Sampling of Non-planktonic Algae (Benthic Algae or Periphyton) 1982

Methods for the Examination of Waters and Associated Materials

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Contents

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Warning to users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specification. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection' Public Health Laboratory Service Monograph Series No 6, HMSO, London.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting and rescue equipment. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

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About this series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains short reviews of the more important analytical techniques of interest to the water and sewage industries. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users — the senior analytical chemist, biologist, bacteriologist, etc to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is a committee of the Department of the Environment. It has seven Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
9.0 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes for booklets in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in the booklet.

L R PITTWELL
Secretary

7 October 1983

Sampling of Non-planktonic Algae (Benthic Algae or Periphyton)/1982

1 General Introduction

The purpose of this paper is to outline and discuss methods of sampling algae which are either attached to, or associated with, submerged surfaces. Sampling is affected by the following considerations.

1.1 Large variations in the density of algae may occur over quite short distances. Replication in sampling may be more important than minor improvements in sampling methods.

1.2 Many methods have been devised for sampling particular types of substratum in shallow lakes, ponds and streams. Some techniques may be generally adapted to particular types of submerged surface but nearly all have disadvantages. Good quantitative methods for sampling deeper lakes and rivers generally involve scuba-diving but are, as yet, not well developed. Individual workers may have to adapt methods to suit local conditions.

1.3 It is rarely possible to estimate the biomass of the algae directly by weighing because of the admixture of dead cells, animals, bacterial and general organic and inorganic detritus. Measurements are usually confined to estimates of chlorophyll a and/or the more time-consuming estimation of cell numbers. In those cases where the direct estimation of dry weight is possible (ie section 7) methods are given.

1.4 The precision of any method cannot be given because so much of the variation normally occurs at the sampling stage. The sources of variation can be established using a nested analysis of variance technique. The principles have been described by Eaton and Moss (1966) for a specific algal sampling problem and general information may be obtained from standard statistical works (eg Sokal & Rohlf, 1969).

Let A samples be taken from a site
For each sample let B subsamples be prepared
And for each subsample let C replicate analyses be carried out
The variance (σ^2) at each stage will be σ^2A, σ^2B and σ^2C
The overall variance of the method will be:

$$\sigma^2 A + \frac{(\sigma^2 B)}{B} + \frac{(\sigma^2 C)}{BC}$$

In subsequent sections it will be seen that subsampling (B) may not always be possible and that analyses (C) will be either estimates of chlorophyll or estimates of cell numbers. Usually the source of greatest variation will be at the sampling stage and, as the most serious constraint is generally time, it is usually better to replicate at the sampling stage and not at subsequent stages. Counting errors may become serious if the cell suspension is not homogeneous. Clumps containing large numbers of a single species are a serious risk and will invalidate the statistical basis of standard counting techniques (Lund, Kipling & Le Cren, 1958). It is then advisable to make a smaller number of counts on several subsamples to reach the desired final count. Bearing these particular difficulties in mind, investigators are referred to General Principals of Sampling and Accuracy of Results 1980, also published in this series.

For many purposes counts of cell numbers of benthic samples are not a practical proposition. Counting of filaments or colonies can be misleading because their variation in size may be due to the degree of homogenisation and have little or no ecological significance. Estimation of percentage cover is quicker and methods given by Backhaus (1968), Kalsvik (1974), Whitton (1979) and Holmes and Whitton (1981) may be more suitable. The estimation of maximum crop sizes is sometimes of particular interest but special care should be taken. Considerable sampling may still be required to establish the timing and location of maximum crops; subjective judgements are notoriously deceptive.

1.5 Investigators are referred to Lund & Talling (1958), Wetzel (1964), Sladeckova (1962), Cooke (1956) for reviews which include discussion of methodology, to Blum (1956), Round (1964) and Whitton (1975) for ecological reviews, and to Schwoerbel (1970) for more detail on a few specific methods.

1.6 Performance and characterisation of the methods

1.6.1 Biota sampled

(a) epipsammic and epipelic algae (see section 2); (b) mixtures of epipsammic, epipelic and epilithic algae (see section 3); (c) & (d) epilithic algae (see sections 4 and 5); (e) epiphytic algae (see section 6); (f) *Cladophora* and other filamentous algae (see section 7); (g) algae attached to artificial surfaces introduced by the investigator (see section 8).

1.6.2 Habitat sampled

(a) Mud, silt and sand (see section 2); (b) fine gravel (see section 3); (c) stones and small rocks (see section 4); (d) large rocks, bedrock and man-made objects (see section 5); (e) macrophytes (see section 6); (f) *Cladophora* in rivers and streams (see section 7); (g) artificial substrata in all water bodies (see section 8).

1.6.3 Type of sampler

Section 2, specialized methods; section 3, corers; section 4, specialized methods; section 5, specialized scrapers; section 6, specialized methods; section 7, visual and cropping methods; section 8, artificial substrata.

1.6.4 Basis of operation

The operator will work from the surface in shallow waters (< 0.5 m). In deeper waters a diver may be necessary. Section 2, removal of algae on sediment surface by suction; section 3, coring; section 4, removal of individual stones, or all stones within a known area; section 5, removal of all algae from substrata, *in situ*; section 6 and section 7, several different methods; section 8, clean artificial surfaces exposed in water for known periods.

1.6.5 Form of data

Sections 2, 3, 4, 5, 6 & 8. Qualitative description of the algal flora; quantitative estimation of numbers or proportions; quantitative estimation of chlorophyll a. Section 7, cover data and estimates of biomass.

1.6.6 Limitations of methods

Sections 2–6, require evaluation; section 7, other algae, notably *Oedogonium*, *Stigeoclonium*, *Ulothrix* and *Vaucheria* may interfere. Depth and/or turbidity of the water may affect visual methods; section 8, bias introduced. Artificial surfaces are different from natural surfaces in age and texture.

