

178/1

DIATOMS AS WATER QUALITY INDICATORS IN BRITISH RIVERS

F. E. Round

Department of Botany, University of Bristol,
Bristol BS8 1UG, England

Initial Report to the Department of the Environment
on work carried out under DOE Contract no. 120021-00-37

CONTENTS

CONTRACT PROPOSAL

ABSTRACT

1. INTRODUCTION
 - 1.1. Prologue
 - 1.2. Initial discussions
 - 1.3. Response by F. E. Round
 - 1.4. Historical
 - 1.5. Discussion of the problem and the habitat
2. THE CONTINENTAL AND OTHER SYSTEM OF INDICES
 - 2.1. Kolkwitz & Marsson
 - 2.2. Zelinka & Marvan
 - 2.3. Lange-Bertalot
 - 2.4. Descy
 - 2.5. Coste
 - 2.6. Schoeman
 - 2.7. Watanabe
3. SITES AND METHODS
 - 3.1. Sites
 - 3.2. River sampling
 - 3.3. Sample preparation
 - 3.4. Identification and counting
 - 3.5. Preparation of indices
4. RESULTS OF SEASONAL SURVEY
 - 4.1. River Wye
 - 4.2. Conclusions
5. RIVER SURVEY
 - 5.1. Introduction
 - 5.2. Zone 1
 - 5.3. Zone 2
 - 5.4. Zone 3
 - 5.5. Zone 4
 - 5.6. Zone 5
 - 5.7. Fine tuning of zones
 - 5.8. Discussion
6. CONCLUSIONS
7. REFERENCES
 - Appendix I. Simplified method and record sheet
 - Appendix II. Method for quantitative removal of epilithon from stones
 - Appendix III. Notes on species
 - Appendix IV. Example of drawings of species
 - Appendix V. Examples of SEM illustrations to assist light microscopic determination
 - Appendix VI. Future work
 - Appendix VII. Report on Welsh river survey by Dr. K. Benson-Evans

CONTRACT PROPOSAL

Objective

To modify the Descy Index method of pollution monitoring to provide a year round, easily usable, guide to UK water quality.

Programme of work

1. To identify a number of rivers, both polluted and unpolluted, draining regions of differing geological characteristics in England, Scotland and Wales and select sampling points.
2. Obtain samples of diatoms and green algae from each selected sampling point at various times of the year, identify and consider in the light of water quality parameters extant at the time.
3. Assess the applicability of the Descy method to British rivers, modify and simplify as appropriate.
4. Develop indices for the various river types considered and refine as necessary.
5. Prepare a photomicrographic and line drawing atlas of the diatoms and related organisms used in the indices.
6. Check the indices developed for all sampling points.
7. Test the system developed on other river systems in conjunction with Water Authorities.

ABSTRACT

The Descy method of assessing water quality of rivers using diatom analyses as submitted to the DoE in 1985 has been shown to work. However, several complicating factors relating to the sampling technique have been discovered. The indices of water quality were biased towards lowland Belgian rivers and much new data has been obtained making the system applicable to the wider range of waters in the British Isles. The large EEC Commission study of river diatoms has been extended into an ongoing study involving French workers and this is resulting in more complex indices (see Appendix VI, Section 7). In addition, German water authorities and research institutes have initiated programmes on river diatom floras, and these need to be taken into account.

To date 67 rivers have been sampled from near their source to estuary (or confluence with another river) and from 470 sites a diatom flora has been obtained. A seasonal survey of two rivers revealed the presence of an epilithic (living on stone surfaces) diatom flora at all times; there are no complicating life cycle reproductive or resting stages. Field sampling is simple and laboratory preparation requires only basic techniques. However, after much sampling using the Continental system, it was discovered that two quite distinct floras were associated with stone surfaces and that these had been combined in all the studies in other countries. This feature explained many of the anomalies which I detected in the literature. Simple methods were devised to assess the two communities separately but this did mean that a complete re-assessment of the Continental work was required. The indices of water quality using the Continental systems do not provide very fine distinctions since numerical indices obscure subtle distinctions caused by environmental factors. The wide survey of rivers revealed that certain species or groups of species were characteristic of certain stretches and these, rather than numerical values, offer to provide the most detailed information on water quality. Five major zones and six subzones have been defined from the diatom analyses. The confusion over the two floras has not helped define this zonation which must be improved by re-sampling some of the sites and separating the floras.

An atlas of the essential species (from drawings, photomicrographs and scanning electron micrographs) is being prepared which will allow non-specialist workers to identify the indicator diatoms. Scanning electron micrographs can be of great assistance to light microscopists when identifying diatoms.

Since 1985, Descy and his co-workers have extended their sampling to countries other than Belgium (including the UK) and have also developed elaborate systems using greater numbers of species. There are several other active groups working in Europe and methods using scanning electron microscopy are being perfected in Germany. Whilst such detailed scientific work is essential to understand the full complexity of the biological system, the techniques can be simplified for routine use - see proposals in Appendix I.

A simplified system of water quality assessment using diatoms is proposed. This can be easily operated by water

authorities yielding rapid results which are just as accurate as those from more complicated surveys. Apart from their use to classify stretches of rivers, the diatom analyses enable the exact extent of acid water reaches (important for fish colonisation), pinpoint the onset of eutrophication, indicate zones of pollution/recovery and the effect of saline incursion. The diatom flora reflects the overall chemical status of the river at any one point, integrating all the daily/seasonal changes.

This is, surprisingly, the first survey of the diatom communities in British rivers and will provide base-line data for the whole range of river types, but much remains to be done and a summary of proposals for future work is provided (Appendix VI).

Work on green algal species ecology in Welsh rivers by Dr. K. Benson-Evans and her co-workers has been continued during the period of this survey and the results reveal many interlocking features. The green algal/diatom/chemical data has been subjected to various statistical/correlative analyses and both sets of data are in a state for the application of further analysis by the most recent canonical community ordination techniques.

1. INTRODUCTION

Biological methods to assess water quality have been used during the last 100 years since it was realised that ultimately it is the effect on organisms which is critical and that this can only be measured by studying the reaction of the organisms themselves; only now is the full impact of deteriorating water quality on fish, seals and humans being realised. Micro-organisms are more widespread and more sensitive to environmental factors than larger organisms. The most abundant photosynthetic group in waters is the diatoms, yet they have received virtually no attention in UK waters in relation to water quality. The present work is the first comprehensive survey in British rivers.

1.1. Prologue

Correspondence between the DoE and Dr. J.P. Descy working in Belgium led to discussion of the possibility of testing on British rivers the methods devised on the continent for water quality assessment using diatom indices. The Belgian, and to a lesser extent, the French (Coste) and German (Lange-Bertalot) indices were outlined in a communication from Dr. Descy and the key points made by him in this and his publications are:-

- a. Periphytic diatoms (in this instance epilithic - i.e. growing on stone surfaces) can be very reliable water pollution indicators.
- b. Diatoms are easy to collect and fast sampling of the rivers is possible.
- c. The diatom flora is widely distributed throughout the length of the water course and in waters of various types. Sampling of an area of stone surface yields millions of cells and errors due to fine surface variations in populations are eliminated.
- d. The diatoms show a wide range of sensitivity to pollution even in cases of moderate pollution.
- e. The diatoms respond rapidly to water quality changes.
- f. The diatom community composition depends mainly upon the chemical characteristics of the water and not on the size and physical features of the rivers.
- g. The quantitative data are very sensitive.
- h. Microscopic counting is accurate and reproducible.
- i. The ecological requirements of diatoms are well known.

1.2. Initial Discussions

The Chairman of SCA sounded out the opinion of several experts prior to the letting of the contract and their comments indicated that:-

- a. Diatoms were indeed abundant in rivers and ought to be used in biological monitoring. One correspondent referred to work showing that the flora was more or less the same whether upper surface or sides of stones sampled, but that there may be differences related to surface characteristics of stones. Problems may arise with identification, but these could be overcome by provision of illustrations.
- b. No systematic work on the diatoms of British rivers was available; diatoms should be used in long-term programmes monitoring British rivers. But Dr. Descy's method should not be used before more intensive knowledge of pollution tolerance of the species was obtained.
- c. The Continental data was based on too few rivers and these were largely wide, slow flowing lowland rivers and many British rivers are not of this type. During sampling of fast flowing rivers, epilithic species can be lost.
- d. The Continental surveys did not extend over the range of geological formations through which British rivers flow.
- e. The taxonomic notes provided by Dr. Descy were an unnecessary complication and doubts were cast upon the "knowledge of the tolerance of individual species".
- f. A simplified system might be devised using a smaller number of species and using the composition of the community and not numbers of diatoms.
- g. There would be a need to provide illustrations and not rely on the floras mentioned by Dr. Descy since they were written in French or German.
- h. There was scepticism of the use of mathematical indices particularly since the basis of some of the values used by Continental workers could not be checked and to a large extent there was no critical evaluation of methods in the Continental papers.
- i. Good quality microscopes would be essential in any laboratories using diatoms in biological monitoring.
- j. It was pointed out that diatoms from at least two benthic communities were present in Dr. Descy's paper. The need to identify and count large numbers 250-500, even 1500, in samples was mentioned.

1.3. Response by F.E. Round

In my initial response to the request to comment on the use of diatoms as indicators of water quality and at a subsequent small meeting with members of SCA, I made the following points:-

- a. Water quality indices must be based on organisms which occur in all waters at all seasons of the year. They should have no complicating resting stages and can be easily identified without the need for specialist training. They must be identifiable by

different workers in different laboratories with no confusion and provide information on levels of pollution and overall nutrient status. The diatoms are the only group of organisms which meet all these requirements and the composition of the diatom flora is the result of the integrated physico-chemical features of the river at each station. However, I pointed out that until some exploratory work on the stone flora of British rivers was completed, we simply would not know what problems (if any) there are. Some problems in identification would almost certainly arise but could be circumvented by the production of illustrated guides. Some work on the taxonomy of some species might be necessary since there was no British work on the benthic river diatom associations.

b. Sufficient rivers must be sampled to establish the applicability of the indices and if necessary to define a small number of indices for use in different geological/physical watersheds. The claim by Descy that diatoms react quickly to pollution needs testing.

c. There must be a fairly detailed assessment of diatom distribution in UK rivers in order to contribute to any discussion of an integrated European approach - to date we have no data from which to argue a case.

d. Correlation with nutrient levels (e.g. nitrate-N) should be attempted to yield a further indicator of changing nutrient status; a number of rivers encompassing a wide range of nutrient status should be sampled.

e. The diatom flora on stones has been used by most overseas workers but this is only one of at least four benthic diatom assemblages in rivers and contamination and/or exchange between the communities may raise problems. Workers never seem to check the material in a live state to determine whether or not only live species are being recorded. It is necessary to check whether or not dead cells from adjacent or upstream communities remain for any length of time in the community.

f. I expressed doubts about the validity of the quasi-mathematical schemes which had been devised and further discussion with other colleagues confirmed my doubts. Data obtained from British rivers should be tested using the continental indices and if possible other non-mathematical indices should be produced for defined geological/nutrient status river groups.

g. A simplified system was desirable and illustrative material needed to avoid the need for complex floras (most being written in languages other than English). I stressed that it would be necessary to use only very distinctive species. A system of recording presence or absence of selected species might be evolved for water works practice.

The outcome of the discussion and written comments was a contract to investigate the use of diatoms as water quality indicators (see over).

1.4. Historical

The first serious attempt to use diatoms as indicators of water quality was the study by Kolkwitz & Marsson (1908) who showed for the first time that water quality determined the composition of the flora. They made no effort to define the microhabitats in which the algae grew and the next important series of papers (Butcher 1932, 1940, 1946) used the technique of placing glass slides in rivers and analysing the growth on these. Unfortunately this is a highly selective technique and many diatoms capable of attachment to natural surfaces do not attach to glass. Nevertheless this pioneering work by a very perceptive biologist gave many clues which should have been followed up, e.g. in Butcher (1932) we find the clear statement "Diatoms comprise undoubtedly the largest and most prevalent single group of algae in a river". In spite of the technique he showed differences between calcareous and non-calcareous rivers, distinguished communities of the oligotrophic and eutrophic zones, distinguished attached (adnate), pedunculate and free-living components, showed that the "river plankton" contained many cells from the benthic habitats, showed that the diatoms undergo seasonal growth patterns (actually colonisation patterns) but that the dominant algae were always present, though occasionally one diatom could suddenly produce a peak of growth, and finally that grazing of diatoms was extremely important (Butcher 1940, 1946). An account of encrusting algae in fast flowing streams (Fritsch 1929) may have given the impression that diatoms were sparse on stones though he did comment that Cocconeis placentula occurred in all the communities.

The most quoted paper on methods is that of Douglas (1958) - the technique is similar to one described in Appendix II; it is only suitable for obtaining very precise quantitative data on relatively clean surfaces. In spite of the excellence of Douglas' sampling technique, the information obtained is of limited value since species were not distinguished - only Achnanthes, Gomphonema and Synedra groups - certainly this technique simplifies counting but obscures data if applied to rivers in general. Moore (1976, 1977) studied all the benthic diatom communities in a broad survey and showed distinct seasonal variations in biomass. Later Jones (1974, 1978) experimented with a method using epifluorescence microscopy for direct estimation of diatom (Cocconeis) populations on stones - this works only on clean stones but does not enable accurate species determinations.

The only other British work of note is that of Marker and a group of workers at the FBA (Marker 1976a,b; Marker & Gunn 1977; Casey, Clarke & Marker 1981; Marker & Casey 1982) who studied natural populations and populations in circulating artificial streams. The approach was mainly physiological/primary productivity biased and mainly on chalk streams - again, the diatoms contributed large amounts of biomass and showed considerable seasonal variation - in contrast to Butcher's earlier comments on chalk rivers. McIntyre (1968) working in the United States also made a detailed study using artificial streams and showed that light and current levels could affect the flora.

Nowhere in the British work is there a full account of the

diatom flora in any benthic habitat, nowhere a comprehensive account of the variations in the diatom flora along the length of any river; nowhere is there any relation of the flora to the chemical conditions.

The major work on the epilithic diatom flora has been undertaken in Europe (see Sladecek) and in the last decade the Belgian, French and German workers have shown that of the biological indicators of river water quality, the diatoms are the preferred organisms - see especially the detailed survey of Leclercq & Maquet (1987) - since only diatoms and bacteria are consistently present throughout river systems.

The system of using indices has grown out of work by Zelinka & Marvan (1961) and Sladecek (1973, 1986), though it is Descy's (1979) work which is most quoted. These indices are based on detailed quantitative studies of individual sites combined with values obtained from information in the literature concerning the occurrence of diatoms under particular ecological conditions. From these, tables have been produced providing a consensus opinion of the tolerance of the diatoms to the environmental factors. The latest and most detailed is that of Leclercq & Macquet (1987); this summarises also the data from the work of Descy (1979), Mounthon & Coste (1984) and Sladecek (1986). All the information is converted to an arithmetic form and each of the 199 species characterised by a value between 1 - 5 (1 indicating a position in saprobiontic and 5 in saprophytic situations) - in other words, in polluted or clean water. All the calculations involve estimates of the dominance of species in the habitat plus a subjective (?) assessment of the value of each species as an indicator. This latter is a complication since it is essentially based on an estimate of the "width" of the species distribution - narrow limits giving "good" and wide limits giving "poor" indicator values. The Leclercq & Maquet (1987) work provides the most complete comparison of chemical invertebrate and diatom indices. Lange-Bertalot (1978) used groups of "differentiating" species to define zones and bases his arguments on the view that it is increasing levels of pollution which limit diatom distributions - whilst this is true, it is equally true that the "stress" of clean water also limits some species. A further interesting study of the diatoms in the outflow streams from sewage works (Scharf 1984) showed that diatoms provide more subtle distinctions of water quality than chemical analysis.

Several studies, e.g. Leclercq (1977) and Fabri & Leclercq (1979 & 1987) in Belgium; Cambra (1987), Sabater *et al.* (1977), Sabater & Sabater (1978) in Spain, have provided data on the fast-flowing upper reaches of rivers. The acid rivers are dominated by Eunotia spp. and the calcareous Spanish rivers by Ceratoneis arcus, Diatoma mesodon and Denticula tenuis. The Spanish workers also discussed the changes downstream as eutrophication and pollution increased - their results are in general agreement with those of other European workers.

Finally to mention two other techniques. In 1948 Margalef proposed a method to obtain the algae from surfaces by coating them with a plastic and stripping this off - it has never been developed. The other technique, much more widely used, is to use artificial substrata (usually glass microscope slides) in the

rivers. This technique does collect diatoms but it has never proved of great value though equally it has rarely been used in an extensive downstream study. Some workers have considered species diversity to be a better reflection of water quality than specific composition of the communities, e.g. Stevenson (1984), whilst the reverse view is expressed by Sullivan (1986), "the most important parameters in assessing water quality using diatom communities were the identity and autecology of the constituent species, in particular the dominant ones". He favours a quantitative indicator species approach which he believes works well. Similar view are expressed by Van Dam (1982), Archibald (1972), Schoeman (1979) and Kobayasi & Mayama (1982).

The above is not a complete survey of the literature - only a selection of works which I have found valuable.

1.5. Discussion of the problem and the habitat

Simply to blindly follow the methodology of other workers did not seem the most productive approach - especially since there was criticism of the continental work. In fact it was not at all easy to determine the exact details of the continental techniques. The aquatic system is extremely complex and on a chemical basis forms an unbroken cline of factors which can only be arbitrarily subdivided. The organisms on the other hand are confined to certain water types and limits exist in nature and do not have to be arbitrarily fixed.

In view of the virtual absence of published data on the benthic diatom flora and from experience of the first few samples, it was decided to undertake a comprehensive survey of the epilithic diatom flora from as wide a standpoint as possible whilst still obtaining data which could be compared with the continental indices. The absence of any detailed taxonomic treatment of the flora required that a systematic study be undertaken using both light and scanning electron microscopy, not for its own sake, but in order to make the ecological data accurate. Identification of species must be precise if any system of indices is to be developed. Scanning electron microscopy has been used in this study only to check the identity of species which exhibit a range of form in order to give confidence to the light microscopic identifications. It is unlikely that it will be used in routine work although a recent South African river has been studied from counts under the SEM and Klee & Steinberg (1987) have used it in a detailed study of planktonic centric diatoms in Bavarian rivers. This is the only study I know which is made by a water authority using SEM but I have just learnt that these same workers are now starting a similar study on the epilithic diatoms of Bavarian rivers.

By definition, the epilithon is the flora (and fauna) attached to stone (rock) surfaces. Reports in the literature indicated that the diatoms comprise the bulk of algae attached to stones though there are times in the year when other algal groups, e.g. Chlorophyta and Cyanophyta, become abundant. Taken over a year's cycle, however, the diatoms are the predominant element of the epilithon. Also by definition the diatoms of the epilithon will be non-motile species. (It is of course

conceivable that actively moving forms could be associated with the surface but in running water this would on theoretical grounds be unlikely - though this would require checking). Attachment involves secretion of mucilage, hence the surface of the stones becomes covered with a gel-like layer. Within this gel two life forms can exist:- (a) adnate forms in which the siliceous skeleton is attached directly on the rock surface (Round, 1981) - there will of course be a very fine layer of mucilage secreted by the diatom, lying between the diatom and the rock surface; (b) the pedunculate forms which are attached to the rock surface by mucilage stalks of varying length, the cells being held above the rock surface.

All species therefore in this epilithic community are drawn from the genera which have the capability of secreting mucilage strictly for attachment. An initial perusal of the literature showed that a large number of species recorded and used in the indices were not of this nature. For example, all the species of Navicula, Nitzschia and Suirella are actively motile forms. In Descy's 1979 publication, at least 56 species are motile and 40 are non-motile attached species. This obviously needed careful investigation. It is just conceivable that some motile species live in the layer of mucilage produced by the attached forms but most live on the layer of silt deposited on the mucilage secreted by the attached epilithon - they are not technically part of the epilithon (see Fig. 1).

Soon after the commencement of sampling it became obvious that all previous studies had been based on gross sampling of sites. The worst example simply took any object hanging into the river and scraped the diatoms off; counting of this material resulted in the identification of 100-500 species - a ridiculous number of species "caught" from the drift in the river. Methods based on diatoms have sometimes been criticised on the grounds that there are too many species in the diatom flora of rivers; this is not true of the epilithon if the casual species are ignored. It is interesting that in Sladecek's (1973) review which lists the diatoms in some detail, the number of invertebrates listed is approximately six times as great.

Experience of many other communities and extensive reading (see Round, 1981) had convinced me that no algal habitat was as simple as is so often assumed. Why should it be - it is the aquatic equivalent of the terrestrial vegetation and just as complex. Faced with this situation I decided to make a simple assumption, i.e. that an ideal river would start in a region with natural acid rain (all rain is normally acid, theoretically at pH 5.6) and that the river water would gradually change - first losing its acidity - picking up alkalinity - showing an increase in nutrients due to farming - the catchment becoming increasingly eutrophic - and the river water finally even slightly saline. At intervals further temporary enrichment or pollution events would interrupt the sequence. The steady increase in nutrients should be accompanied by changes in the diatom flora and these could be plotted and the ideal river/ideal diatom flora be used as a basis for further studies. Theoretically, the diatoms should react to changing conditions producing a series of overlapping pulses of different species (Fig. 2). Such pulses have in fact been predicted from plots of the occurrence of individual species

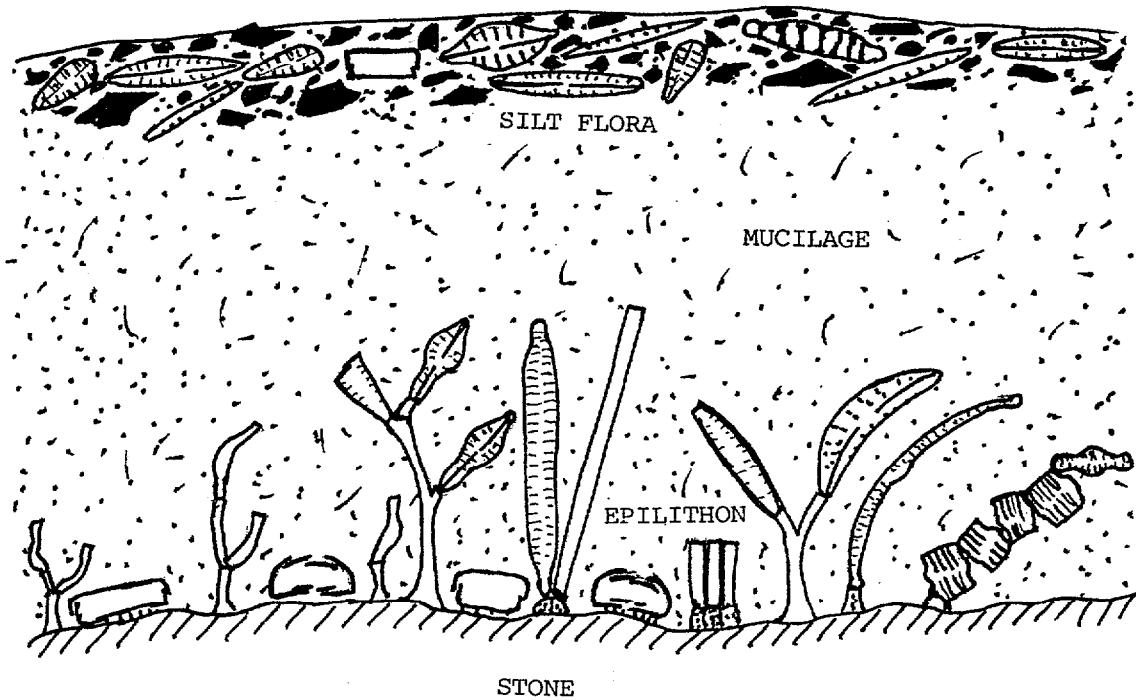


Fig. 1. Diagrammatic representation of the epilithon attached to the stones, the mucilage around these extending into an upper layer in which silt is deposited (black blocks) amongst which the surface silt flora lives.

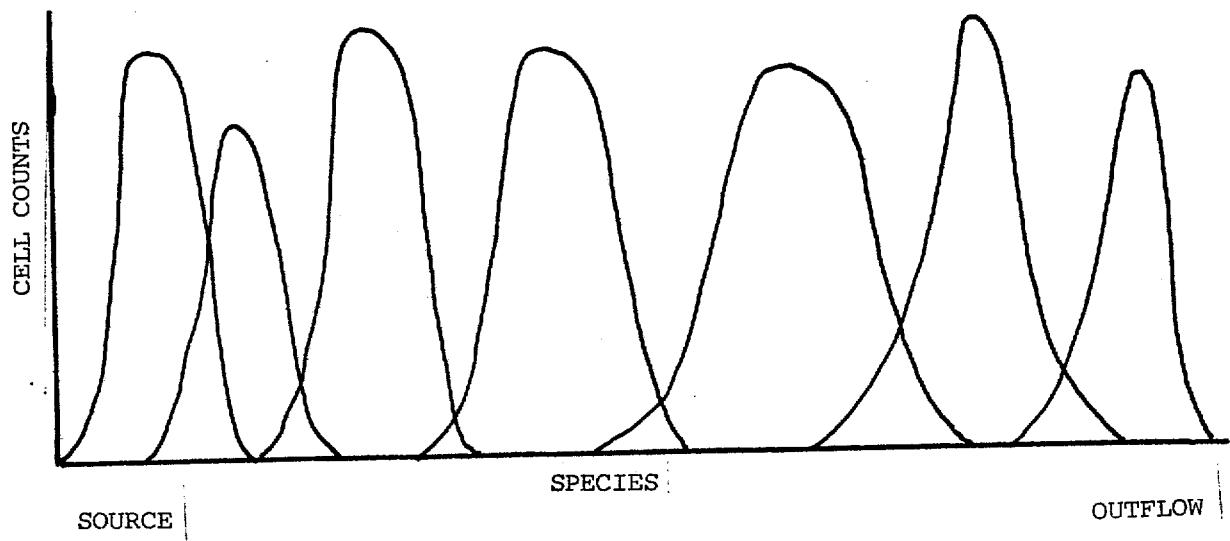


Fig. 2. Hypothetical overlapping pulses of species down a river.

against factors such as pH., alkalinity, chloride content, etc. (see Descy 1984). However, since many environmental features are fluctuating - some in unison, others independently, the situation is likely to be complex and especially so where isolated inputs occur. The two regions of extreme conditions, i.e. close to the source and towards the outflow, might be expected to have well-defined peaks whereas in the mid-reaches, more widely tolerant species may produce overlapping distributions. My basic concept in Fig. 2 is reflected in the much more complex diagrams of Statzer & Higher (1986) for recurrent invertebrate communities, the zonation of which they conclude is controlled by flow rate. The diatoms may have one enormous advantage over the invertebrates in that they occur in the boundary zone where they will be relatively unaffected by flow rate. The "ideal river" should steadily increase in nutrient status and not be perturbed by any exceptional events (large industrial sites, etc.). It was likely that such a river would arise in mountainous country in the West. Two such rivers close enough to Bristol which could also serve as seasonal "controls" were chosen - the Wye and Usk. The lowland rivers should also fit into the "ideal" but their upper reach should slot into the lower eutrophic section of the "ideal" river.

In addition, experience in diatom ecology had convinced me that all initial observations should be on live material and not on acid cleaned material mounted on slides. The reasons for this were (a) studies of lake epilithon have shown that here the community structure is complex (like that of a terrestrial forest reduced to a microscopic scale) and the major features of this community could only be determined by observation of live material; (b) counts of cells in acid cleaned material assume that all cells were living at the time of sampling; (c) such material may contain contaminants from upstream communities or from adjacent non-epilithic communities - both may possibly be detected if live material were observed; (d) observation of live material is necessary to (i) check persistence or invasion of cells, (ii) check grazing or parasitism of cells, (iii) detect sexual reproductive stages, (iv) check death of cells through nutrient limitation or toxicity, (v) indicate periods of seasonal variation should this occur. All these features were considered possible complicating factors when using diatoms as water quality indicators - but seem not to have been considered by most other workers. They may not affect final proposals but certainly required detailed consideration at the developmental stage.

A decision had to be made on where and at what intervals the rivers should be sampled. To make sampling possible within reasonable time limits, it was decided to collect from as near the source as possible to near the estuary wherever the river could be approached at bridges, etc. and at roughly equal distances (approximately 10-20 miles) apart. At the same time, general notes would be made on the type of river and its surrounding catchment.

To take the above features into consideration, it was realised that it would be necessary to transport stones back to the laboratory. A further useful feature emerged from this decision. Seven to ten stones were usually selected from each

sampling site, but these proved not to be always of the same rock type, so in the laboratory the major rock type was selected for sampling and the others discarded. This reduces or eliminates possible variation in the flora on different types of rock. Blin et al. (1980) found little variation on three different rock types after the initial colonisation period - their results differ in another way from European studies in that Epithemia sorex was a dominant in the community - this feature alone made necessary a study of U.K. rivers for comparison with Continental European which might have different species.

The technique utilised by most workers on rivers varies slightly but essentially involves the removal of the diatom flora from stone surfaces, either by using an in situ sampling device or by lifting the stones from the river and sampling the surface in the field or in the laboratory. This is followed by preparation of the material for light microscopy followed by identification and counting of species (usually counts of 500 valves per sample) and finally using the counts in some mathematical expression which can be linked to water quality measurements.

It soon became obvious that sampling the stone surface was not quite as simple as other workers had maintained. It can be made simple, e.g. by simply brushing everything off an area of the upper surface. This works excellently on stones with a coating of primary colonisers and no contaminating macroalgae (such as Cladophora or Lemanea) or mosses or liverworts. However, in addition to these macroscopic plants there is often a layer of silt on the stones and coating this a layer of diatoms (see Fig.1). To take this situation into account the surface layer can be washed off by gentle rubbing and spraying with distilled water - this sample is then treated separately - the photographs Figs 3, 4, & 5 left-hand columns show examples of this flora. Beneath this is a layer of mucilage which is produced by the diatom growth and often fills the space between the branching upright stalks of diatoms. This layer can be removed under the tap, "washing" the stone with the palm of the hand. Finally the un-mucilaginous surface is rubbed vigorously and washed off - this contains the primary epilithic flora (Figs 3, 4 & 5 right-hand columns) which is quite different to the surface silt flora. The combination of these two floras explains the appearance of non-motile and motile species in the list of species used by continental workers. Subsamples were always checked under the microscope at this stage - whether cells were live or dead noted and any parasitism or grazing noted. Presence of other algae and anything else of interest also was noted.

