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The Use of Chironomid Pupal Exuviae for Biological Surveillance of Water Quality

TECHNICAL MEMORANDUM No·18

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THE USE OF CHIRONOMID PUPAL EXUVIAE FOR BIOLOGICAL SURVEILLANCE OF WATER QUALITY

by

R. S. WILSON AND J. D. McGILL

Department of Zoology, University of Bristol

The report introduces a new method of biological water quality surveillance applicable throughout the whole length of a river, both in shallow and in deep water sections.

DEPARTMENT OF THE ENVIRONMENT
Water Data Unit
Reading Bridge House
Reading
RG1 8PS
United Kingdom

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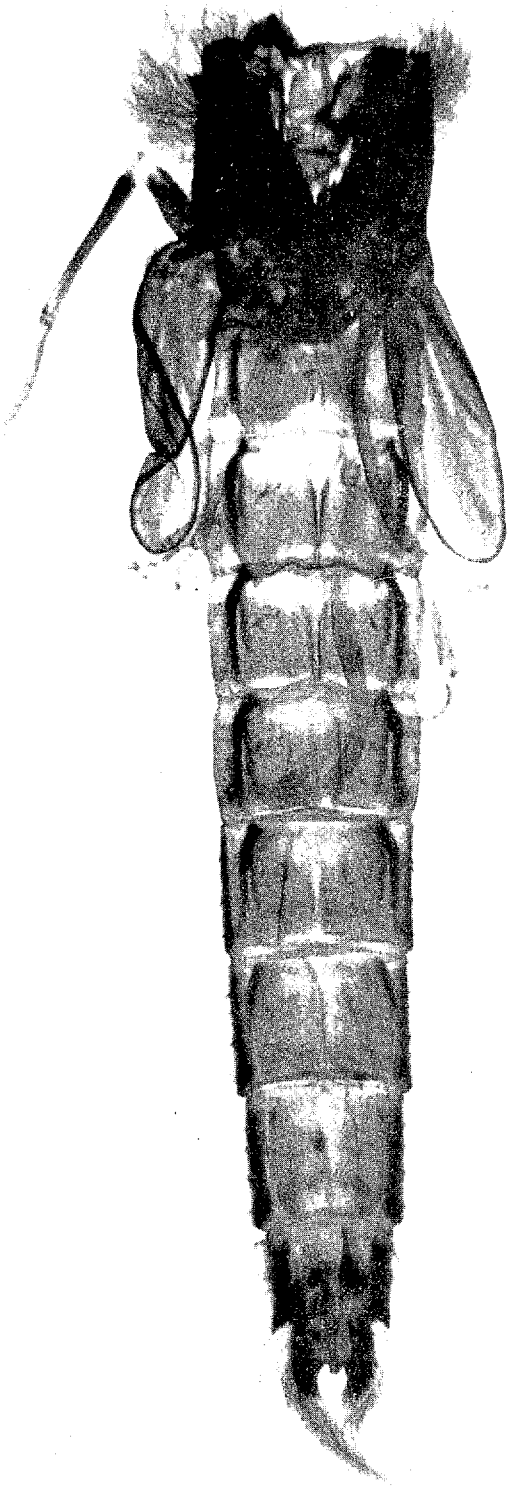


Plate 1 Chironomus riparius *(L.) pupal exuviae*

1 INTRODUCTION

It is generally accepted that changes in water quality may be detected through long-term alterations in the composition of a river's benthic macroinvertebrate community[1, 2]. The usefulness of such benthos surveillance depends on the frequency and method of collection and on the sensitivity of the analytical procedures employed[3, 4].

Various methods may be used in sampling, but the most common is that of "kick-netting" on shallow riffle areas. This practice is restricted to water courses with suitable shallow reaches, and is therefore limited in application. Nevertheless, it currently forms the basis of most biological assessment procedures. There remains the major problem of sampling deep, muddy, lowland sections of rivers. No currently recognised sampling methods are capable of adequately handling these areas: grabbing is inefficient and difficult, while artificial substrates are liable to be swept away and at best give an artificial indication of the natural fauna. Furthermore, insufficient attention has been paid in the past to the natural faunal variations in a river, such as occur through normal changes in substrate and temperature and flow regime. It is inappropriate to apply the same criteria to all sections, and there is a great need for some method of biological assessment which can give comparable data throughout the length of a river from source to mouth.

This Technical Memorandum is based on work sponsored by the Water Data Unit (Department of the Environment). It gives an outline of a new, widely applicable surveillance method, together with practical examples of its use, which should prove of interest to the water authority biologist. The method outlined in this memorandum can be used to examine all streams and rivers as well as canals and lakes. It is easily carried out from the bank along shallow or deep reaches, although a boat may be used to advantage in deep water where the banks are steep or treacherous. It also provides data directly comparable between sites along the whole length of a river. The method concentrates on a single group of aquatic organisms – the Chironomidae (Diptera, Insecta) – or more strictly, on the distribution of their pupal skins or exuviae.

2 THE CHIRONOMIDAE

This important aquatic family has over 450 species in Great Britain[5], and is particularly well suited for use in biological assessment as it contains a large number of different species each with its own environmental requirements. The larval stages are found in almost every conceivable fresh and brackish water habitat, and many individuals and species occur in the same locality. The winged adults permit a wide distribution of each species, while movement of the relatively sedentary larvae is governed by the local environment throughout their development.

2.1 Life history

The general life history of the chironomids has recently been reviewed[6] and only a brief account of the life cycle is given here:–

The egg. The gelatinous egg masses are laid in water, either at the water surface, or attached to aquatic vegetation, or other firm substrata. The eggs usually hatch within a few days.

The larva. On hatching, first instar larvae are released from the egg but remain within the egg mass until it disintegrates. After a migratory phase, the larvae establish themselves in a suitable habitat. A wide variety of life-styles may be assumed and larvae of different species are distributed in the environment in relation to the type and proportions of aquatic microhabitats available. They may be algal feeders, detritus feeders, filter-feeders, or leaf-miners; many of them are tube-dwellers; some common forms are carnivores. All chironomid species pass through four larval instars before pupating.

The pupa. At the end of the larval period the larvae transform into pupae. The pupal stage lasts approximately 3 to 4 days during which the pupa normally remains inside the larval tube. When the adult insect is ready to emerge, the pupa rises to the surface of the water to "hatch" or eclose. The emergent adult midge flies away leaving the pupal exuviae floating on the surface. These exuviae drift with the current and collect along the river bank in eddies or on obstructions such as weed beds in the river. Such aggregations of pupal exuviae therefore form an "integrated" sample of all the chironomids emerging at the time.

The adult. Adult male chironomids form swarms which are readily seen on summer evenings dancing up and down in the still evening air. These swarms often comprise very large numbers of midges, and have been known to cause a nuisance to humans although they do not bite. The swarm acts as a species-specific attraction to females which approach it and are mated. The females then return to the water surface to lay their eggs and complete the life cycle.

The life cycle of the Chironomidae is summarised in Figure 1.