1.6.7 Efficiency of methods

Requires evaluation.

1.6.8 Logistics of sampling

Requires evaluation.

2 Mud, Silt and Sand

2.1 Introduction

Three different population types may exist on these substrates.

(a) True epipelic algae are motile forms which migrate diurnally through the sediment. They include pennate diatoms, some blue-green algae and various flagellates.

(b) The soft sediments may become more stabilised by non-migratory populations.

(c) Epipsammic algae (attached to sand grains): these are really small forms (< 10 μ) firmly attached to the surfaces of sand grains.

2.2 Sampling techniques

2.2.1 The sediment is delimited by pressing a perspex cylinder (9 cm ID) into the sediment, leaving a substantial collar above the sediment (Eaton & Moss, 1966). The inlet tube from an evacuated aspirator is carefully drawn over the surface of the sediment to remove the upper few millimetres of sediment.

2.2.2 It may be impractical to remove all the algae from a known area in deeper waters where visibility is obscured or manipulation of equipment difficult. A vacuum technique similar to 2.2.1 may be used without the perspex collar to give a qualitative assessment of the flora present. Numbers may only be used to estimate the proportions of algae present.

2.2.3 Firm sediments may be sampled using a Gilson corer/FBA automatic mud sampler (Elliott & Tullett, 1978) and more diffluent sediments with a Jenkin corer (Mortimer, 1942). The latter is particularly useful in deeper waters where the mud-water interface within the core remains relatively undisturbed. The upper lid is removed immediately after sampling and the 0.5 cm of mud, containing most of the living algae, siphoned off.

2.3 Preparation of sample for microscopic evaluation

In many situations the concentration of algae in the sediment may be sufficient for direct counting. Care must be taken to ensure that the sediment remains evenly suspended when subsamples are removed. The narrow orifice of a pipette will restrict the transfer of the larger particles. The end should be removed and the pipette recalibrated. The sediment should be diluted sufficiently so that the algae may be seen easily between, or attached to, the sediment particles. True motile epipelic forms should be seen clearly but small algae attached to sand grains may be obscured and their numbers underestimated.

2.4 Preparation for chlorophyll analysis

A subsample of the sediment is filtered through glass-fibre filters (either 7 or 9 cm, Whatman GF/C), using a Hartley funnel and Buchner flask. Fine sediments tend to clog up small filters rapidly (<7 cm) and it may be necessary to use larger filters to obtain sufficient pigment for analysis. The sample is partially dried in the dark, and then extracted in methanol or acetone. The choice of solvent is critical and reference must be made to the booklet Chlorophyll a in Aquatic Environments 1980, also published in this series. The sample is then refiltered and the filter washed with the appropriate solvent. The filtrate and washings are combined and made up to a known volume. A small error will be introduced by not knowing the water content of the extract. A much greater error would be introduced by drying out the sample completely before extraction because desiccation, even in the dark, leads to chlorophyll breakdown.

2.5 General comments

Many types of corers are available but have largely been developed for the sampling of benthic invertebrates (Kajak, 1971; Elliott & Tullett, 1978). They normally remove an unnecessarily large amount of sediment because live algae will be confined to the upper half centimetre or so, unless the sediment has recently been disturbed.

More sophisticated methods are available for the separation of epipelic and epipsammic algae (Eaton & Moss, 1966; Moss & Round, 1967). The former paper gives a clear account of sampling and subsequent separation of the epipelic algae from the mud. These algae have a diurnal rhythm and migrate to the surface of the sediment in the morning. The method involves trapping the algae in lens tissue as they reach the sediment surface and removing the tissues before the algae migrate down again.

3 Fine Gravel

3.1 Introduction

Fine gravel includes particles between 2 mm and 20 mm but there may be considerable quantities of finer material below the surface. Grabs and dredges are usually unsuitable, partly because the area of the substratum removed is difficult to estimate but mainly because loosely attached algae may be washed off during sampling.

3.2 Sampling techniques

3.2.1 In shallow waters a wide open-ended metal corer can be used to remove samples of gravel of suitable dimensions (Ladle, pers. comm.). A thin base plate must be slid underneath before the core is removed, to prevent the sample falling out because it is unlikely that a self closing corer would form an adequate seal. Alternatively, the gravel may be removed by hand and the finer sediments and detached algae pumped, or sucked, out of the corer.

3.2.2 Corers, such as those described by Shapiro (1958), may be used to freeze the cores, *in situ*, before removal. Freezing may destroy some of the algae.

3.3 Preparation of sample for microscopic evaluation

Direct microscopy will be of limited value because of the size of the particles. Moreover the stones will be too small for brushing and scraping techniques to be useful (see 4.2). Many algae will be very firmly attached, but as the algal populations develop, it is possible to detach at least part of the microflora on a reciprocal shaker. An ultrasonic probe may also be used to dislodge the algae most closely attached from stones. The power setting and duration of exposure should be carefully monitored to avoid cell disruption due to mechanical or thermal effects. If necessary, more than one exposure should be used to optimize recovery. Standardization may be very difficult due to differences in population types.

The algae and finer sediments may be washed through a 1 mm sieve and may now be treated as in 2.3.

3.4 Preparation for chlorophyll analysis

Do not subsample. Remove as much water as possible from the sample. Separate the finer from the coarser material using a 2 mm sieve. Filter the finer material through a glass-fibre filter (such as Whatman GF/C); place this material together with the larger stones in a wide-neck vessel with a tightly fitting lid and extract in 90% acetone or methanol.

To test the efficiency of the methods described in 3.3 extract the chlorophyll from the two fractions separately and calculate the proportion of pigment remaining on the larger stones.

4 Stones and Small Rocks

4.1 Introduction

This includes material which is >2cm but still small enough to be removed from the water. The stones can be made of flint, limestones, sandstones and a variety of igneous and metamorphic rocks. Most of the algae grow over the surface as true epilithic algae but some endolithic forms grow within the softer limestones and in the weathered surface layer of harder rocks. Removal of endolithic algae by the methods outlined in 4.3 is not quantitative.