All the systems devised rely on "indicator species" though these are disguised by the arithmetical treatment - each species "indicates" a degree of tolerance to oxygen level or nutrient level or pollution level, etc. In the literature the "indicator value" is derived partly from correlation with chemical/physical data and partly from comments in the literature usually based on data from cleaned samples and this results in the impression that many diatoms are widely distributed and little value as indicators. This type of consensus ecology is unsatisfactory and must be replaced by more precise data derived from careful sampling, consideration of live assemblages, measurement of

Fig. 3. The flora on stones from the River Ribble. The silt flora (left column) is dominated by Navicula lanceolata with a small amount of Navicula gregaria. The true epilithon (right column) is rich in Reimeria sinuata and small Navicula species indicative of extreme eutrophication. In this, and the succeeding figs 4 and 5, the consistency of the diatom flora in the two microhabitats is shown in the three random micrographs from each slide.

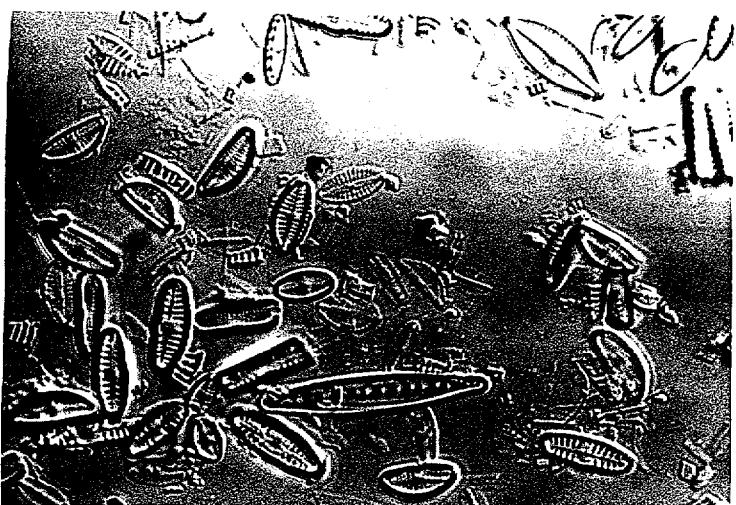
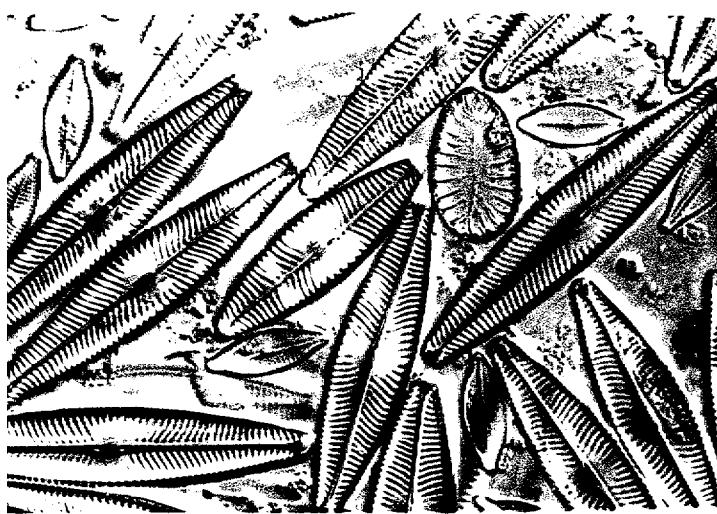
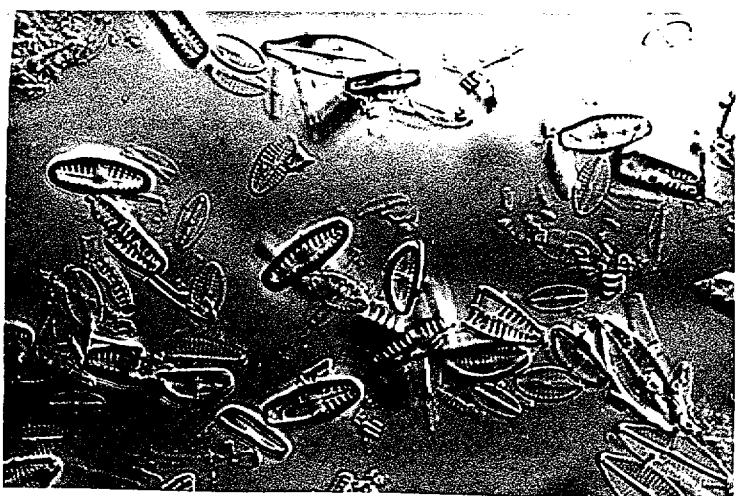
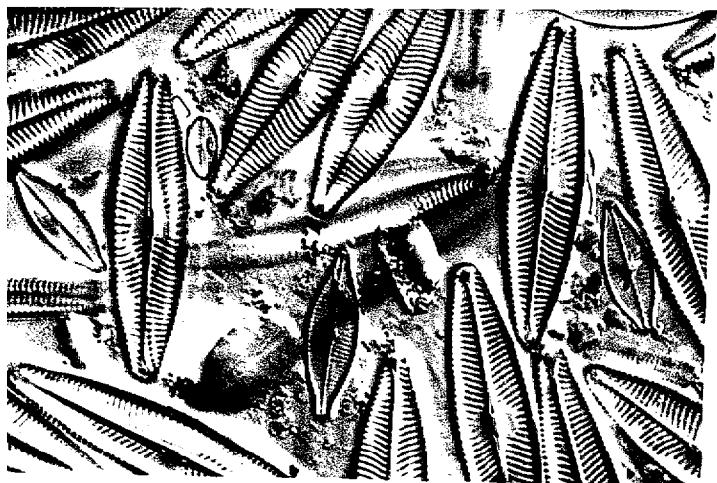


Fig. 4. As for Fig. 3 but from the River Kent. The silt flora (left column) is dominated by Navicula gregaria. The true epilithon (right column) is dominated by Navicula species - probably a new species.

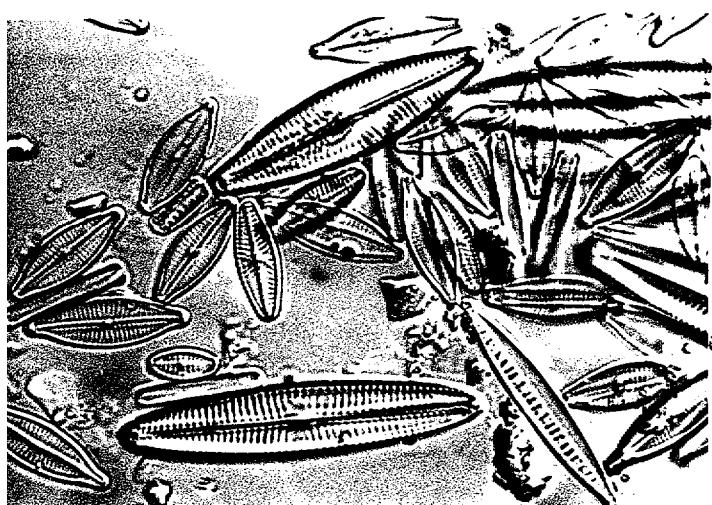
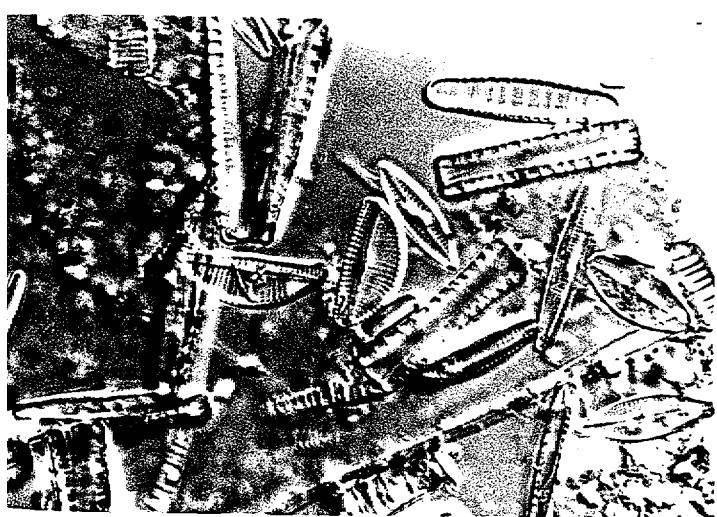
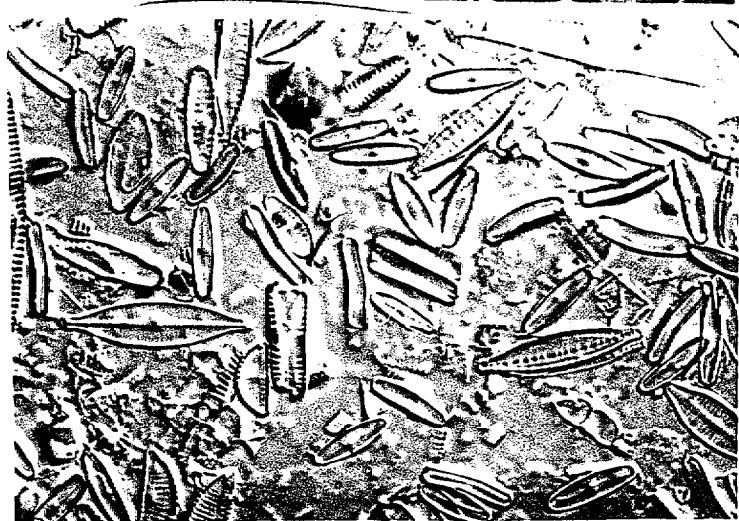
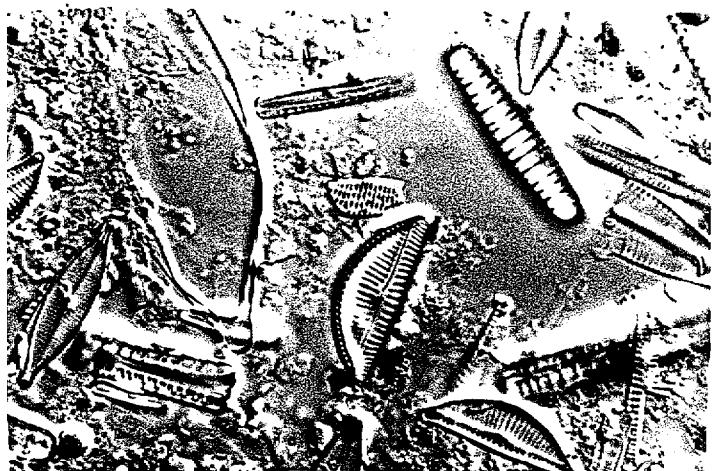
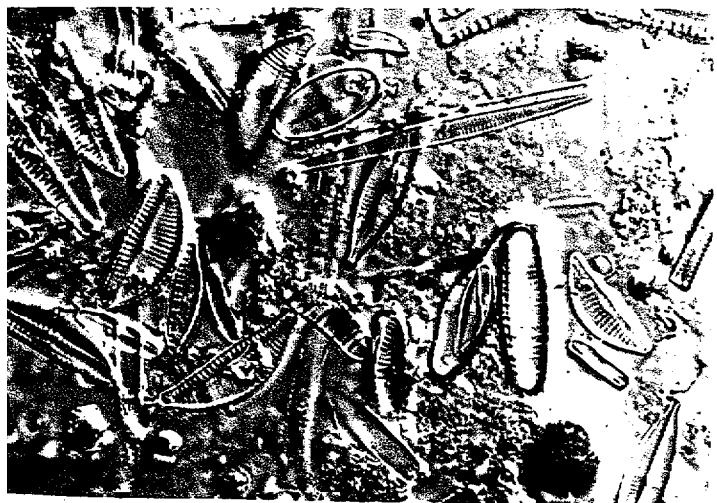


Fig. 5. As for Fig. 3 but from the River Manifold. The silt flor (left column) has a Diatoma species, with Cymbella sileciaca and Navicula gregaria present. The true epilithon (right column) has Achnanthes minutissima dominant.



factors at the rivers, etc. The indicator species are allied to cell counts so given a further arithmetic function - this is carried to an extreme in some Japanese work (Watanabe *et al.* 1986).

The problem of counting also arises - how many cells (valves - see Fig. 6) should be counted - the more cells counted the more species added to the list; this only adds confused data into the system, since the casual, even washed in, species are drawn into any calculations. In fact these casual species are irrelevant to the state of the river at any one site. Only the dominants need be considered - in rich samples these are obvious in a single microscope field and it is never necessary to count more than 100 valves. The spurious accuracy from greater counts is meaningless when some of the problems of counting are realised, e.g. valves do not always come apart so counts are a combination of single valves or of two valves counted as one (since every diatom cell has two valves, some workers divide the counts by two to give a count of cells - but this presupposes that all the cells have separated into individual valves); some cells are always seen in girdle view and cannot always be identified in this view; in the case of genera like Tabellaria, care must be taken to count only valves and not girdle bands (see illustrations of Tabellaria where there may be 5-10-15 girdle bands to one valve); cells are sometimes fragmented and therefore difficult to count accurately - arbitrary systems are then applied (e.g. three-quarters or more of a valve counts as one, apices of needle-like forms can be counted and divided by two: but see above); there are usually a few valves which lie awkwardly and cannot be allocated with certainty to species; a few valves in any sample are often difficult to assign to a particular species. Nevertheless in almost every slide made it is possible to list the dominants even though cell counts will vary considerably. Counts should however be made but used with caution. Experience may reveal that some rare species are useful indicators and intensive work on single rivers may indicate further peculiarities.

To return to the problem of how many valves (cells?) should be counted? Most workers suggest 500. Schoeman (1973) found that counting between 400 and 800 gave differences of only 1-2% and so settled on 400. My experience is that there is little point in counting more than 100 since this gives a perfectly adequate estimate of the dominants considering all the variables listed above - it also eliminates the long lists so prevalent in many works. In actual fact, when most workers data is examined, it turns out that in spite of the large numbers counted only the few dominants are used in the final preparation of the indices. The diagrammatic illustrations (Fig. 7) show what some single fields look like in the light microscope - the upper field has a Navicula species dominant and the lower has Achnanthes dominant, Reimeria and Amphora subdominant. The micrographs (Figs 3 - 5) which were taken randomly on each of six slides also show how similar the microscope fields are for both the silt flora and the true epilithon. There are, of course, contaminants from either flora in the other but these clearly do not influence any overall count.

Much data in the literature indicates wide ranges for many diatom species but this is almost certainly due to detection of

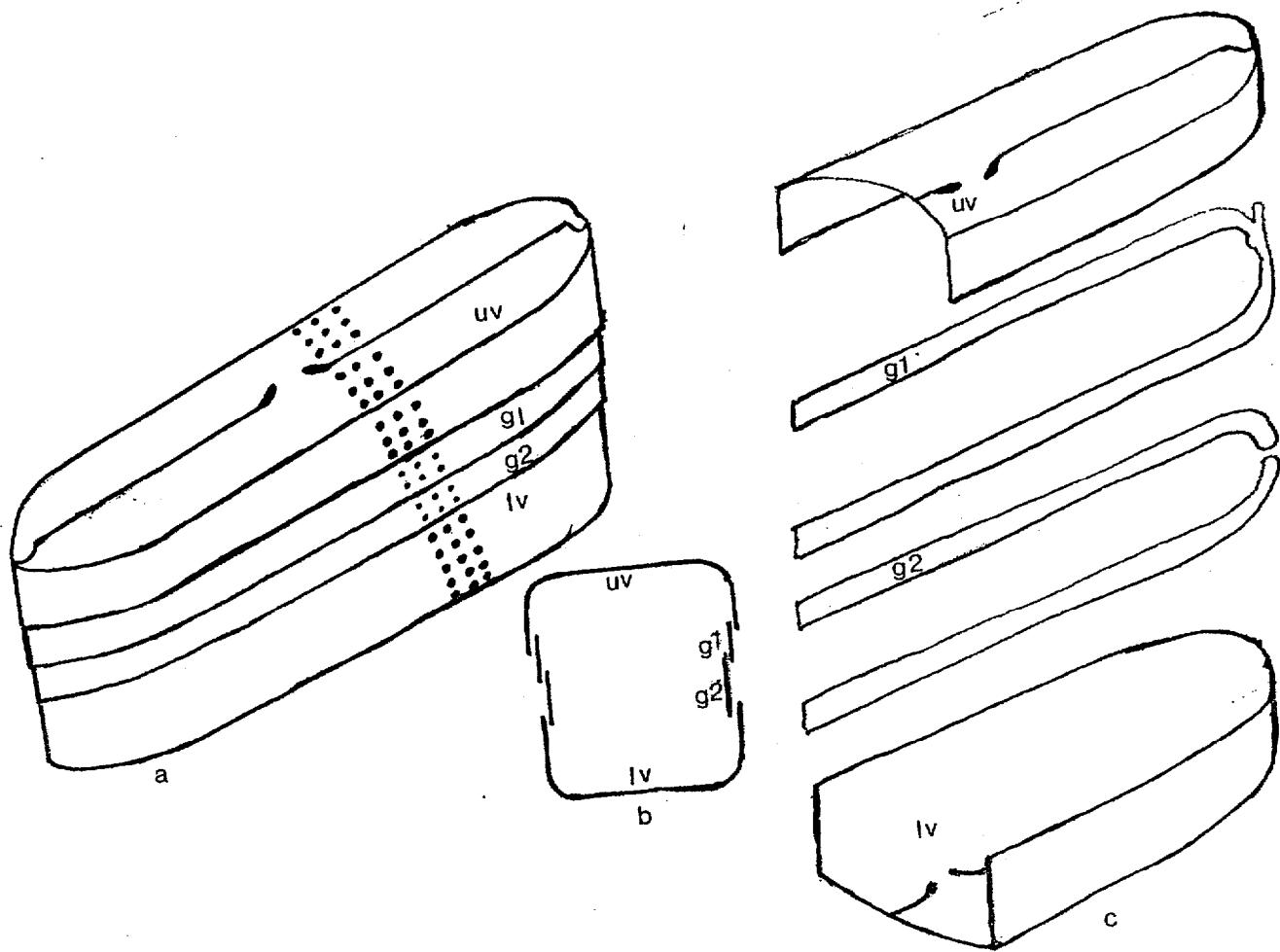
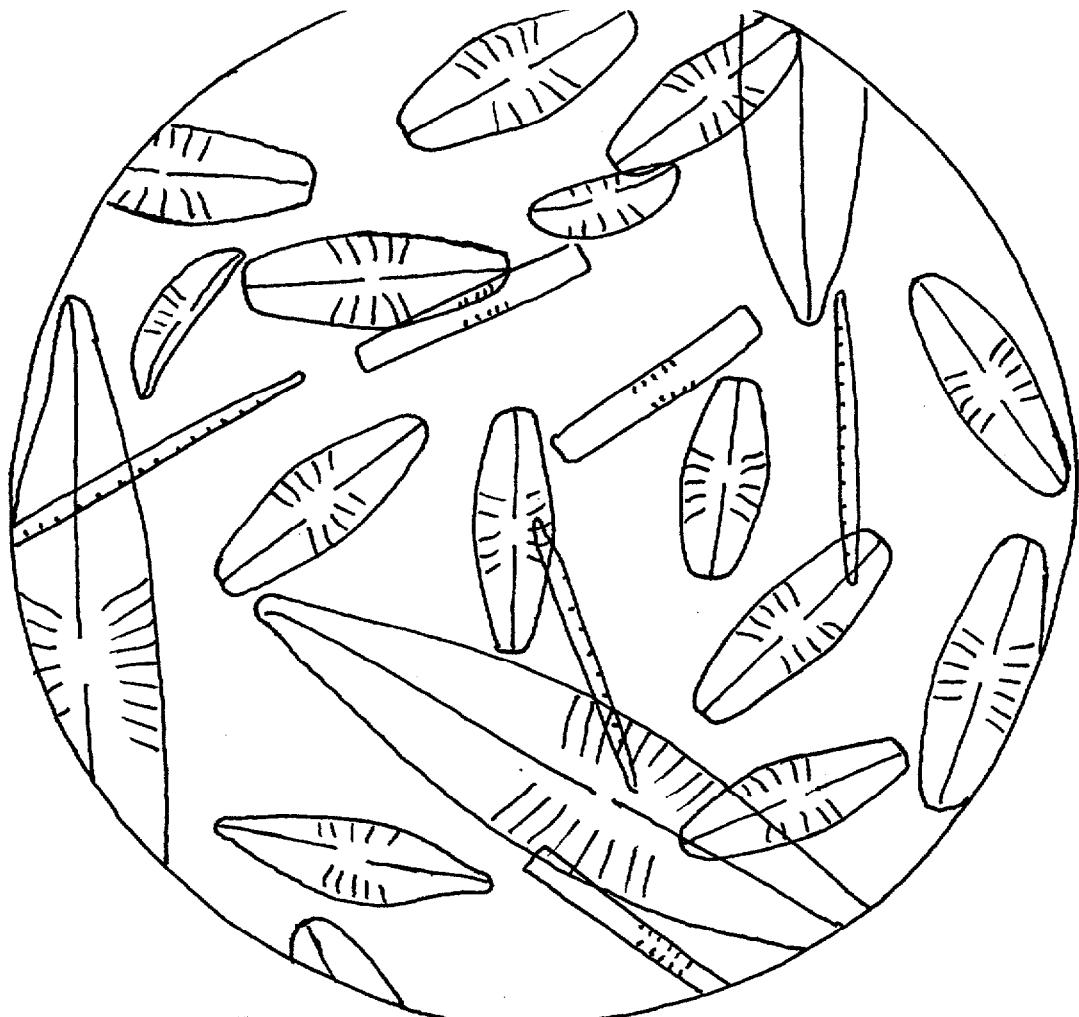
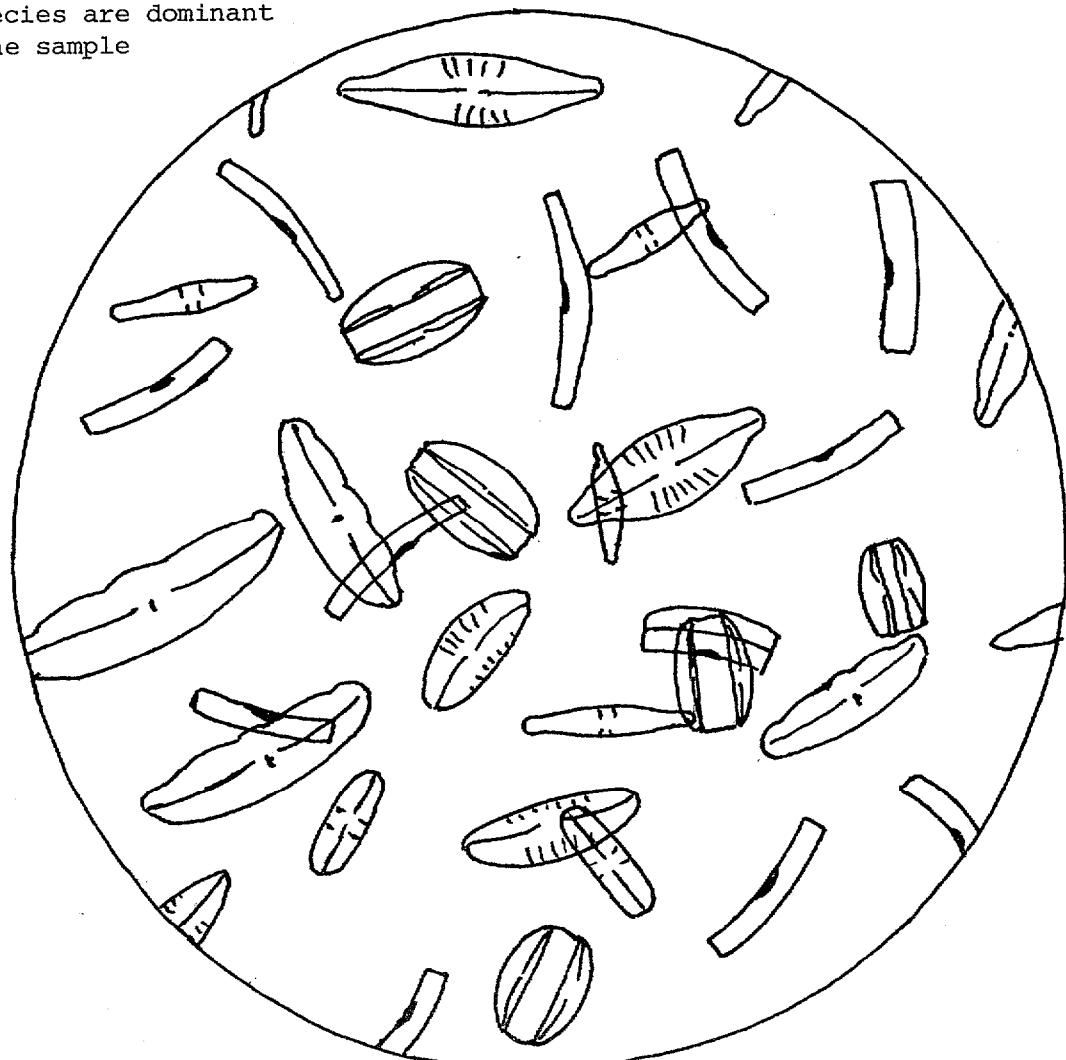


Fig. 6.
a. A diatom cell showing the upper (U) and lower (L) valve
and the two middle valves (G1, G2).
b. Cross-section of the diatom.
c. The diatom cut in half and expanded.



A

Fig. 7. Two fields
drawn directly from slides,
a few species are dominant
in any one sample



B

species (probably present in small numbers) living in suboptimal conditions or of dead frustules washed in from other communities. It is thus important to determine the species ranges over optimal conditions for growth. This will improve the indices considerably. The compilations based on literature surveys (e.g. Lowe 1974) should be used with extreme caution since the tendency is for authors to copy one another.

2. THE CONTINENTAL AND OTHER SYSTEMS OF INDICES

A brief survey is provided and further details and discussion are available in Descy & Coste (1987, 1988).

2.1. Kolkwitz & Marsson

The earliest system regularly quoted is that of Kolkwitz & Marsson (1908). This utilised algae amongst which diatoms figure prominently. It is now of limited value and in their lists only a few diatoms are loosely associated with stone surfaces, e.g. Nitzschia palea is of importance in the strongly mesosaprobic water, Diatoma vulgare, Achnanthes minutissima, Gomphonema parvulum, Nitzschia dissipata, Suriella ovalis in the weakly mesosaprobic and Tabellaria flocculosa, Meridion circulare, Fragilaria virescens, and Cymbella minuta (= C. ventricosa) in the oligosaprobic zone. These are roughly in the position we would expect from modern studies. Even this earliest attempt at classification of waters using bacteria, fungi and algae suffers from excessive listing of species and no real appreciation of community structure.

2.2. Zelinka & Marvan (1961)

These workers provide a detailed listing of diatoms classified according to their distribution across the spectrum from polysaprobic to xenosaprobic together with an estimation of the "indicator value" of each species. Their calculations are made using the technique which Descy later followed. This is a useful compilation but like most other lists it does not provide sufficient information about the optimal distribution of the species and there is no distinction between epilithic forms and the non-epilithic.

2.3. Lange-Bertalot (1978, 1979 and in Descy & Coste 1977, 1988)

This is a classification of diatoms into three groups of sensitivity to pollution: 1 = the very tolerant (i.e. occurring in polluted waters); 2 = the tolerant, and 3 = the intolerant (i.e. occurring in unpolluted waters). The number of species in group 1 is very small (Amphora veneta, Gomphonema parvulum, Navicula accomoda, Navicula atomus, Navicula cloacina, Navicula frugalis (Syn. N. subminuscula), Navicula goeppertia, Navicula minima, Navicula saprophila, Navicula seminulum, Navicula simplex, Navicula twymaniana, Navicula veneta, Nitzschia communis, Nitzschia ganderheimensis, Nitzschia palea, Nitzschia umbonata, and Synedra ulna). In my experience most of these are only abundant in very polluted waters but some, e.g. Gomphonema parvulum, Navicula veneta and Synedra ulna extend into less polluted waters. Also the small Navicula and Nitzschia species do not need to be identified but can simply be grouped in initial assessments. A study in Japan (Kobayasi & Mayama 1982) found that this method gave similar results to those for the R. Main.

2.4. Descy

The system used by Descy (1979, 1980, 1987, 1988) employs a mathematical formula whereby percentage occurrences of diatoms in the samples are multiplied by "indicator" factors 1-3, i.e. (1) for bad indicators and (3) for good indicators; these are then summed. The products are then multiplied by "sensitivity factors" where (1) is the most tolerant of pollution and (5) is the least tolerant; these are then summed. Finally the second summation is divided by the first. The final number (between 1-5) gives the range from "heavily polluted" (1) to clean water (5).

Taking Descy's example of a site polluted by domestic sewage, where only four species were present, viz:

		Factors		
		Indicator	Sensitivity	
(a)	<u>Achnanthes lanceolata</u> :	0.9%	$x_1 = 0.9$	$x_3 = 2.7$
(b)	<u>Gomphonema parvulum</u> :	0.5%	$x_1 = 0.5$	$x_2 = 1.0$
(c)	<u>Navicula accomoda</u> :	78.7%	$x_3 = 235.1$	$x_1 = 235.1$
(d)	<u>Nitzschia palea</u> :	19.7%	$x_2 = 39.4$	$x_1 = 39.2$
	Others	0.2%	-----	-----
			275.9	278.2

The Descy index is 278.2 / 275.9 = 1.0

The system seems to work but only in a rough manner (it has been criticised by Leclercq & Maquet, 1987) and I have great trouble with the 1-3 indicator value - it is not clear on what data this is based and looking down Descy's table I would not agree with his allocation of many species.* Leclercq & Maquet point out that Descy does not include some of the poorly represented species and neglects the small Navicula species which are characteristic of the polluted (highly organic) sites. They criticised the values placed on some species, particularly of those species found in the waters of median quality. They found that the values placed on the species are derived from estimates in the literature which are too vague and not checked against the chemical status of the waters. These have been my criticisms. To take some examples: Amphora pediculus, Diatoma vulgare, Nitzschia dissipata and Reimeria sinuata are classified as 5 alongside Meridion circulare but whilst these are all sensitive (i.e. absent from the extreme polluted sites), the first four all tolerate a very considerable degree of eutrophication and what

*Footnote: In the report of a workshop on diatoms as indicators (Proceedings 9th International Diatom symposium 1986) the comment is made "These systems [indices] are based on broad associations that result in rather broad generalisations", and further on, "It was not, however, considered valid to suppose that recognition of and decisions concerning problems of pollution should ever be based on a single mathematically expressed pollution scale, as is often sought by those only interested in rapid estimations of water quality".

many would classify as moderate pollution and Meridion circulare will not tolerate any pollution.