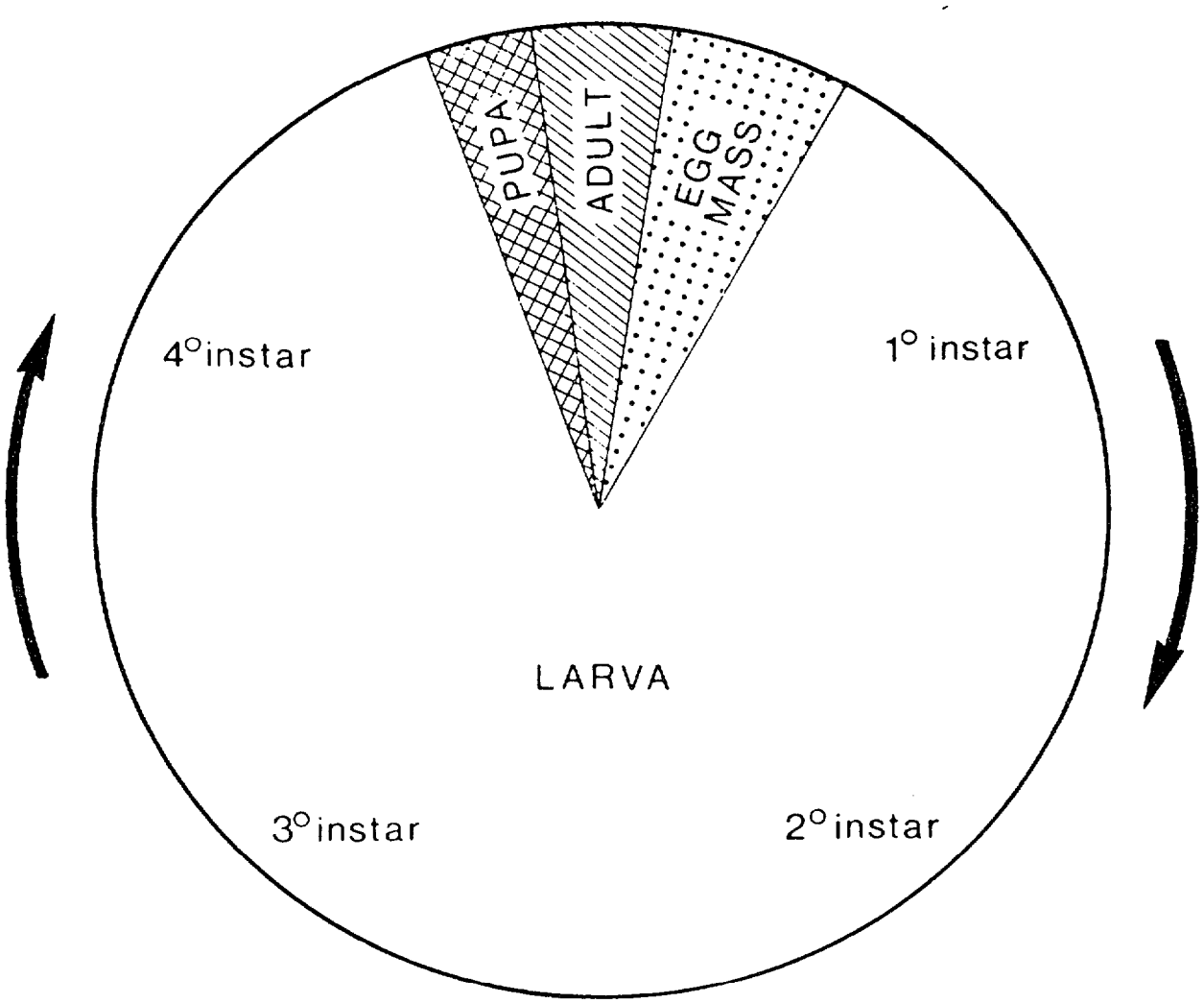


Figure 1 Life-cycle of the Chironomidae showing the approximate relative duration of each stage

2.2 The pupal exuviae

All aquatic chironomids undergo eclosion at the water surface leaving the pupal exuviae floating for about 24 to 48 hours. In rivers these exuviae may travel downstream for distances which vary depending on the flow rate, the degree of turbulence, and the state of the weed growth. Moved by wind and water currents, they collect with the general flotsam of the stream in eddies and behind obstructions, on weed beds or simply against the windward bank (Figure 2). They may be collected from these locations using a simple pond net.

The collection of chironomid pupal exuviae has long been recognised as a quick and easy means of studying the spatial distribution and phenology of the family[7, 8, 9, 10]. Only recently however has attention been directed toward correlating such collections with water quality[11, 12].

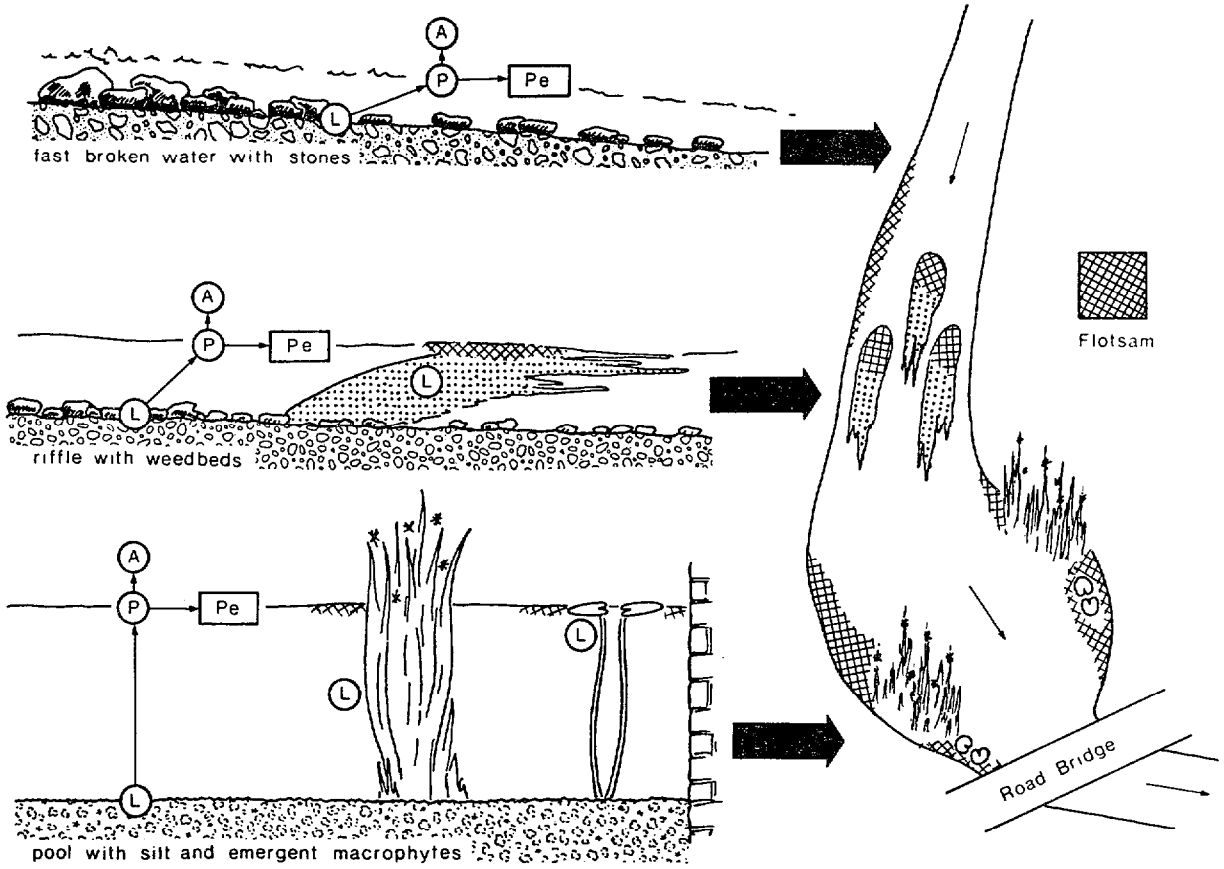


Figure 2 Diagram showing how chironomid pupal exuviae originate from river habitats

2.3 Identification

Previously the great potential of the Chironomidae in the biological assessment of water quality has been offset by taxonomic difficulties, and especially by problems of larval identification[13].

Pupal exuviae are much easier to identify to species or to species group than are the larvae, many of which are superficially very similar. It is often necessary to rear larvae through to the adult stage for positive identification. Many exuviae on the other hand have characteristic forms and markings which enable an experienced investigator to recognise them in mixed samples even with the naked eye.

Figure 3 illustrates typical exuviae from the four principal sub-families or tribes. Further taxonomic details and keys for identification to genus and species will be given in a subsequent

