figure 1. Upper part: Map of the English Lake District showing the drainage basin of Windermere (shaded). Lower part: Bathymetric map of Blelham Tarn and of its plastic enclosures called tubes A and B. (From Lack & Lund 1974).

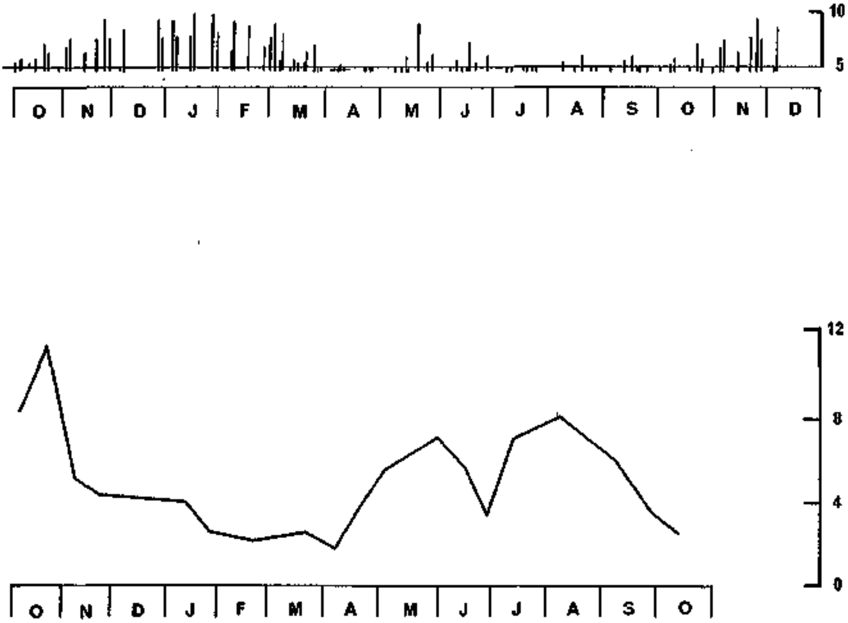

Figure 2. Upper graph: growth of *Asterionella formosa* from 1971 - 1974 at 4000 - 6000 lux and 18 ± 2°C for 7 days. Lower graph: growth of *Microcystis aeruginosa* in 1974 - 1975 at 1300 ± 100 lux and 20 ± 2°C for 286 hours (from 16 and 15 respectively). Growth of *Asterionella* expressed as doublings of the inoculum (vertical scale, $\log_2$) and of *Microcystis* as harvested dry weight.