Essentially Descy's system is using the sensitivity of the species to pollution - only a few are "insensitive" in his sense, i.e. tolerant of pollution. [There is a peculiar semantic problem here, probably due to French to English translation. I would use the term "tolerant" to mean widespread - i.e. tolerating a range of conditions]. In fact all the diatoms in Descy Groups 3, 4, 5 are incapable of surviving pollution but admittedly show decided preferences for water of decreasing nutrient content, pH, alkalinity, etc. Certainly the calculation ending up with values around 5 will indicate a water which is unpolluted but much more information could be obtained by using the indicator species in a more defined manner. In fact of the species in Descy's Group 5 several are quite capable of living in richer waters - species such as Amphora pediculus and Diatoma vulgare and these are clustered with the species from absolutely clean water. The system is not designed to indicate subtleties of water quality but merely the range between polluted and clean water. The species list is also packed with diatoms having no real relationship to stone surfaces.

The Descy index system in the paper passed to me by the DoE for evaluation is in fact not the most useful of Descy's work. This is a 1984 paper (*Ecologie et distribution des Diatomées benthiques dans le Bassin Belge de la Meuse*) from which can be extracted the ranges of water chemistry over which 70 common diatoms occur (see Table 1). Instead of using numerical indices, indicator species or groups of species can be clustered and related to average chemical conditions or ranges of chemical conditions or optima of chemical conditions. But which chemical features are best to correlate with the diatoms? Many workers have used alkalinity and Table 1 has been constructed based on alkalinity. Whilst writing this report I received the 1st and 2nd Report of further Continental work by Descy & Coste - these contain more information on the tolerance levels of the diatoms but end in favour of the Grid system of Coste which is even more complex.

Table 1. Taken from my analysis of Descy (1984) data based on total alklinity

	Alkalinity (Mean value)
<u>Eunotia tenella</u>	8.2
<u>Eunotia exigua</u>	16.1
<u>Diatoma mesodon</u>	15.0
<u>Tabellaria flocculosa</u>	14.0
<u>Achnanthes microcephala</u>	22.6
<u>Gomphonema olivaceiodes</u>	29.7
<u>Cymbella minuta</u>	71.2
<u>Cymbella sinuata</u>	82.0
<u>Fragilaria capucina</u>	51.1
<u>Fragilaria vaucheriae</u>	81.4
<u>Meridion circulare</u>	55.6
<u>Navicula cryptocephala</u>	91.6
<u>Cocconeis placentula</u>	92.8
<u>Gomphonema parvulum</u>	94.4
<u>Amphora pediculus</u>	148.3
<u>Diatoma vulgare</u>	142.7
<u>Navicula gregaria</u>	123.7
<u>Navicula lanceolata</u>	108.2
<u>Nitzschia dissipata</u>	121.2
<u>Surirella ovata</u>	132.0
<u>Synedra ulna</u>	121.2
<u>Gomphonema olivaceum</u>	153.1
<u>Navicula goeppertia</u>	181.3
<u>Navicula veneta</u>	166.3
<u>Navicula accommoda</u>	214.0
<u>Navicula subhamulata</u>	201.6

Using data from the Meuse (Descy & Coste 1988) and plotting the Descy index against nitrite does show the effect of increasing eutrophication/pollution (Fig. 8) but it is clearly not a very sensitive index and the Coste system is said to be better - Leclercq & Maquet (1987) comment that the Descy system gives "plus optimiste" values and thus underestimates pollution.

2.5. The Coste Method (1976, Descy & Coste 1987, 1988)

Two groupings are used to classify diatoms, and in the latest version (Descy & Coste 1988) into 8 groups of species clustered according to their sensitivity to pollution (Group 1 are basically clean water diatoms and Group 8 occur in polluted water), and four subgroups of species clustered according to their occurrence in the upper, mid, lower and estuarine zones of rivers (in Descy & Coste, 2nd report 1988). This system is so complex I have not been able to disentangle the details. In any case, it depends on the use of a very large number of species (in the first trials 618 spp. later reduced to 223), most of which positively do not occur in the epilithic flora. It is however suggested in the Descy & Coste report as being the preferred method.

Fig. 8 shows a plot of some results from this standardised grill index and the earlier 1984 version for a series of sites on the R. Meuse - there is a general correlation with nitrite values which I have extracted from their data and plotted as a rough measure of water quality. The conductivity, chloride, orthophosphate and ammonia all increase and dissolved oxygen decrease at approximately the same point as the rise in nitrite (large arrow on Fig. 8). However, out of the 13 stations, five do not follow the nitrite whether the earlier or later grill index is used. There is in my opinion no way it could be used by water authorities. The standard Descy index is plotted in Fig. 8.

The allocation of species to classes of "euryoecious" (= wide ecological amplitude), and "stenoecious" (= "better indicators" is how this group is described - but really they are species having a narrow ecological amplitude) is again, like the Descy system, a subjective allocation, unless there is some published basis for this subdivision. In fact, the data do not seem to agree with the concepts, e.g. the species confined to the polluted sites (i.e. stenoecious) are in the euryoecious group. I simply do not believe one can group 618 species in this way - research would be needed on every species to determine its range of tolerance - reliance on statements in the literature are very dubious - some of the comments are based on distribution in lakes, in tropical waters, etc. and almost all are based on occurrence (usually in acid cleaned material) and not on abundance in the habitat.

2.6. Schoeman (1973).

This author has many useful comments - in particular he stresses that indicator species have to be considered in relation to the diatom association - the mere presence of a species is not

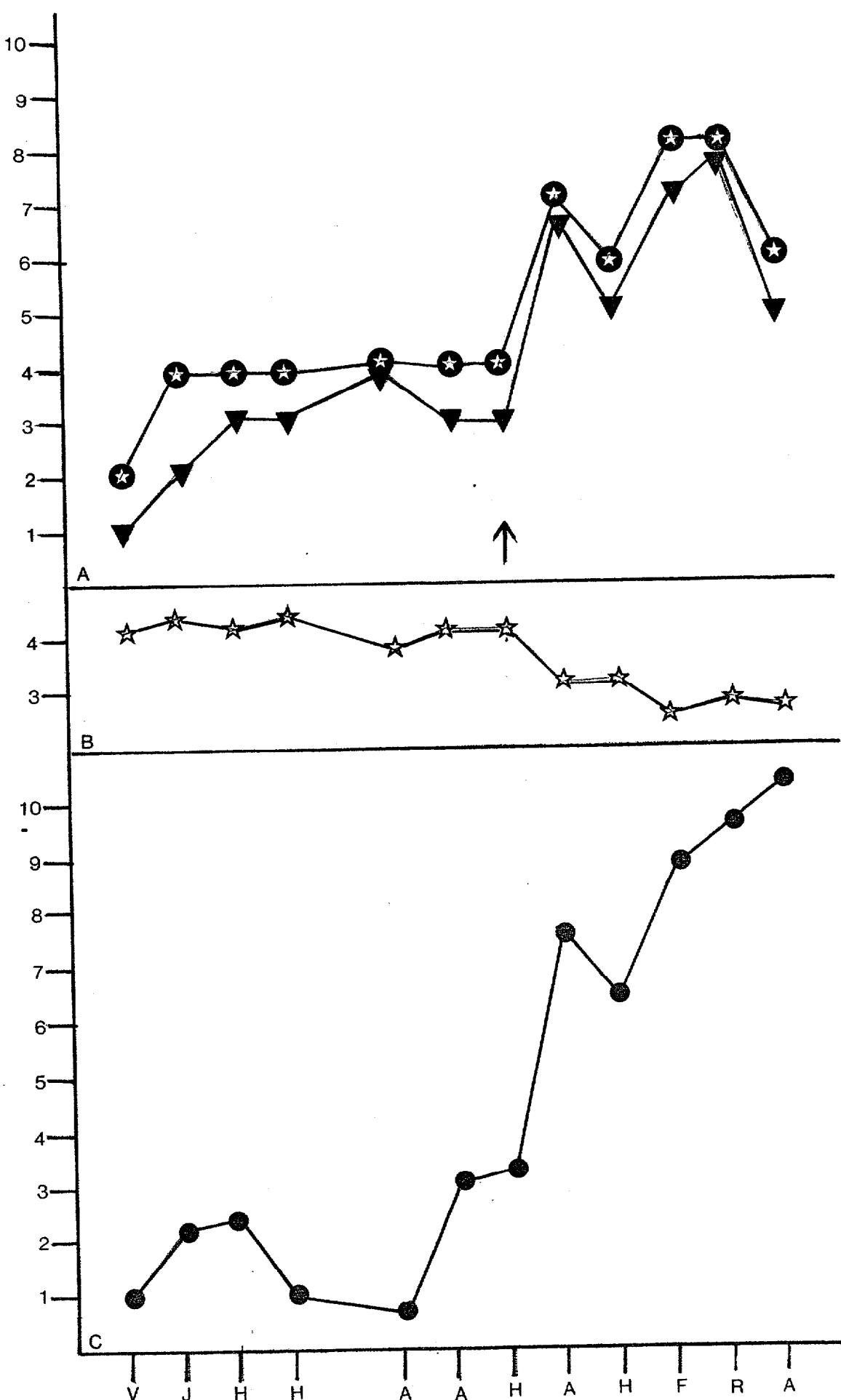


Fig. 8. Data from Descy & Coste for sites on the River Meuse downstream from (v) through (a).

a. Two variations of the indices derived from the Coste grid system. The numbers have been reversed to conform with the increasing nutrient values downstream.

b. The Descy index which ranges between 2.5 and 4.5.

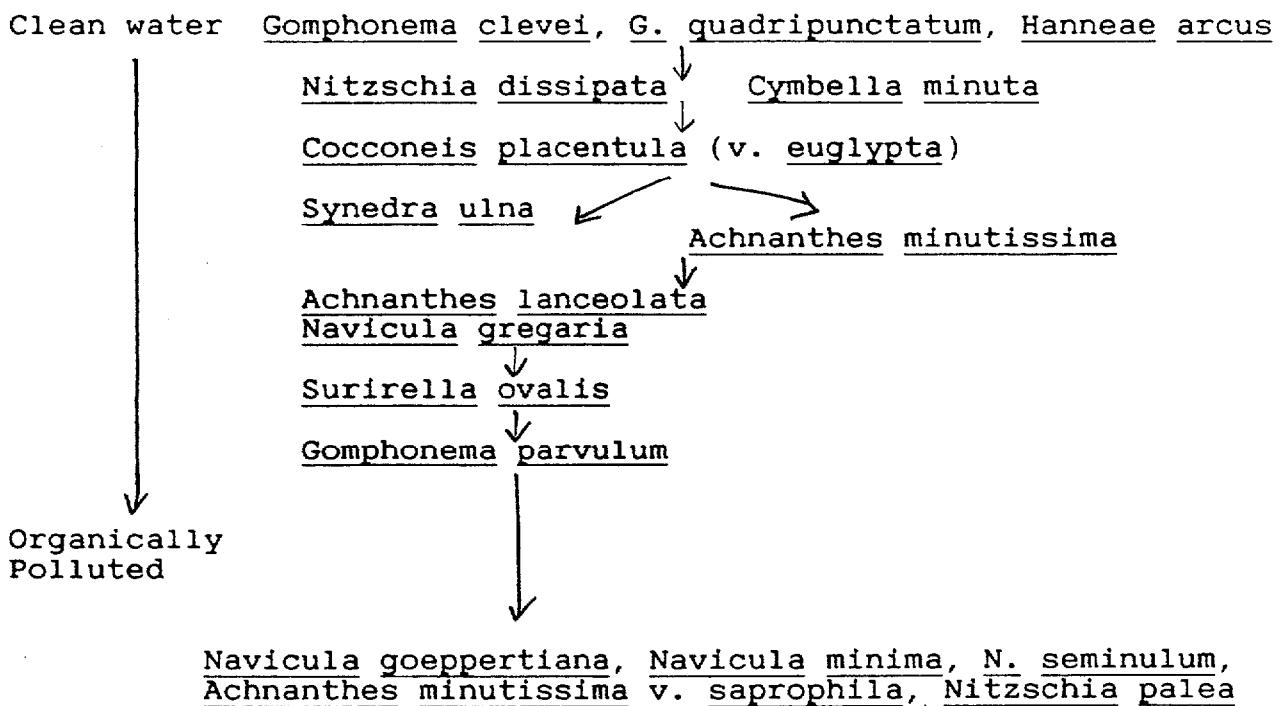
c. The nitrite values for the river stations.

of indicator value - it is only when the species is present as a "substantial percentage" of the association that it is of value as an indicator species. He also stressed that a wide variety of habitats exist in a river - in "each of these we find a different association". He also points out that classification of diatoms in relation to pH values is only of value if, once again, the abundance of the species is considered. It is striking that in his analysis of approximately 300 samples there is usually an overwhelmingly single dominant species and 2-5 sub-dominants exactly as in my experience (though Schoeman's samples were from a wider range of habitats than mine). The analysis of Schoeman's data is difficult owing to the method of presentation but many of its general features are confirmed by my findings.

2.7 Watanabe and co-workers

Watanabe (1988, 1986 and earlier papers, but difficult to follow the Japanese English, and the 1988 paper is not at all clear) has attempted to relate diatom distribution to organic pollution (BOD₅) and has devised rather complex indices. However, using only his graphical representation it is possible to extract the species which are tolerant to pollution. This work is yet again confused by the inclusion of species which are only rare outside their major zone of occurrence but making allowance for this one can discern a sequence from clean water through to extreme pollution as in Table 2.

Table 2. Extracted from Watanabe (1988) using only species common in the U.K. The data does not extend into highly acid waters.



3. SITES AND METHODS

3.1. Sites

Table 3. List of rivers sampled to the end of July 1988.
Number of stations in brackets.

R. Wye (12) Sampled once a month for 12 months	R. Avon (Hampshire) (12)
R. Usk (10) " "	R. Dart (6)
R. Thames (upstream from Oxford) (7)	R. Tweed (12)
R. Avon (Warwickshire) (14)	R. Conwy (7)
R. Avon (Gloucestershire) (11)	R. Piddle (13)
R. Towey (9)	R. Duddon (9)
R. Tamar (9)	R. Exe (10)
R. Ogmore (9)	R. Stour (Hampshire) (7)
R. Kennet (7)	R. Itchen (5)
R. Dee (Cheshire) (10)	R. Test (6)
R. Elan (3)	R. Ystwyth (11)
R. Mole (6)	R. Rheidol (5)
R. Honddu (2)	R. Monnow (7)
R. Arrow (7)	R. Lugg (8)
R. Teme (Herefordshire) (8)	R. Corve (6)
R. Kent (7)	R. Lambourn (8)
R. Gowan (3)	R. Sprint (4)
R. Wenning (4)	R. Lune (10)
R. Lea (7)	R. Hindburn (2)
R. Hodder (5)	R. Ribble (9)
R. Teifi (7)	R. Frome (Dorset) (9)
R. Eden (10)	R. W.Cleddau (5)
R. Teign (Devonshire) (7)	R. E.Cleddau (5)
R. Avon (Devonshire) (4)	R. Ellen (5)
R. Taw (9)	R. Stour (Kent) (6)
R. Moffat Water (4)	R. Esk (6)
R. Nith (7)	R. Annan (8)
R. Nidd (1)	R. Wharfe (6)
R. Coln (6)	R. Windrush (7)
R. Tees (11)	R. Swale (9)
R. Welland (5)	R. Ure (6)
R. Don (7)	R. Aire (12)
R. Irwell (2)	R. Mersey (2)
	Sankey Brook (2)

3.2. River sampling

On each river samples were collected from sites as near the source as possible and at random intervals to near the estuary. The saline regions were not sampled. Seven to ten stones were selected, consciously selecting stones which visually had a growth of diatoms on relatively flat upper surfaces. Where possible the stones were taken in riffles - those being shallower, easier to sample and recommended by other workers. The flora of stones does differ somewhat between slow-flowing and rapid-flowing regions but this feature needs further study - the dominants will nevertheless enable a good estimate of water quality.

3.3. Sample preparation

Stones of similar (geological) type were selected. If silt was present, the surface of this was moistened and gently rubbed and the diatoms washed off (an undefined area*) into a centrifuge tube.** The stone surface was then washed under the tap, rubbing with the palm of the hand until the silt/mucilage layer was removed. [As can be seen from Fig. 1, there may be some loss of epilithon at this stage and I believe it is necessary to make more studies of this aspect of sampling. But if simplicity is all that is needed and a typical flora obtained for each site, then the simpler technique may be sufficient]. Finally, the stone surface was again rubbed to remove the firmly attached epilithic flora which was washed into a second centrifuge tube. [This often requires considerable pressure on the stone surface, resulting in fracture of cells and a system of brushing may prove desirable]. Tubes were allowed to stand overnight and the supernatant poured off. To the sediment an equal amount of concentrated potassium permanganate solution was added and the sample left overnight. Then an equal amount of concentrated hydrochloric acid was added, the tubes sealed with cling film and placed in an oven at 60°C until the liquid turned colourless. The samples are then washed with distilled water by five repeated centrifugations (or until no acid remains). A small amount (adjusted with distilled water to a suitable concentration of diatom valves to give an even spread of valves on the cover glass) is then pipetted onto a coverglass, allowed to dry and mounted in a high refractive mounting medium (e.g. Naphrax). The mounting medium is then dried out by gentle heat on a hotplate.

All glassware must be scrupulously cleaned between sampling since diatom valves left behind will confuse future counts. Disposable pipettes should be used when transferring material to cover glasses.

3.4. Identification and counting

This is best done under x100 oil immersion lens. The number counted can be fixed by the individual (see p. 000 for a discussion of some aspects). If absolute counts are required (i.e. cells per unit area of stones, see Appendix II). Identification can be done from a set of illustrations (see Appendix IV). Since it is preferable to count the silt/mucilage flora and the firmly attached epilithon separately it is

Footnotes:

* see Appendix II for a method involving defined area sampling.

** The continental method of removing all the silted material and epilithon often results in slides which are thickly spread with silt as well as diatoms; this makes identification and counting difficult. The effect is even more disastrous if it is necessary to prepare samples for scanning electron microscopy as the fine silt covers the diatom valves.

desirable to find a way of combining the two sets of data obtained from each site. This would save having to consider the two sets separately. However, it is not quite so simple since the non-silted sites in any river support only the epilithic flora and so comparison of these sites with the silted sites does require separation of the two floras in the silted samples. If repeated sampling is to be done on material from specific sites on specific rivers to check temporal changes it is desirable to prepare record sheets (see Appendix I for a suggested system) with the lists of species encountered at each site and in this way it is easy to detect changes. Slides should be stored so that sites can be re-checked in the future.

3.5. Preparation of indices

See the discussion on p. 18. Since the clean water diatoms are not listed in Descy (document to DoE) I have assigned the sensitivity value 5 and the indicator value 3 to these.

4. RESULTS OF SEASONAL SURVEY

For the purpose of this report data from only a fraction of the sites is presented. This is sufficient to answer all the major points in the contract. The final analysis of all the data will form a rather massive account.

4.1. River Wye

The river Wye was sampled monthly (except when prevented by high winter flow) over an annual cycle. 12 stations were visited. Data for one station on the River Wye is presented in table 4 and figs 9 and 10.

The first point to note is that a diatom flora was present on stones at all sites on all sampling dates. This almost universal occurrence of diatoms is confirmed from the data collected in various months of the year from the other rivers. Only very rarely have stones been found to be devoid of diatoms - these have usually been in fast-flowing reaches of mountain streams (e.g. of the R. Duddon) - though even this may be a seasonal effect since the sampling time on the River Duddon was early spring and in such a rapid flowing river the flora may not have had time to develop after winter scouring. The data for the Wye show that each site has its distinctive flora and that this is maintained throughout the year. However the proportion of species in the percentage counts at each site varies considerably - as is to be expected since species grow at different rates and have different seasonal optima. Fig. 10 shows the seasonal variation of the major species at Builth in the River Wye. Although the material was not prepared in a way to give cells per square mm (cm) it is possible by counting along a fixed transect (or a fixed number of fields) to give a crude estimate of seasonal change (Fig. 9). A method such as that described in Appendix II would certainly show that these seasonal variations are even larger than I determined. Sampling has been at approximately 10 mile intervals on rivers and only rarely and incidentally has any close sampling been attempted. However one interesting set of observations was made at Site II on the River Wye - this station on the main river is clearly an acid site with Eunotia exigua, Peronia erinacea and associated species (Zone 1 - see below). A few meters downstream, a small side stream enters the river and stones from this side stream were occasionally sampled - they proved to have Achnanthes minutissima dominant (i.e. a dominant normally found in the nutrient rich zone 3 of rivers). This stream flowed past Forestry Commission houses and buildings and a small number of sheep folds, etc. and clearly this produces sufficient effluent to change this small upland acid stream into a Zone 3 type reach - this proved an excellent example of the sensitivity of diatoms to perturbation of the environment. The small input was insufficient to modify the next downstream station.

The upland stations on the R. Wye are characterised by Eunotia exigua and Tabellaria flocculosa/Anomoeoneis vitrea, Peronia erinacea (especially at Site 2) and Surirella linearis. All are given a classification 5.3 in Descy & Coste (1988) which produces an index around 5 - i.e. clean water, though each station is slightly different and this is where the mathematical

system falls down. If all species are classified as 5.3 the index provides no useful information; additional species having, for example, a 4.3 classification must be present to modify the index.

Putting the data (Table 4) from the station at Builth Wells into the Descy index gives indices over the year varying between 3.5 and 4.4, i.e. moderate eutrophication to slight pollution. Thus in spite of the great variation in the percentage values of individual species during the year the state of the river is much the same - nevertheless, the fluctuations in the index may be due to fluctuating water quality. The lower stations on the Wye, e.g. at Bredwardine, produces a Descy index ranging through 2.5 - 3.5 - 4.3 which on his classification indicates heavy pollution through middle pollution to slight pollution or heavy eutrophication (the latter would be my estimate). The low index figure occurred only once during the year and probably indicates a pollution event.

Table 4. Percentage counts of the main species at the Builth station on the River Wye.

Species	Date											
	A	M	J	J	A	S	O	N	D	M	A	
<i>Achnanthes minutissima</i>	52	29	55	45	17	48	22	15	20	21	24	
<i>Cocconeis placentula</i>	6	4	12	14	15	20	5	5	10	47	50	
<i>Cymbella minuta</i>	6	30	7	9	16	2	9	2	11	5	3	
<i>Reimeria sinuata</i>	5	8	6	19	41	1	8	3	21	13	18	
<i>Navicula lanceolata</i>	18	2	3	3	2	10	23	50	18	7	2	
<i>Navicula phyllepta</i>	6	2	3	2	3	16	24	20	6	4	2	
<i>Nitzschia dissipata</i>	3	4	0	0	0	0	0	3	0	0	0	
<i>Surirella ovata</i>	4	18	3	2	0	0	7	0	2	2	1	
<i>Achnanthes lanceolata</i>	0	2	0	0	3	0	0	0	0	0	0	
<i>Fragilaria vaucheriae</i>	0	0	5	0	0	0	0	0	0	0	0	
<i>Gomphonema parvulum</i>	0	0	0	0	1	2	0	0	0	1	0	
Month	A	M	J	J	A	S	O	N	D	M	A	
Descy Index	3.9	3.7	4.4	4.2	4.3	3.5	3.6	3.7	4.1	3.9	4.1	

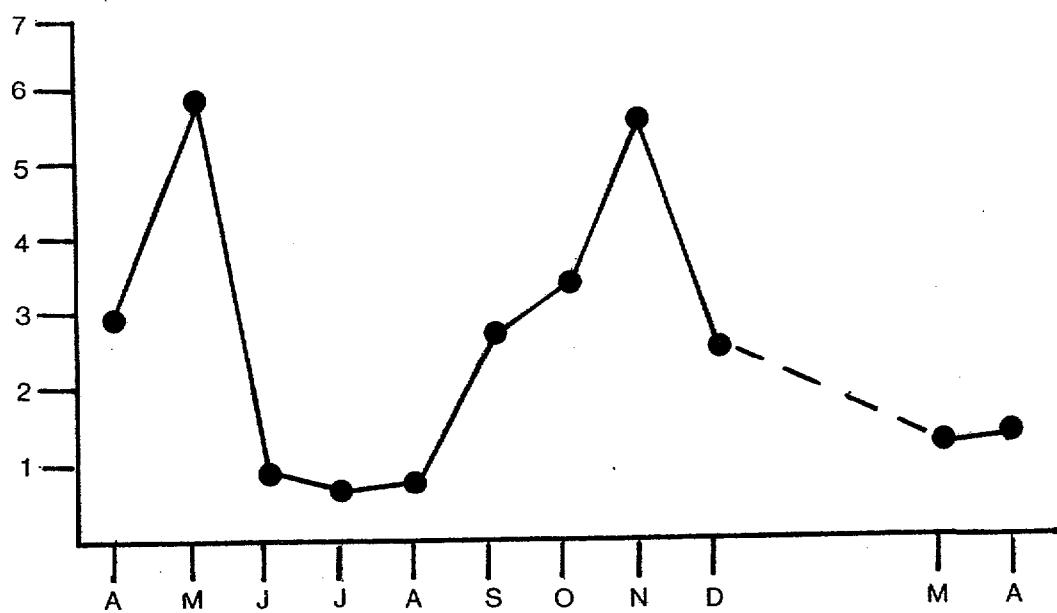


Fig. 9. The seasonal occurrence of diatoms in the River Wye epilithon at Builth Wells. These are total numbers in five microscope fields.

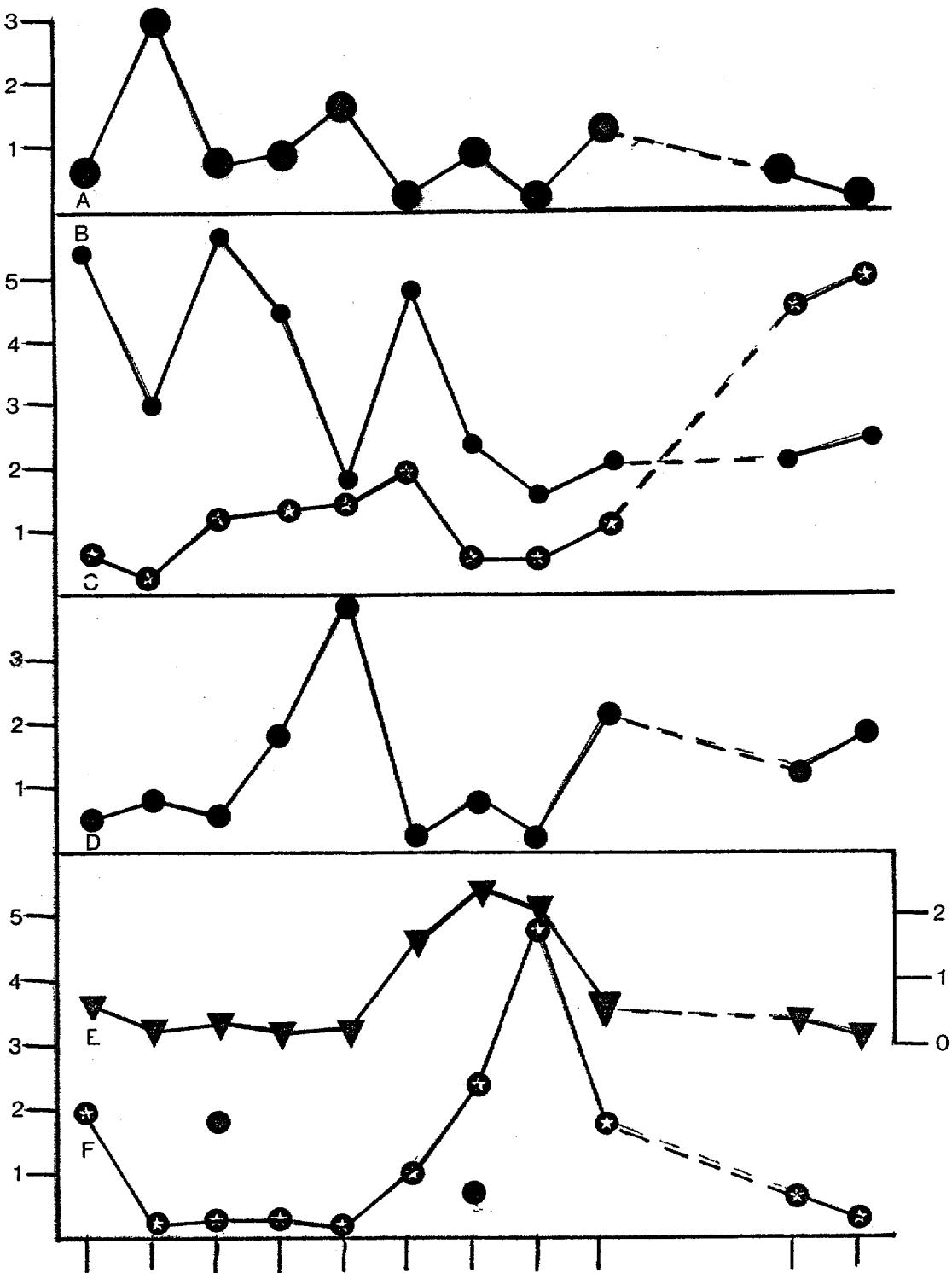


Fig. 10. The seasonal variation of percentage occurrence of diatoms in the River Wye at Builth Wells. (a) *Cymbella minuta*; (b) *Cocconeis placentula*; (c) *Achnanthes minutissima*; (d) *Reimeria sinuata*; (e) *Navicula phyllepta*; (f) *Navicula lanceolata*.