4.2 Sampling methods

4.2.1 Method A

Remove individual stones at random. It is important that there is no subjective selection involved. This is most satisfactorily achieved by selecting the approximate location at random (within half a metre) and making the final location arbitrarily with a pointer.

4.2.2 Method B

Quadrats may be used to delimit areas of the river bed. All the surface stones within the area are removed. The quadrat will need to be weighted to stay in place.

The area of the quadrat is largely a compromise between the need to obtain a representative sample of the substrate but still sufficiently small for rapid analysis. Generally an area between 100–300 cm^2 will suffice.

4.3 Preparation of sample for microscopic evaluation

4.3.1 Treatment of entire sample

(1) Shake up the stones in water. Loosely attached forms come off and may be separated through a coarse sieve.

(2) Now use a test tube brush or a tooth brush to remove more firmly attached algae.

(3) In addition there may be very firmly encrusted forms, such as *Hildenbrandia, Ulvella* and lime-encrusted algae, growing under the loosely attached forms. These may be removed by scraping with a sharp scalpel or by abrasion with a wire brush. Inevitably some of the algae are destroyed by the abrasion.

4.3.2 Treatment of part of sample. Method I

Algae may be removed from a known area of substrate surface using a more complex brushing technique (Douglas, 1958; see Fig. 1a). The brush is made of a steel rod with a hole about 5 mm deep drilled into the end, into which stiff nylon hairbrush bristles (0.5 mm diameter) are cemented and protrude 3–6 mm. The area to be brushed off is delimited by the neck of a 50 ml polythene bottle with the bottom sawn off. This is stiff enough to be held tightly in one hand without being crushed and yet soft enough to grip the surface of a wet stone or the smooth surface of an irregular stone. The algae outside the sampling bottle are brushed, washed off the stone and discarded. The brush is inserted and the area delimited by the neck scrubbed clean. The polythene bottle is removed and the detached algae are washed off. The brushing and washing is carried out in a sorting tray so that none of the sample is lost.

4.3.3 Treatment of part of sample. Method II

This summarizes a method described by Ertl (1971). It is designed to overcome the serious disadvantage of 4.3.2 where material may be lost because the polythene bottle does not mould itself to rough surfaces.

The sampler consists of two metal or plastic cylinders, one 5–10 cm long with a known base area (5–10 cm^2), the other about 1 cm shorter and 2 cm larger in diameter (Fig. 2). The cylinder with the smallest diameter is inserted into the larger and fixed to it by two or three bars. The free space between the cylinders is filled with plasticine or similar material (e.g. blue-tac). The sampler is pressed firmly against the substrate to be sampled and the plasticine is forced down onto the substrate. This thoroughly isolates the known sampling area of the inner cylinder. The periphyton in the area is removed with a sharp scraper and the detached organisms removed with a pipette (with the end cut off), using a small volume of filtered water obtained from the sampling site. The organisms which are still attached to the substrate can be removed by a brush with stiff bristles.

4.4 Preparation for chlorophyll analysis

4.4.1 Algal suspensions prepared in 4.3 may be filtered through glass-fibre filters such as (GF/C) and extracted in 90% acetone or methanol as in 2.4 above.

4.4.2 If the stones are not too large, the chlorophyll may be extracted directly.

(1) Water should be carefully drained off the stones and filtered through glass-fibre filters.

(2) Place the stones and filter in a wide-necked tight-fitting screw top bottle.

(3) Extract the pigments in the appropriate solvent.

4.5 General comments

Corers are unsuitable except with the smallest stones in this size range. With either sampling method 4.2.1 or 4.2.2 loosely attached material may be washed off and it may be necessary to place the stones in a container under water before removing them completely. Associated fine sediments are not removed.

Douglas (1958) does not recommend the use of wire brushes because too much detritus is removed but more recent experience suggests that very vigorous abrasion is required in many localities.

Before the cell suspension, prepared in 4.3.1, is used for cell counts the larger lumps should be gently broken up with a spatula or a low speed homogeniser or ultrasonic probe. This must be done carefully — the object is to reduce the size of the aggregated particles or algal cells, not to macerate the cells themselves.

A stripping technique may be used for removing algae from the surface of submerged objects (Margelef, 1949). The substratum is coated with a solution of collodion and allowed to dry. The collodion is stripped off with the algae adhering and may be examined directly under the microscope. This method is only suitable for thin layers of algae and will also distort or destroy the more delicate algae. Chlorophyll methods cannot be applied subsequently.

Epifluorescence may be used for the direct examination of algae on stones (Jones, 1974). This technique has the advantage that the algae are examined live, *in situ*, where their mutual relationships may be studied. There are, however, three major disadvantages. First, algae must occupy little more than a monolayer or they will obscure each other. Second, the stone surfaces must be fairly flat because the working distances of most high-power objectives are small. Third, only small areas can be examined and the clumped distribution of many algae will introduce a bias only overcome by a long series of random counts.

5 Large Rocks, Bedrock and Man-made Objects

5.1 Introduction

This category includes rocks, masonry, concrete, wooden posts and other man-made structures which are too large to be removed from the river. This method is tentative, taking the most effective ideas of Douglas (1958) and Ertl (1971), but the efficiency is not established. The original methods are illustrated in Figs 1b and 2 and the suggested alternative in Fig. 3.