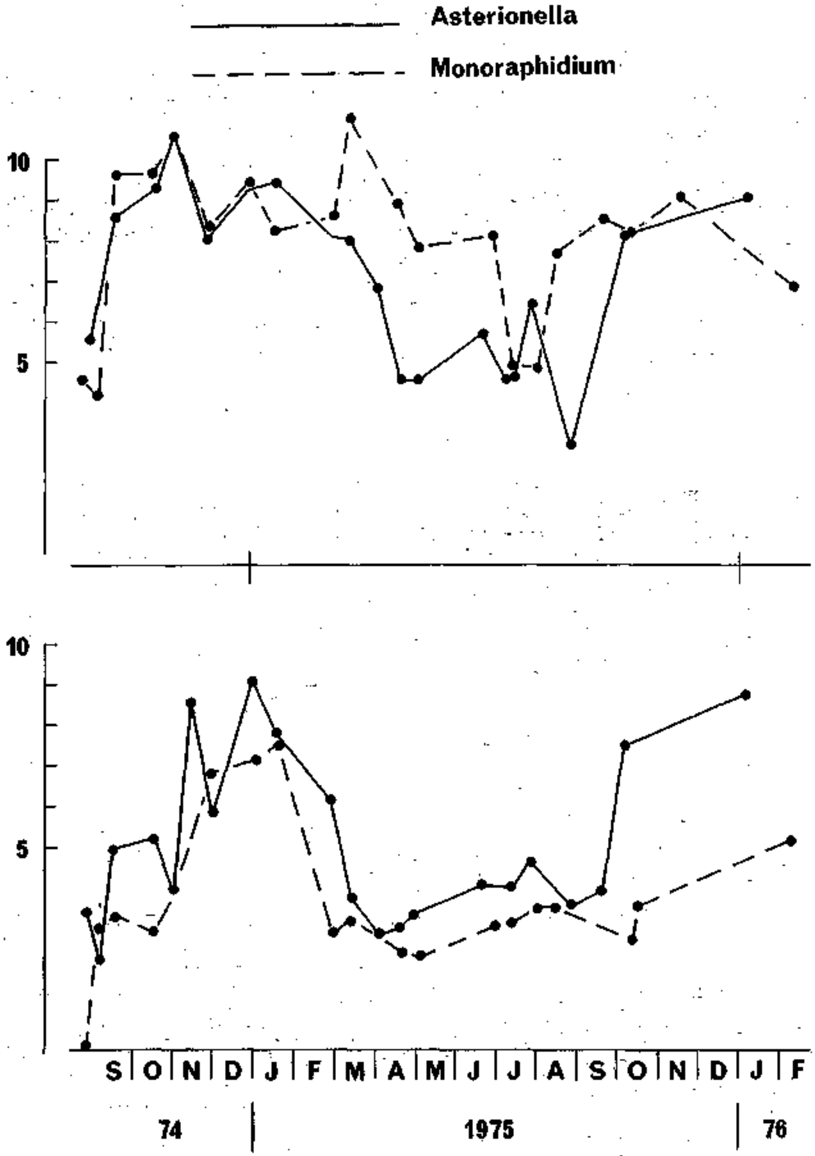

Figure 3. The growth of *Asterionella formosa* (continuous line) and *Monoraphidium contortum* (broken line) in filtered water from Blelham Tarn, 1974 - 1975, expressed as doublings of the population over a 7-day period at 18 ± 2°C and 4000 - 6000 lux. Upper graph : 100 µg PO_4P l^{-1} added to the filtered water; lower graph : no additions. Vertical scale : growth on a $\log_2$ basis.