Isolated black dots on the lower graph are values for *Surirella ovata*.

4.2. Conclusions

A flora is virtually always present on the stone surfaces. It undergoes considerable seasonal fluctuation in cell numbers but rather less in species composition. It is rare to find a silt/mucilage flora and sparse epilithic flora. The Descy indices show that there is a steady downstream change from 5 through an average of 4 at Builth to 3.5 at Bredwardine. One might add here that it is very rare to encounter an anomalous flora at a site - however much the proportions of species vary the species tend to be those characteristic of the zone. Also it is rare to find river planktonic species though occasionally a bloom in the river deposits cells onto the stones. Equally, lake/reservoir plankton is rare and when observed a check with the map has always revealed the presence of an upstream lake or reservoir source.

Periods when few cells were found corresponded, not with periods of high flow and low light levels - the former would lead to scouring and the latter to low growth and hence slow recruitment - but with lower summer flows, and at this time observation of live material revealed a period of active grazing. Protozoa and larval stages of invertebrates were responsible and many dead cells (almost certainly voided from protozoal cells and larval guts) were noted. These dead cells were almost entirely members of the silt associated flora. This animal activity was confined to a short period in spring (confirmed on other rivers) when growth of diatoms was also at a maximum. The results emphasise the extreme seasonality of the animal fauna on the stone surfaces. NB: this effect would not be recorded if only cleaned samples were investigated and it does demonstrate that the two floras are not affected to the same extent by grazing. This is another feature which makes a nonsense of attempting extremely accurate counting.

5. RIVER SURVEY

5.1. Introduction

The sampling was designed to obtain the same kind of data as that used by Descy and only as the work proceeded and, more strikingly, as the data was analysed, was it realised that apart from the uppermost clean waters and the moderate to extreme polluted zones, the flora of the eutrophic region was grossly complicated by the inclusion of the silt and true epilithic flora. This does not affect the derivation of numerical indices, but it does produce very complicated patterns in the major zone of most rivers. Nevertheless, study of the data reveals that first and last occurrences of species can be used to categorise successive reaches of rivers (this is a technique used by palaeontologists working for example with deep sea cores where it has proved most valuable). Widespread species can if necessary be excluded from consideration.

Each reach or zone can be compared with others of its kind to build up a generalized picture and from it a set of indicator species rather than a single species - this in effect is applying the techniques of the higher plant sociologists. The character species can then be compared with the water quality characteristics for each zone.

Species richness increases downstream even when the simplest method of counting is used.

The epilithic flora at any one site is dominated by 2-8 species (i.e. species with percentages between 5-70%). The method of presentation has been devised to give as clear a picture as possible without the complication involved in listing all the species at a site - rarely more than 30.

From the data it is possible first to define five major zones. (1) The clean headwater stretch; (2) a zone of slight but increasing nutrient enrichment; (3) a zone of gross enrichment; (4) a zone of incipient pollution; (5) a zone of extreme pollution. Diagram Fig. 11 is a summary of my initial subdivision taking all the data into account.

The majority of Western rivers encompass merely zones 1-3.

The sites on lowland rivers tend to fall into type 3.

Zone 4 can occur inserted into zone 3 and is often the only perturbation.

Zone 5 is rare except in a few rivers experiencing industrial pollution.

As might be expected, zones 1 and 5 are the most clear-cut with indicator species which are virtually absent from the other three zones. Zones 2 and 4 tend to have definite indicator species but sometimes these are accompanied by species of zone 3. The latter is the largest and most complex zone and requires further detailed studies involving the separation of the silt and true epilithic flora.

These zones and their indicator species will now be discussed in detail.

5.2. Zone 1.

Waters at the head of the Western rivers will be nutrient poor/acidic draining the western (mountainous) regions and these have the most distinctive diatom flora.

The dominant indicator and almost the only species in the most extreme reaches of this type is Eunotia exigua (SEM 1) which in many actreams can achieve almost total dominance (e.g. in the Rivers Dart, Exe, Conway, Towey, Wye, Usk and Elan and Derbyshire Derwent above Howden Reservoir. An E. exigua zone probably occurs in the headwater streams draining most of the western acid uplands/mountains but time has not permitted sampling the highest reaches of many rivers. A survey over 12 months of streams entering the reservoir Llyn Brianne in central Wales has revealed an E. exigua community (Table 5, see p. 00), but intensive study has shown that this community is not uniform and can be divided into several associations of species. The subtle relationship between these associations and the drainage basin of each stream has only been partially analysed.

In some zone 1 sites Achnanthes microcephala is abundant but there is a problem with this species since it has been absorbed into A. minutissima by some workers and I originally took this view. But I now believe it is a distinctive entity characteristic of this and perhaps Zone 2 but samples will need to be re-checked. The taxonomic problem makes analysis of the data in the literature difficult.

5.3. Zone 2

This is characterised by the addition of Hannaea arcus and Fragilaria capucina. Peronia fibula sometimes also starts in this zone. Tabellaria flocculosa can still be frequent but Eunotia exigua is dying out. These species are common where some upland farming is obvious in the catchment and as soon as the farming becomes more extensive, the flora is dominated by Achnanthes minutissima. A nice example occurs in four stations above Findhorn Bridge on the Findhorn (Scotland). Achnanthes minutissima is dominant accompanied by Fragilaria capucina and a small amount of Hannaea arcus - but these sites are interesting for the addition of Synedra ulna to each. This needs further study since S. ulna is quoted in most publications as a diatom of polluted waters. In the Scottish Dee H. arcus is also present throughout.

On some rivers the uppermost site visited was characterised not by Zone 1 species but by the Zone 2 species; in some instances this simply reflects the fact that I did not get high enough up the river, e.g. the uppermost station (and next lower one) of the R. Eden had Hannaea arcus accompanying Achnanthes minutissima, but the landscape suggested that sampling started below the E. exigua zone. In the River Tweed, however, there

were no zone 1 or 2 stations, i.e. Eunotia exigua and Hannaea arcus were absent. Sampling of the R. Ogmore region revealed that the eastern branch had no zone 2 species but in the western branch Hannaea arcus was present indicating clear water. After the confluence Hannaea was unable to grow.

5.4. Zone 3

This is the major zone of most rivers sampled and will need to be sub-divided. Achnanthes minutissima carries over into this zone but tends to decline downstream in percentage occurrence, e.g. in the Scottish Dee (57%-66%-65%-47%-73%-90%-58%-36%-24%-18%) and in the Tweed (73%-68%-64%-25%-3%-9%-7%-0%-17%-0%). In the Moffat Water/R. Annan, A. minutissima is dominant in the four upper stations and recurs lower down once - a presumably clean station. The more eutrophic the river, the less the percentage of Achnanthes minutissima and in some lowland rivers it is virtually absent (e.g. the Hampshire Stour). Initially the high (above 50%) Achnanthes minutissima region might be designated zone 3a.

The next important species to appear is Cymbella minuta, e.g. in the Tweed (8%-0%-7%-60%-47%-70%-81%-67%-79%-47%-76%).

Three other species appear in the lower zone - Cocconeis placentula, Reimeria sinuata and Amphora pediculus. For example, in the Scottish Dee, Cocconeis placentula has the following distribution (0-0-0-0-0-0-27%-21%-29%) (R. sinuata only occurs at the last but one station and Amphora pediculus is absent). In the River Exe, Cocconeis placentula is intermittent below the Zone 1/2 and in the River Spey there is a similar distribution (0%-0%-0%-4%-5%-25%-21%-32%). In the Hampshire Avon Cocconeis placentula has the following distribution (3%-0%-0%-6%-12%-0%-0%-13%-25%-10%), in the River Exe (0%-0%-0%-30%-0%-0%-34%-0%-0%), and in the River Tweed (0%-0%-0%-0%-7%-0%-0%-0%-0%-18%). This zone can be designated Zone 3c and the mid-region with Cymbella minuta abundant Zone 3b. In these rivers this zone 3c is the final zone and in some rivers it stretches throughout a considerable length.

The distribution of Amphora pediculus in the Hampshire Avon is (0%-0%-46%-42%-35%-29%-10%-62%-24%-4%), in the Hampshire Stour (66%-6%-35%-11%-60%-44%) and the Wiltshire Coln (37%-36%-24%-18%-10%-32% before it joins the River Thames), it is absent or only present at the lowest station in the Rivers Exe, Ribble, Tweed and Eden, in the Aire it is present only at the slightly polluted stations 3, 5, 6 & 7 and in the Tweed only at the lowermost station. It is virtually absent from the Ogmore, Annan, Scottish Dee, Dart, Findhorn and Spey. Since Amphora pediculus is often only present in the lowermost zone we might term this 3d. However, this zone can actually encompass whole small southern nutrient-rich rivers, e.g. Hampshire Avon, Stour and Coln. Another species which tends to occur in the lower reaches is Diatoma vulgare, e.g. in rivers Eden in the Derbyshire Derwent and Dove.

Reimeria sinuata is present in Zone 3b (Moffat Water/ Annan/Derbyshire Derwent, Tamar, Eden) and in other rivers in the

zone 3c (e.g. Scottish Dee, Dart, Exe, Ogmore, Spey, Teifi, Towey, Tweed). In several rivers this species has sporadic occurrence in the 3a/b zone and higher percentages in the 3c zone but in the final (3d) stations it is often absent. There are two distinct forms of this species (based on size but differing in structure when observed by SEM) and the samples require a more detailed study. This may also be a species which is more closely linked to the type of substratum (possibly sandstone, e.g. in the Nith, Esk and Monnow) than the other zone 3 species - it is virtually absent in the lowland/calcareous rivers, though the larger form may occasionally occur here, e.g. in the River Itchen.

The above data can be summarised in a simple diagram (Fig. 11) and clearly a whole river can encompass all zones. Alternatively a river may include only one zone, e.g. small mountain streams running into the sea may simply be dominated by zone 1 species or a lowland river dominated throughout its length by diatoms of zone 3d (e.g. the Rivers Coln and Hampshire Stour).

It must be stressed that these zone 3 subdivisions are extremely tentative - the data is grossly affected by the frequent occurrence of a silt flora (see Figs 3-5). Complete separation of the two floras will almost certainly improve the subzoning.

5.5. Zone 4

This zone of incipient pollution is characterised by the dominance of Gomphonema parvulum, a species which also often appears in small amounts in the lowermost zone 3 stations (e.g. R. Annan) and downstream of some towns. Krammer & Lange-Bertalot (1986) note its occurrence in the outflows from sewage treatment stations but indicate its wide occurrence from moderately polysaprobic to a-mesosaprobic. In spite of their comments on the number of morphological forms and possibility of separate populations in waters of differing type, I have found it common only in incipient pollution sites, e.g. in the River Don. However, the whole Gomphonema parvulum complex will have to be analysed very carefully since I am certain that it is an aggregate of species. The other major feature of this zone is the absence of the characteristic zone 3 species, Amphora pediculus, Cocconeis placentula and Reimeria sinuata. The stones are often covered with oily silt which supports rich, sometimes pure populations of Navicula lanceolata and Navicula gregaria whilst Navicula veneta, Navicula phyllepta can be frequent.

5.6. Zone 5

This zone only occurs on a few rivers and is characterised by the large Navicula goeppertia and the small hyaline Navicula species (N. atomus, N. pelliculosa etc.), together with Gomphonema parvulum, Navicula gregaria and Achnanthes lanceolata var. (ovalis?). Amphora veneta and Gomphonema augur are rare in the samples but virtually confined to this zone. Nitzschia species are often abundant but they are probably associated with the silt and are not truly epilithic (e.g. in a sample from

Sankey Brook running into the Mersey, when the stones are washed the Nitzschia species virtually disappear) - of these the easily recognisable Nitzschia communis and Nit. perminata are good additional indicators. In many schemes the most polluted zone is characterised by a large list of Nitzschia species but it is not necessary to try and identify species of this the most difficult genus of the diatoms. A species mentioned in the Continental literature as characteristic of this zone is Navicula accomoda but I have only found it in one British river. This could be due to slow dispersal to the British Isles or to the absence of such a high degree of pollution as found in Continental rivers. This zone is the easiest to recognise since the zone 3 species are all virtually absent and Navicula goeppertiana and Amphora veneta are unmistakable species.

5.7. Fine tuning of zones

The final analysis of all the data for all the zones will lead to more definitive indicators characteristic of sub-zones. As an example, the following is taken from some clean sites in Wales around Llyn Brianne. Although all these sites are classified as zone 1, they can be subdivided on a floristic basis. Table 5 gives the basic data (excluding rare species).

Table 5. The average percentage distribution over one year of diatom species on stones in the streams around Llyn Brianne.

Sites	2	3	4	5	6	7	8	1	9	10	11	12	13	14
Diatoms														
<u>Achnanthes austriacus</u>	6	-	7	-	-	14	17	18	4	2	-	-	-	-
<u>Eunotia exigua</u>	12	-	14	5	38	18	14	51	60	55	61	57	23	33
<u>Eunotia vanheurckii</u>	-	2	59	73	42	58	50	20	8	18	2	-	-	-
<u>Eunotia rhomboidea</u>	-	-	3	-	8	-	2	-	15	8	11	16	12	16
<u>Eunotia curvata</u>	-	-	-	-	-	-	-	-	-	-	6	3	24	2
<u>Tabellaria flocculosa</u>	-	-	9	5	6	2	2	1	11	13	12	8	26	37
<u>Achnanthes minutissima</u>	32	36	-	-	-	-	-	-	-	-	-	-	-	-
<u>Reimeria sinuata</u>	24	-	-	-	-	-	-	-	-	-	-	-	-	-
<u>Cymbella minuta</u>	7	2	-	-	-	-	-	-	-	-	-	-	-	-
<u>Diatoma mesoda</u>	6	4	-	-	-	-	-	-	-	-	-	-	-	-
<u>Meridion circulare</u>	5	-	-	-	-	-	-	-	-	-	-	-	-	-
<u>Fragilaria capucina</u>	2	11	-	-	-	-	-	-	-	-	-	-	-	-
<u>Hannaea arcus</u>	1	-	-	-	-	-	-	-	-	-	-	-	-	-
<u>Peronia fibula</u>	1	-	1	-	-	3	-	1	-	-	10	6	-	-
<u>Gomphonema</u>	-	26	-	-	-	-	-	-	-	-	-	-	-	-
<u>Anomoeoneis brachysira</u>	-	-	-	-	-	-	-	-	-	-	3	10	4	-

The squares around the species percentages delimit four associations. Sites 2 and 3 are unexpected in this region. The presence of Reimeria sinuata indicates an input of some nutrient since this species is normally found in the lower half of rivers - it is this kind of discovery which, when fully analysed, will hopefully provide a clue to the chemical condition which governs the occurrence of this species. A similar situation exists for Gomphonema clevei and Cymbella minuta. The occurrence of

Achnanthes minutissima and Fragilaria capucina places these two stations just outside the predominantly Eunotia exigua reach which is the acid/nutrient poor region - the most pristine running waters in the British Isles. The five sites 1, 9-12 belong in this section but even here there are subtle distinctions in that at stations 11 and 12 the very characteristic Peronia fibula is present. Stations 4-8 have Eunotia exigua but this is overpowered by another very small Eunotia - E. vanheurckii. Stations 7, 8 and 1 have characteristically high values for Achnanthes austriaca. Finally, stations 13 and 14 are characterised by the higher occurrence of Tabellaria flocculosa. Absence of species is also diagnostic, e.g. Tabellaria flocculosa is present at all sites except the unusual sites 2 and 3.

This subdivision was made two years ago and whilst I believed it to be a perfectly valid example of "environmental control" of the diatom populations, I had no idea whether or not it was unique to the area. Recently I received a paper by Leclercq & Fabri (1987) based on more samples than mine (430 samples from 72 stations over 3 years) but on the same E. exigua community (in the Ardenne - Belgium) where they found a strong correlation with geology and physico-chemistry. Their survey extended over the E. exigua community proper into the slightly nutrient enriched Fragilaria capucina community. In the E. exigua community they were able to distinguish five "trophic variants" and in the F. capucina association three variants and a slightly polluted variant (Table 6 is extracted from their data). Whilst not identical (e.g. Tabellaria flocculosa occurs only rarely in one association of the Ardenne and Peronia not at all), the similarities are so close as to give great confidence in the use of such techniques. It was very encouraging to find the same species in both regions and the same apparent anomalies (e.g., the occurrence of Cymbella minuta, Reimeria sinuata, Diatoma mesodon only in isolated instances) and the species which indicate slight pollution (I would call it eutrophication) present in very small amounts in the Ardenne (Navicula lanceolata, Cocconeis placentula, Gomphonema olivaceoides, Reimeria sinuata and Achnanthes lanceolata); these in greater percentages characterise my Zone 3 sites. Carrying the comparison further, I would designate the five E. exigua types as sub-associations of my Group I, the Fragilaria capucina as Group IIa, the more enriched stations with Hannaea arcus as Group IIb, and the slightly eutrophic as Group III. These fit in another way into my river zones, which show the sequence E. exigua - Fragilaria capucina / Hannaea arcus - Achnanthes minutissima - Cymbella minuta - Cocconeis placentula / Reimeria sinuata. Thus the same sequence of enrichment is detectable even at a level where the real eutrophic indicators (Cymbella minuta, Cocconeis placentula, Synedra ulna, Reimeria sinuata, Navicula lanceolata) are only just present and the even stronger indicator of eutrophy (Amphora pediculus) is absent. Subtleties such as the occurrence of Achnanthes austriacus in three of my sites is also reflected in Leclercq & Fabri's fourth association. A short paper on diatoms in rivers in the Tatra Mountains also has these variants (Kawecka 1981) showing that the same indicators are widespread.

These studies confirm the subtle association of species with

equally subtle (and as yet undetermined) environmental (chemical features). They also illustrate the value of using the associations and not mathematical indices (these would all give a figure around 5 in the Descy system). The associations are finely tuned to the environment.

Table 6.

The distribution of species in nine variants of the Eunotia exigua and Fragilaria capucina communities in the Ardenne (adopted from Leclercq & Fabri (1987). Values for the diatoms are percentage occurrence. Alkalinity increases from associations 1 - 9. IIa, IIb, IIIb refer to positioning of these associations in my zones.

Associations	1	2	3	4	5	6	7	8	9
Diatoms	I					<u>IIa</u>		<u>IIb</u>	<u>IIIb</u>
<u>Eunotia exigua</u>	100	90	75	70	40	30	5	5	-
<u>Eunotia rhomboidea</u>	-	15	10	5	-	-	-	-	-
<u>Eunotia curvata</u>	-	-	10	-	-	-	-	-	-
<u>Anomoeonis serians</u>	-	-	10	-	-	-	-	-	-
<u>Fragilaria capucina</u>	-	-	5	-	5	15	35	10	-
<u>Achnanthes austriaca</u>	-	-	15	-	-	-	-	-	-
<u>Pinnularia hilseira</u>	-	-	-	-	5	-	-	-	-
<u>Achnanthes minutissima</u>	-	-	-	-	35	15	45	40	40
<u>Gomphonema parvulum</u>	-	-	-	-	5	5	5	5	5
<u>Tabellaria flocculosa</u>	-	-	-	-	-	5	-	-	-
<u>Hannaea arcus</u>	-	-	-	-	-	-	10	5	-
<u>Cymbella minuta</u>	-	-	-	-	-	-	-	10	-
<u>Diatoma mesodon</u>	-	-	-	-	-	-	-	5	-
<u>Fragilaria vaucheriae</u>	-	-	-	-	-	-	-	5	-
<u>Gomphonema clevei</u>	-	-	-	-	-	-	-	5	-
<u>Synedra ulna</u>	-	-	-	-	-	-	-	5	-
<u>Navicula lanceolata</u>	-	-	-	-	-	-	-	-	5
<u>Cocconeis placentula</u>	-	-	-	-	-	-	-	-	10
<u>Gomphonema olivaceoides</u>	-	-	-	-	-	-	-	-	5
<u>Reimeria sinuata</u>	-	-	-	-	-	-	-	-	5
<u>Achnanthes lanceolata</u>	-	-	-	-	-	-	-	-	5

This shows how much detail can be extracted from these studies where and when it is necessary to provide such fine subdivisions. The work on this zone illustrates how finely tuned the diatoms are to their environment.

5.8. Discussion

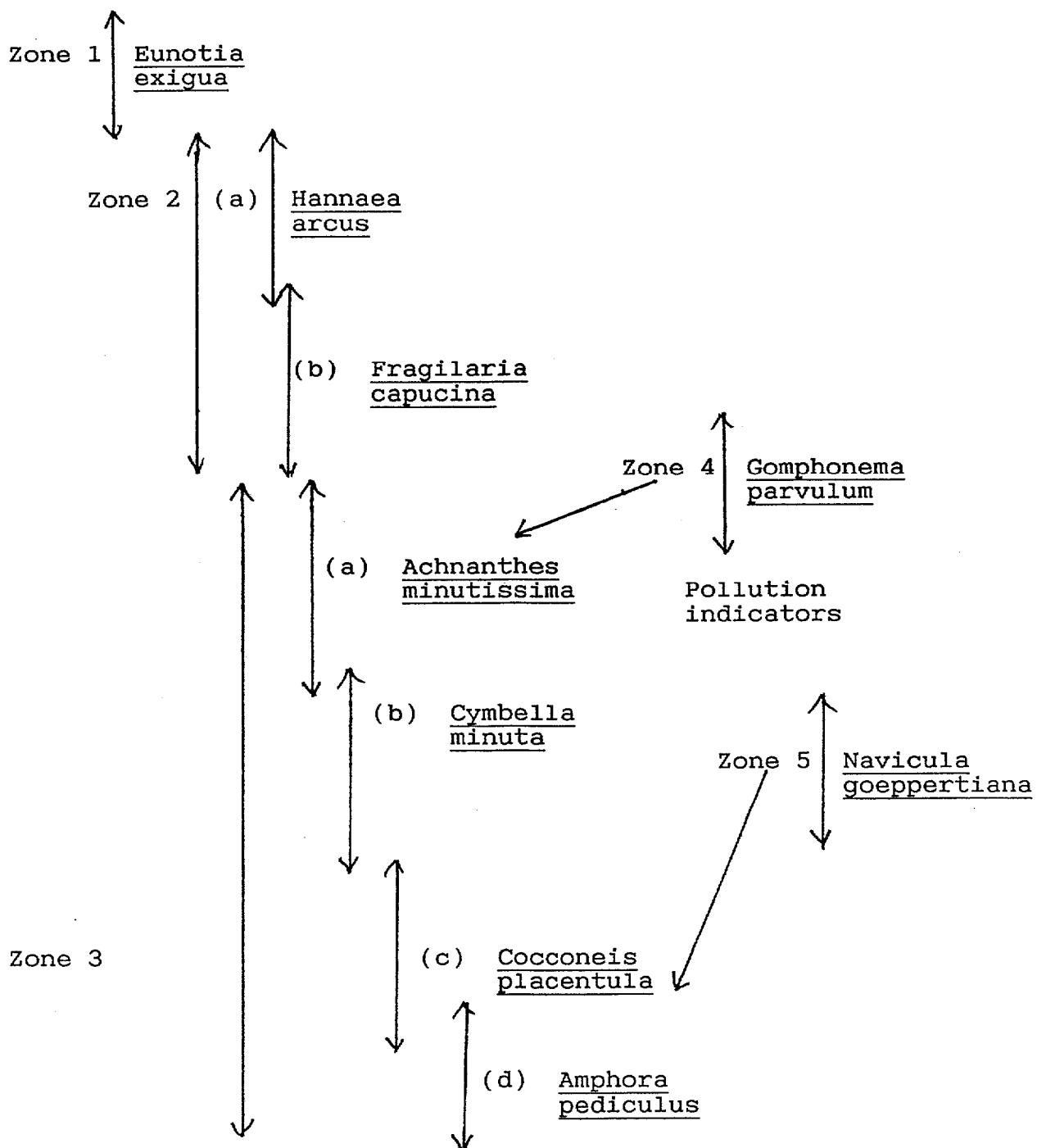
The above system is based on a small number of easily recognisable diatoms, viz.

- Zone 1, Eunotia exigua, Tabellaria flocculosa, Peronia fibula.
- Zone 2a, Fragilaria capucina
2b, Hannaea arcus
- Zone 3 (a), Achnanthes minutissima
- Zone 3 (b), Cymbella minuta
- Zone 3 (c), Cocconeis placentula
- Zone 3 (d), Amphora pediculus
- Zone 4, Gomphonema parvulum
- Zone 5, Navicula goeppertiana

With the exception of Navicula goeppertiana the above species are all attached to the rock surfaces by mucilage pads or stalks. Some do form low branching colonies but apart from the Navicula goeppertiana they are non-motile forms (N. goeppertiana requires further study - it may be an exceptional Navicula and immobile).

Thus only 11 major species are necessary to provide a basic sub-division. Zones 1, 2, 4 and 5 can be easily and rapidly identified without complex mathematical treatment of the data. The number of species could certainly be increased since there are situations where one of these major indicators gives way to another species. The subdivision of zone 3 requires much more detailed study to produce a refined system. A further feature is that the upper stations and the polluted stations are characterised by high percentage counts of one (two species), i.e. the stress results in the elimination of many species and thus the excessive growth of one (two) in the absence of competitors for space and nutrients. The intermediate stations and particularly the lowermost stations on almost all rivers have a rich flora with 4-5-6-7 species competing for dominance. Nevertheless at these stations there is always a dominant and it usually falls in with the idealised pattern in Fig. 11, i.e. Amphora pediculus dominant at the lowest station. If the species in the succession in Fig. 11 are compared with those extracted from Descy (1984) in Table 1 it will be seen that they occur in the same order and thus represent a sequence correlated with nutrient status. If these names are inserted onto the hypothetical series of pulses in Fig. 1, then Fig. 12 is the result. However, this is clearly only the beginning of the analysis and much finer distinctions will be possible as work proceeds.

Fig. 11. A preliminary zonation of British rivers based on the dominant diatoms in the epilithon (excluding the silt flora).