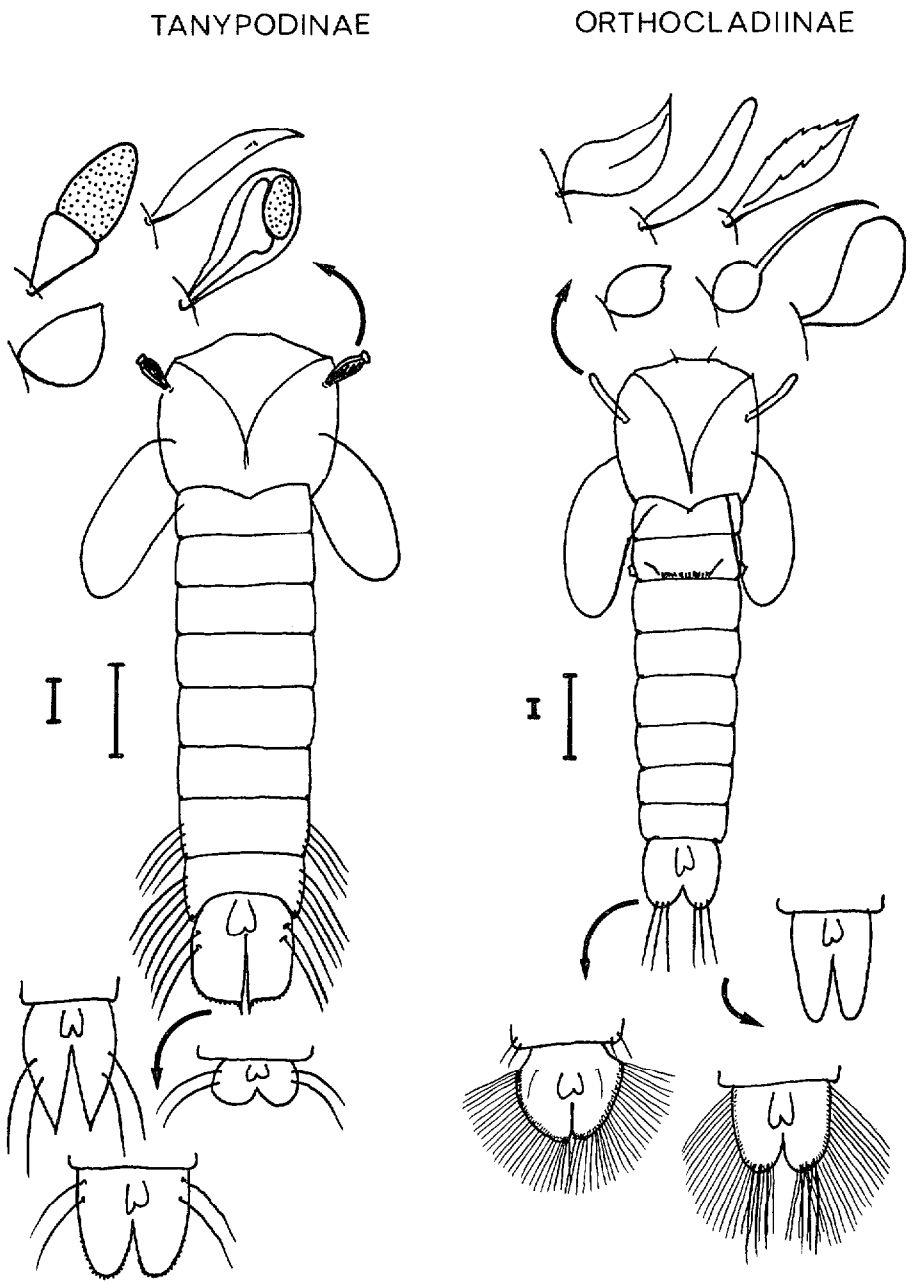
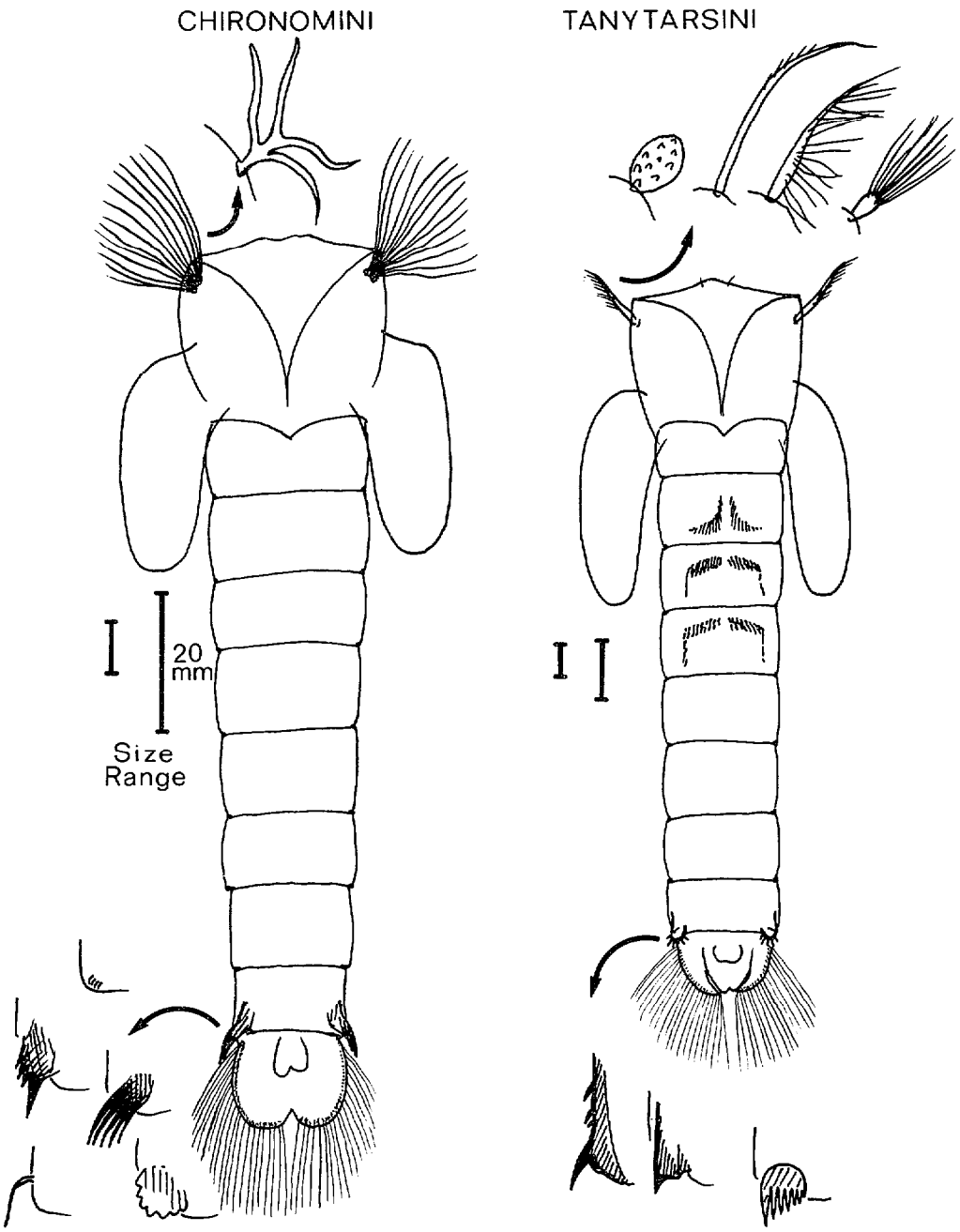


Figure 3 Drawings of typical exuviae, representative of the four principal sub-families or tribes, to show the main features used in identification. Variations in thoracic horn and terminal segment morphology are also shown.

memorandum, together with information on which forms are known to be tolerant of various types of environmental stress.

In the assessment method proposed here, the identification of the exuviae may be taken to a number of levels of complexity, the simplest of which is merely to distinguish between the different types without allocating any formal names to these types. If they are thus distinguished, the exuviae in the sample may be compared with those in samples from other localities and be related to them by diversity or similarity indices. This procedure may be sufficient in the majority of investigations, but when supported by the identification of sub-families and tribes, or of certain key types, a great deal more information may be obtained from the samples. The method can therefore be tailored to suit the individual requirements of any monitoring programme.



3. FIELD TECHNIQUE

The sampling method is simple. Collections of floating scum or flotsam which contain the exuviae are easily made with a fine-mesh, long-handled pond net. The mesh should not be coarser than about 250 microns pore diameter, so as to retain the smallest specimens. Plates 2 and 3 show the localities where flotsam accumulates in different types of stream, and demonstrate the ease with which they may be collected.

The pupal exuviae are affected principally by water currents in small and medium sized rivers, and only to a lesser extent by wind. They collect in the same place as do the general flotsam of the stream such as twigs, leaves and other debris. On eroding or riffle reaches the exuviae accumulate in bank-side eddies and on the upstream edge of weed-beds (Plate 2). On deeper lowland courses the action of the wind has more effect, and accumulation of flotsam should be looked for on the exposed bank (Plate 3). Even when not easily seen, an adequate sample of exuviae may often be taken by collecting in all the likely places.

Samples are placed in polythene bags and clearly labelled both inside and outside, and then fixed with a few millilitres of forty percent formalin in the field. They may be stored indefinitely before sorting. A flow diagram depicting in full both field and laboratory procedures is given in Figure 4.

4. LABORATORY TECHNIQUE

In the laboratory the sample material should be washed through a 4 mm sieve to remove twigs, leaves and other large debris and into a 250 micron retaining sieve. It is recommended that the material in the 4 mm sieve is rinsed at least three times in separate washes of water in a bowl before discarding. The washings can be tipped into the retaining sieve.

The material in the retaining sieve is then transferred to a bowl and suspended in a volume of water (5 litres) from which an initial assessment may be made of the numbers and types of exuviae. If the sample is a poor one containing only 100 to 200 exuviae, then the whole sample should be sorted. If, however, as often happens during the spring and summer, the sample contains thousands of exuviae, then a sub-sample must be taken. The initial sorting procedure can be done most easily with the naked eye. The sample to be sorted is placed in a white pie dish or similar receptacle under a good light and the exuviae picked out with a pair of fine forceps (Figure 4).