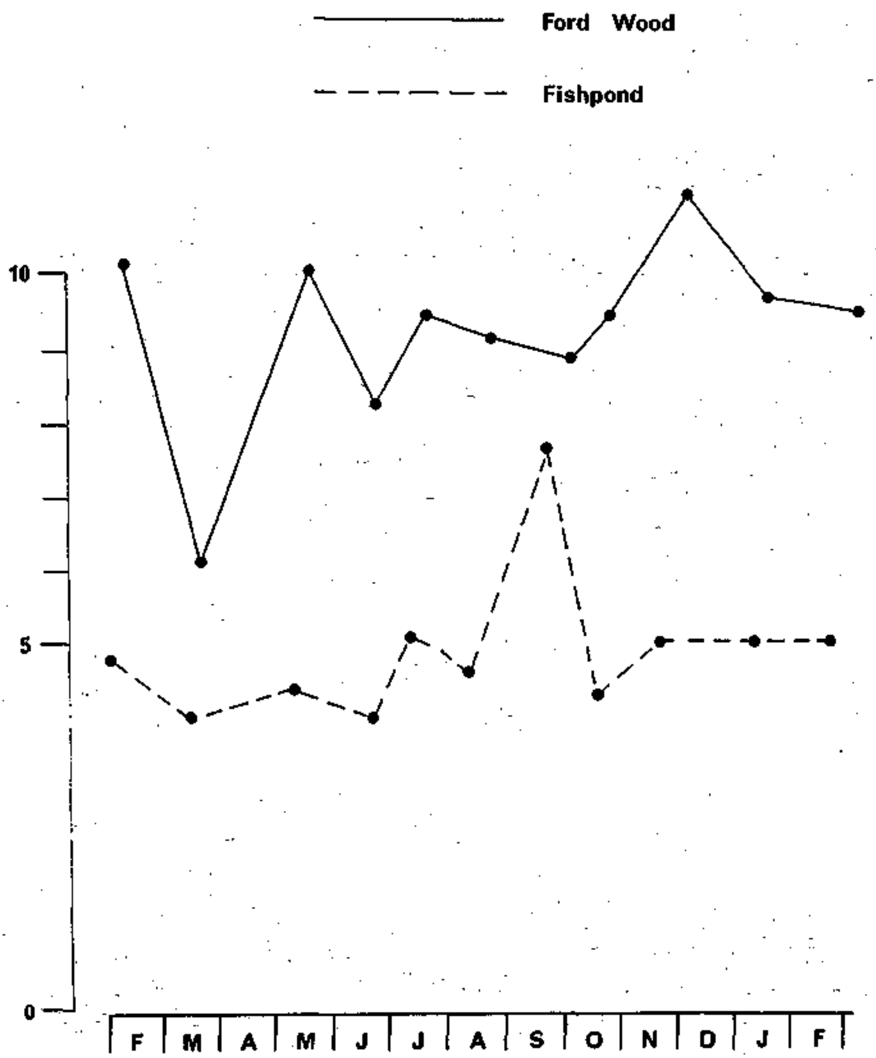

Figure 4. The growth of *Asterionella formosa* in filtered water from two inflows to Blelham Tarn, expressed as doubling of the population over a 7-day period at 18 ± 2°C and 4000 - 6000 lux. Continuous line : Ford Wood Beck. Broken line : Fishpond Beck. Vertical scale : growth on a $\log_2$ basis.