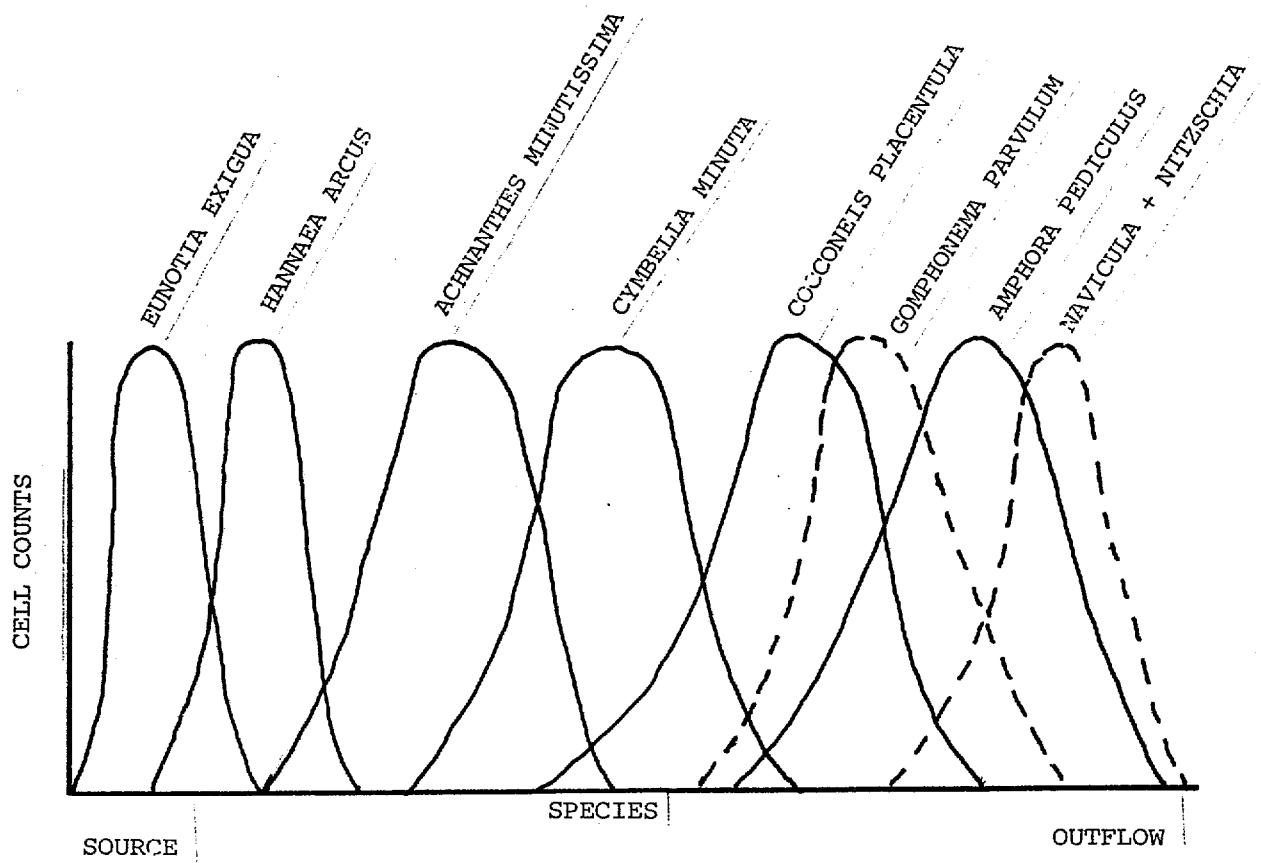


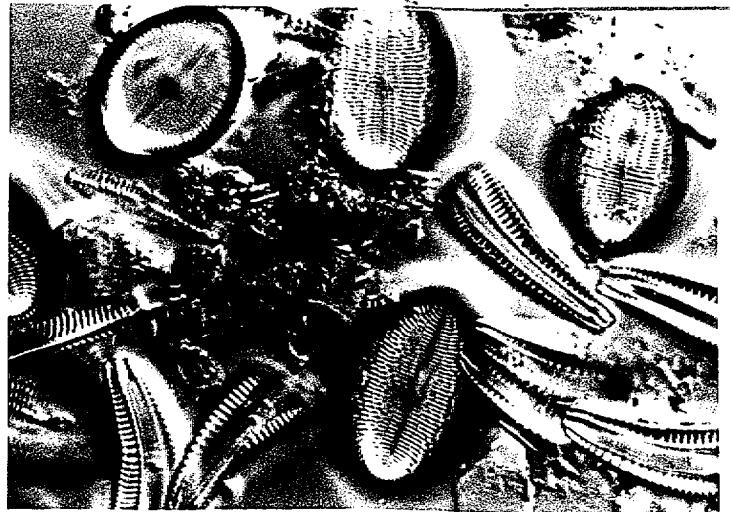
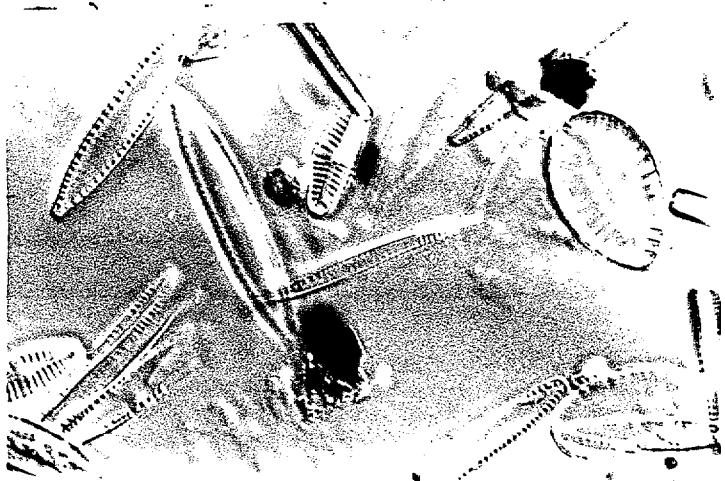
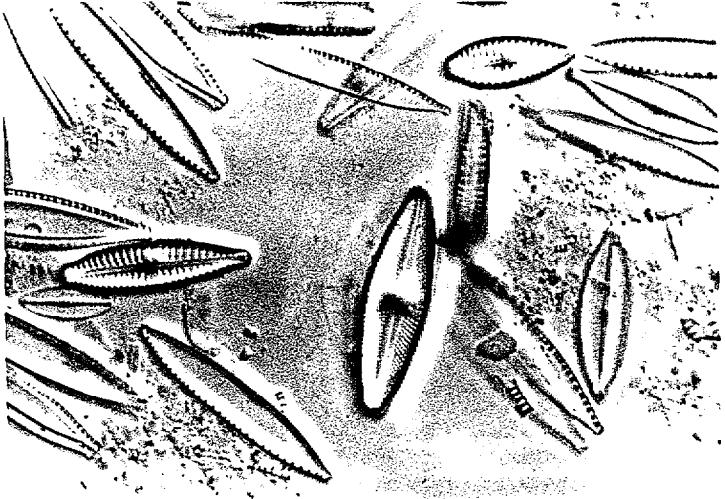
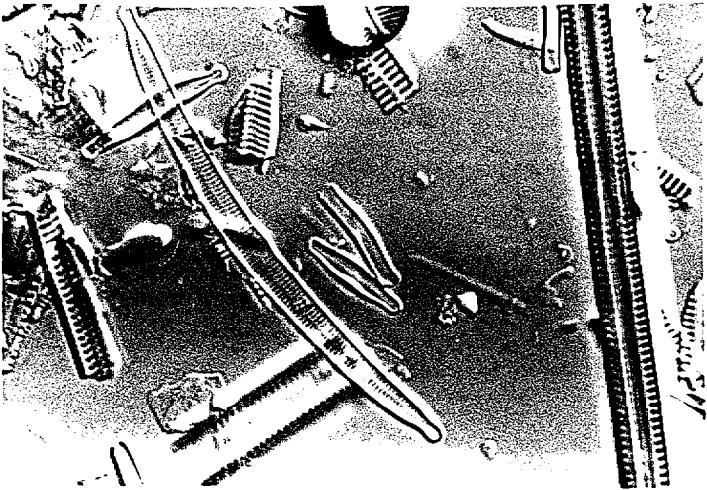
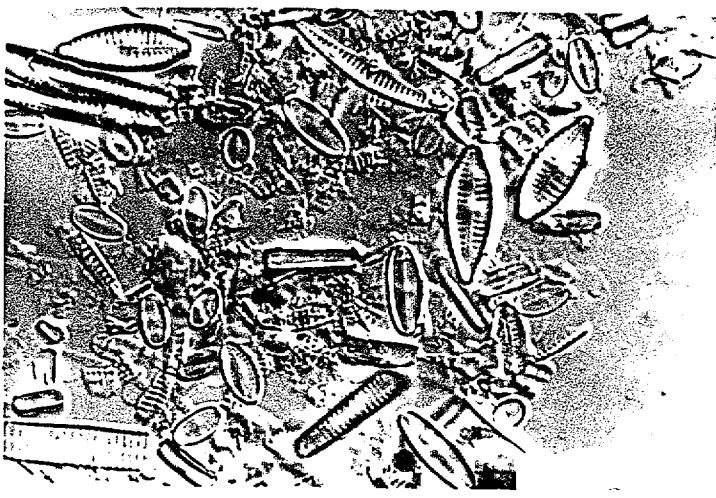
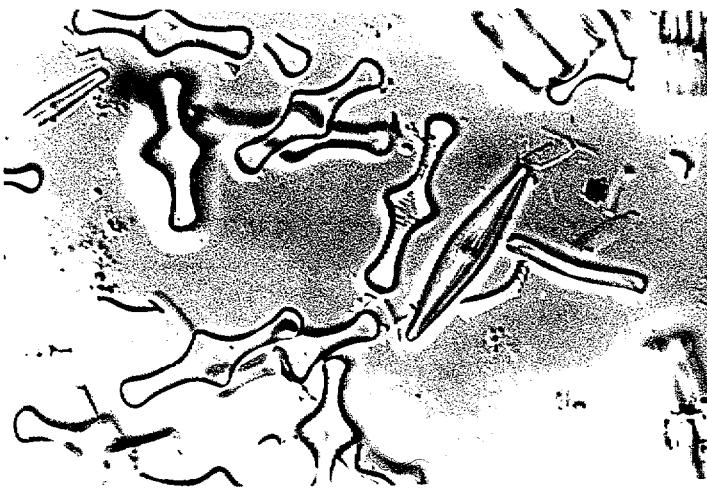
Fig. 12. | Species succession plotted on the hypothetical scheme outlined earlier in Fig. 2.

The analysis of the data presented was complicated by at least two features, (a) during the early sampling the complexity imposed by the existence of both an epilithic flora and an associated silt flora was not fully appreciated and (b) when this was realised and sampling of both achieved separately, this raised problems in combining the data - combination of the later samples was necessary in order to try and make them comparable to the earlier samples. Further studies are necessary to improve sampling - e.g. should all samples be treated in a two-stage manner - first washing the silt flora off and then removing the true epilithon or is it possible to use only the true epilithic flora and discard the silt flora - or indeed may the silt flora alone provide a better set of indicators at least for the zone 3 and possibly 4 & 5. I doubt whether it would provide much data on zones 1 and 2 though this requires further sampling. As will be seen from Fig. 1, it is desirable to experiment further with sampling since at the intermediate washing stage a distinctive set of species may be isolated and used as indicators.

The Continental, Japanese and South African analyses have all involved a large number of species - the minimum for an index seems to be Descy & Coste's (1988) 83 species but in the latest report he abandons his earlier index and uses the grid system of the French worker Coste involving 223 species selected from a full grid of 618 taxa. These numbers of species are quite unnecessary. However the question will undoubtedly be raised - why are not such numbers recorded in this survey? - are British rivers so different from those of the rest of the world? - is the sampling at fault?, etc. etc. In answer to these questions it can first be pointed out that the epilithon by definition is an attached, non-motile community and if this is sampled then only if there is massive contamination will motile species presumably derived from the sediment flora (the epipelon) and of non-motile/non-attached phytoplankton be encountered. At least 21 of Descy's 83 belong in this non-epilithic category and 2 belong to the epiphytic flora on Cladophora (see Fig. 13). This is an extremely conservative estimate since it leaves species of Navicula and Nitzschia in; the habitat of these is almost certainly not the epilithon but I need to sample the non-epilithic flora more intensively. In addition, in Descy's list there are at least 15 species which occupy the silt flora of the stones and another 4 or 5 which are varieties which may not have great significance though this requires further study. This brings Descy's flora down from 83 to 42-43 which is still high if only the real epilithon is sampled. However, in his list there is no mention of the pure water species Eunotia exigua, Tabellaria flocculosa, Hannaea arcus, Achnanthes austriaca or Brachysira vitreae. The very nice fit between the data for zones 1/2 and that of the much more extensive study of Leclercq & Fabri (1987) is almost entirely due to the fact that in these upper stations the added complexity of a silt flora is generally absent.

When the final details are settled, a flora of at most 20-30 species will be adequate to provide excellent indicators for water quality purposes. Fig. 13 brings together representative microphotographs of the epilithic flora of six different zones illustrating the distinct diatom flora in each. It is interesting to note that Descy lists his selection of species and

Fig. 13. Examples of the epilithic flora of an upland clean stretch (Zone 1) of the Scottish Dee (top left) with Tabularia flocculosa, Achnanthes microcephala and Brachysira vitrea. Mid-left: From the River Findhorn with Hannaea arcus - a typical Zone 2 assemblage. Bottom left: From the River Derwent with Diatoma vulgare, Navicula lanceolata and Fragilaria vaucheriae (a Zone 3 silted site). Top right: From a slightly polluted site on the River Don with Gomphonema parvulum and numerous Navicula spp. Mid-left: From the River Mersey - polluted, with Navicula goeppertiae and Nitzschia spp. Lower right: From Sankey Brook. A polluted site, with Nitzschia spp. dominant. Centre bottom: The flora epiphytic on Cladophora - Rhoicosphenia curvata and Cocconeis pediculus dominant.



those of Lange-Bertalot, Coste and Sladcecek and only 23 species are common to all four lists, and of these only some 7 are ones used in the above scheme (plus the clean water species which Descy does not list). Lange-Bertalot (1979) mentions about 50 species providing 99% of all the individuals and Patrick & Roberts (1979) quote figures of 1-4 species only providing over 10% of the counts in five streams. In a study of the diatom flora on different faces of stones in rivers, Cazaubon & Loudiki (1986) had to make very precise estimates of the epilithic flora and it is interesting that their results reveal a flora of only 40 species and of these only 9 were abundant - the first four dominants all being attached species (viz. Achnanthes minutissima, Hannaea arcus, Cymbella minuta and Reimeria sinuata) found in many British rivers. This study also showed that even at the level of distribution on the stone surface relative to direction of water flow the diatom species had distinct preferences: another example that they are very precise indicators of environmental conditions.

Is the sampling technique at fault since so few species are recorded? Searching the slides for all the rare species and varieties could increase the records though it is doubtful if the total would reach 618. Where then are all these species growing? The answer is that almost all the planktonic species are washed in (a few are river planktonic) and deposited on the sediments - these were found at isolated sampling times when it was obvious that a plankton bloom was involved. Many other species are motile and must belong to the sediment community either of the river sediments or washed in from lake/pond/soils. The relationship between the silt flora on the stones and the river sediment community (epipelon) needs careful analysis. The epipelon may add information of great value in estimating water quality or may itself provide an even more ideal set of indicator species. Sampling this community is not difficult and the substrate is nearly always present and indeed in all the slow flowing reaches and in the polluted reaches it is much easier to sample than the epilithon. Other sources of the "618 flora" will be the flora of the macroscopic aquatic plants (this has both attached and silt living components) and the attached flora of sand grains. These communities also need study to determine whether or not they can be utilised - especially in regions where the epilithon or epipelon are difficult to sample, e.g. in the regions of gravel deposits in some southern rivers.

Finally, there is no doubt that counting large numbers of cells in each sample enormously increases the records of species. Is, then, data being lost? Probably some data is lost but, comparing the results with those of others, in all cases the species with percentage occurrence above 5% in the counts rarely exceeds five or six and it is these which are providing the basic indicators in all the systems which have been devised. In many samples (and for instance in the examples quoted by Descy to illustrate his index - Navicula accomoda 78.7%, Nitzschia palea 19.7), only these two species have percentage occurrence above 5% and in such instances a single microscopic field provides this information!! (see Fig. 7 and photomicrographs 3-5).

In the proposal it was suggested that a simpler system could be devised and a grid record sheet would be adequate as in Table

6. The data from all the river stations are in a form suitable for mathematical analysis and the use of the latest Fortran programme for canonical community ordination (CANOCO) by partial detrended correspondence analysis, PCA, and redundancy analysis (Ter Braak 1986) is being considered.

6. CONCLUSIONS

1. The species in British rivers are the same as those used in the Descy index (as supplied in a paper to the DoE and Descy 1979); the Descy Index can therefore be calculated for British river sites. The species list needs extension to include the species from the large numbers of British rivers arising in mountains. The newer Descy & Coste (1988) systems of indices will also work. However the following are serious objections:

- a. The systems use too many species and accurate identification of such a number would be difficult for many workers.
- b. The indices do not enable sufficient distinctions to be made between sites.
- c. The indices confuse species from several associations.
- d. The systems do not provide information which would enable inter-comparison of stretches of rivers; it is essentially a classification based on the sensitivity of species to pollution.
- e. The system of water quality based on a 1-5 arithmetical calculation is merely a scale of decreasing eutrophication and obscures much valuable data which can be obtained from "breaks" in the distribution of species.

2. There is an epilithic diatom flora in all rivers sampled and it is often the dominant flora of the stone surfaces. Removal of the flora can be achieved by very simple techniques, though I now believe these should be improved and standardised.

3. The diatom epilithon undergoes considerable seasonal development but this does not affect the use of the flora to indicate quality since the major taxa are always present.

4. The survey has revealed that all previous workers have confused at least two different communities on the stone surfaces. A simple method of sampling these separately is provided.

5. The epilithon can be subdivided into a series of indicators for clean, low nutrient status waters (my zone 1); waters of increasing nutrient status (zone 2); waters becoming extremely eutrophic (zone 3); waters with incipient pollution (zone 4); waters of extreme pollution (zone 5). The zone 3 waters are the most abundant and can be subdivided into 4 subzones based on the dominant diatoms. This is a broad classification which is however preferable to the continuous scale of the arithmetically based indices.

6. Each zone and subzone still comprises a complex of waters and with further work more subtle distinctions on water quality will be possible.

a. Water quality changes in the acid/clean water zone can be monitored and the sites of excess acidity identified. The transition zone can be defined and improvements in water quality checked by monitoring the extension of this zone. The seasonal variation in the index at the major stations analysed in the R. Wye indicates broadly similar water quality over the year but it is necessary to check these changes very meticulously with water chemistry characteristics. A more striking example occurred at Bredwardine on the Wye where the Descy index indicated a single pollution event.

b. The diatom floras show that the major overall water quality problem is associated with eutrophication. Gross pollution such as occurs in parts of the Rivers Main/Rhine in Germany are apparently rare in British rivers (but more sampling is required in some areas).

c. Improvement in water quality would involve the downstream extension of the Achnanthes minutissima zone, elimination of the Gomphonema parvulum association and reduction in the Amphora pediculus zone.

7. Although not completely analysed there are some broad geographical variations in the epilithic flora. These need further study and analysis.

8. A simplified classification (the above zones) is provided as an alternative to the continental systems. The difficult species have been removed from this proposed system and counting simplified.

9. A preliminary illustrated flora based on light and electron microscopy is in preparation.

There is no doubt that the continental workers' views that diatoms are excellent indicators of water quality is correct; some new work on acid streams has shown that very fine distinctions can be detected.

In order to get a general picture for the British Isles I undoubtedly made problems for myself. The data enables broad generalisations concerning diatom associations associated with downstream changes but the sampling was not at close enough intervals to detect perturbations in the flora due to isolated inputs. The light microscope observations have often given the impression of different forms of the same diatom species in different rivers but checking with the scanning electron microscope has removed many of these doubts, though there are still many problems to be investigated.

It is easier to master the flora if one river (or one zone - as in the Leclercq & Fabri work) is studied intensively. There should be no difficulty for a worker using diatoms as indicators provided a start is made with a few sites visited regularly.

Chemical data from River Boards has still to be related to the diatom analysis but I would prefer to extract all the information I can from the diatom survey and devise species associations recognised in their own right before being influenced by chemical data. The breaks between diatom associations can then be used to subdivide the chemical data in a biologically meaningful way.

I now also need to cluster the data from each zone of each river - a process which may (if zone 1 is typical) yield more detailed subdivisions to relate to chemical conditions. Before doing this, I want to re-analyse some of the sites in the light of the further experience of the diatom systematics gained during the last few months of checking species. The data is such that it can be entered into computerised analysis systems such as the latest canonical community ordination system of Ter Braak (1980).

A series of publications is now planned based on the last three years data plus some additional sampling to check critical points. An initial subdivision of the information into papers suggests the following titles:

1. Review of the epilithic diatom flora in rivers and its use in water quality assessment.
2. The structure and sampling of the epilithic habitat with respect to diatoms.
3. The zonation of rivers based on the diatom flora of the epilithon.
4. The relationship between epilithic diatom zonation and water chemistry in British rivers.
5. The effect of geography/geology on the distribution of epilithic diatoms in British rivers.

7. REFERENCES

- ARCHIBALD, R.E.M. 1972. Diversity in some south African diatom associations and its relation to water quality. Water Res. 6, 1229-1238
- BLINN, D.W., FREDERICKSON, A. & KORTE, V. 1980. Colonisation rates and community structure of diatoms on three different rock substrata in a lotic system. Br. phycol. J. 15, 303-30
- BUTCHER, R.W. 1932. Studies in the ecology of rivers II. The microflora of rivers with special reference to the algae on the river bed. Ann. Bot. 46 813-861
- BUTCHER, R.W. 1940. Studies in the ecology of rivers IV. Observations on the growth and distribution of the sessile algae in the River Hull, Yorkshire. J. Ecol. 28 210-233
- BUTCHER, R.W. 1946. Studies in the ecology of rivers VI. Algal growth in certain highly calcareous streams. J. Ecol. 33, 268-283
- CAMBRA, J. 1987. Flore et vegetation algologiques des eaux epicontinentals de la Reserve Naturelle des vallees d'Ordesa (Pyrenees aragonaises). Candollea 42, 475-490
- CAZAUBON, A. & LOUDIKI, M. 1986. Microrepartition des algues epilithiques sur les cailloux d'un torrent Corse, le Rizzanese. Annals Limnol. 22, 2-16
- COSTE, M. 1976. Contribution a l'ecologie des diatomees benthiques et periphytiques de la Seine: Distribution longitudinale et influence des pollutions. Soc. Hydrotechn. France III (9), 1-7
- DESCY, J.-P. 1979. A new approach to water quality estimation using diatoms. Nova Hedwigia, Beiheft 64, 305-323
- DESCY, J.-P. 1984. Ecologie et distribution de diatomees benthiques dans le bassin Belge de la Meuse. Inst. Roy. des Sciences Nat. de Belg. Documents de Travail No. 18, 25 pp. 70 pl.
- DESCY, J.-P. & COSTE, M. 1987. Utilisation des diatomees benthiques pour l'évaluation de la qualité des eaux courantes. Premier Rapport d'Avancement to UNCED (Unité d'Ecologie des eaux douces). 24 pp. plus 2 Appendices.
- DESCY, J.-P. & COSTE, M. 1988. Utilisation des diatomees benthiques pour l'évaluation de la qualité des eaux courantes. Deuxieme Rapport. 48 pp. plus 3 Appendices.
- DOUGLAS, B. 1958. The ecology of the attached diatoms and other algae in a small stony stream. J. Ecol. 46, 295-322
- FABRI, R. & LECLERCQ, L. 1979. Flore et vegetation algales des ruisseaux de la Reserve naturelle demariale des Hautes

Fagns (province de Liege, Belgique). Min. de l'Agric. Admin. des Eaux et Rorets. Trav. 11, 47 pp.

JONES, J.G. 1974. A method for observation and enumeration of epilithic algae directly on the surface of stones. Oecologia 16, 1-8

JONES, J.F. 1978. Spatial variation in epilithic algae in a stony stream (Wilfin Beck) with particular reference to Coccconeis placentula. Freshwater Biology 8, 539-546

KAWECKA, B. 1981. The ecological characteristics of diatom communities in the mountain streams of Europe. In Proc. 6th Diatom Symposium (ed. R. Ross), pp. 425-33. O. Koeltz, Koenigstein

KLEE, R. & STEINBERG, C. 1987. Kieselalgen Bayerischer Gewässer. Informationsberichte Bayerisches Landesamt für Wasserwirtschaft. 4/87. 194 loose-leaf pages

KOBAYASI, H. & MAYAMA, S. 1982. Most pollution-tolerant diatoms of severely polluted rivers in the vicinity of Tokyo. Jap. J. Phycol. 30, 188-196

KOLKWITZ, R. & MARSSON, M. 1908. Okologie der pflanzlichen Saproben. Ber. Deutsch. Bot. Ges. 260, 505-519

LANGE-BERTALOT, H. 1978. Diatomeen-Differentialanten anstelle von Leitformen: ein geeignetes Kriterium der Gewässerbelastung. Arch. Hydrobiol. Suppl. 51, Algol. Stud. 21, 393-427

LANGE-BERTALOT, H. 1979. Pollution tolerance as a criterion for water quality estimation. Nova Hedwigia, Beih. 64, 285-304

LECLERCQ, L. 1987. Vegetation et physico-chimie des eaux des rivières naturelles du Nord du massif, Ardennats (Belgique). Typologie des peuplements de diatomées. Cryptogamie, Algologie, 8, 157-171

LECLERCQ, L. & FABRI, R. 1982. Flore et vegetation algales des eaux oligotrophes du Bassin de la Schwalm (Belgique, Province de Liege). Bull. Soc. Roy. Belg. 115, 53-68

LECLERCQ, L. & MAQUET, B. 1987. Deux nouveaux indices diatomique et de qualité chimique des eaux courantes. Comparison avec différents indices existants. Cah. Biol. Mar. 28, 303-310

LECLERCQ, L. & MAQUET, B. 1987. Deux nouveaux indices chimique et diatomique de qualité d'eau courante. Application au Samson et à ses affluents. Comparison avec d'autres indices chimiques, biocénétiques et diatomiques. Inst. Roy. Sc. Natur. Belg. Documents de travail 38, 113pp.

LOWE, R.L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. National Environmental Res. Center EPA-690/4-74-005. 334 pp.

- MARGALEF, R. 1948. A new limnological method for the investigation of thin layered epilithic communities. Hydrobiologica 1, 215-216
- MARKER, A.F.H. 1976. The benthic algae of some streams in southern England. I. Biomass of the epilithon in some small streams. J. Ecol. 64, 343-358
- MARKER, A.F.H. 1976. The benthic algae of some streams in southern England. II. The primary production of the epilithon in a small chalk-stream. J. Ecol. 64, 359-373
- MARKER, A.F.H. & CASEY, H. 1982. The population and production dynamics of benthic algae in an artificial recirculating hard-water stream. Phil. Trans. R. Soc. Lond. B.298, 265-308
- MARKER, A.F.H. & GUNN, R.J.M. 1977. The benthic algae of some streams in southern England. III. Seasonal variations in Chlorophyll a in the seston. J. Ecol. 65, 223-234
- MCINTYRE, C.D. 1968. Structural characteristics of benthic algal communities in laboratory streams. Ecology 49, 520-537
- MOORE, J.W. 1976. Seasonal succession of algae in rivers. I. Examples from the Avon, a large slow-flowing river. J. Phycol. 12, 342-349
- MOORE, J.W. 1977. Seasonal succession of algae in a eutrophic stream in southern England. Hydrobiologia 53, 181-192
- MOUTHON, J. & COSTE, M. 1984. Qualite hydrobiologique de la Loire. Etat de reference du site du Villerest. CEMAGREF, Groupement de Lyon, Division qualite des Eaux, Peche et Pisciculture. Lyon (France). 167 pp.
- PATRICK, R. & ROBERTS, N.A. 1979. Diatom communities in the middle Atlantic States, U.S.A. Some factors that are important to their structure. In Proc. 5th Symp. on recent and fossil diatoms (ed. R. Simonsen), Nova Hedw. Beih 64, 265-283
- ROUND, F.E. 1981. The ecology of algae. Cambridge University Press. 653 pp.
- SABATER, S. & SABATER, F. 1985. Diatom assemblages in the River Ter. Arch. Hydrobiol. 111, 397-408
- SABATER, S., SABATER, F. & TOMAS, X. 1987. Water quality and diatom communities in two Catalan rivers (N. E. Spain). Wat. Res. 21, 901-911
- SCHARF, W. 1984. Kieselalgen als Indikatoren fur die Beurteilung der Ablaufqualitat von Klaranlagen. Forum Studie-Hygiene. Zeitschrift fur Forschung und Technik in der Wasser-, Boden- und Luft-Hygiene 35, 268-273
- SCHOEMAN, F.R. 1973. A systematical and ecological study of the diatom flora of Lesotho with special reference to the

- water quality. National Institute for Water Research.
C.S.I.R., Pretoria. 355 pp. 10 pl.
- SCHOEMAN, F.R. 1976. Diatom indicator groups in the assessment of water quality in the Juskei-Crocodile river system (Transvaal, Republic of South Africa). J. Limnol. Soc. Southern Africa 2, 21-24
- SCHOEMAN, F.R. 1979. Diatoms as indicators of water quality in the upper Hennops River. J. Limnol. Soc. South Africa 5, 73-78
- SLADECEK, V. 1973. System of water quality from the biological point of view. Ergebn. Limnol. 7, IV, 1-218
- SLADECEK, V. 1986. Diatoms as indicators of organic pollution. Acta Hydrochim. Hydrobiol. 14, 555-566
- STATZER, B. & HIGLER, B. 1986. Stream hydraulics as a major determinant of benthic invertebrate zonation patterns. Freshwat. Biol. 16, 127-139
- STEVENSON, R.J. 1984. Epilithic and epipellic diatoms in the Sandusky River, with emphasis on species diversity and water pollution. Hydrobiologia 114, 161-175
- SULLIVAN, M. 1986. Mathematical expression of diatom results: Are these "Pollution Indices" valid and useful? In Proc. 8th Diatom Symposium (ed. M. Ricard), 772-776. Koenigstein: O. Koeltz.
- TER BRAAK, C.J.F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67, 1167-1179
- VAN DAM, H. 1982. On the use of measures of structure and diversity in applied diatom ecology. Nova Hedwigia, Beih. 73, 97-115
- WATANABE, T., ASAII, K. & HOUKI, A. 1988. Numerical water quality monitoring of organic pollution using diatom assemblages. In Proc. 9th International Diatom Symposium (ed. F.E. Round), pp. 123-41. Biopress, Bristol & O. Koeltz, Koenigstein.
- WATANABE, T., ASAII, K., HOUKI, A., TANAKE, S. & HIZUKA, T. 1986. Saprophilous and eurysaprobic diatom taxa to organic water pollution and diatom assemblage index (DAIpo). Diatom 2, 23-73
- ZELINKA, M. & MARVAN, P. 1961. Zur Praxisierung der biologischen Klassifikation der Reinheit fliesender Gewässer. Arch. Hydrobiol. 57, 389-407

APPENDIX I

A simple brief outline system suitable for NRA use.

Select 5 stones from the river site. Choose flat stones (preferably from riffles), if possible with a dark brown or mucilaginous coating. Transfer to the laboratory in a closed plastic box.

In the laboratory, wash off the diatoms from the silt layer and collect in a beaker. Then wash the stone under the tap to remove all the silt flora. Finally rub or brush the stone surface rigorously to remove the firmly attached diatoms - collect these in a beaker.

Transfer 10 ml of each sample to separate centrifuge tubes. Centrifuge or stand overnight. Pour off supernatent liquid. Add 1 ml saturated potassium permanganate solution. Shake and leave overnight. Then in fume cupboard add 1 ml conc. hydrochloric acid. Seal the tubes with cling-film and place in a warm oven (or water-bath) at approximately 60°C until the sample becomes colourless. Cool and add distilled water, centrifuge. Repeat addition of distilled water and centrifugation 5 times. The final sample will have a grey cloudiness depending on the quantity of diatoms. If too grey (too dense a diatom sample) dilute with distilled water. Place sufficient of this sample onto a coverglass to give a well spread sample. Dry and then mount the coverglass in Naphrax on a labelled slide (label with site, date, etc.).

Dry on a hotplate in the fume cupboard overnight.

Identify and count the first 100 diatoms observed at random on the slide using the accompanying illustrations and table to record the species.

NB: (1) The table may need modification depending on the diatom flora in particular sites;
(ii) The distinction into further zones is being developed;
(iii) The relationship between these diatom zones and various chemical parameters will be provided by further studies.

Table 6. A suggested record grid for counting the diatoms in the epilithon.

Station	1	2	3	4	5	6
<i>Eunotia exigua</i>						
<i>Eunotia rhomboidea</i>						
<i>Eunotia vanheurckii</i>						
<i>Eunotia curvata</i>						
<i>Tabellaria flocculosa</i>						
<i>Anomoeoneis brachysira</i>						
<i>Achnanthes austriaca</i>						
<i>Achnanthes microcephala</i>						
<i>Hannaea arcus</i>						
<i>Fragilaria capucina</i>						
<i>Achnanthes minutissima</i>						
<i>Cymbella minuta</i>						
<i>Coccconeis placentula</i>						
<i>Amphora pediculus</i>						
<i>Reimeria sinuata</i>						
<i>Synedra ulna</i>						
<i>Achnanthes lanceolata</i>						
<i>Surirella ovata</i>						
<i>Fragilaria vaucheriae</i>						
<i>Gomphonema parvulum</i>						
<i>Navicula spp.</i>						
<i>Navicula subminuscula</i>						
<i>Navicula pelliculosa</i>						
<i>Navicula accomoda</i>						
<i>Navicula goeppertiae</i>						
<i>Nitzschia communis</i>						
<i>Nitzschia palea</i>						
<i>Amphora veneta</i>						

APPENDIX II

Areal sampling

If it is necessary to obtain figures for the number of cells per unit area, a known area of stone surface must be sampled. This can be achieved in the field by techniques described by Descy and others. For stones transferred to the laboratory I have developed a suitable technique. Sections (rings) (5 mm deep) are cut from glass tubing and the cut edge coarsely ground. The stone is allowed to dry and then a glass ring is glued (using a rubber based adhesive such as UHU) to the stone surface. A number may have to be used because of the great variation in colonisation over stone surfaces. When the ring is firmly attached (allow at least a day for the glue to dry) the area inside the stone can be wetted and brushed (a dentist's drill brush has been used) and the aqueous suspension pipetted into a centrifuge tube. It is necessary to do this repeatedly and to wash the brush into the centrifuge tube. Tests need to be done to ensure that all cells are removed from the stone surface. The material can then be cleaned by the standard method and cover glasses prepared with a standard amount of the final washed material. It is a lengthy process and accuracy can only be achieved by care at all stages and by taking sufficient samplings from the stone surface. Its disadvantages are that live cells cannot be observed; it is time consuming and unnecessary unless extremely detailed floral composition is needed.