Figure 5 is a graph showing the number of exuvial types found in samples containing different total numbers of specimens. There is a marked inflection in the pattern corresponding to a sample size of approximately 200 exuviae. Therefore, a sub-sample of 200 exuviae will give a good representation of the exuvial types present in the sample, although lower numbers may be adequate to demonstrate the proportions of the major types. In practice, approximately 120 to 150 exuviae should be counted and sorted to show the pattern of types present, and provide data for the calculation of similarity or diversity indices. The simplest sub-sampling procedure involves stirring the sample in a bowl of water, so that the exuviae are mixed evenly throughout the water volume. Then a small sieve, normally a nylon mesh tea strainer, is scooped once through the bowl to collect the sub-sample. The volume of water in the bowl should be such that the scoop removes approximately 120 to 150 exuviae. All the skins in the scoop should be counted. If insufficient exuviae are present, then a second scoop must be made and again counted in its entirety. When a list of species present is required, however, as for whole river surveys using single sets of samples, approximately 500 skins should be examined per sample to ensure that as many as possible of the species present are recorded.

5. SAMPLE ANALYSIS

The analysis of chironomid exuvial samples relies on the operator being able to detect visible differences in size, colour, form and pattern of the exuviae. At the simplest level, a single type or a restricted number of types may be removed from a series of samples and its or their relative abundance compared to the other types present, or to the total numbers of exuviae in each sample. The most convenient method of doing this is to work out the percentage abundance of the types under consideration.

At a more advanced level of analysis, the operator may separate the whole sample into recognisably different forms, and allocate to each a type number. This can be done without any knowledge of the actual specific names of the exuviae. The relative abundance and diversity of the sample, and its similarity to other samples analysed in the same way, may then be calculated. It may be that this level of analysis will prove to be the one most useful to the water authorities for routine work. Further information into the nature of the sample may be gained by distinguishing between the sub-families and tribes as indicated in Section 2.3. This gives a useful background against which the abundance of selected types may be displayed.

It is thought that only rarely would an operator be concerned with the detailed species composition of his sample, although certain key species could with profit be identified. Identification to genus will normally provide the greatest taxonomic detail required in practice. Keys to the genera and certain species or species groups of chironomid pupal exuviae are in preparation and will be available in the near future.

Key species are those which have some "indicator" value, for example *Chironomus riparius* (L.) (Plate 1). Work is currently in hand to locate key species associated with both organic and toxic pollution.

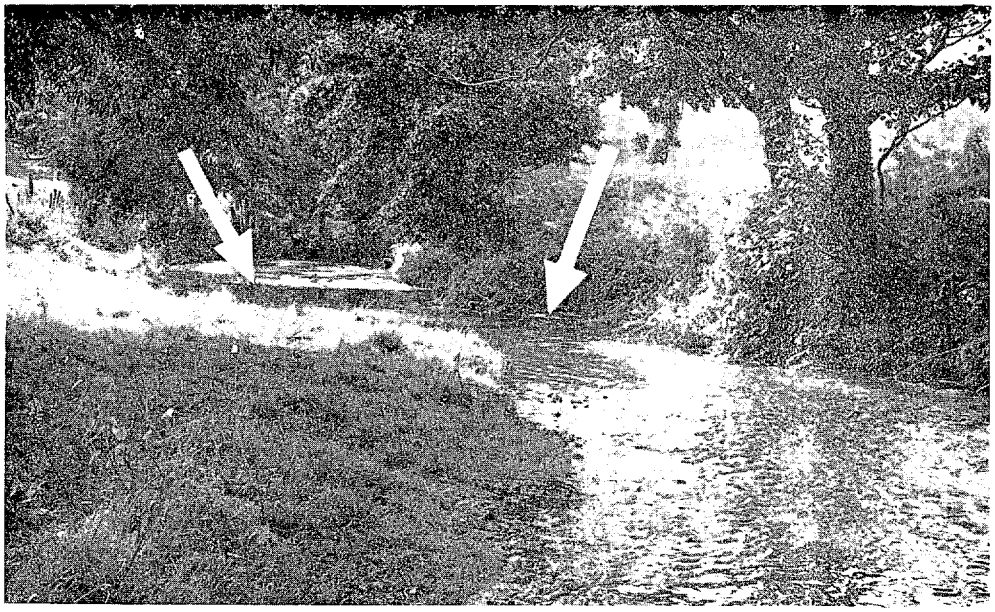
6. DATA PRESENTATION

When the samples have been analysed, it is recommended that the data be presented:

a. as *bar charts* showing the percentage composition of types. If the analysis has been taken only to the identification of key species, then these may be shown against the percentage composition of sub-families and tribes (Figure 6 and 7). This presentation allows a visual comparison to be made between samples. As far as possible the order of plotting should follow the Check List for British Insects[5];

b. As a set of *diversity indices* calculated from the percentage composition of types[14, 15], or as a set of *similarity indices* where each sample is compared to the top station (for example by totalling the lowest of each pair of percentages for each type in the two samples in question)[16]. These will reveal the points at which significant changes occur down the river.

c. as *graphs* demonstrating the ecological valency of the species sampled; for instance whether they are primarily associated with eroding or depositing substrata, and whether they are pollution tolerant or intolerant.