5.2 Sampling methods

The area to be sampled is delimited by a metal tube approximately 2–5 cm^2 in cross-sectional area (Fig. 3). Another tube approximately 2 cm larger in diameter fits over it and is fixed by bars. The inner casing is narrowed off at the top and a length of wide rubber tubing attached, to prevent too much water passing through the top. Sufficient space must be left at the top to allow free movement of the brush over the sampling area. The brush or scraper is a narrow bore tube with either the scraper or bristles cemented around the hole with epoxy-resin. It fits through the inner casing of the sampler where it narrows to the apex. The sampler is placed over the area to be studied and plasticine or similar material is pressed down between the inner and outer casing. Thick growths are best removed with a rod covered by a rubber sleeve, thinner growths with a nylon brush but the removal of encrusted growth will require the use of a sharp scraper and a stiff wire brush. The scraper must be sharpened frequently. Detached material is removed by applying a vacuum to the specimen tube, which remains out of the water. For full details see Douglas (1958).

5.3 Preparation of the sample for microscopic evaluation

Most of the preparation of a cell suspension has already been accomplished out of necessity because the substrata could not be removed from the water. The material will still contain lumps which must be broken up before cell counting (see 4.5).

5.4 Preparation for chlorophyll analysis

Algal suspensions prepared in 5.2 are filtered directly through glass-fibre filters and extracted in methanol (see 2.4).

5.5 General comments

Errors, due to incomplete removal of crustose algae, are probably more severe than in 4.3.2 and 4.3.3, because of the difficulties of operating under water, and for the same reason it is not possible to test the efficiency of the method. Some material **will always be left behind** but the size of the error will depend on the population type and substrata. Gale

(1975) has recommended the use of a dentist's ultrasonic scaler. It is undoubtedly very effective but it is relatively expensive and slow to use; for general survey work the increased efficiency will probably not be worthwhile.

6 Epiphytic Algae

6.1 Introduction

Sampling of epiphytic algae is complex because of the variable habit of the macrophytes and the environments in which they grow. The nature of the epiphytic growth can vary enormously, from closely adhering material to very loose diffluent growths. Many of these forms are not strictly attached at all but are growing in the protection offered by the larger plants. There is no clear separation between the different forms of epiphytic growth so the investigator must use his own judgement over the suitability and precision of different methods. The nature of the information required will affect the choice of method.

6.2 Sampling methods

6.2.1 This method applies to shallow water (<50 cm) of lake margins or rivers and streams. All the macrophytes are removed from a known area (0.02–0.05 m^2 — see also 4.2) or, if only portions of the macrophyte are removed, results can only be expressed in terms of the surface area of the host. Plants which grow vertically out of the water (e.g. *Berula, Scirpus* and *Hippurus* are readily removed in this way. Trailing plants in shallow, flowing water (e.g. *Ranunculus, Myriophyllum* and *Potamogeton*) are removed with rather more damage to the macrophyte stand. Plants must be detached carefully. The denser the macrophytes, the more likely that loosely attached epiphytes will be lost. It is sometimes more satisfactory to place the sample in a polythene bag before removing it completely from the water.

6.2.2 In deeper waters, or with dense macrophyte stands, it is more satisfactory to remove portions of material (i.e. stems and leaves) from different depths. Densities of the macrophytes should be estimated separately (see another publication to be issued in this series).

A specialist reed cutter has been described by Knudson for use in deeper stands (Fig. 4) but even this instrument is limited to a depth of 0.5–1.0 m because of water clarity. For details of the cutter refer to Knudson (1957).

6.3 Preparation of sample for microscopic evaluation

6.3.1 Proceed through the following steps but do not use more of them than is necessary to remove the epiphytes.

(1) The most loosely attached algae will be washed off with a fine jet of water.

(2) Somewhat more adhering forms will be detached by agitation in a reciprocal shaker.

(3) A nylon brush and/or careful scraping will remove most algae from stiff stems and broad leaves.

(4) More resistant forms are partially removed from the host tissue during immersion in an ultrasonic cleaning bath.

After careful homogenisation to break up clumps of cells without destroying the algae themselves, samples of the suspension may be counted as in 2.3.

6.3.2 Algae may also be counted directly using standard transmission microscopy providing the tissues are thin. This is a tedious process because the algae will be irregularly distributed, and care must be taken that representative areas have been examined. This is only possible by making a small number of counts in a large number of fields chosen at random.

6.4 Preparation for chlorophyll analysis

Algal suspensions prepared in 6.2.2 are filtered directly through glass-fibre filters and extracted in methanol (see 2.4). Care must be taken not to include portions of the macrophyte tissue.

6.5 General comments

The efficiency of these methods is very variable. Bear in mind that the efficiency will vary from macrophyte to macrophyte and throughout the year as the population structure changes. None of these methods is very satisfactory on finely divided leaves (particularly *Myriophyllum* and the submerged leaves of *Ranunculus*).

7 Quantitative Measurement of *Cladophora* in Watercourses

7.1 Introduction

Cladophora is a widely-distributed, usually attached, filamentous green alga. Under some circumstances, it is capable of massive entwined growths with a high nuisance value.

Cladophora forms part of the normal stream flora and its mere presence should not be taken as an indication of enrichment or pollution. In its normal form it is relatively small-celled, multi-branched and forms clumps only a few centimetres in overall dimensions. Virtually unbranched forms may also be found in many situations, including unpolluted waters. Many other filamentous green algae occur in rivers and *Ulothrix, Oedogonium* etc. are unbranched. Where excessive growths occur the filaments may be several metres long, the cells relatively large, branching may be much less frequent and dense mats or skeins as much as 30 m long are formed and may change the number and distribution of other species. Nuisance growths are stimulated by nutrient (especially phosphate) rich conditions (Pitcairn & Hawkes, 1963; Bolas & Lund, 1974). It is usually advisable to record approximate cell dimensions (a range) and frequency of branching as well as the quantity of the alga. For identification, see Bellinger (1974).

These dense mats, known colloquially as "blanket weed" may occur in amenity water and be associated with changes to fauna and flora of the waters. Diurnal fluctuations in oxygen concentration are associated with all plant growth. Excessive growth or the presence of much *Cladophora* will exaggerate these fluctuations and may lead to deoxygenation in the autumn when decay occurs.