APPENDIX III Notes on species - Brief examples of the details that can be provided

Although at the early discussion stage one correspondent suggested that Descy's comments on species were superfluous, I believe they can be made useful, and the following is a very preliminary extract from my notes. These will be continued and enlarged and I believe will be of assistance when river authorities have to take the diatoms into account.

Achnanthes

Achnanthes minutissima is the most abundant species on stone surfaces in many rivers especially the nutrient poor western sites. It is virtually absent from lowland eutrophic and calcareous rivers. In rivers where it is abundant in the upper reaches, it often dies out in the eutrophic zone. In its typical form it is easily recognisable by the curved girdle view and in valve view by the slightly swollen central region tapering to slightly capitate ends. However there is much confusion in the literature. Lange-Bertalot has combined A. minutissima var. cryptocephala, A. microcephala and A. linearis with A. minutissima. But A. minutissima var. jackii has been retained. Descy keeps A. minutissima var. cryptocephala separate and this seems desirable from my light microscope observations. It is a variety which is only abundant in the clean zone 1 (as A. microcephala in many publications and in my counts). It can be distinguished by SEM but I have to confess that it is difficult to distinguish short cells of var. microcephala from A. minutissima in the SEM. I have started to study these in detail from the various rivers but have not yet solved the problem. There are several other difficult Achnanthes in the A. linearis/A. exilis groups; these occur in the more eutrophic regions and need to be studied using the SEM. It has been suggested by some workers that these small Achnanthes be left out of counts. However, there are several objections to this: (1) at many stations they are the dominants and so the characterisation of the association would not be possible; (2) it would lead to confusion in the literature; (3) the various forms may be important in characterising certain waters; (4) a subjective decision would have to be made each time an Achnanthes was seen. It may be better simply to list them as Achnanthes spp.

Achnanthes lanceolata. This species is absent from the acid nutrient poor rivers and only becomes frequent in alkaline rivers. It is easily recognised by the "hoof" mark on the side of the valve. There are many varieties. The striation is quite pronounced and in the SEM this is obvious from the multiseriate nature of the pore fields. A small almost circular form is abundant only in polluted sites (Fig. SEM 33). The var. rostrata is often abundant where the water is extremely eutrophic whereas the species itself is characteristic of chalk streams.

Achnanthes austriaca (=Navicula rotaena). This I have kept in Navicula since I have not seen any specimens in the SEM where there is one raphe-less valve. It is a common form in the zone 2

waters.

A general feature of Achnanthes which has to be coped with is the recognition of the raphe valve and the raphe-less valve (Figs 00). After a little experience the two forms can be learnt and referred to one species. A more difficult problem is that the raphe-valve can at first sight be identified as a Navicula species (cf. the problem with A. austriaca). In girdle view the Achnanthes cell is always bent but Navicula is never bent.

Amphora

Only commonly represented by one species growing on stones. The genus is normally motile and does not even colonise the silt of stones.

Amphora pediculus. This smallest of the Amphora species is a true epilithic species, the cells attaching individually to the stone surface along with Reimeria sinuata. These two must be capable of survival beneath layers of mucilage and silt. There are several varieties of A. pediculus (Lee & Round, 1988) but there is no need to distinguish these in river surveys. It is absent from zones 1 and 2 and from 4 and 5. Intermittent in zone 3 but with a tendency to be more abundant in the lowermost 3d. The species is small and at first sight might appear difficult to identify but there is no other diatom with which it can be confused. The smallest forms tend to lie in girdle view on slides and appear round.

Amphora veneta. A larger form - the central striae are visible under oil immersion. It is only found in the zone 4 and is a good indicator of incipient pollution but it has never been found in quantity.

Anomoeoneis

This genus is very rarely found - occurs only in eutrophic waters. It should not be confused with Brachysira species which in the older literature are placed in this genus. Anomoeoneis is typically a form of tropical saline lakes.

Hannaea

The more recent name for Ceratoneis. This is a quite unmistakeable genus, curved and with a central expansion.

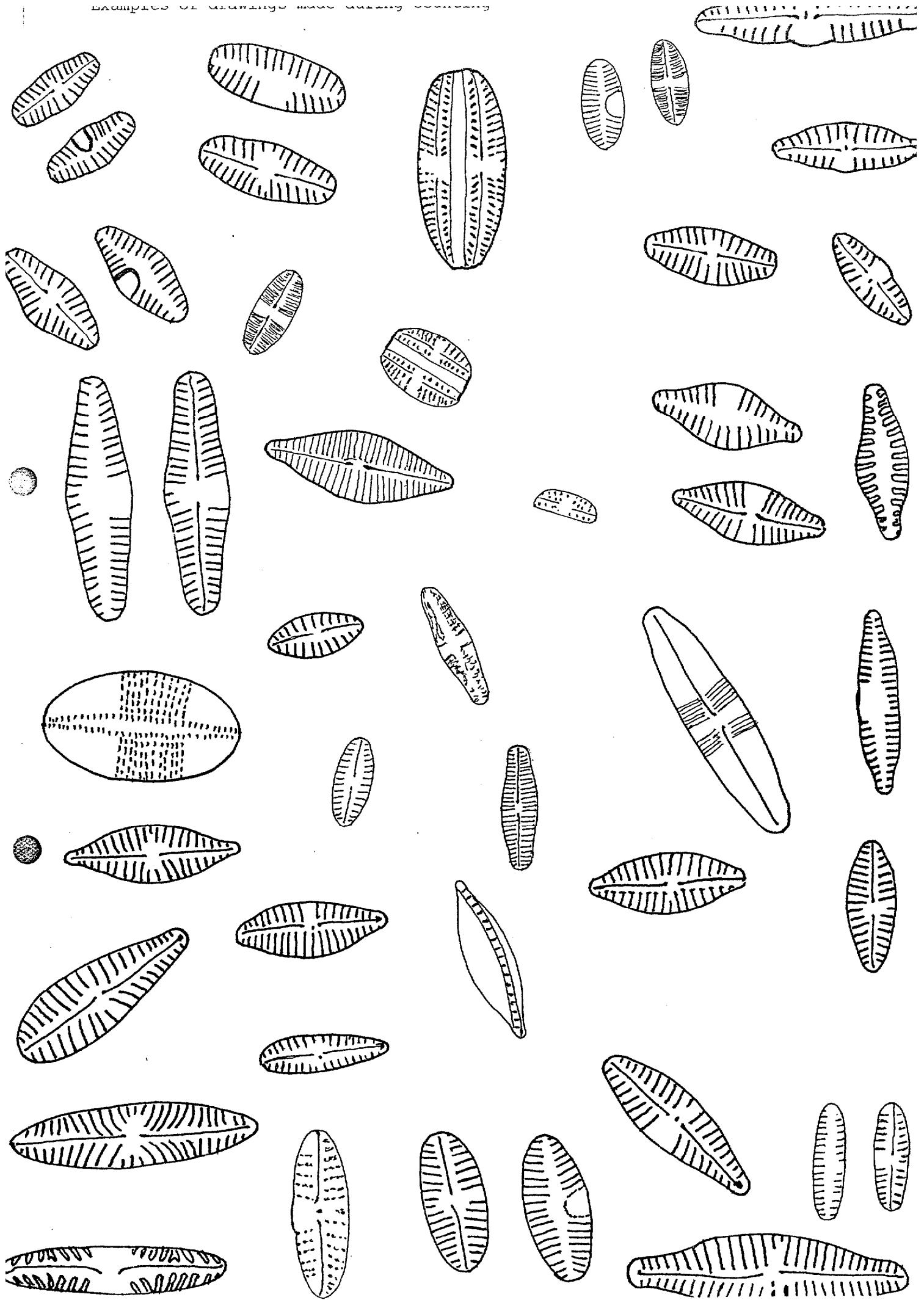
H. arcus. Found in a variety of forms which may simply be size variants of the species though they have been given varietal names. Certainly a very long thin form has been found only in the River Spey. Occurs in zone 2 almost exclusively. Where found lower down a river it is almost certainly a good indicator of the input of clean waters. In the case of R. Findhorn this species characterises the whole of the upper reach above Findhorn Bridge to the final deserted farm.

Meridion.

This genus is generally associated with running waters but most often in springs. It does occur on stones but sporadically. Certainly it is only found in clean waters but is not common enough to give a good indication of water quality.

APPENDIX IV Examples of drawings of species

Whilst identifying and counting samples from each epilithic site, camera lucida drawings have been made and one example from the River Frome, Dorset, is reproduced here. These will be broken up and arranged into individual genera and probably put alongside enlargements of individual species from microphotographs such as are indicated in Figs 3 - 5, 11.



APPENDIX V Examples of Scanning Electron Micrograph illustrations to assist light microscopy determination

A small selection of scanning electron micrographs is appended. These can often be used to assist identification at the light microscope level but are also essential for my checking of species identification.

Key to SEM pictures

1 - 9. Zone 1-2 species

1. Eunotia exigua (inside). 2. Eunotia vanheurckii. 3. Diatoma mesodon. 4. Meridion circulare (inside). 5. Hannaea arcus. 6. Navicula rotaena (large cell) - two others unknown. 7-9. Tabellaria flocculosa. 7. Valve. 8. Half a cell, valve uppermost with its five girdle bands. 9. A single girdle band.

10 - 17. Zone 3 species

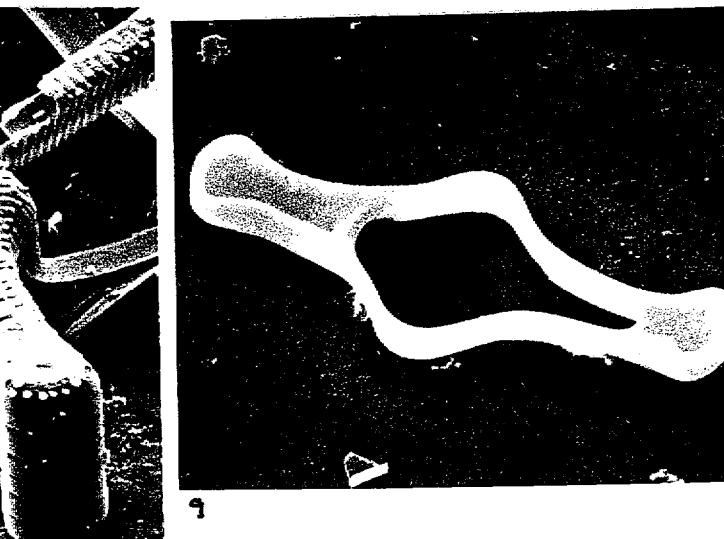
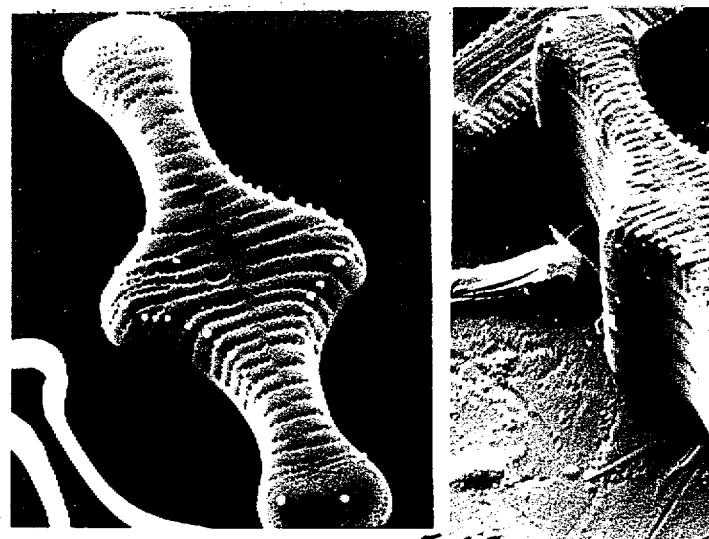
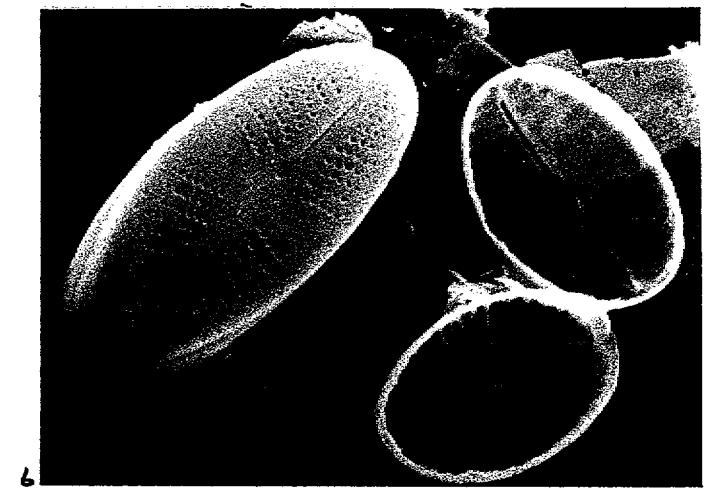
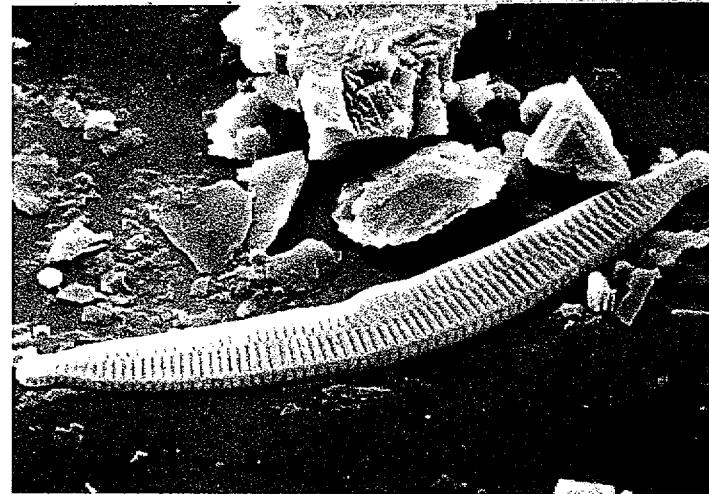
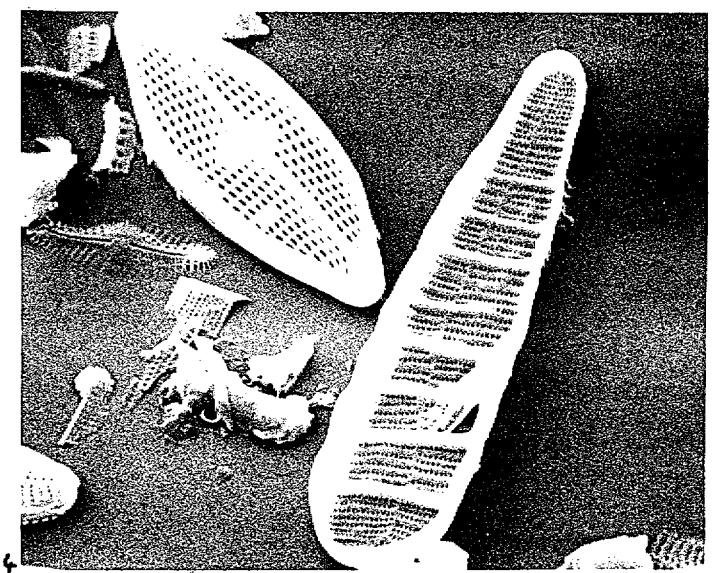
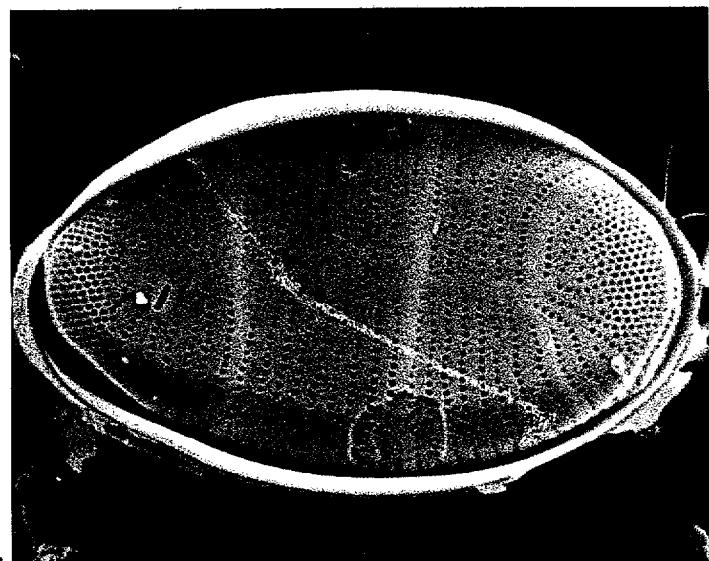
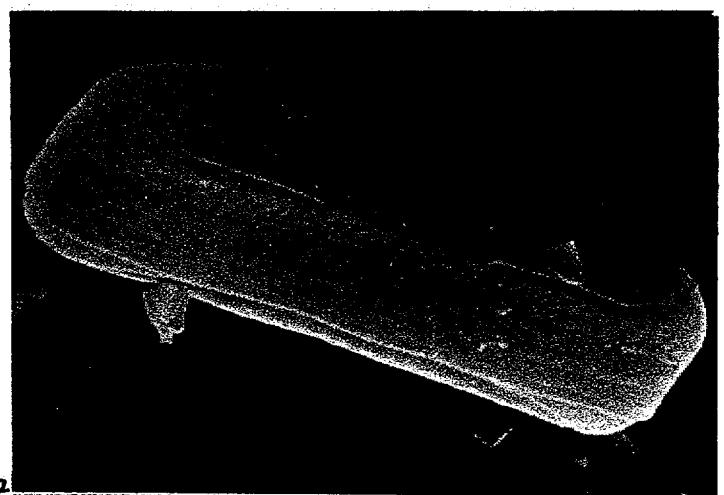
10. Coccconeis placentula. 11. Coccconeis placentula variety. 12. Reimeria sinuata - cell complete - two valves and three (?) girdle bands. 13. Reimeria sinuata, one valve. 14. Gomphonema angusta. 15. Gomphonema tergestinum. 16. Fragilaria vaucheriae. 17. Cymbella minuta - two small valves; Cymbella silesiaca (?) large valve.

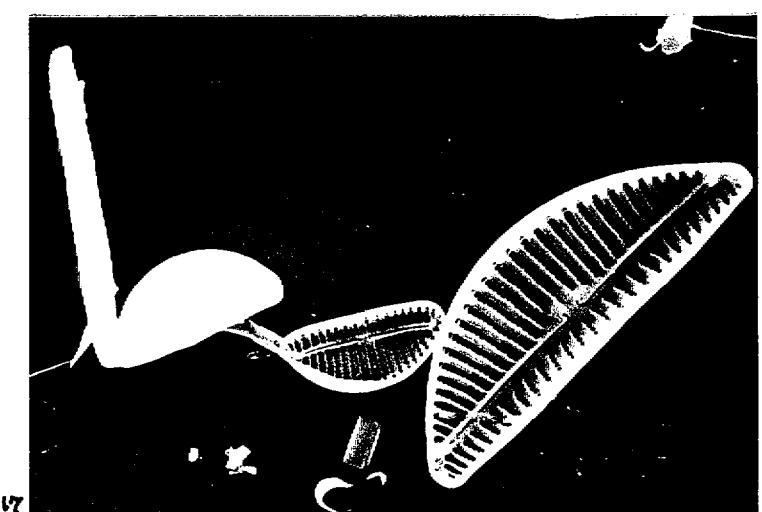
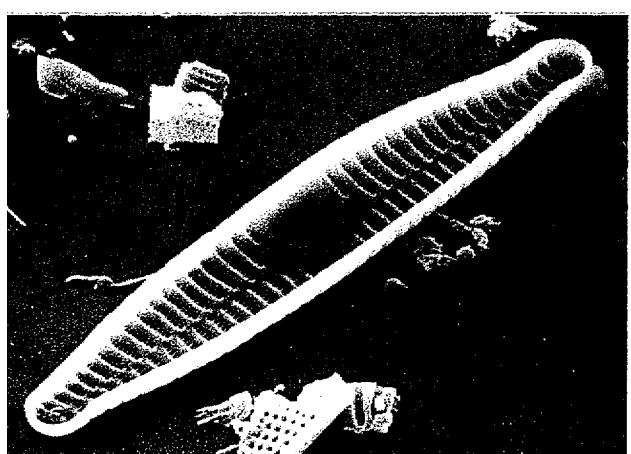
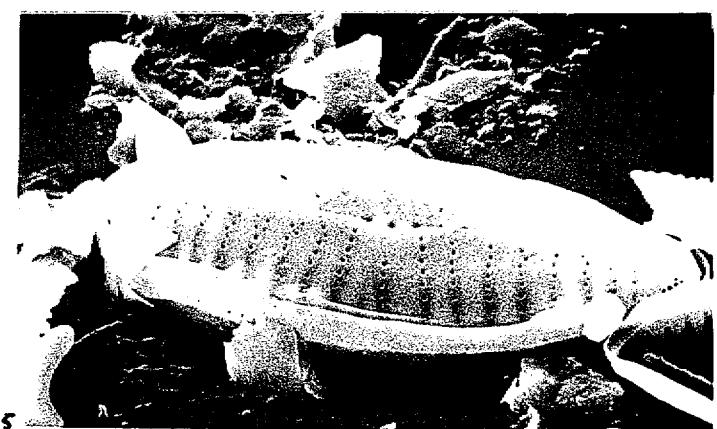
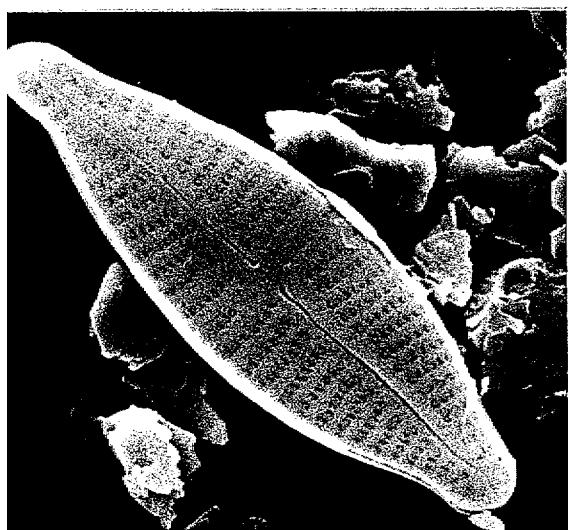
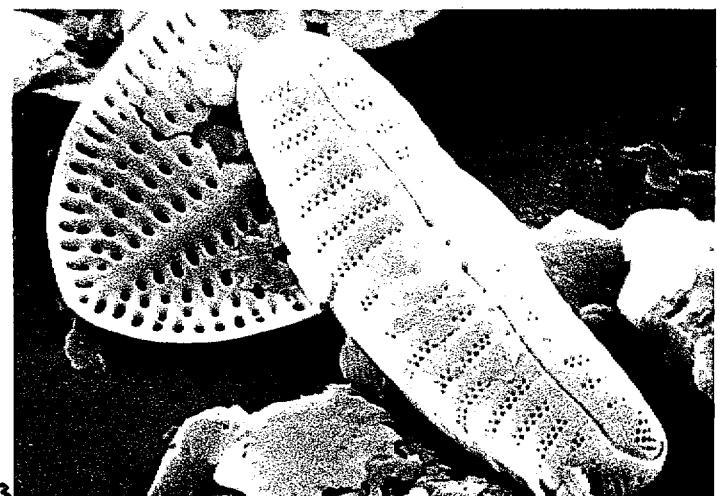
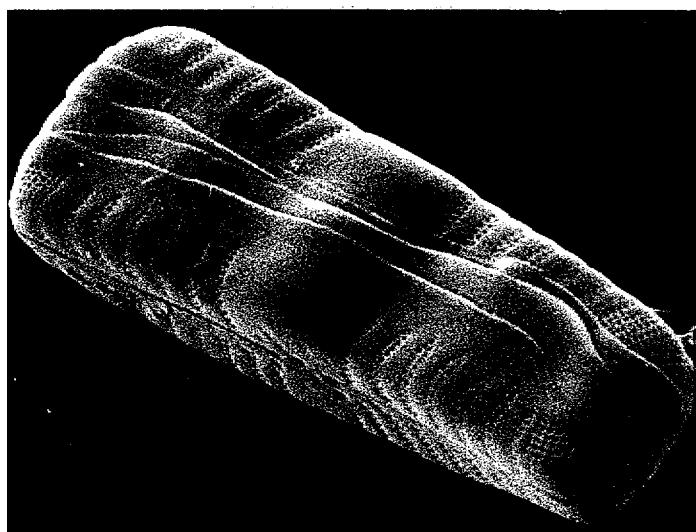
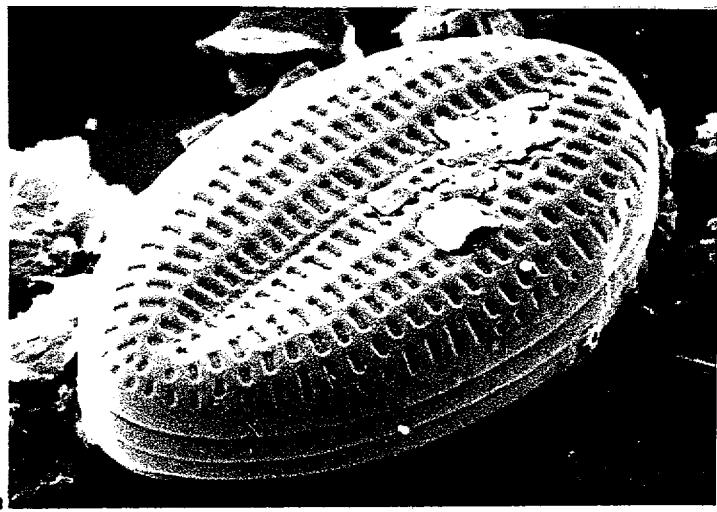
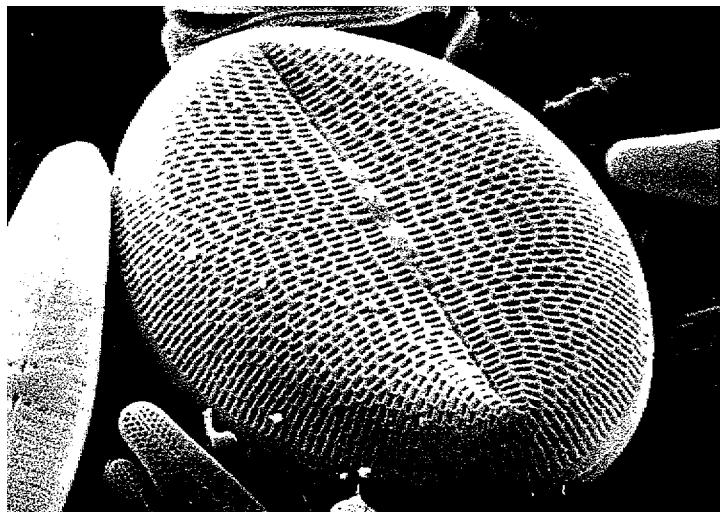
18 - 23. Zone 3 species; 24 - 25. Zone 5 species.