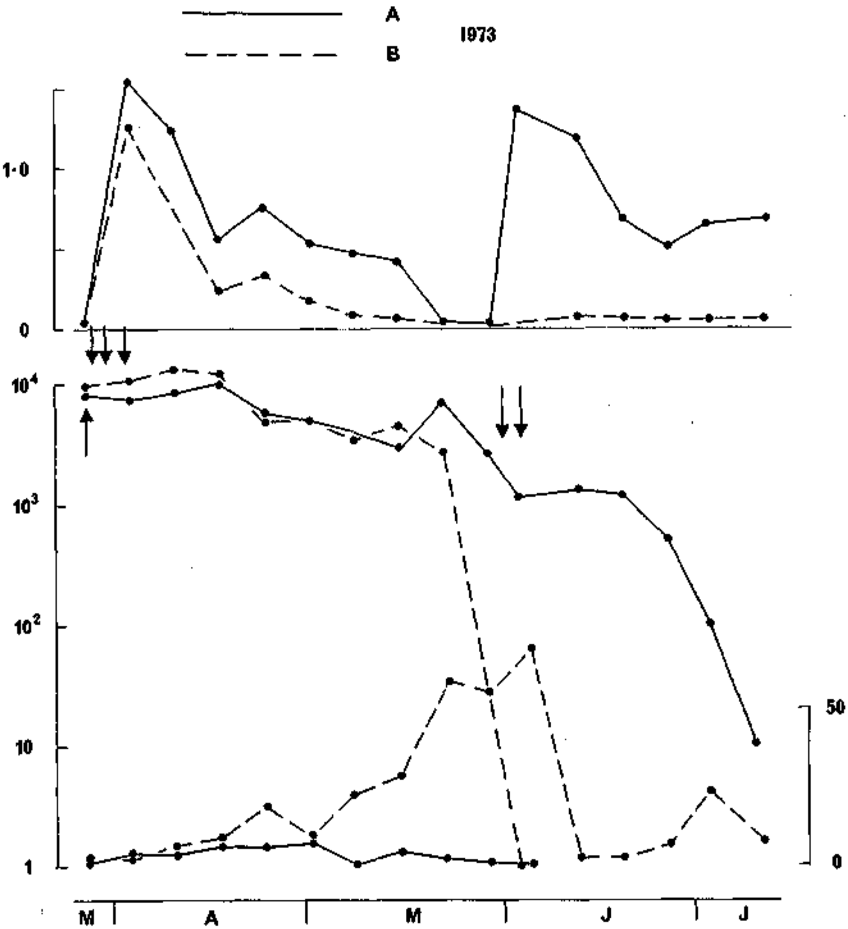

Figure 5. Blelham Tarn. Fertilization of tubes A & B with silicon and phosphorus in the spring and early summer of 1973. Upper graph: concentration of soluble reactive silicon (SRS) in the 0 - 5 m column of tube A (continuous line) and that of tube B (broken line). Middle graph: total live diatoms in the 0 - 5 m column of tube A (continuous line) and that of tube B (broken line). Lower graph: total length of filaments of *Oscillatoria agardhii* var. *isothrix* in the 0 - 5 m water column of tube A (continuous line) and of tube B (broken line). Arrows below upper horizontal axis, pointing downwards, from left to right. : first three arrows, dates of addition of silicon to both tubes. Next two arrows, at lower level, dates of addition of silicon to tube A alone. *Arrow on left hand side, pointing upwards, date of addition of phosphorus to tube B alone. Left-hand vertical scales : upper, SRS, mg l^{-1}; lower, diatoms cells ml^{-1} (log_{10} scale). Right-hand vertical scale : total length of *Oscillatoria* filaments, mm ml^{-1}.