Blanket weed, largely consisting of *Cladophora,* may cause problems in water treatment works where uncovered, slow-sand filters are used. Mats of weed, although not always seriously affecting the hydraulic behaviour of the filters, increase cleaning costs. After draining filter beds, the mats have to be removed quickly because, *inter alia,* they represent one of the few natural extra-enteritic situations where *Escherichia coli* (an organism usually indicative of faecal pollution) is known to multiply.

7.2 Sampling of Cladophora

The first two methods have the advantage of minimal disturbance of the growing *Cladophora* and relative speed. Since standing crops can alter rapidly there may often be justification for using a programme of frequent visual measures in preference to a limited number of biomass estimates. Photographic records of growths are a valuable addition to visual estimates and should be kept where practicable.

The third and fourth methods are based on cropping and, although they may be required in some circumstances, they are suitable only when the effects do not invalidate future observations.

7.2.1 Method I. Visual, rapid and very approximate

A preliminary assessment of *Cladophora* in a body of water, when little time is available, may be made from bridges and accessible banks, using visual estimates of the percentage cover of the river bed, together with an approximate estimate of the maximum size of clumps or masses. This method is only roughly quantitative and areas near bridges may be atypical and should be used with caution, even if they are the only readily accessible points.

A suggested scale is as follows:

Present. Observed but less than 10% cover

Frequent. About 10–30% cover

Abundant. About 30–50% cover

Very abundant. 50% or more cover

Clump or skein sizes:

Maximum dimensions under 10 cm. Very small
" " 10–50 cm. Small
" " 50–2000 cm. Moderate
" " over 2000 cm. Large

7.2.2.a Method IIa. Visual

A 50 m length of stream, as typical as possible, should be selected and pegged out at 1 m intervals downstream and ½ m intervals across the stream. If possible, several sites should be selected at random. The network of quadrats produced is defined by wires or thick twine and each is assigned a number. At least 25 such 1 m x ½ m quadrats are then selected using random number tables. The area covered by *Cladophora* within each is estimated visually, or using a ruler, if preferred.

Results are recorded as percentage cover. "Clump" sizes should also be recorded.

7.2.2.b Method IIb. Visual

A 50 m length of stream is divided into quadrats as described in method IIa above.

All quadrats are then examined and the dominant habitat (i.e. gravel, *Cladophora*, macrophyte etc., expressed in terms of percentage of stream bed covered) noted and mapped on a plan of the quadrats.

Results are presented as a histogram showing percentage of quadrats in which *Cladophora* is dominant.

In common with IIa this method is particularly suitable for regular (e.g. fortnightly) measurements showing seasonal changes, growth increments and the effect of spates etc.

7.2.3 Method III. Biomass estimate

Large sampling errors are usually associated with biomass methods and, consequently should be undertaken and interpreted with caution.

Quadrats are defined and selected as in method II above.

Sampling is then carried out from random areas within the quadrats using an Aston Cylinder Sampler (Thorpe & Williams) consisting of a metal cylinder with a serrated base which, when screwed into (i.e. worked into, rather than pushed into) the substratum, encloses an area of 0.1 m^2. Water flows into the sampler through a perforated plate facing upstream and out through a sampling net attached to an opening in the downstream part of the sampler (Fig. 5).

All the *Cladophora* enclosed in the sampler is detached by hand and carried into the net by the flow. It is then transferred from the net to suitable containers for transport to the laboratory and treatment as described in 7.3. Preservation of the algae is aided if the samples are kept cooled and processed the same day.

7.2.4 Method IV. Biomass estimate

The area of watercourse to be sampled is marked off as in method II and at least five quadrats selected using random number tables.

The downstream half of the selected areas is then enclosed by a stop net constructed of Netlon or similar strong plastic mesh with approximately 1 cm openings and the entire *Cladophora* is removed as completely as possible, tearing by hand and working from the downstream end, upstream. *Cladophora* collected in the stop net is gathered and added to the crop.

The biomass of other plants may be estimated in a similar way but should be sampled separately, one species at a time.

Crops collected are transferred to suitable containers for transport to the laboratory and drying with minimum delay (see 7.3).

7.3 Treatment of samples (for 7.2.3 and 7.2.4)

The sample is washed in tap water and large invertebrates and other debris may have to be picked out individually. This is very important and can be time consuming. Where *Cladophora* is mixed with other algae, small random samples are taken for estimates under the microscope of percentage *Cladophora* present.

Samples may be placed in individual nylon or cloth bags and then spun (5 mins) using a domestic spin drier with a cloth lining. The spinning is only intended to remove superficial river water so that the spun weight approximates to a true fresh weight. There should be no drying in the real sense of the word. It is then spread thinly on suitable mesh racks, on which it is dried to constant weight at about 40°C under infra-red lamps, radiant heaters or even convectors. Alternatively, the algae may be dried at 100°C (24 h). These methods are quite different and will give distinctly different values so for any study only one of them should be used. A separate drying room should be used, if possible, because the smell can be considerable. After drying, samples should be kept for 1 h at room temperature in polythene bags (to prevent reabsorption of water) before weighing. The results are recorded as grams per metre^2, percentage biomass, or total crop as appropriate.

Care must be taken that the drying stage is reached and completed as rapidly as possible to avoid decomposition. Thin and even distribution on the drying racks is important in this respect. Subsampling of the spun weed to obtain spun weights and dry weights without drying the whole sample is not recommended. Large errors can be introduced by subsample variation which is only overcome by considerable replication and speed of operation. The procedure should be checked carefully if subsampling is used.

7.4 Appendix

7.4.1 Note regarding *Cladophora* on slow sand filters

The determination of the biomass of *Cladophora* on slow sand filters is a twofold problem. Firstly, it may be useful to know the quantity that is present on a filter that is about to be cleaned. Secondly, it is often useful to know the biomass on a filter that is in operation, and therefore the estimation must be carried out without draining the filter. Blanket weed on slow sand filters is rarely a pure population of *Cladophora* and usually contains a mixture of many species of filamentous algae, and associated epiphytes. Neither the species composition nor the biomass of algae are evenly distributed on slow sand filters and sampling programmes must take this into account.