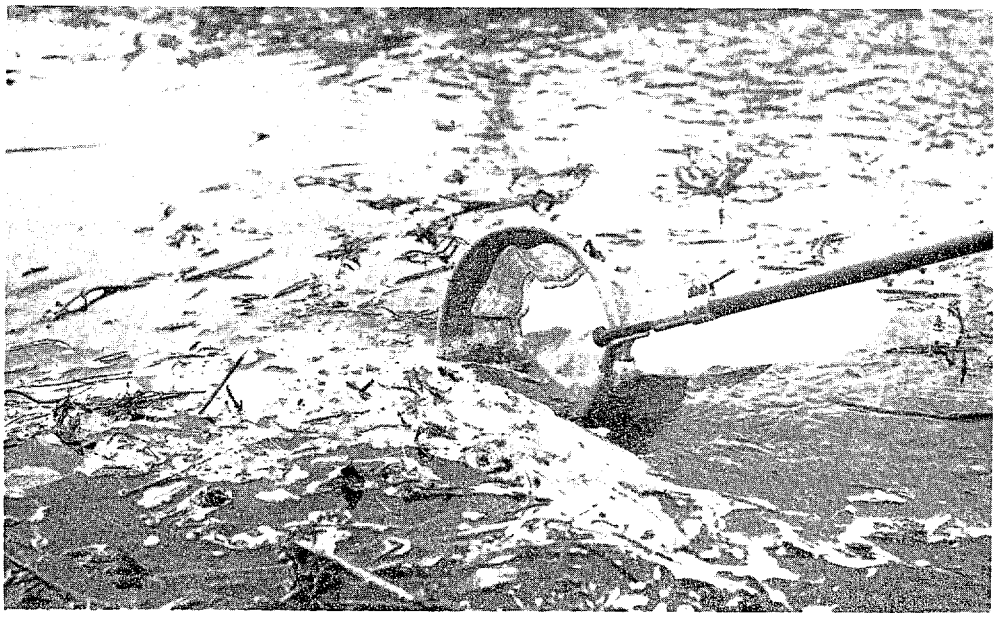


Plate 2 Sites in shallow water where exuvial collections may be made

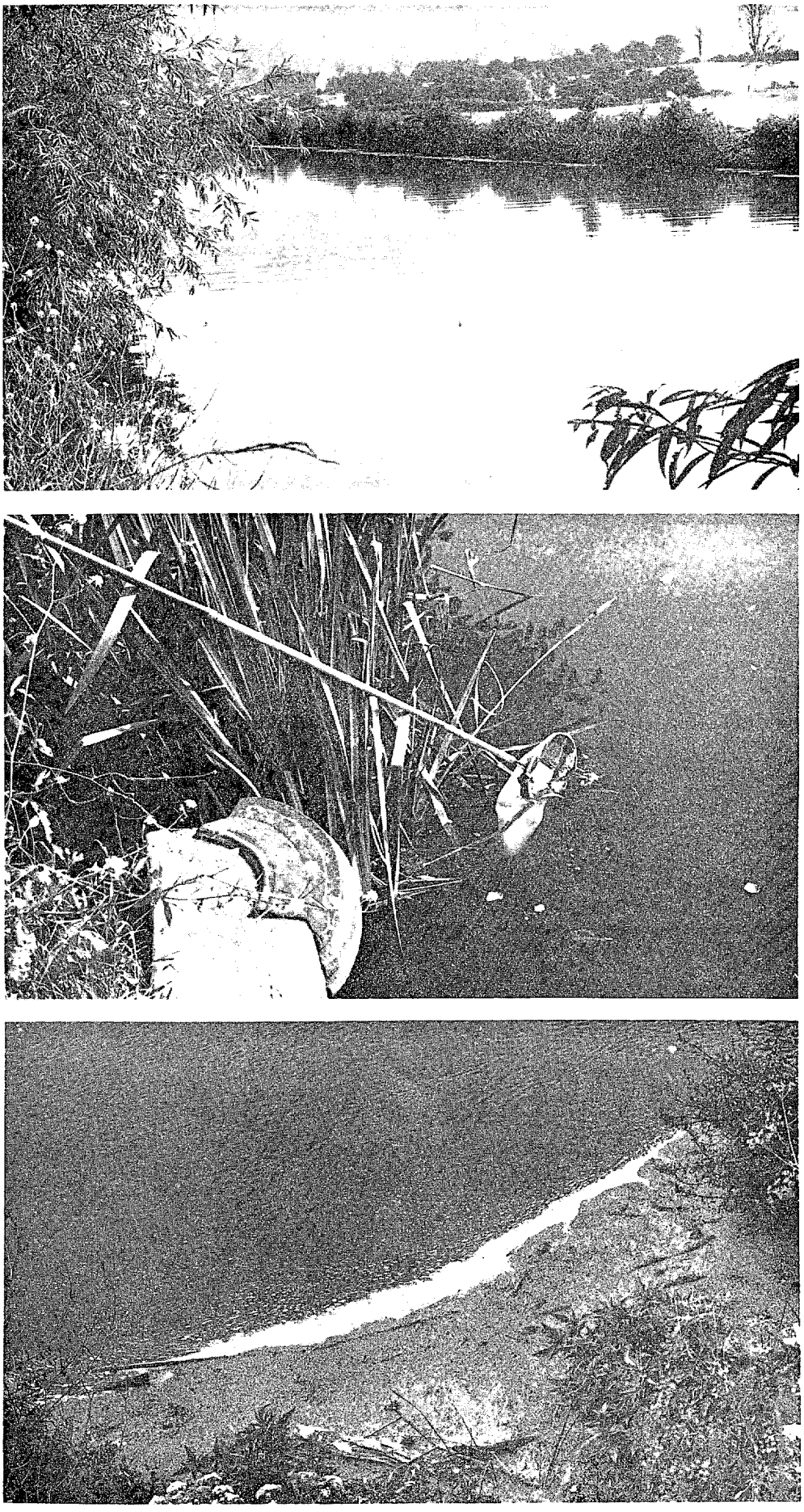
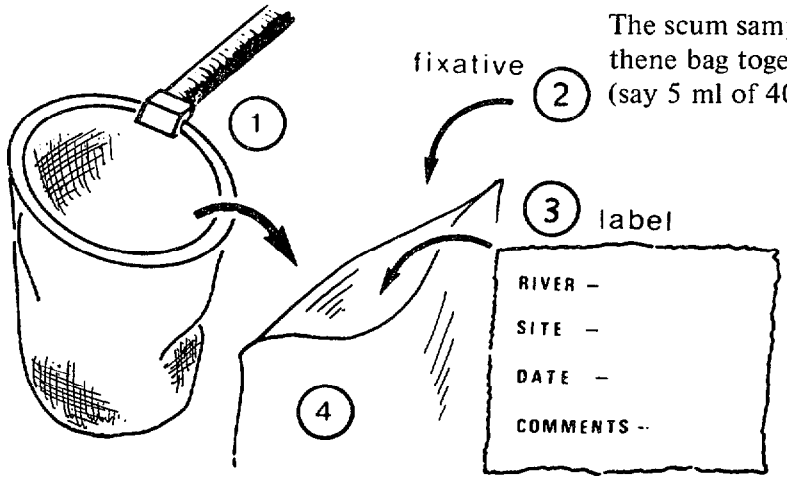


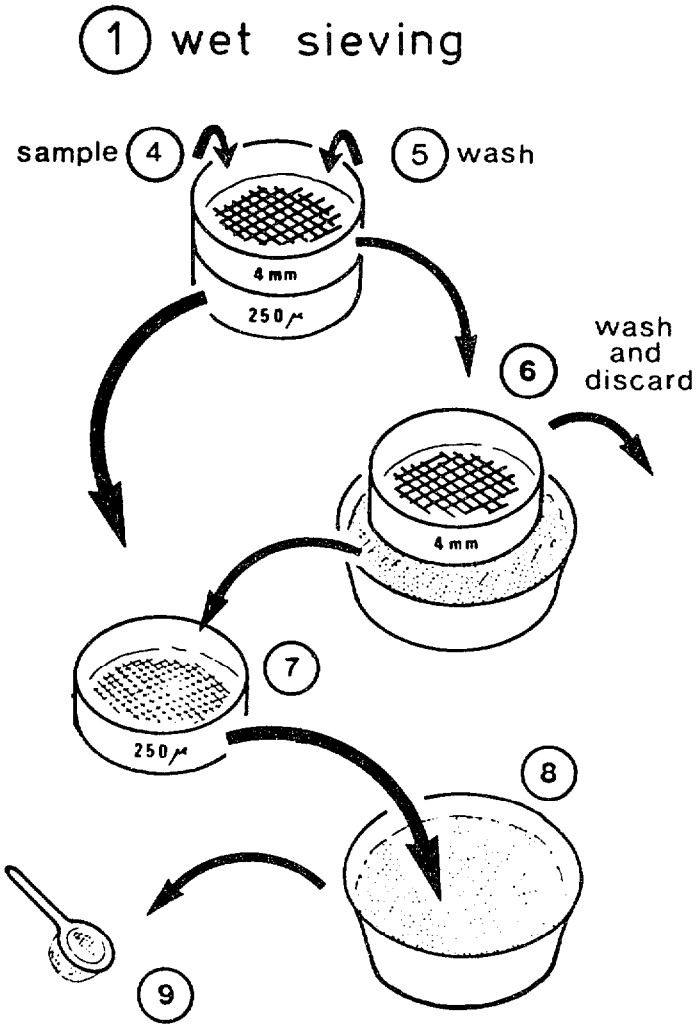
Plate 3 Sites in deep water where exuvial collections may be made

FIELD TECHNIQUE



The scum sample (1) is placed in a polythene bag together with a fixative (2) (say 5 ml of 40% formalin). and label (3).

LABORATORY TECHNIQUE



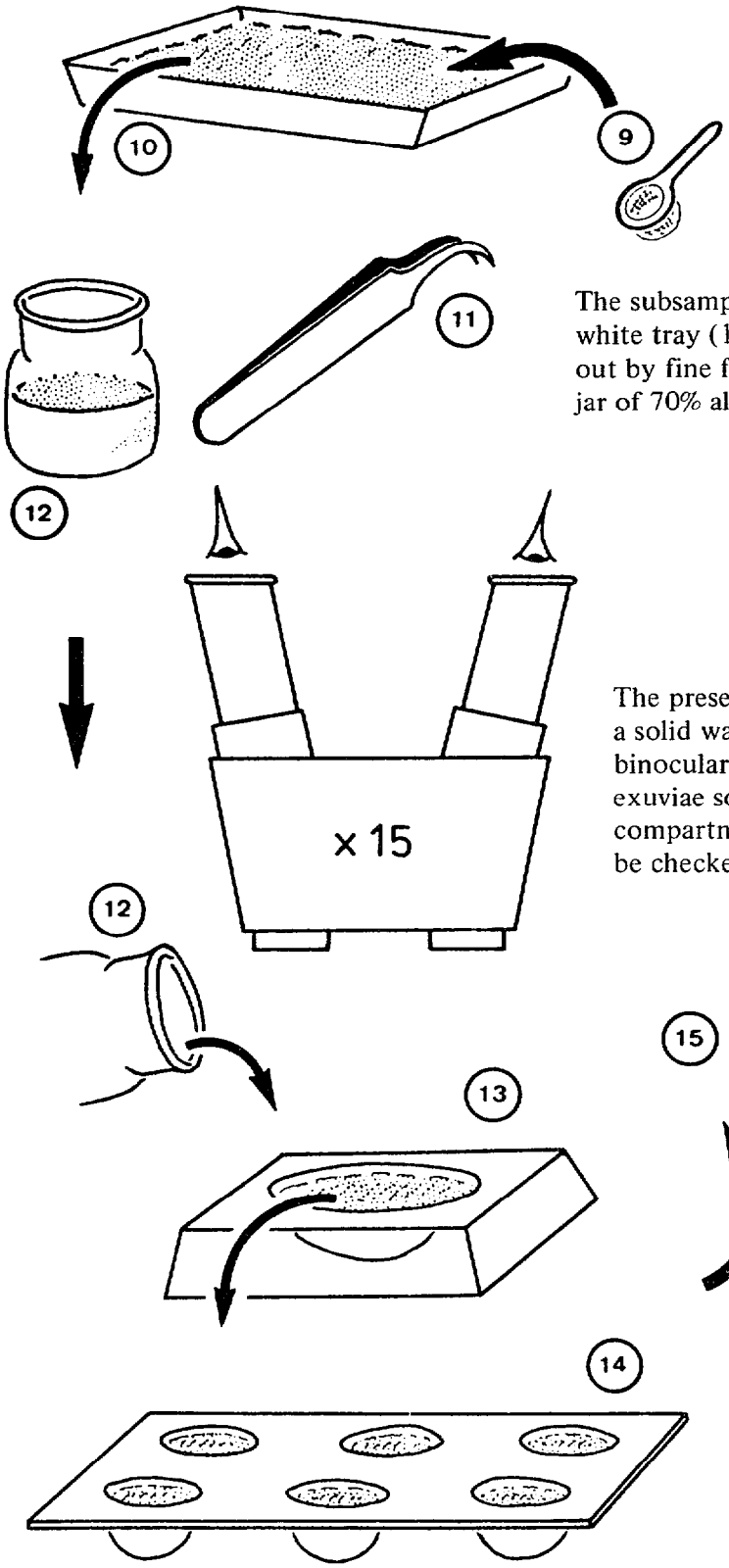
In the laboratory, the sample is placed in a 4 mm sieve (4) and washed through into a 250μ retaining sieve (5).