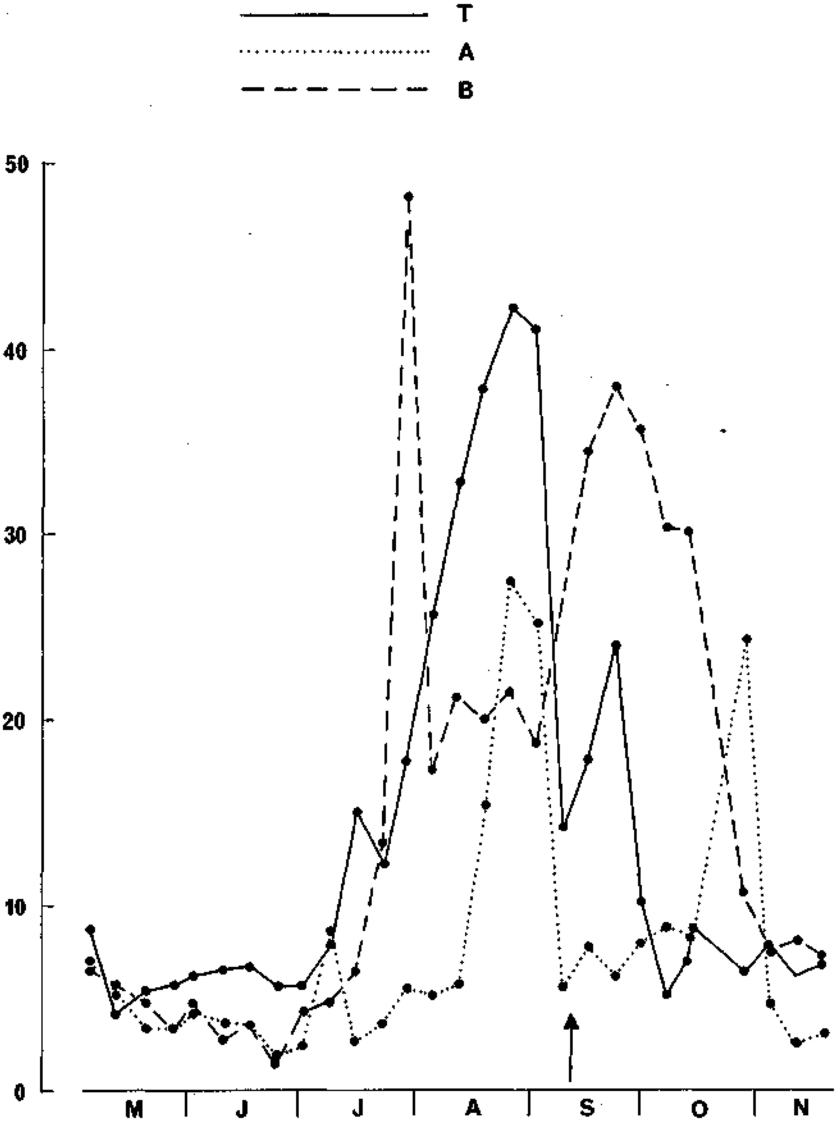

Figure 6. Blelham Tarn. Weekly fertilization of tubes A and B from the end of April to late November, 1975, with phosphorus, nitrogen and silicon and of tube B on 11 September (arrow above horizontal axis) with ferric iron and EDTA. Vertical axis : concentration of chlorophyll *a* (µg l^{-1}), without allowance for phaeophytin, in the 0 - 5 m water columns of the lake (continuous line), tube A (dotted line) and tube B (broken line).