The principal management problems of blanket weed are related to the mass that has to be raked off the bed before the filter is cleaned by mechanical skimming. This mass may be simply measured by recording the number and size of loads of wet weed that are removed.

If it is necessary to know the spatial distribution of weed over the filter surface, a suggested sampling scheme is as follows. As soon as the filter is drained, samples (0.1 m^2) are cut with a knife and are gathered manually. The number and position of these samples would be chosen on the basis of a grid whose dimensions would depend on the sampler's assessment of a particular filter bed at the time of sampling. Samples should be treated as in 7.3 above.

There is no straightforward way of estimating biomass of blanket weed of a filter bed in operation without adversely affecting the filtering characteristics.

7.5.2 Notes on the quantitative measurement of other filamentous algae

The same principles may be used to assess the cover and biomass of other filamentous algae in rivers. However, most are more delicate than *Cladophora* and more care is needed in cropping and washing. *Vaucheria* is particularly susceptible to damage and cannot be readily washed; sand and silt are frequently trapped between the filaments and will interfere with direct gravimetric estimates.

Similar methods may be used to assess cover in the shallow margins of lakes (see another booklet to be issued in this series) but methods have yet to be developed for quantitative assessment.

8 Artificial Substrata

8.1 Introduction

The difficulties of sampling diverse and uneven surfaces where benthic algae grow have bedevilled investigators for years. Methods, which try to avoid this problem, have been in existence for over 50 years; they involve using artificial substrata on which the algae are allowed to colonize naturally. The most common substrata used are glass microscope slides. These have the advantage that the algae may be examined directly under the microscope. Their disadvantage lies in the possibility that the flora growing over the surface of the glass may differ either qualitatively or quantitatively from the natural microflora. The flora growing on a stable glass slide is more likely to be representative of the flora of a flat stone than a mobile soft sediment or growing submerged macrophytes. Depending on the immersion period the flora may also be more representative of the colonization phase rather than the established flora. Moreover there is evidence that differences and similarities vary with the season and depend on the composition of the flora. Diatoms are usually over-represented, whereas crustose green and blue-green algae are less well represented and filamentous forms, like *Cladophora* and *Vaucheria*, are hardly represented at all.

Other workers have used plastic foils which, after exposure, may be cut up into segments for different analyses. Plastic sheet may also be suitably lacerated to simulate, physically, some macrophytes.

In spite of these difficulties artificial substrata serve a very useful comparative function between water bodies. It is essential, however, that identical experimental techniques are adopted throughout the comparison.

Examples of methods which have been developed are Butcher (1932); Hohn (1954); Patrick, Hohn & Wallace (1954); Pieczyńska (1964); Szczepańska (1967); Backhaus (1968); Friedrich (1973); Klasvik (1974). For reviews of the literature on artificial substrata, investigators are referred to Newcombe (1950); Castenholz (1961); Sládečková (1962) and Sládeček & Sládečkovã (1964).

8.2 Methods

8.2.1 Apparatus, designed for immersion in streams and rivers, must minimize interruption of the current. They should be as flat as possible and posts or rods used to secure them to the river bed should be placed some distance away so that debris which collects does not disturb algae growing on the slides. Slides are removed either at regular time intervals (i.e. at weekly intervals) if the rate of colonization is being studied, or after a longer time, i.e. 30 days (see Friedrich, 1973) or sometimes an even longer immersion period if the established flora is being studied.

Some of the classical work on river ecology was carried out using a simple flat apparatus, holding microscope slides, and secured to the river bed by chains (Butcher, 1931; see Fig. 6B). Multiple slide holders may be used (Friedrich, 1973). Slides are placed in pairs so that when removed they both have one relatively clean surface, making microscopic examination easier. One slide represents the upper surface, the other the lower (Fig. 6D).

Specially designed concrete blocks with the tapering edge facing upstream present far less of a barrier to the current (Klasvik, 1974; see Fig. 6A). Plastic sheets are secured to the concrete with adhesive strips. After sampling, the plastic sheet may be cut up for different analyses.

8.2.2 Catherwood diatometers are specialist racks holding batteries of slides for use in rivers (Patrick, Hohn & Wallace, 1954). The slides are held vertically to prevent the excessive deposition of organic and inorganic detritus (Fig. 6F). There have been many modifications (see for example APHA 1980). In Fig 6F the vertical slide rack is only illustrated symbolically.

8.2.3 In lakes, the slides still have to be secured but the danger and difficulties of current encountered in rivers are minimal. Slides are secured vertically or horizontally at a number of depths (Fig. 6 C & E). Benthic algae are largely confined to the littoral region of the lakes, so care should be taken not to expose the slides too far from the shore. If the epiphytic algae of marginal macrophyte stands are being studied the slides should be supported within the stand; this ensures a local supply of epiphytes for colonization and also ensures that the algae growing on the artificial surfaces are subject to the same light climate as the natural epiphytic populations.

8.3 Removal of artificial substrata

Slides are removed from the holder and placed in a vessel filled with water from the same water body. Samples should be kept cool in transit back to the laboratory.

8.4 Preparation of samples for microscopic evaluation

Some materials may be examined direct. Others will be too thick and will have to be scraped off and dispersed before examination. Plastic or polyethylene substrata may be cut up into portions for different types of examination and analysis.

8.5 Chlorophyll analysis

Generally samples may be immersed directly in methanol to extract the chlorophyll without removing the artificial surface first. It is not advisable to use the same sample for subsequent microscopic examination because only the most robust algae will be recognisable after direct immersion in 100% methanol.