The coarse fraction in the 4 mm sieve is washed 3 times in a bowl of water (6) by dipping the sieve in an out, and the washings added to the fraction in the 250μ sieve (7). The coarse fraction is now discarded.

The fraction in the 250μ sieve is resuspended in a bowl of water (about 5 litres) (8), and a subsample taken by sweeping a small teastrainer (9) once through the material in the bowl after stirring thoroughly.

Figure 4 Sequence of field and laboratory operations

(2) sorting



The subsample is dispersed in water in a white tray (10) and the exuviae picked out by fine forceps (11) and placed in a jar of 70% alcohol and 2% Glycerol. (12)

The preserved sample (12) is examined in a solid watchglass under a low power binocular microscope (13), and the exuviae sorted into types in a multi-compartment tray (14). They should be checked again (15) before counting.

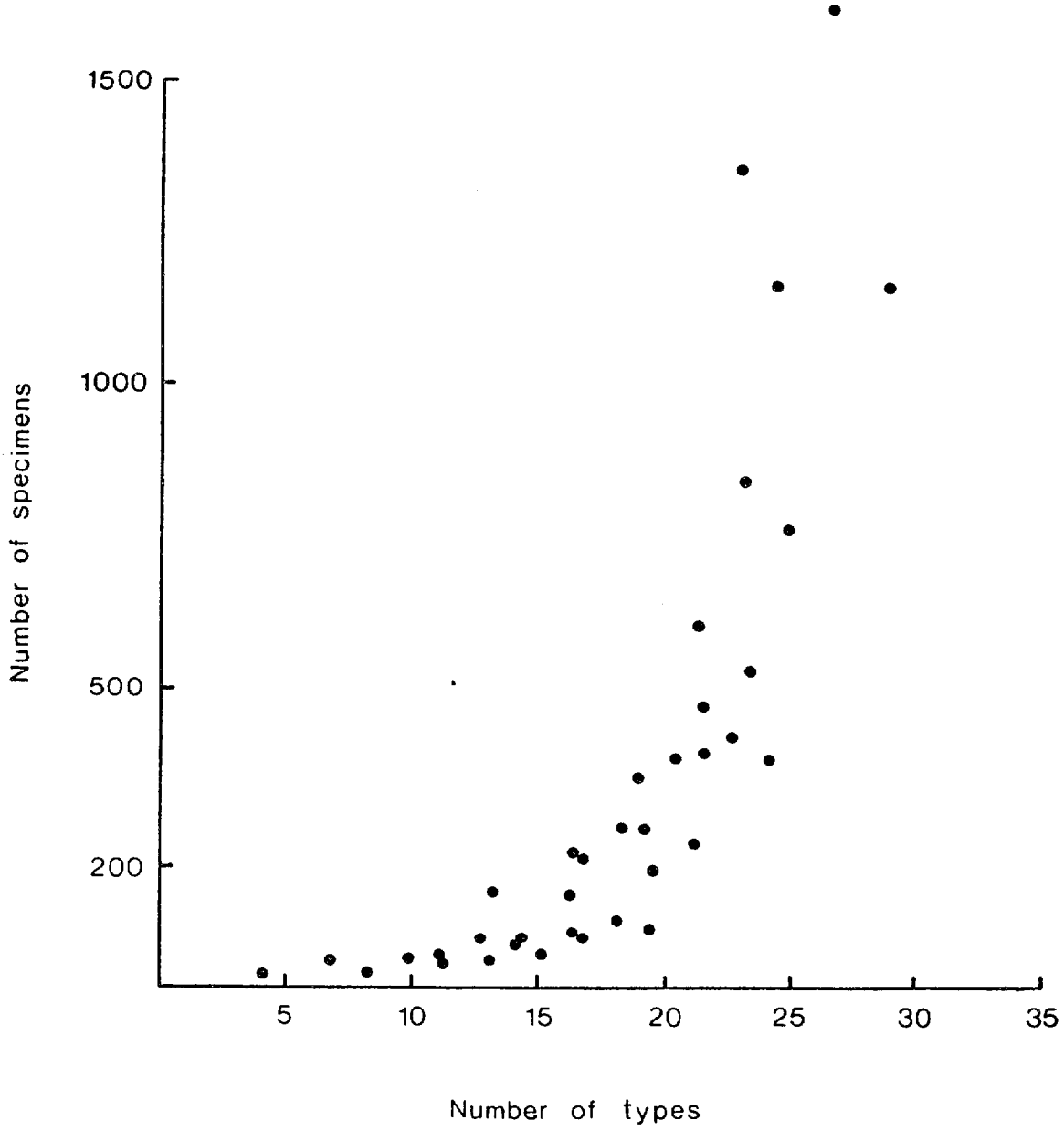


Figure 5 Graph of number of exuvial types found in samples of different size

7. PRACTICAL EXAMPLES OF THE METHOD

7.1 The investigation of localised discharges

Localised discharges or perturbations in a stream may be assessed by taking a closely spaced series of samples along the section of the stream in question. The effect of the discharge on the river may then be evaluated by analysing the changes above and below the point of discharge. A measure of the polluting effect is given by the degree of change between the communities immediately above and below the discharge and by the distance downstream over which this change extends. It should be remembered, however, that the overall nature of the river may alter over the same course, especially if the distances involved are, say, a mile or more. Care should be taken, therefore, when interpreting the results.

This method works, for example, where the midge, *Chironomus riparius*, which is associated with heavy organic loadings, is present in association with a point discharge of sewage. *Chironomus riparius* is frequently the dominant species remaining in the polluted zone, and at high densities it is regarded as indicative of enriched conditions[1, 2]. In the river Chew, Avon, merely being able to recognise this one exuvial type was enough to assess the effects of the discharge, in a situation where other sampling methods could not be applied (Figure 6)[12].

Another example, which involves a different set of exuvial types, is the effect on the river Severn of the inflow from the organically loaded river Chelt, near Cheltenham, Gloucestershire. The Severn at this point is a wide (60m to 80m) fast-flowing river with steep soft mud banks and was sampled by the authors using a boat made available by the courtesy of the Severn-Trent Water Authority. Figure 7 shows the results of this one-day survey and reveals a marked change in the exuvial pattern downstream from the Chelt inflow for about 1km to 2km. The dominant type here was *Eukiefferiella claripennis* (Lundbeck), probably associated with filamentous weed growth in this section.

It is clear that different rivers or sections of rivers will show different biological responses to organic loading, depending on the quality of effluent, the volume flow of the effluent and the river, and on the character of the river. A fast-flowing stony river will respond differently to a slow-flowing muddy river, even to a similar pollutional load.

7.2 Whole river surveys

Instead of taking a series of samples from a limited river section, pupal exuviae may be collected from points of access along the entire length of a river to obtain a measure of the pollutional status of its various reaches. Suspect sections are those where the chironomid community contains an unusually high proportion of pollution-tolerant forms.

Each sample should consist of large numbers of exuviae, integrated from along extended lengths of river bank. Over 500 exuviae should be examined to obtain, on the basis of presence and absence of taxa, a good indication of the entire chironomid fauna from that section of the water course.

This application of the exuvial technique was carried out by the authors on a series of polluted river systems in August 1978, and the results from two of these are presented in Figure 8. Both rivers were known to be polluted by a mixture of toxic and organic discharges.