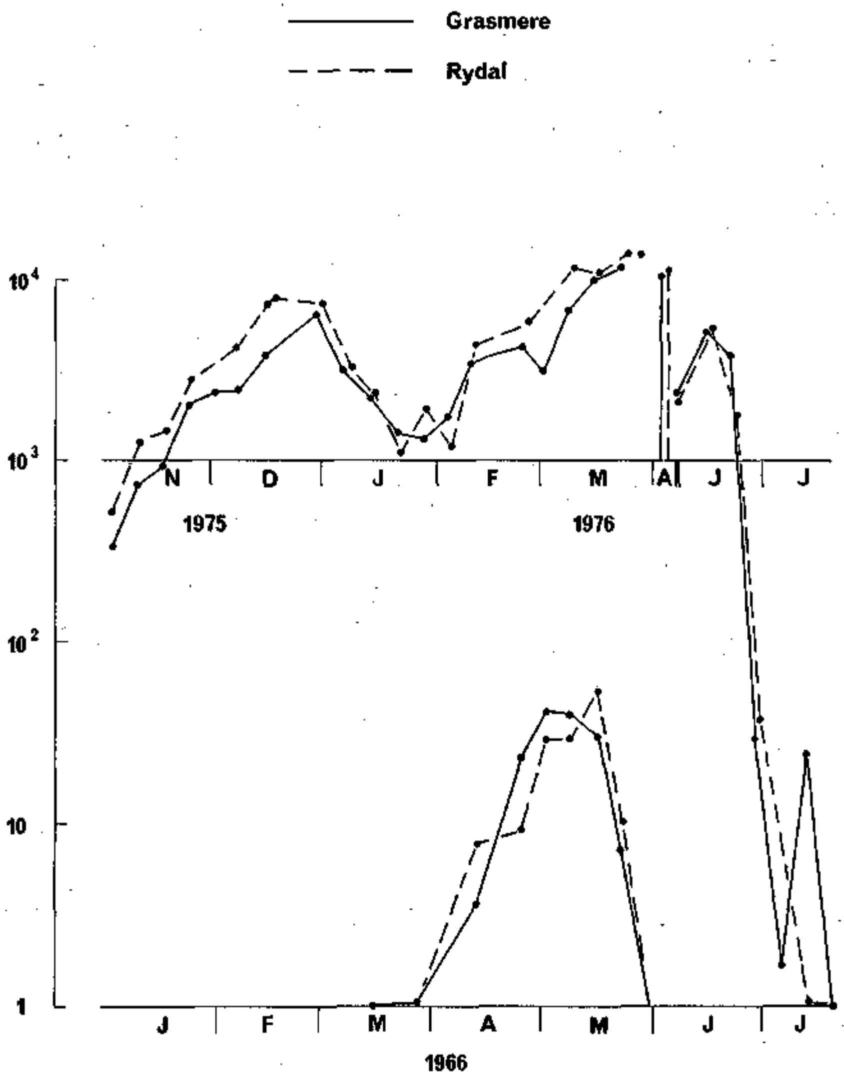

Figure 7. Abundance of *Asterionella formosa* in Grasmere (continuous line) and Rydal Water (broken line). Upper graph : from 17 November to 4 August, 1975 - 1976 when the diatom was abundant. Lower graph : from 3 January to 5 September, 1966, when it was rare. Vertical scale : live cells ml^{-1} in the 0 - 5 m water column (log_{10} scale).
Note. In 1976 there were only two counts (vertical lines) between the end of March and the beginning of June. The lines marking the decrease of these populations from late June to the beginning of August pass down to the horizontal axis of the lower graph.

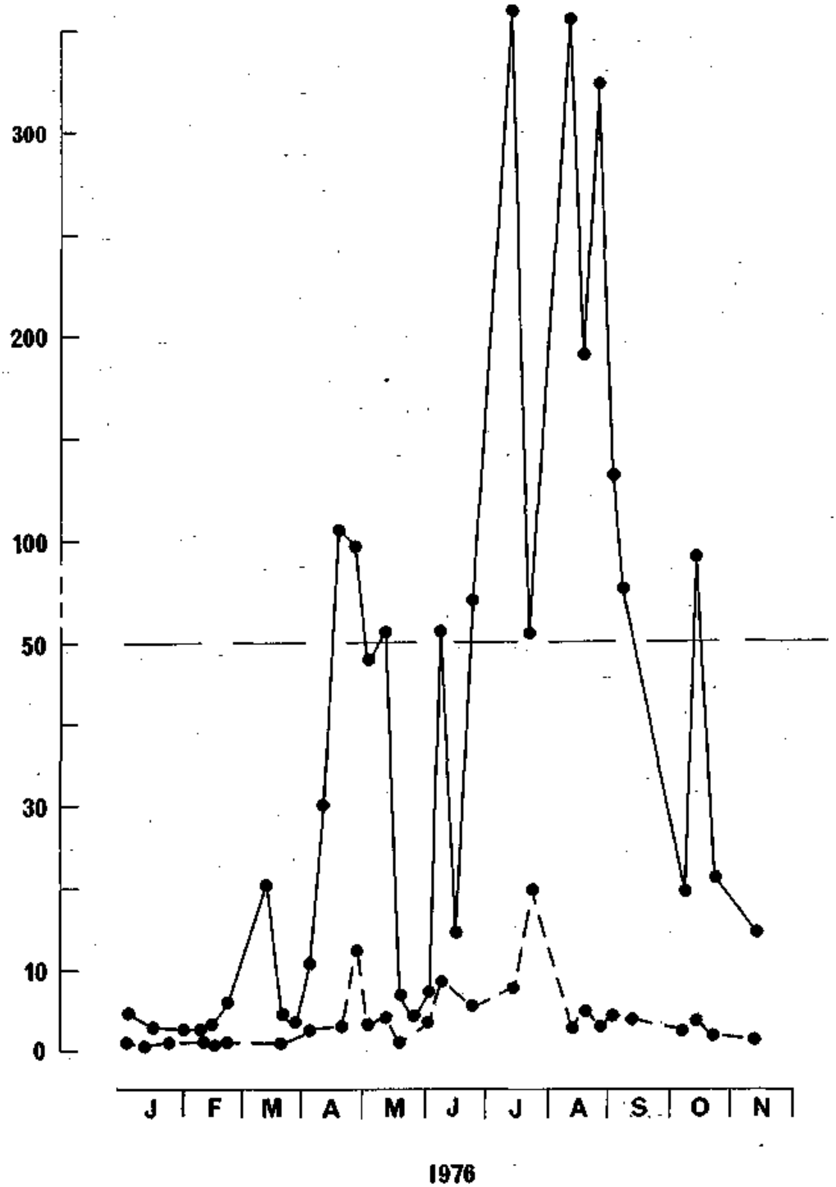

Figure 8. Elterwater, 1976. Chlorophyll *a*, without allowance for phaeophytin, in the 0 – 5 m water column of the inner (continuous line) and outer (broken line) basins. Vertical scale : chlorophyll *a*, $\mu g\ l^{-1}$. Note change in scale above 50 $\mu g\ l^{-1}$.