9 General Method for the Estimation of Attached Diatom Populations

Diatoms can be removed from their substratum by carefully controlled treatment with hot dilute hydrochloric acid (Tippett, 1970). The complex carbohydrates which form the mucilaginous and gelatinous attachment materials are hydrolysed before the two silica valves of the diatom separate and before the host plant material breaks up. The advantage of this method is that the diatoms then form a homogeneous suspension and the diatoms, which were either living (or recently so) at the time of sampling, may be distinguished from those which were dead, because the collapsed contents will still be retained within the valves. The optimum concentration of hydrochloric acid is determined:

(1) Make up concentrations of hydrochloric acid between 1 and 5%.

(2) Boil samples of attached algae in a range of those acid concentrations for 10 mins. Cool quickly.

(3) Separate the diatoms from the substrate with a 1 mm mesh sieve. Rinse. Wash the diatoms free of HCl by centrifugation at 1000 g for 5 mins. The diatom material will still contain fine detrital particles.

(4) The optimal acid concentration is determined by inspection of the substrate and the diatom suspension. For future work select that concentration which gives maximum removal from the substrate with minimum destruction of the diatoms.

(5) Diatom numbers can be estimated by standard counting techniques (see another booklet to be published in this series).

(6) Chlorophyll a cannot be estimated because the acid degrades the pigments.

9.1 Appendix

A more sophisticated method has recently been described by Gough & Woelkerling (1976), and involves the shaking of plant material in dilute acetic acid in a series of 45 s treatments. The method is specifically designed for the removal of all epiphytes, but no account is given of potential destruction or distortion of the more delicate species.

9.2 Hazard

Formaldehyde should not be used to preserve the algae because HCl reacts to form volatile bischloromethyl ether or methyl chloromethyl ether ($ClCH_2OCH_2Cl$ and $ClCH_2OCH_3$) which are known carcinogens.

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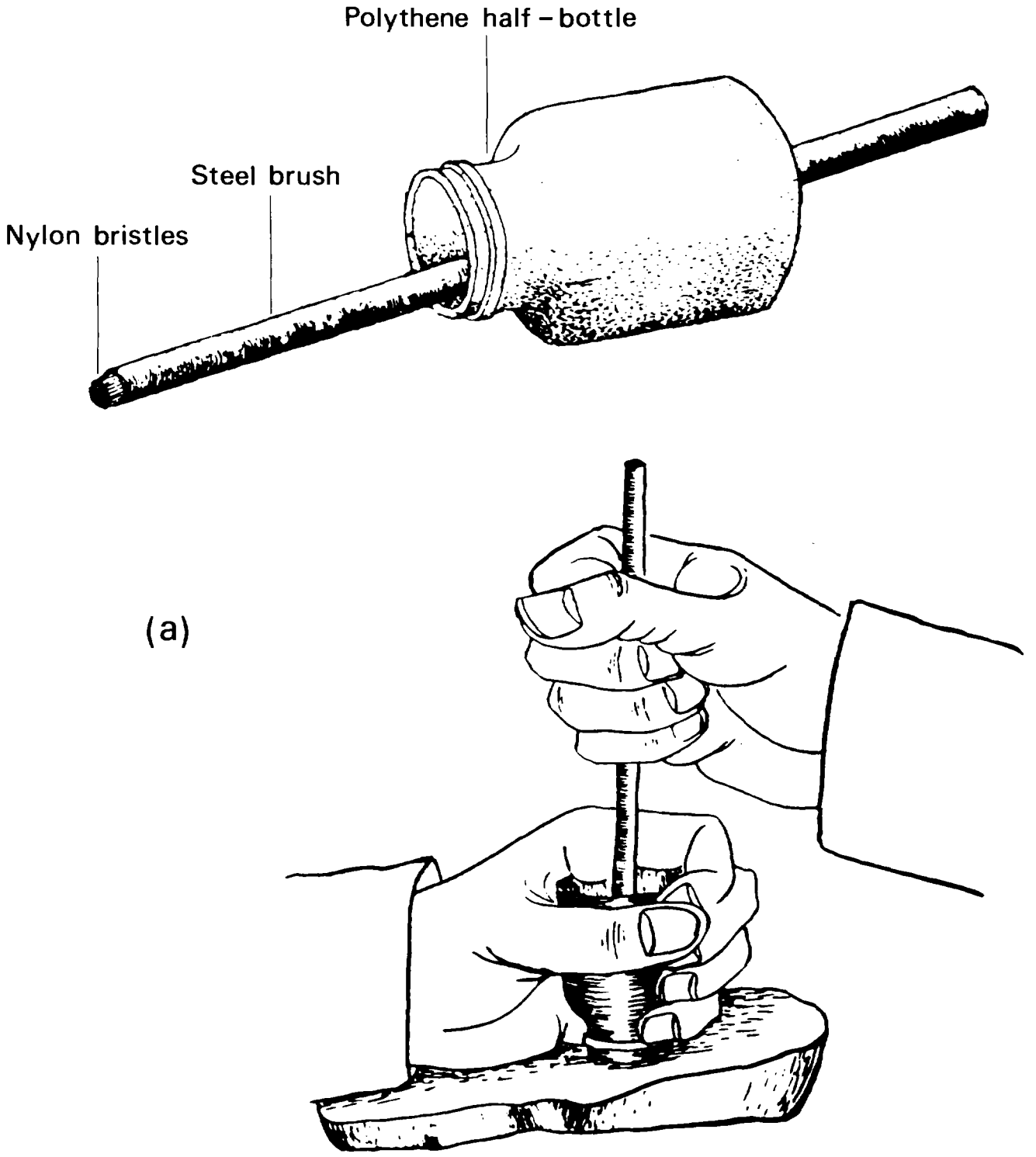
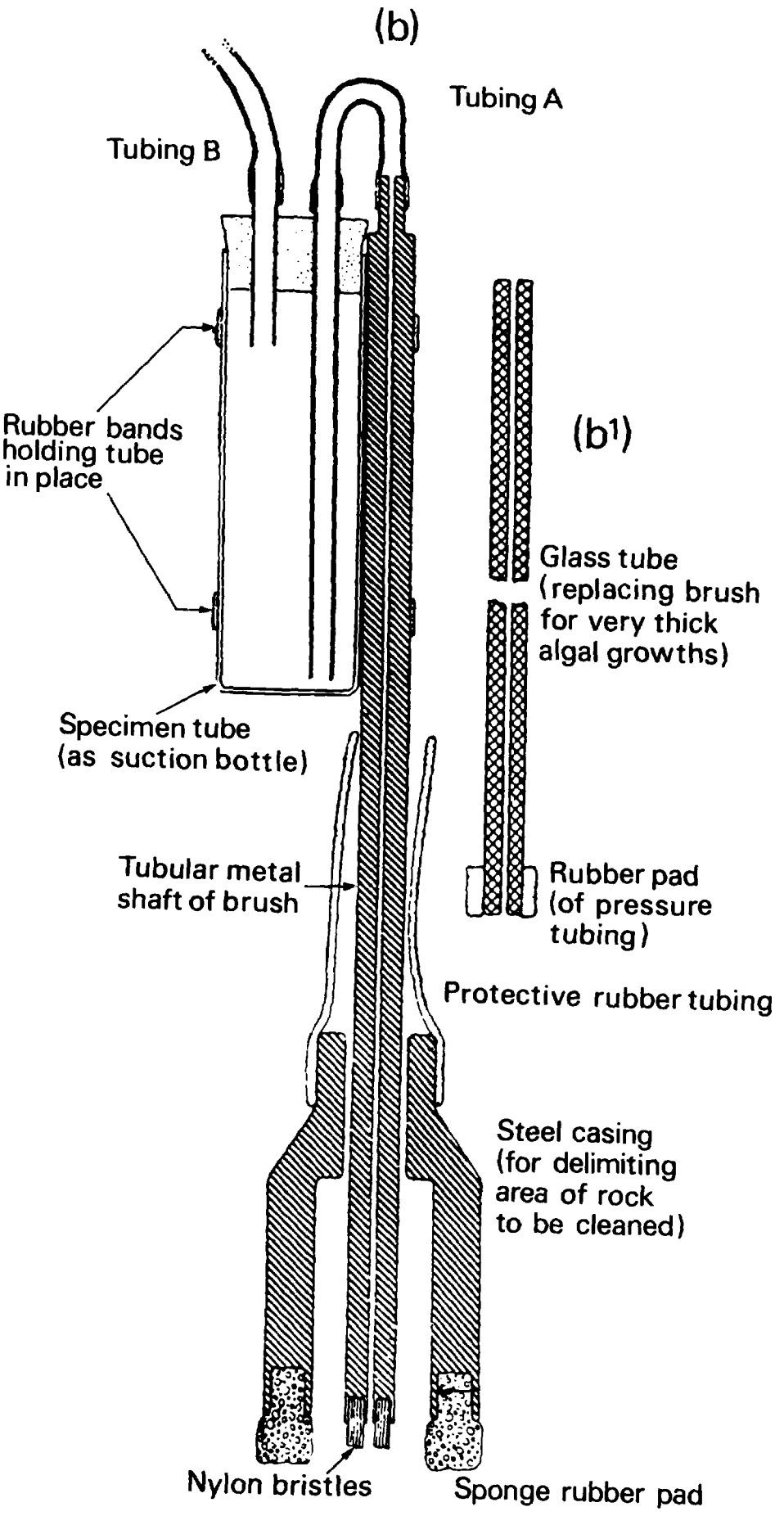