The first was a small fast-flowing stream, the Willow Brook, Northamptonshire, sampled on 10th August 1978, and known to be polluted near its source by effluents from a steel works and a sewage treatment plant. The major pollutant besides high concentrations of nitrate, phosphate and ammonia, has in the past been dissolved zinc from the steel-making process, which has been known to reach a concentration of 25 mg Zn l^{-1}.

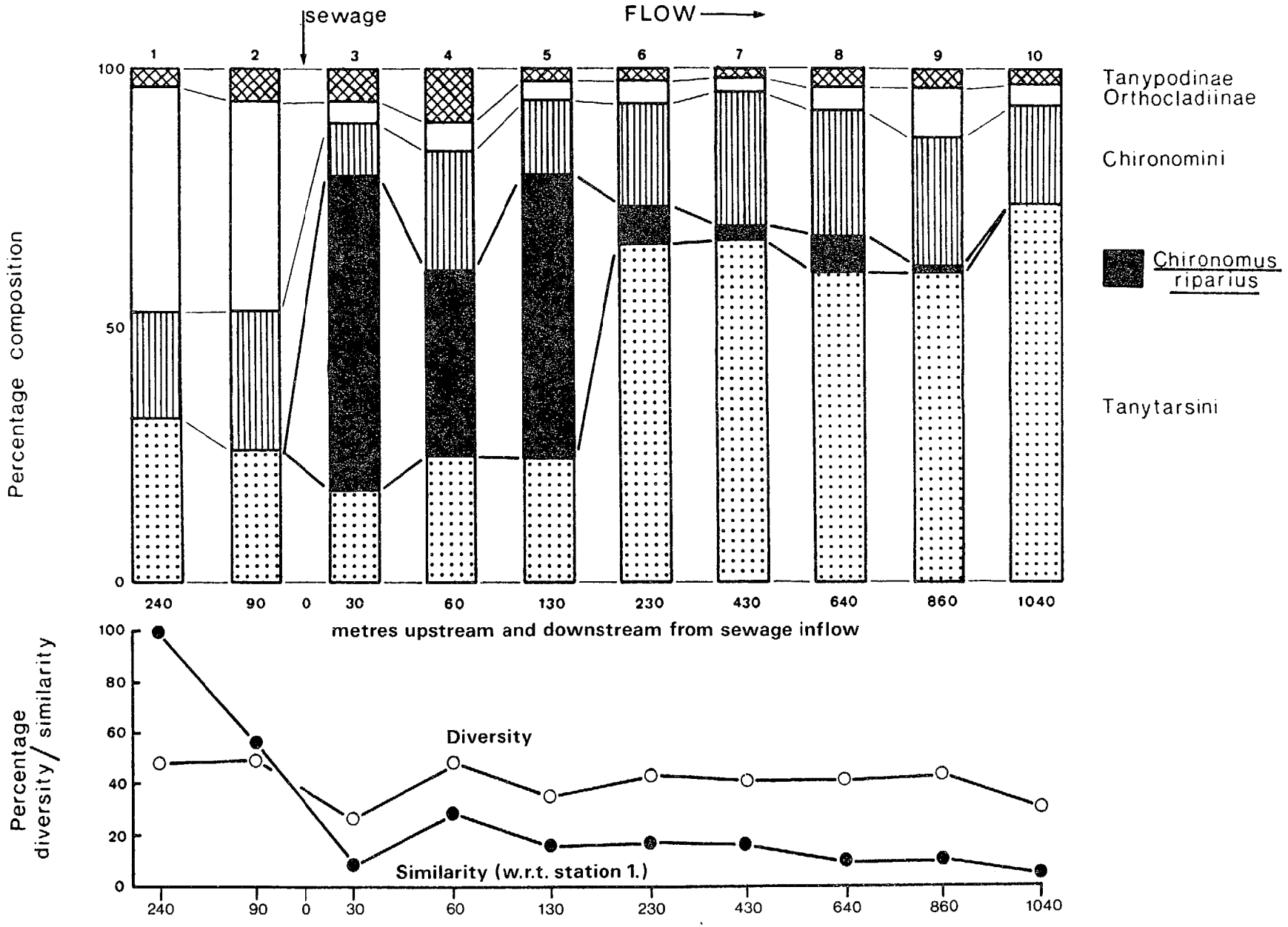


Figure 6 Analysis of samples from the river Chew
Bar charts showing percentage of Chironomus riparius *(L.), and of subfamilies and tribes, in a set of samples taken on 4.8.76 at Chew Stoke on the river Chew, Avon. Also shown are the diversity indices and similarity indices for each sample.*

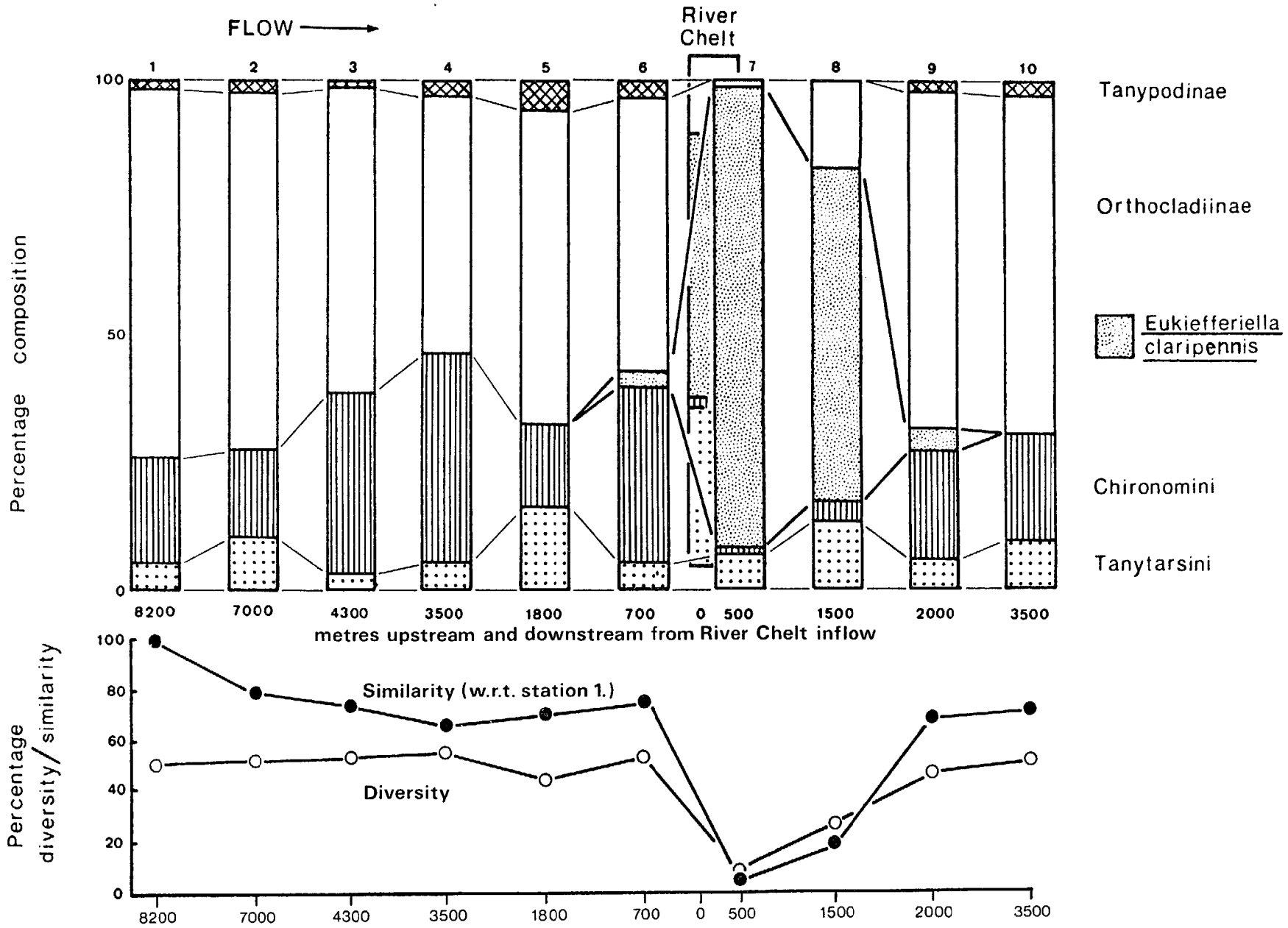


Figure 7 Analysis of sample from the river Severn
Bar charts showing percentage of Eukiefferiella claripennis *(Lundbeck) and of subfamilies and tribes, in a set of samples taken on 21.6.77, on the river Severn above Gloucester. Also shown are the diversity and similarity indices for each sample.*

The pollutional status of the Willow Brook and the effects of the discharges on the fish and invertebrate fauna of the stream, together with improvement in quality since the introduction of new effluent treatment processes, has recently been investigated[17]. The study illustrated an overall reduction in the impact of the pollutants as concentrations decreased with passage downstream. The concentration of zinc was reduced markedly during passage through the lakes which lie along the course of the stream.