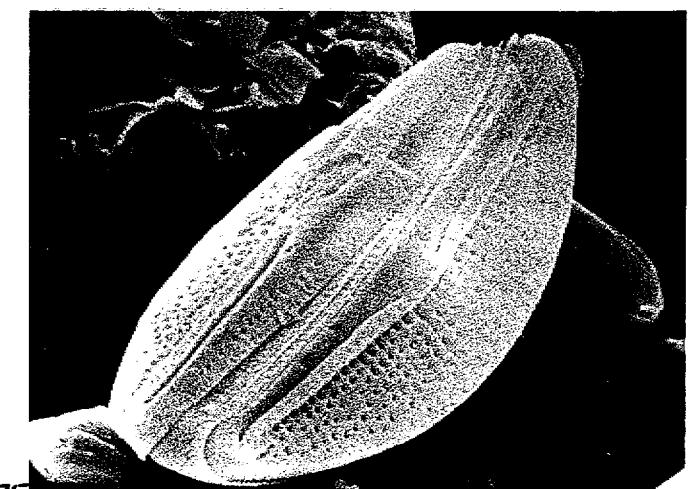
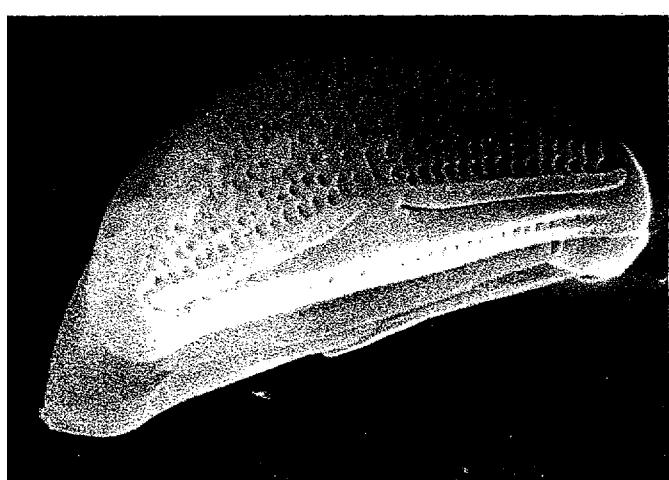
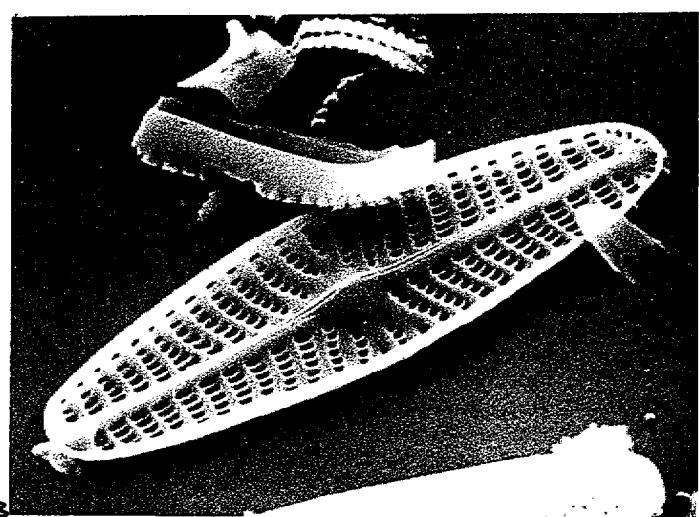
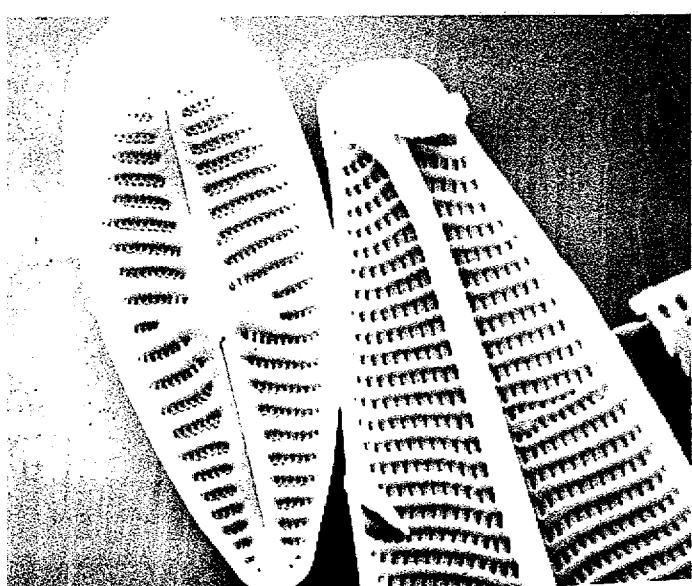
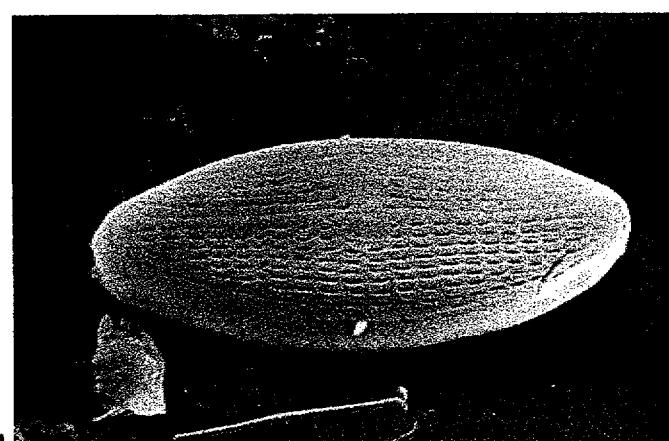
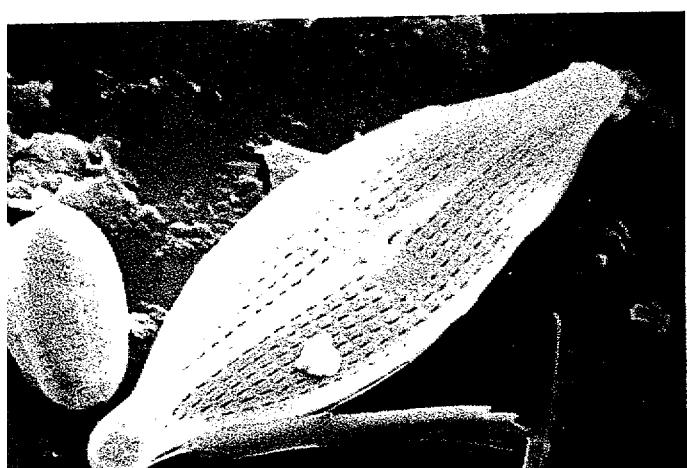
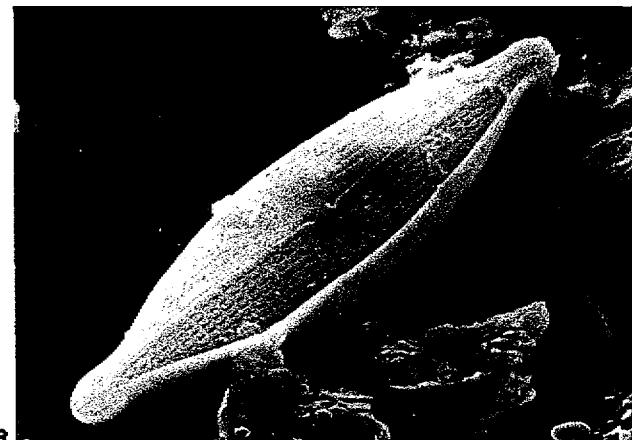
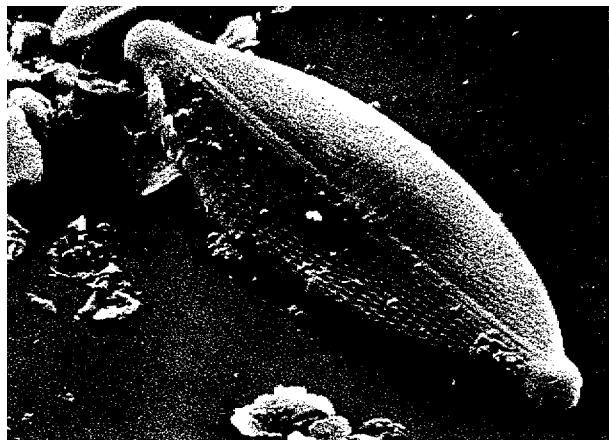
18. Navicula cf. salinicola. 19, 20. Navicula cf. gregaria. 21. Navicula cf. phyllepta. 22. Gomphonema olivaceum (left valve), Navicula lanceolata (part of valve). 23. Navicula cf. schoenfeldii. 24, 25. Amphora veneta.

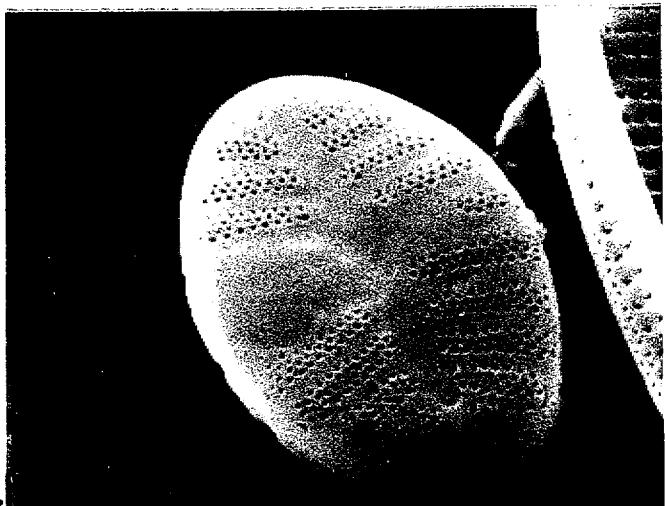
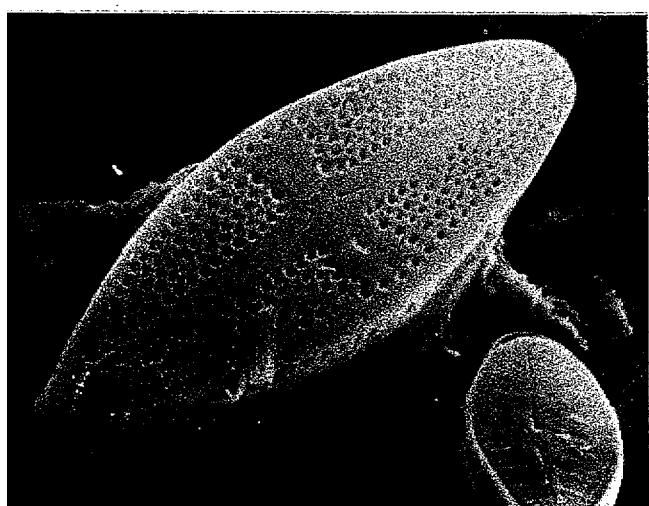
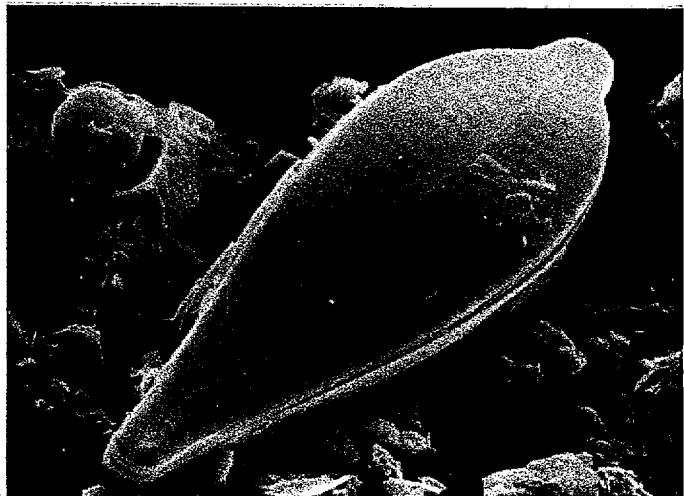
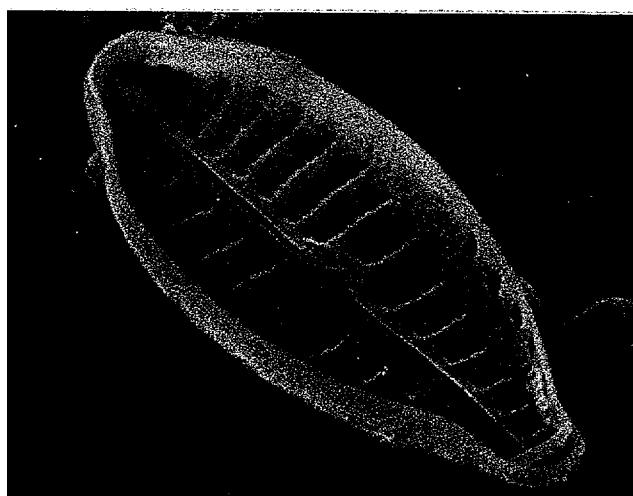
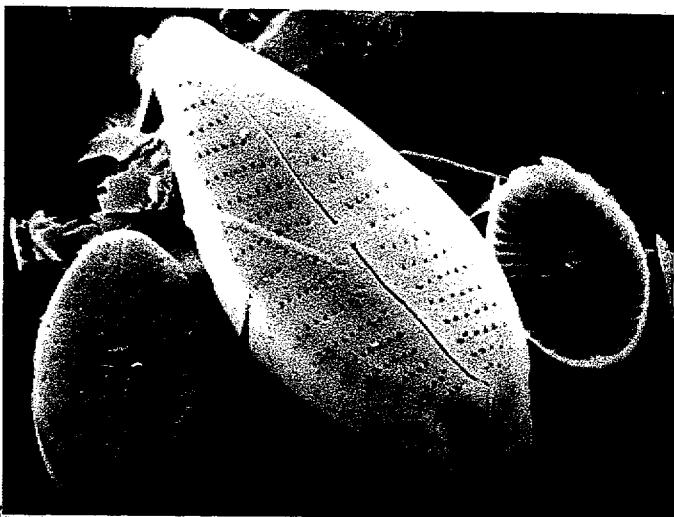
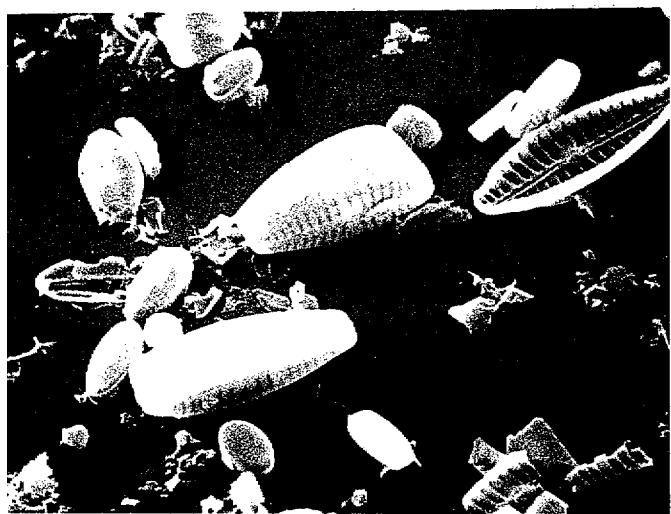
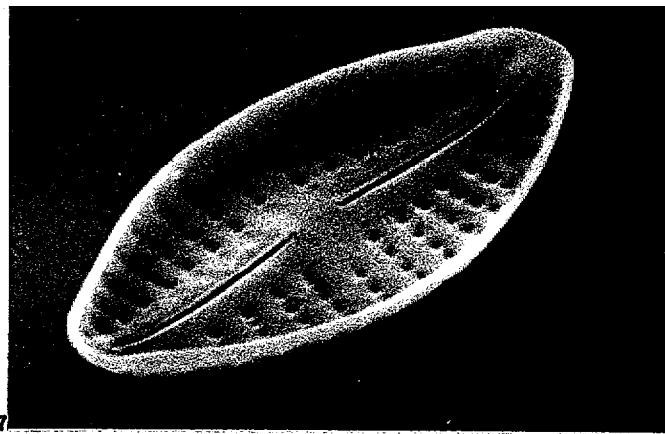
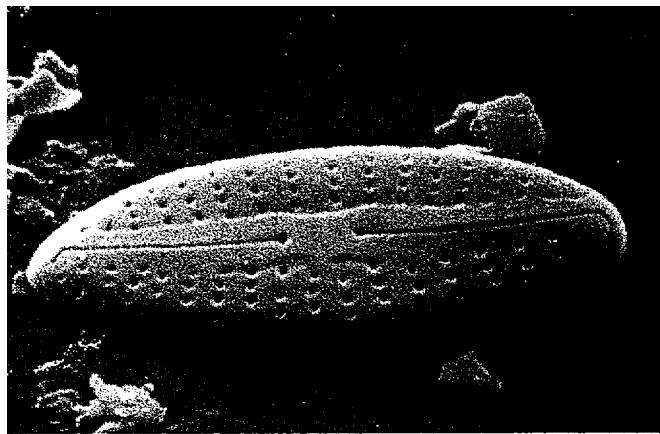
26 - 33. Zone 4-5 species.

26, 27. Navicula subminuscula (outside and inside view). 28. Lower power field of Gomphonema parvulum and several small Navicula species. 29. Gomphonema parvulum and two small Navicula species. 30. Gomphonema parvulum (inside). 31. Gomphonema turris. 32. Navicula goeppertiana. 33. Achnanthes lanceolata (v. ovalis?).









APPENDIX VI Suggested Future Plans

As a preliminary it is necessary to point out that this report is the only piece of work on the diatom epilithon of British rivers (compared with work on the Continent going back to the beginning of the century) and the flora is as, or more, widespread than the invertebrate flora which has been studied extensively, the most recent UK work involving five workers over more than five years. Nevertheless, I believe the data provides a basic framework. Though there are gaps and much more needs to be done, the outcome will be an improvement on the Continental system. The work to date has shown that the Continental view that the diatom epilithon is widespread and highly suitable for water quality assessment is true.

Brief plans involve:-

1. Refining of the data based on exclusion of the silt flora from the true epilithon to provide the simplest possible indicator system for water quality and detection of pollution. It is necessary to discover if the presence of a silt layer affects the underlying epilithon. Also, selection of non-silted stones at all sites down a river may provide a solution - up until now the tendency has been to select stones with a visual brown diatom flora and these tend to be silted. The zone 3 flora requires intensive study to define the exact indicator value of species. Evidence from the more widespread studies on zones 1 & 2 encourage the view that extensive work on zone 3 species will be profitable.
2. Completion of the assessment of the effects of geographical/geological features on the flora and construction of indices.
3. Study of further sites of extreme pollution, probably incorporating data from diatom communities other than the epilithon.
4. Correlation of all the diatom data with chemical data.
5. Improvement of the illustrations of the critical species.
6. Testing of the system above and below a variety of discharges and determination of the distance required for recovery.
7. The latest Descy & Coste proposals for the EEC also involve the diatom flora of the epiphytic (plant surfaces) and epipellic (sediment surfaces) communities. Since elements of these floras also complicate most previous studies, it is essential that these be given the same attention as the epilithon. The epipelon may prove to be a more suitable flora to use for water quality - there is almost no data available for British rivers, but methods evolved for lake studies by one of us (FER) can be used to provide an uncontaminated set of data. The sampling technique is easier than that for stone surfaces.
8. Study of these other communities is also necessary to aid the refinement of the indices based on the epilithon.

APPENDIX VII Report on Welsh river survey by Dr. K. Benson-Evans
Plant Sciences Department, University College, Cardiff.

Part of the grant under DoE Contract no. 120021-0037 was used to support continuation of an intensive survey of algae in Welsh river sites with the intention of applying statistical techniques to the accumulated data. Data has been obtained especially on green algae and the relationship between these and diatoms have been studied.

INTRODUCTION

Several methods for the estimation of epilithic algal biomass have been described in the literature. Almost all involve scraping the algal growth from the surface of stones, rocks, and other submerged objects.

In order to overcome the patchy distribution of algal growth, five stones were collected randomly from transects across each river. The algal growth was scraped off the stones and the scrapes were mixed. Permanent and wet mount slides were prepared and the algal biomass was counted and expressed as cells mm⁻².

Silting of submerged substrata in slow-flowing rivers and build-up of undesirable growths, e.g. sewage fungus in organically polluted streams, poses problems. This is due to the fact that silt accumulation and sewage fungus colonisation hinder the colonisation of representative epilithic algal communities. Species not tolerant to silt or pollution rarely colonise such sites (Esho & Benson-Evans 1983).

NATURAL HARD SUBSTRATES

Stones, rocks, cobbles, boulders, etc. are the best natural substrata for studying epilithic algal communities.

The importance of hardness of the substrata in biological colonisation of bottom materials in streams has already been reported by Macan (1974), Whitton (1975) and many others.

The size of the substrata has been reported to play an important role in determining the degree of colonisation (Douglas 1958), the larger, more stationary stones being more readily colonised than the unstable, smaller stones. This is due to the effect of current in scouring algal material as the stones roll on the river bed.

Chemical interaction between the substratum and the colonisers was reported by Parker *et al.* (1973) who found that Monostromata quaternarium prefers iron rich substrata, whereas Hydrurus foetidus in the same stream bed always selected sandstones and limestones rich in silica. Nienhuis (1980), on the other hand, pointed out that the chemical composition of hard substrata is probably unimportant, since the nutrients which the algae need originate from the water. He also stated that it is not clear whether the inorganic components of the substrata play

a part in algal settlement and growth but emphasised that the physical structure of the hard substrata is more important. Antoine & Benson-Evans (1985a) found higher algal cell densities on sandstones rather than on silt stones, the highest being recorded on coarse sandstones and the lowest on red silt stones. This effect was mainly due to the greater roughness of the surface of coarse sandstone. The Chlorophyta contributed about 26.76% of the total biomass colonising coarse sandstones and only about 5.27% of that colonising red silt stones. Coarse sand stones were also the most favourable substrata for the germination of carpospores and advanced mature shoots of the red alga Lemanea Bory (Thirb & Benson-Evans 1983).

AIMS OF THE WORK AT THE PLANT SCIENCE DEPARTMENT, UNIVERSITY COLLEGE, CARDIFF.

1. In order to back up the collections of algal samples made from a wide area of the British Isles by Professor F.E. Round, Botany Department, University of Bristol, it was agreed that work should be concentrated on the South and Mid-Wales area for which 20 years of physico-chemical and algal data were already available.
2. Additional samples were to be collected from some new sites within the area.
3. Community analyses were to be carried out on data from (1) and (2) above, together with correlations of physico-chemical data and the algae. The seasonality of individual species was to be established.
4. Since earlier work had indicated that physical factors such as current velocity and substrate appear to be more important in affecting colonisation and growth of epilithic algae than water chemistry, these factors were to be studied in more detail in order to evaluate them prior to suggesting a method for estimating water quality using algae as indicators. These factors were to be studied both in the field and the laboratory.

METHODS

Harvesting and preservation of benthic algae

The technique used for harvesting the epilithic algae is summarised as follows:

A hollow square box was pressed closely to the surface of the stone and the algae growing on the area around the box were scraped off. The box was moved away and algae were scraped off carefully from the isolated area with a razor blade and transferred into a 30 ml sampling tube on which the 25 ml level was marked. A few drops of Lugol's solution were added and the final volume was made up to 25 ml using de-ionised water (Young 1945, Furet 1979, Hadi 1981, Esho 1983, Antoine 1984).

Preparation of permanent slides

A clean microscope slide was placed on an electric hot plate adjusted at 75-80°C. The concentrated algal sample was

thoroughly shaken to ensure even distribution of the algal cells. Using a micropipette, 0.05 ml of the sample was transferred to the centre of the slide. When the drop had dried, a drop of concentrated nitric acid was placed on it and left to dry. A small amount of Naphrax was placed on a clean coverslip and the coverslip was then inverted onto the dried drop. Two slides were made for each sample, and each slide was labelled.

Wet mount preparation (fresh samples)

The sample was thoroughly mixed, and a small drop (0.05 ml) was placed on a clean microscope slide, covered with a coverslip and left for a few minutes to settle before examination. This method enabled an estimate of the proportion of "living" and "dead" algal cells. In local rivers the proportion of the latter is extremely low. In such fresh preparations there is usually a substantial under-estimation of very small cells.

Counting algal cells

The microtransect method for counting diatoms in the permanent preparation was used, and the marker or reference diatom method was used for counting algal cells other than diatoms in wet mount preparations, as suggested by Furet (1979). The number of diatom frustules were counted across a complete microtransect (across the measured diameter of the drop). Two microtransects were enough.

A wet mount slide was made from the same sample and cells of all algae other than the diatoms together with the reference (marker) diatom were counted along microtransects using a 10X and 40X objective. The relevant equation was then applied throughout in order to directly relate the two counts (see Appendix A).

According to Carroll (1981) who carried out an intensive study of the distribution of diatom valves under the coverslip, there is a similar distribution of valves on coverslips taken from different sites and from the same site at different times of year. He also found that the area within 6 mm of the edge of each coverslip showed the most variation and should be ignored but random traverses in the central 10 mm area were reliable. For greater accuracy he calculated a correction factor that can be applied.

Artificial substrates

According to Tuchman & Blinn (1979) and Tuchman & Stevenson (1980), artificial substrata can be used for comparing their algal communities with those colonising the natural substrata.

Perspex slides were used by Hadi (1981) and Antoine & Benson-Evans (1985). The slides were screwed onto building bricks and the bricks were anchored to the river bed at many sites. Colonised slides were removed every month and replaced by clean ones during the study period.

The algal growth on the upper surface of the slide was scraped off. Scraps for counting were suspended in 24 ml of dilute Lugol's iodine solution. The diatoms were cleaned with

hot nitric acid and counted by the microtransect method of Edmondson (in Vollenweider, R.A. 1971) and the other algae by the diatom tracer method as described by Furet (1979).

Current

Current is one of the important controlling factors for the growth of benthic algae in running water systems. It is the current velocity which controls spatial distribution of the different algal species within streams and rivers. Blum (1957) and Whitford (1960) have shown that the algal flora inhabiting the rapids of streams differ from those growing in the pools of the same streams.

Recently, Antoine & Benson-Evans (1982) and Thirb & Benson-Evans (1983), studied the effect of current velocity on the growth of the benthic algal populations and of Lemanea, respectively, under controlled laboratory conditions. In these two investigations, algal populations and Lemanea were seeded on rock blocks, in the Rivers Ithon and Usk, respectively, before the blocks were subjected to different current velocities in laboratory channels. The methods for preparing the blocks and for the construction of the channels are fully described below and illustrated by these authors. Antoine & Benson-Evans (1982) showed an inverse relationship between the chlorophyll-a content of epilithic algae and the current velocity. This was attributed to the fact that loose-lying algae, such as filamentous and coccoid green algae, become dislodged from the blocks maintained in the higher current velocities.

This observation was augmented by the fact that the growth of almost all species of the Chlorophyta and of Oscillatoria tenuis Ag. (Cyanobacteria) was inversely related to the current velocities (Antoine & Benson-Evans 1982). A similar observation has also been reported by McIntire (1966). However, the species of the Bacillariophyta did not show any uniform reaction to the current velocities, but rather a pattern of ten different responses to the current velocity was noted (Antoine & Benson-Evans 1982). Such different patterns of distribution were related to the different sizes and powers of adhesion of the various diatom species.

The growth of Lemanea, on the other hand, was directly related to the current velocity in the laboratory channels, where the highest biomass was recorded at the highest current velocity. This agrees with the field observations where this red alga grows in shaded, fast-flowing water and the highest biomass is always recorded throughout late autumn and winter; growths of Lemanea almost disappear during the summer months.

Natural and artificial substrata

Stones from many rivers were collected and cut into blocks of 25 x 100 x 25 mm using a diamond saw. The blocks were cleaned with distilled water and sterilised at 200°C for 2 hours. The blocks were then glued to a wooden trough using Araldite waterproof adhesive. The troughs with the blocks were fixed onto the river bed by means of iron stakes (Antoine & Benson-Evans 1982, 1984).

On each occasion the blocks were left in the river for five weeks to allow for their colonisation by the natural algal population. After that period the blocks were transferred immediately to the laboratory.

Modified Bristol's medium (BBMP) was prepared as described by Cox & Bold (1966). Forty litres of the BBMP solution were prepared, the pH was adjusted to that of the river water and mixed with another forty litres of the river water. This mixture was then autoclaved, cooled and placed in a reservoir from which it was pumped into the laboratory channels. This mixture was replaced by a fresh preparation every week.

The apparatus used (designed by Thirb & Benson-Evans 1982) consisted of three plastic channels with flat bottoms, 7.5 cm wide and 5.5 cm deep. The flow of water into each channel was adjusted by using screws to loosen or tighten the feeding tubes. This regulated the amount of water pumped into the channels in order to obtain a constant velocity in each. Each channel received a continuous flow throughout the investigation period. The medium passing through these channels was collected in a horizontal drain and then returned to the reservoir.

The channels were kept in a thermally controlled growth room at a temperature similar to that of the river water on the day of collection of the blocks. Light was supplied from six fluorescent tubes at an intensity of 7.35 W m⁻². The photoperiod was 16 hours of light and 8 hours dark. Five blocks were put into each channel for four weeks, after which the algal growth was scraped off the block for counting of all algal cells.

Analysis of data

The water quality and algal data collected from Welsh rivers by the Cardiff workers over the past twenty years were statistically analysed using the MINITAB statistical package. Correlations between the algae and pH, DO, BOD and SS have also been analysed.

The analyses of data from the rock blocks used in the laboratory channels and for the study of stone sizes were carried out using a Principal Component Analysis SPSS X package.

A LIST OF RIVERS IN MID AND SOUTH WALES

1. River Wye
2. River Usk
3. River Ely
4. River Taff
5. River Ogmore
6. River Kenfig
7. River Llynfi
8. River Ewenny
9. River Alun
10. River Thaw
11. River Ystwyth
12. River Rhymney
13. River Rheidol
14. River Nant Fawr
15. River Ebbw Fawr
16. River Severn
17. River Ebbw Fach
18. River Ithon
19. River Elan
20. River Sirhowy
21. River Iraw
22. River Dwyfor
23. River Rassau
24. River Afon Lwyd
25. River Gymore
26. River Carn

FUTURE WORK

- a. Further studies on the effect of stone size and of rock types, other than those already used experimentally, on the presence/absence and growth of benthic algae in both natural and controlled laboratory streams.
- b. Further studies on the effects of different current velocities in relation to the new rock substrata and stone size (see above).
- c. Further studies on the algal flora of artificial and natural substrates.
- d. Computer analyses of 25 years data from rivers of S.E. Wales to establish the seasonality of algal species, and further relationship with physico-chemical data from numerous sites on polluted and unpolluted streams and rivers.

REFERENCES

- Antoine, S.E. and K. Benson-Evans. 1982. The effect of current velocity on the rate of growth of benthic algal communities. *Int. Rev. ges. Hydrobiol.* 67: 575-583.
- Antoine, S.E. and K. Benson-Evans. 1985a. Colonisation rates of benthic algae on four different rock substrata in the River Ithon, Mid Wales, U.K. *Limnologica* 16: 307-313,
- Antoine, S.E. and K. Benson-Evans. 1985b. Phycoperiphyton development on artificial substrata in the River Wye System, Wales, U.K. Part 1: Biomass variation. *Acta Hydrochim. Hydrobiol.* 13: 277-296.
- Antoine, S.E. and K. Benson-Evans. 1986. Part 2: population dynamics. *Acta Hydrochim. Hydrobiol.* 14: 347-363.
- Carroll, D.M. 1981. The Effect of Coal Industry Wastes on the Diatoms of a Number of Small Rivers in S.E. Wales. Ph.D. Thesis. University of Wales.
- Furet, J.E. 1979. Algal Studies of the River Wye System. Ph.D. Thesis, University College, Cardiff, University of Wales.
- Hadi, R.A.M. 1981. Algal Studies of the River Usk. Ph.D. Thesis, Department of Plant Sciences, University College, Cardiff, University of Wales.
- Thirb, H.H. and K. Benson-Evans, 1982. The effect of different current velocities on the red alga Lemanea in a laboratory stream. *Arch. Hydrobiol.* 96: 65-72.
- Vollenweider, R.A. 1971. A Manual on Methods for Measuring Primary Production in Aquatic Environments. 2nd Print. IBP Handbook No. 12. Blackwell Scientific Publications, Oxford and Edinburgh.
- Young, O.W. 1945. Limnological investigation of periphyton in Douglas Lake, Michigan. *Trans. Amer. microsc. Soc.* 65: 1-20.