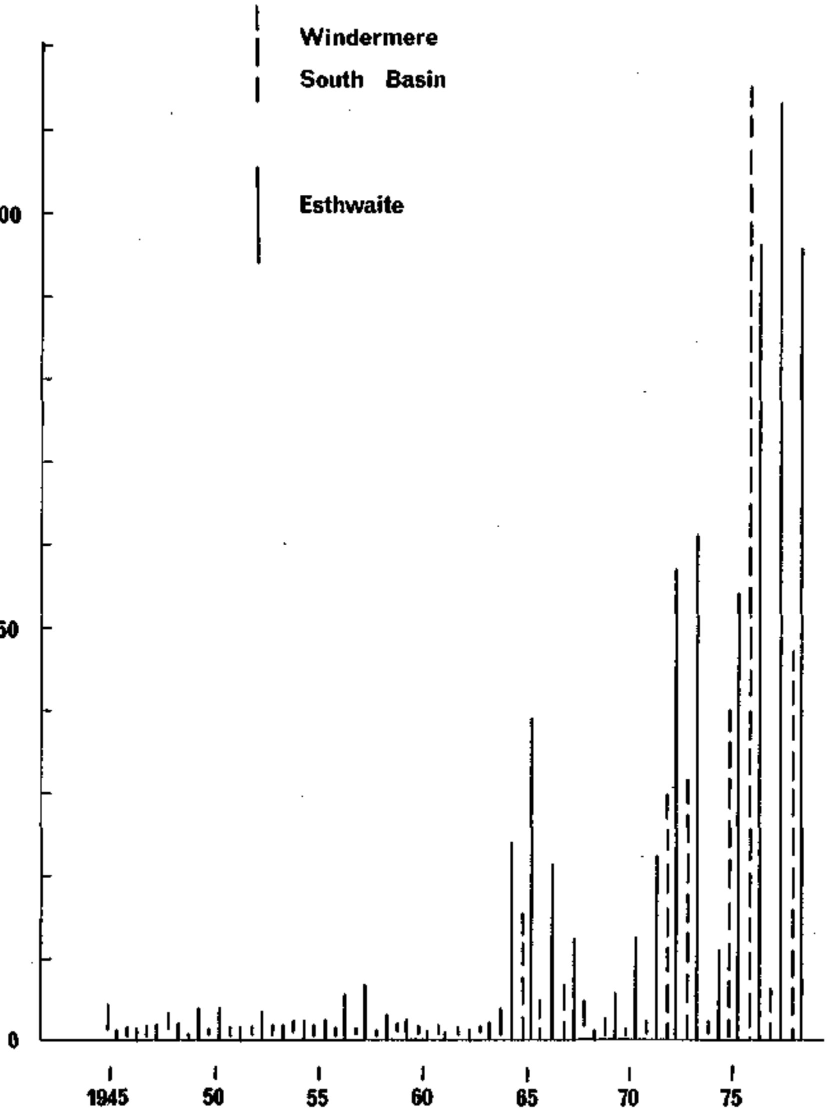

Figure 9. Esthwaite Water and Windermere, South Basin. The average weekly abundance of *Ceratium hirundinella*, 1945 - 1978 inclusive, in the 0 - 5 m water column of Esthwaite Water (continuous lines) and 0 - 5 m (1945 - 1962), 0 - 10 m (1962 - 1 June 1964) and 0 - 7 m (2 June, 1964 onwards) water columns of Windermere, South Basin (broken lines). Vertical scale : Esthwaite Water, cells ml^{-1}; Windermere, South Basin, cells 5 ml^{-1}. Concerning 1978, see text.