Figure 8A shows the results of the authors' exuvial survey along the length of the Willow Brook. The graph plots the number of intolerant species as a percentage of the whole fauna, in relation to distance downstream from the river source. It can be seen that there is an increase in the number of intolerant forms with increasing distance from the input of pollutants. The chironomid fauna is severely restricted near the source. Overall, there is a trend for species diversity to increase with distance downstream, the rise in the intolerant part of the chironomid fauna being greatest below each of the lakes.

The other example, Figure 8B, was a similar survey carried out along the river Churnet (Derbyshire) on 14th August 1978. Initially clean in its upper part, it is known to be affected by a number of polluting discharges in its middle reaches, and shows recovery with distance downstream.

The exuvial survey also revealed this sequence. The river shows a clean water fauna, with a high proportion of intolerant forms in its upper reaches, followed by an overall reduction in species diversity across the region of polluting in-flows, attributable to the exclusion of intolerant forms from both erosional and depositional environments. With distance downstream from this region, the fauna shows progressive recovery, so that by the time it reaches its confluence with the river Dove, it has regained its former status.

It is evident from these examples that the exuvial technique can provide a rapid biological assessment of entire river systems on the basis of a single set of field collections, taken on one day at an optimum time of year (late May to September).

The importance of the exuvial method is that whatever the scale of operation, it can reveal *points of change* in the river fauna, and follow the restitution of the normal fauna downstream. In this way, the magnitude of effect of the pollution load on the river itself can be demonstrated. Knowledge of the actual species of chironomid involved and their detailed ecology is *not necessary* for the method to be of practical use. As competence in identification and knowledge of the ecology of the species of chironomid is developed or becomes available, then a finer and more detailed interpretation can be put on the sample data.

8. DISCUSSION

Certain objections may be raised against the method as applied here: notably that it uses only a single taxonomic group of the stream biota; that it is subject to marked seasonable effects; and that it deals with samples derived from an undefined area of stream.

Perhaps the first of these objections is the most cogent, although all other practical monitoring methods suffer at least in part from the same difficulty. A complete faunal analysis, although ideal, presents insuperable problems with sampling methods, taxonomic expertise and time available, that rule it out as a practical proposition for stream monitoring. Limiting the sampling to macroinvertebrates, or to fish, or to diatoms, or to any other restricted group of organism such as chironomids, is justified provided the group can be shown to respond to the conditions being investigated in a clear and measurable way.

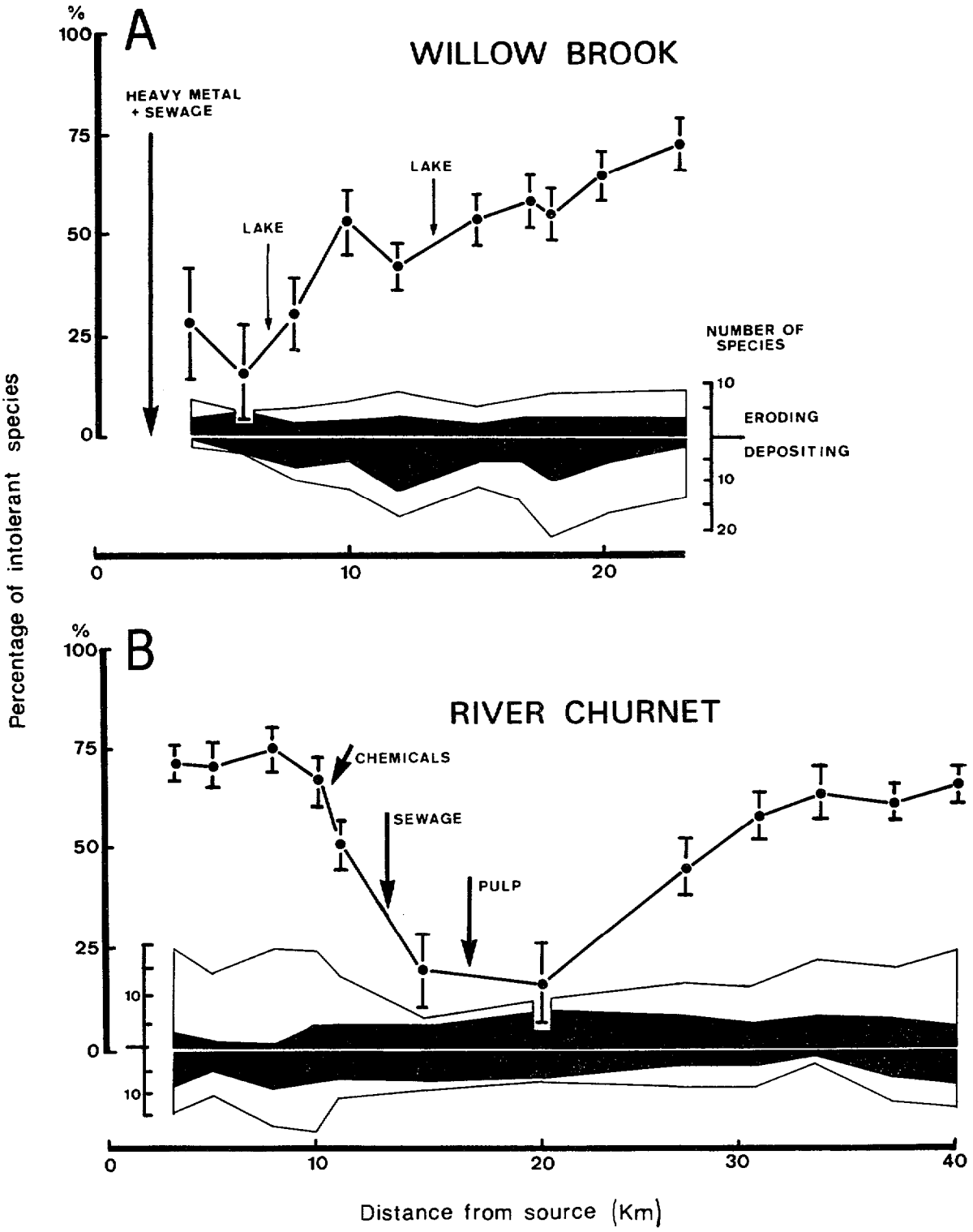


Figure 8 *Analysis of samples from the Willow Brook and the river Churnett*
Graphs of the percentage of pollution intolerant species and numbers of species associated with eroding and depositing substrata in sets of samples taken along the Willow Brook (A) and the river Churnet (B) in August 1978. The limits are those which would occur if one too few or too many intolerant species was counted. In the eroding and depositing graph, the black represents the pollution tolerant species.

Turning to the second objection, it is fair to point out that seasonal changes occur in all aquatic organisms[18, 19], and must be allowed for in evaluating any biological samples. The best time of year for exuvial samples is during the summer, say from late May to September, after the spring emergence peak has passed, and when the emergence pattern settles down to a reasonably steady state[10, 11]. This restriction in sampling period is not a serious limitation to the method since many pollutional effects are greatest during the warmer weather.

The objection that the scum or flotsam sample may contain exuviae from an undefined area upstream of the sampling point is important. Pupal exuviae have been shown to remain intact and undecomposed for about one to two weeks depending on the river conditions[7, 11]. This period of time would allow them to float many miles downstream. Experiments on the river Chew[11], however, have shown that floating material travels only 400 m down the river, and that most exuviae in a sample derive from only 50 m to 100 m upstream. These results have been confirmed on other rivers. It has also been shown that exuviae float for from 24 to 48 hours before sinking, so that each sample integrates over time as well as distance.

The drifting exuviae are swept from the water surface by wind, and become trapped along the bank, in eddies, and by reedbeds and other obstructions. Indeed this effect is fundamental to the scum sampling method, as it concentrates the exuviae ready for netting. It also permits easy sampling of all types of water body including both fast-flowing, shallow streams, and slow, deep, muddy rivers. It is equally applicable to lakes, and possibly to estuaries.

In conclusions, it is clear that the exuvial method has great potential in the field of biological assessment of pollution. By virtue of the ease and speed of sampling and its relevance to all sections of any river, the method can also be of real value in typing and classifying rivers, and in following long-term change from year to year. The authors envisage that the method could be adopted by water authority biologists as a useful extension of their present sampling technique, and would obtain results where current techniques are difficult or imposssible to apply.

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