RESULTS

Correlations between algal species for 20 years of data

Some diatoms and green algae show a highly significant correlation with each other to form distinct community associations as can be seen from the data given in Table 1.

Correlations between algae and physico-chemical factors

To date, correlations between the algae recorded and counted over the past 20 years in the South and Mid Wales area have been analysed to look for any correlations with pH, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Suspended Solids (SS) as these are the most commonly used criteria of water quality. Table 2 indicates those species that were found to be either significantly, very significantly or highly significantly either positively or negatively correlated with these physico-chemical factors. Further work needs to be carried out with nitrogen and phosphate data, silica, heavy metals, chlorides, etc.

Effects of current velocity

An analysis of the effect of current velocity on the 36 most common algal species in the R. Wye system, using a SPSS X package (Principal Component Analysis) has shown that the bulk of these species were negatively correlated with current velocity. Only Navicula veneta, Fragilaria vaucheriae, Cymbella ventricosa and Cyclotella meneghiniana showed some positive correlation, and these not very significantly. See Fig.1 and Table 3.

A further study of the effect of current velocity on algae colonised in the Nant Carn and kept in the laboratory re-circulating channels at different velocities was carried out over a period of nine weeks. Blocks of Pennant sandstone were used throughout and lower velocities used than in the previous work by Antoine and Benson-Evans (1982). The velocity of current was the most important factor influencing the growth of the seeded algal species with poor growth at the fastest and slowest velocities (0.62 and 0.0022 m s⁻¹) and the best growth at the medium to low velocities of 0.22 and 0.18 m s⁻¹ respectively. The samples were dominated by Achnanthes linearis. Diatoma hiemale var. mesodon, Cocconeis placentula and the coccoid green algae reached a maximum in the first week followed by the flagellate green algae in the second week. Other species increased over the nine week period. Meridion circulare appeared to be indifferent to the current velocity.

From this and previous work with variations in current velocity and substrate it seems that these must be taken into account before any indicators of water chemistry can be utilised.

Effect of stone size

Blocks of sandstone were cut to give 18 pieces each of three different sizes: small, medium and large. Three of each size were submerged as previously (Antoine and Benson-Evans 1982) at

each of 6 sites on the River Wye system. One block was left at each site for the month of May, a second for the months of May and June and the third for the three months of May, June and July. To date, chlorophyll extractions have been carried out using the 'box' sampling technique described in the methods (2.9 cm side). The results are shown in Figs 2, 3 and 4. Cell counts have still to be done for comparison, and to indicate seasonality.

TABLE 1 RESULTS OF CORRELATIONS BETWEEN ALGAL SPECIES FOR 20 YEARS OF DATA

SPECIES	SIGNIFICANTLY CORRELATED	VERY SIGNIFICANTLY CORRELATED	HIGHLY SIGNIFICANTLY CORRELATED
	$p < 0.01$ $r = 0.525$	$p < 0.005$ $r = 0.563$	$p < 0.001$ $r = 0.639$
<i>Chnanthes anceolata</i>			<i>Cymbella sinuata</i> <i>Gomphonema olivaceum</i> <i>G. parvulum</i> <i>Navicula cryptocephala</i> <i>Nitzschia palea</i> <i>Surirella ovata</i> <i>Synedra ulna</i> <i>Chlamydomonas spp.</i>
<i>Chnanthes nearis</i>		<i>Fragilaria vaucheriae</i>	
<i>eratoneis</i>		<i>Fragilaria virescens</i>	
<i>rcus</i>			
<i>occconeis lacentula</i>		<i>Diatoma vulgare</i> <i>Gomphonema angustatum</i> <i>Navicula avenacea</i> <i>Navicula venata</i>	<i>Cymbella ventricosa</i> <i>Melosira varians</i> <i>Scenedesmus quadricauda</i>
<i>occconeis lacentula var euglypta</i>		<i>Cyclotella meneghiniana</i>	<i>Ankistrodesmus falcatus</i> <i>Scenedesmus dimorphos</i>
<i>ymbolle sinuata</i>		<i>Synedra ulna</i>	<i>Gomphonema olivaceum</i> <i>G. parvulum</i> <i>Navicula cryptocephala</i> <i>Surirella ovata</i> <i>Chlamydomonas spp.</i>
<i>ymbolle ventricosa</i>		<i>Navicula avenacea</i> <i>Synedra ulna</i> <i>Navicula veneta</i>	<i>Diatoma vulgare</i> <i>Gomphonema angustatum</i> <i>Melosira varians</i> <i>Scenedesmus quadricauda</i>
<i>Diatoma hiemale</i>		<i>Cyclotella meneghiniana</i>	
<i>Diatoma vulgare</i>	<i>Synedra ulna</i>	<i>Meridion circulare</i> <i>Melosira varians</i>	<i>Tabellaria flocculosa</i>
<i>Eunotia exigua</i>			
<i>Fragilaria vaucheriae</i>		<i>Fragilaria virescens</i> <i>Ankistrodesmus falcatus</i> <i>Scenedesmus dimorpha</i>	
<i>Gomphonema angustatum</i>			<i>Navicula veneta</i> <i>Melosira varians</i> <i>Scenedesmus quadricauda</i>

CORRELATIONS BETWEEN ALGAL SPECIES (CONTINUED)

SPECIES	SIGNIFICANTLY	VERY SIGNIFICANTLY	HIGHLY SIGNIFICANTLY
	$p < 0.01$	$p < 0.005$	$p < 0.001$
	$r = 0.525$	$r = 0.563$	$r = 0.639$
Gomphonema livaceum		Navicula veneta Chlamydomonas spp.	Gomphonema parvulum Meridion circulare Navicula avenacea N. cryptocephala Nitzschia palea Surirella ovata Synedra ulna
Gomphonema arvulum		Navicula veneta	Navicula cryptocephala Nitzschia palea Surirella ovata Synedra ulna Chlamydomonas spp.
Meridion circulare		Navicula avenacea N. cryptocephala Synedra ulna	Navicula cryptocephala N. veneta Synedra ulna
Navicula avenacea			
Navicula cryptocephala		Chlamydomonas spp.	Navicula veneta Nitzschia palea Surirella ovata Synedra ulna
Navicula veneta		Melosira varians	Synedra ulna Scenedesmus quadricauda
Nitzschia palea			Surirella ovata Synedra ulna Chlamydomonas spp
Surirella ovata		Synedra ulna	Chlamydomonas spp
Cyclotella meneghiniana			Scenedesmus dimorphos
Melosira varians		Scenedesmus quadricauda	
Ankistrodesmus falcatus			Scenedesmus dimorphos

2 RESULTS OF CORRELATIONS USING THE MINITAB STATISTICAL PACKAGE

FACTOR	SIGNIFICANTLY CORRELATED	VERY SIGNIFICANTLY CORRELATED	HIGHLY SIGNIFICANTLY CORRELATED
	$p < 0.01$ $r = 0.525$	$p < 0.005$ $r = 0.563$	$p < 0.001$ $r = 0.639$
	KEY + = positively correlated, - = negatively correlated		
pH	Amphora ovalis + Eunotia exigua - (acidic)		Tabellaria flocculosa - (acidobiontic)
DO		Diatoma hiemale var mesodon +	Cocconeis placentula var euglypta + Cyclotella meneghiniana + Ankistrodesmus falcatus + Scenedesmus dimorphus +
BOD	Cymbella ventricosa -	Achnanthes lanceolata + Gomphonema parvulum + Nitzschia palea + Chlamydomonas spp. +	
SS			Cocconeis placentula var euglypta + Cyclotella meneghiniana +

KEY pH = Hydrogen ion concentration
 DO = Dissolved Oxygen as mg l^{-1}
 BOD = Biochemical Oxygen Demand, as mg l^{-1}
 SS = Suspended solids as mg l^{-1}

SYMBOL	VARIABLE	COORDINATES	SYMBOL	VARIABLE	COORDINATES	SYMBOL	VARIABLE	COORDINATES
1	CUR	(- .82297, - .56809)	2	CHL	(- .95654, - .29161)	3	GR	(- .99944, - .03359)
4	DIA	(- .99719, - .07490)	5	BIOM	(- .99999, - .00365)	6	AFAL	(- .98531, - .17080)
7	AGRA	(- .98303, - .18343)	8	CANG	(- .99442, - .10552)	9	CGLO	(- .99917, - .04080)
10	CGLOM	(- .99882, - .04862)	11	CLAN	(- .97839, - .20677)	12	HFLAC	(- .81036, - .58594)
13	PVUL	(- .97349, - .22872)	14	PRVIR	(- 1.00000, - .00249)	15	SBIJ	(- .96068, - .27755)
16	USUB	(- .98964, - .14359)	17	OTEN	(- .99993, - .01188)	18	CLEN	(- .41704, - .90889)
19	MVAR	(- .39852, - .91716)	20	ALAN	(- .05304, - .99859)	21	ALIN	(- .93496, - .35476)
22	APER	(- .85865, - .51256)	23	CPLAEUG	(- .80702, - .59053)	24	CPLALIN	(- .98555, - .16936)
25	CSIN	(- .96940, - .24548)	26	CVEN	(- .32237, - .94661)	27	DHIE	(- .99611, - .08807)
28	DVUL	(- .99564, - .09328)	29	FVAU	(- .15574, - .98780)	30	GPAR	(- .04144, - .99914)
31	NAVE	(- .98607, - .16636)	32	NVEN	(- .46475, - .88544)	33	NAMPH	(- .02527, - .99968)
34	NPAL	(- .87046, - .49224)	35	PVIR	(- .23455, - .97210)	36	RCUR	(- .95914, - .28292)
37	SOVA	(- .85865, - .51256)	38	SPUL	(- .95914, - .28292)			

FACTOR SCORE COEFFICIENT MATRIX:

	FACTOR 1	FACTOR 2	
CUR	- .02970	- .05162	
CHL	- .03491	.02593	
GR	- .03683	- .00416	
DIA	- .03663	- .00587	
BIOM	- .03681	- .00072	
AFAL	- .03645	- .01682	
AGRA	- .03638	- .01798	
CANG	- .03672	- .01080	
CGLO	- .03683	- .00482	
CGLOM	- .03682	- .00555	
CLAN	- .03624	- .02014	
HFLAC	- .02921	- .05328	
PVUL	- .03608	- .02216	
PRVIR	- .03682	- .00129	
SBIJ	- .03507	- .02464	
USUB	- .03658	- .01431	
OTEN	- .03682	- .00215	
CLEN	- .01439	- .08353	
MVAR	- .01564	- .08516	
ALAN	- .00301	- .09232	
ALIN	- .03404	- .03179	
APER	- .03107	- .04645	
CPLAEUG	- .03033	- .05541	
CPLALIN	- .03646	- .01669	
CSIN	- .03595	- .02370	
CVEN	- .01287	- .08780	
DHIE	- .03658	- .00709	
DVUL	- .03655	- .00757	
FVAU	- .00678	- .09143	
GPAR	- .00258	- .09236	

Table 3

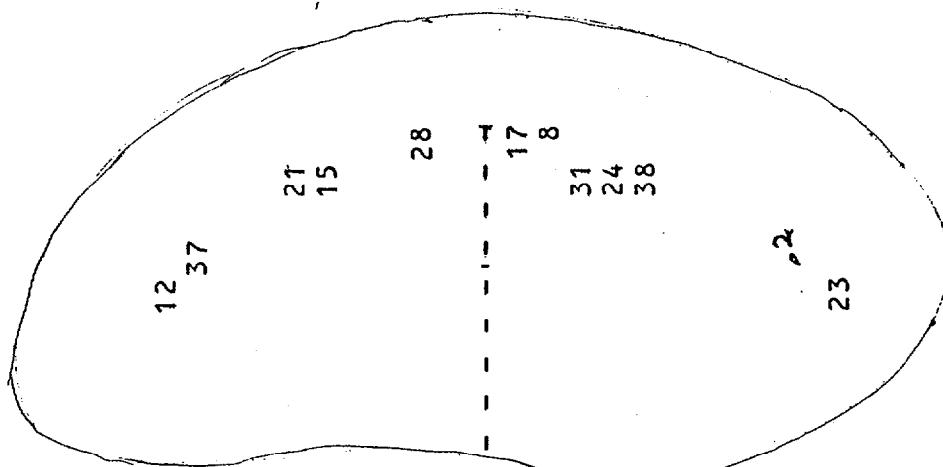
.99982
.01897
-.01897

HORIZONTAL FACTOR 1 VERTICAL FACTOR 2

35

3033

19



1

Fig. 1

3218

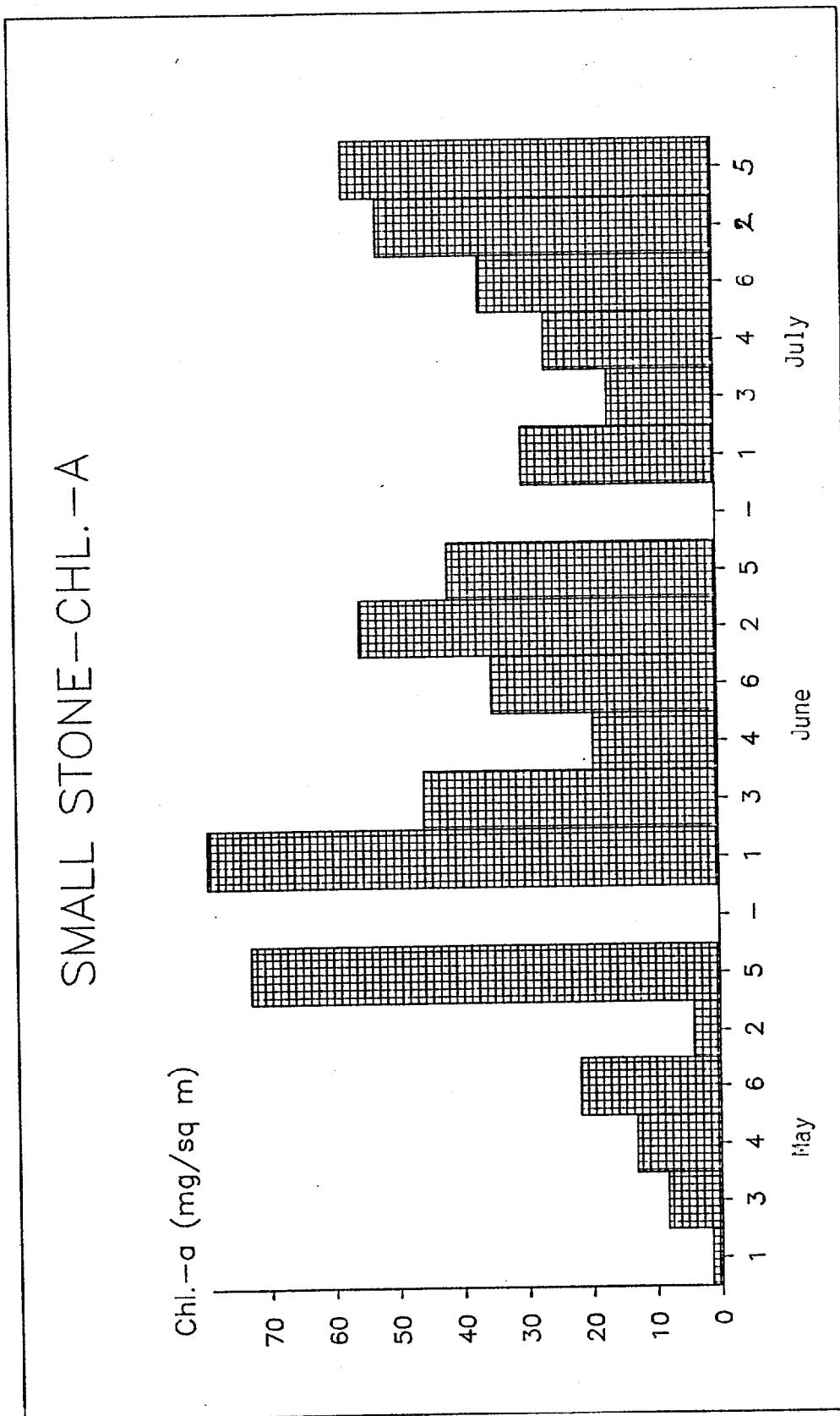


Fig. 2
1-6 = Sites on the River Wye

MEDIUM STONES-CHL.-A

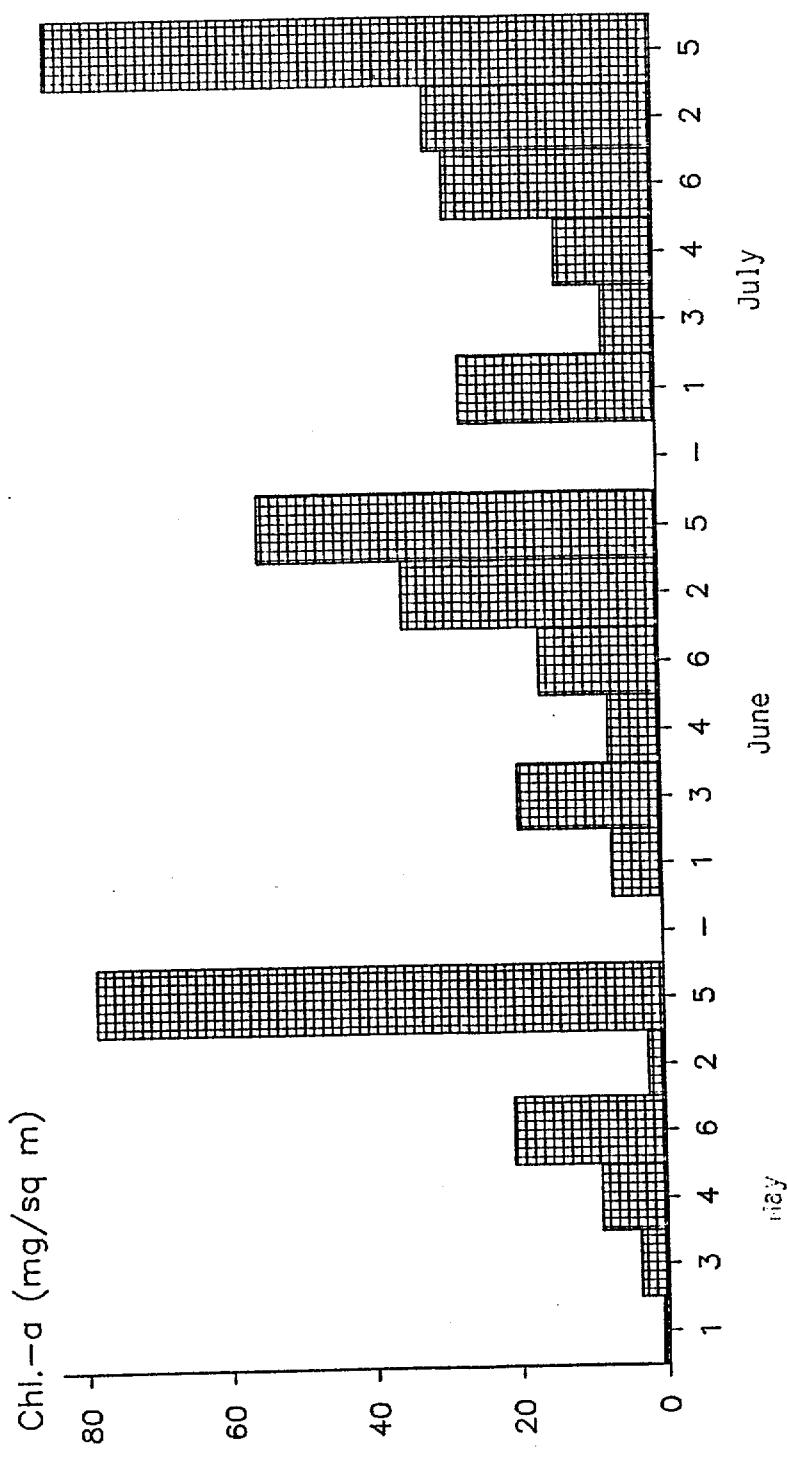


Fig. 3
1-6 = Sites on the River Wye

LARGE STONES-CHL.-A

Chl.-a (mg/sq m)

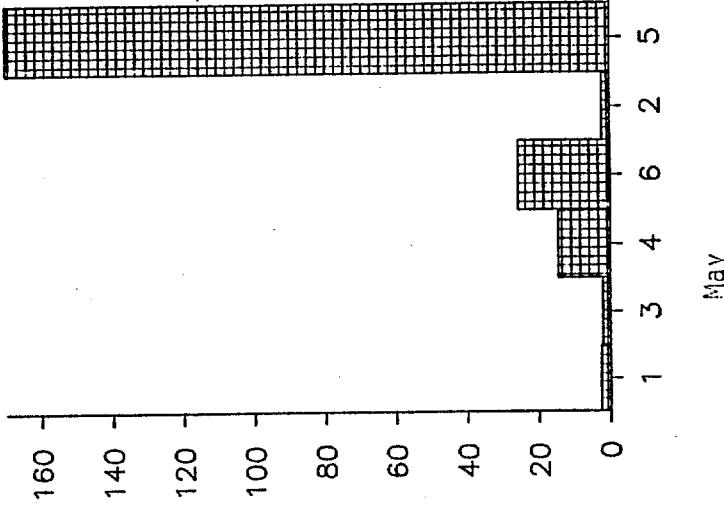


Fig. 4
1-6 = Sites on the River Wye

Table 4. List of species of green algae in Welsh rivers

CHLOROPHYTA

CHLOROCYCALES + TETRASPORALES

Sterococcus limneticus

. superbus

arteria cordiformis

. klebsii

. obutsa

haetopeltis orbicularis

Chlamydomonas reinhardtii

. angulosa

. globosa

. mucicola

. polypyrenoideum

. snowii

. sphagnicola

Chlorogonium acutiformis

. elongatum

. fusiforme

. euchlorum

Disporea crucigenioides

Eudorina elegans

Gloeocystis ampla

G. gigas

G. major

G. plantonica

G. vesiculosa

Gloeoomonas kupfferi

Gonium pectorale

G. sociale

Haematococcus lacustris

Palmella mucosa

Pandorina morum

Polytomella agilis

Schnizochlamys compacta

S. gelatinosa

Sphaerocystis schroeteri

Spirogonium chlorogonicides

Spondylomorum quaternarium

Tetraspora cylindrica

T. gelatinosa

T. lacustris

T. lamellosa

Tetrasporidium javanicum

Uva incurva

Volvox

Chlorococcales

Achanthosphaera zachariasii

Actinastrum hantzschii

A. hantzschii var. fluviatile

A. hookerii

Ankistrodesmus acicularis

A. acicularis var. mirabilis

A. bibraianus

A. braunii

A. chodati

A. convolutus

A. falcatus

A. fractus

A. gracilis

A. spiralis

A. subcapitatus

Botryococcus braunii

B. protuberans

B. sudeticus

Characium acuminatum

C. ambiguum

C. debaryanum

C. hookerii

C. limneticum

C. marinum

C. obtusum

C. ornithocephalum

C. pringsheimii

C. rostratum

C. sieboldii

Chlorella ellipsoidea

C. miriata

C. pyrenodosa

C. vulgaris

Chlorococcum humicolum

C. infusionum

Chodatella

Closteriopsis longissima

Coelastrum cambricum

C. microporum

C. microscopicum

C. reticulatum

C. scabrum

C. sphaericum

Crucigenia irregularis

C. quadrata

C. rectangularis

C. tetrapedia

Dactylococcus infusionum

Dictyosphaerium ehrenbergianum

D. pulchellum

Dimorphococcus lunatus

Elakothrix viridis

Eremosphaera viridis

Excentrosphaera golenkinia

E. viridis

Franceia droescheri

Gloeotaenium loitelsbergerianum

Hydrodictyon reticulatum

Kentrosphaera gloeophila

Keriochlamys styriaca

Kirchneriella contorta

K. elongata

K. lunaris

K. ooesa

subsolitaria	<i>Q. lacustris</i>	<i>T. lunula</i>
perheima ciliata	<i>Radiococcus sp.</i>	<i>T. minimum</i>
practinium pusillum	<i>Scenedesmus abundans</i>	<i>T. muticum</i>
radiatum	<i>S. acuminatus</i>	<i>T. quadratum</i>
oraphidium contortum	<i>S. arcuatus</i>	<i>T. regulare</i>
convolutum	<i>S. armatus</i>	<i>T. tumidulum</i>
minutum	<i>S. bernardii</i>	<i>T. trigonum</i>
tortile	<i>S. bijugatus</i>	<i>Treubaria setigera</i>
nochloris sp.	<i>S. brasiliensis</i>	<i>Westella botryoides</i>
chrocytium agardhianum	<i>S. crassus</i>	
limneticum	<i>S. denticulatus</i>	<u>CHAETOPHORALES, ULOTHRICHALES AND ULVALES</u>
lunatum	<i>S. dimorphus</i>	<i>Aphanochaete repens</i>
chrocytium obesum	<i>S. helveticus</i>	<i>Glidingia marginata</i>
cystis borgei	<i>S. incrassatulus</i>	<i>S. minima</i>
crassa	<i>S. longus</i>	<i>Chaetonema irregulare</i>
elliptica	<i>S. microspina</i>	<i>Chaetopnora incrassata</i>
eremosphaeria	<i>S. obliquus</i>	<i>Chodatia tetrallantoidae</i>
gigas	<i>S. opoliensis</i>	<i>Coleochaete orbicularis</i>
gloecystiformis	<i>S. perforatus</i>	<i>C. scutata</i>
lacustris	<i>S. quadricauda</i>	<i>C. soluta</i>
natans	<i>S. rostrata</i>	<i>Cylindrocapsa conferta</i>
nodulosa	<i>S. setiferus</i>	<i>C. geminella</i>
parva	<i>S. spinosus</i>	<i>Draparnaldia glomerata</i>
pusilla	<i>S. tenuispina</i>	<i>Elakatothrix viridis</i>
rupestris	<i>S. tetradesmiformis</i>	<i>Enteromorpha clathrata</i>
solitaria	<i>Schroederia judayii</i>	<i>E. compressa</i>
modictyon viride	<i>S. setigera</i>	<i>E. intestinalis</i>
diastrum biradiatum	<i>Selenastrum bibraianum</i>	<i>E. lingolata</i>
boryanum	<i>S. capricornutum</i>	<i>E. linza</i>
duplex	<i>S. gracile</i>	<i>E. paradoxa</i> var. <i>tenuissima</i>
duplex var. clathratum	<i>S. minutum</i>	<i>E. prolifera</i>
duplex var. typicum	<i>S. westii</i>	<i>E. ralfsii</i>
obtusum	<i>Sphaerocystis schroeteri</i>	<i>E. ramulosa</i>
simplex	<i>Tetraedesmus smithii</i>	<i>E. torta</i>
tetras	<i>Tetraedron caudatum</i>	<i>Entocladia polymorpha</i>
anktosphaeria gelatinosa	<i>T. duospinum</i>	<i>Epiladlia flustrae</i>
adrigula closterioides	<i>T. hastatum</i>	<i>Geminella minor</i>

Gomontia polyrhiza
Heterotrichopsis viridis
Hormidiopsis ellipsoideum
Hormidium flaccidum
H. fluitans
H. nitens
H. rivulare
H. subtile
Microspora amoena
M. elegans
M. floccosa
M. loefgrenii
M. pachyderma
M. quadrata
M. stagnorum
M. tumidula
M. willeana
Monostroma fuscum
M. grevillei
Percursaria percursa
Phaeophila wittrockii
Pleurococcus vulgaris
Prasiola crispa
P. stipitata
Protococcus viridis
Protoderma viride
Pseudodclonium submarinum
Schizomeris leibleinii
Spongomorpha aeruginosa
S. bombycinæ
Stichococcus bacillaris
S. subtilis
Stigeocladium loricatum
S. nanum
S. pachyderma
S. tenue
Trentepohlia aurea

Ulothrix aequalis
U. cylindricum
U. moniliformis
U. pseudoflaccia
U. speciosa
U. subtilissima
U. tenerrima
U. tenuissima
U. variabilis
U. zonata
Ulva lactuca
Uronema elongatum
Urospora brangioides
U. penicilliformis

OEDOGONIALES

Bulbochaete mirabilis
B. nana
B. regalis
Oedogonium braunii
O. capillare
O. capilliforme
O. curtum
O. fragile
O. microgonium
O. oviforme
O. striatum

SIPHONOCLADALES

Cladophora albida
C. fracta
C. fracta var. lacustris
C. glomerata
C. hutchinsiae
C. pellucida
C. rupestris
C. sericea

Pithophora oedogonia
Rhizoclonium fontanum
R. hieroglyphicum
R. riparium

CODIALES

Bryopsis hypnoides
3. plumosa
Codium tomentosum

CONJUGATOPHYTA

Arthrodeshmus incus
Closterium acerosum
C. ehrenbergii
C. lanceolatum
C. leibleinii
C. lunula
C. moniliferum
C. parvulum
C. setaceum
C. turgidum
C. venus
Cosmarium botrytis
C. cucurbita
C. granatum
C. laeve
C. meneghinii
C. reniforme
C. subtumidum
C. tetraophthalmum
Cylindrocystis brebissonii
Euastrum denticulatum
E. oblongum
Micrasterias truncata
Mougeotia abnormis
M. calcarea
M. capucina

M. cyanea
M. genuflexa
M. laetevirens
M. laevis
M. pulchella
M. robusta
M. scalaris
M. varians
M. viridis
Netrium oblongum
Penium cylindrus
Spirogyra affinis
S. borgeana
S. borythenica
S. circumlineata
S. communis
S. crassa
S. daedoleoides
S. fluviatilis
S. gracilis
S. grevilleana
S. inflata
S. mirabilis
S. nitida
S. reflexa
S. scrobiculata
S. singularis
S. suecica
S. varians
S. weberi
Staurastrum anatinum
S. hexacerum
S. punctatum
Zygnea conspicuum
Z. decussatum
Z. insigne
Z. leiospermum