Figure 1 Sampling apparatus for epilithic algae used by Douglas (1958).
(a) The apparatus and technique for sampling a stone removed from the water.



(b) The apparatus for sampling submerged rock, and (b¹) The replacement for the brush for very thick growths.

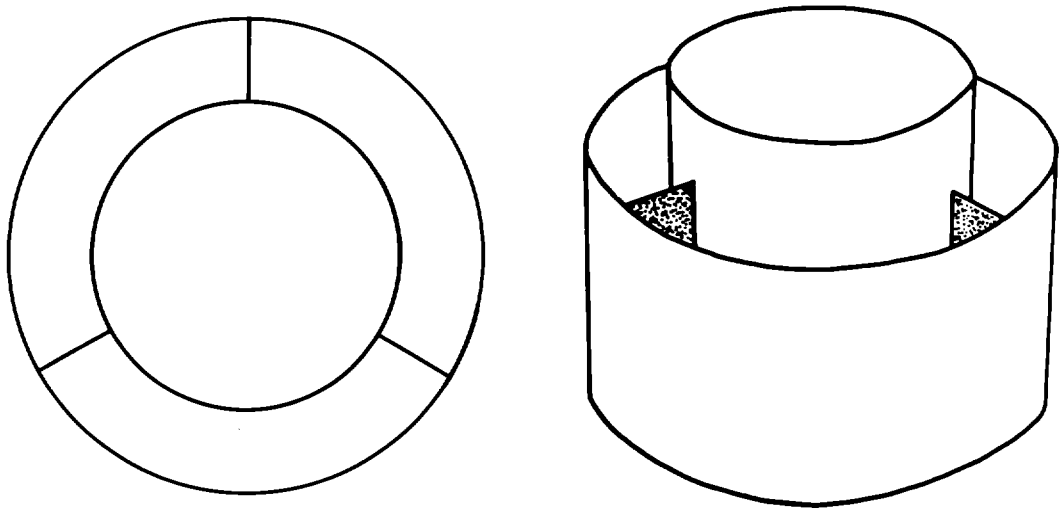


Figure 2 Periphyton sampler used by Ertl (1971).
Typical dimensions
Inner cylinder 1.75 cm radius height 5–10 cm
Outer cylinder 2.75 cm radius and 1 cm shorter
Separation is maintained by 3 bars

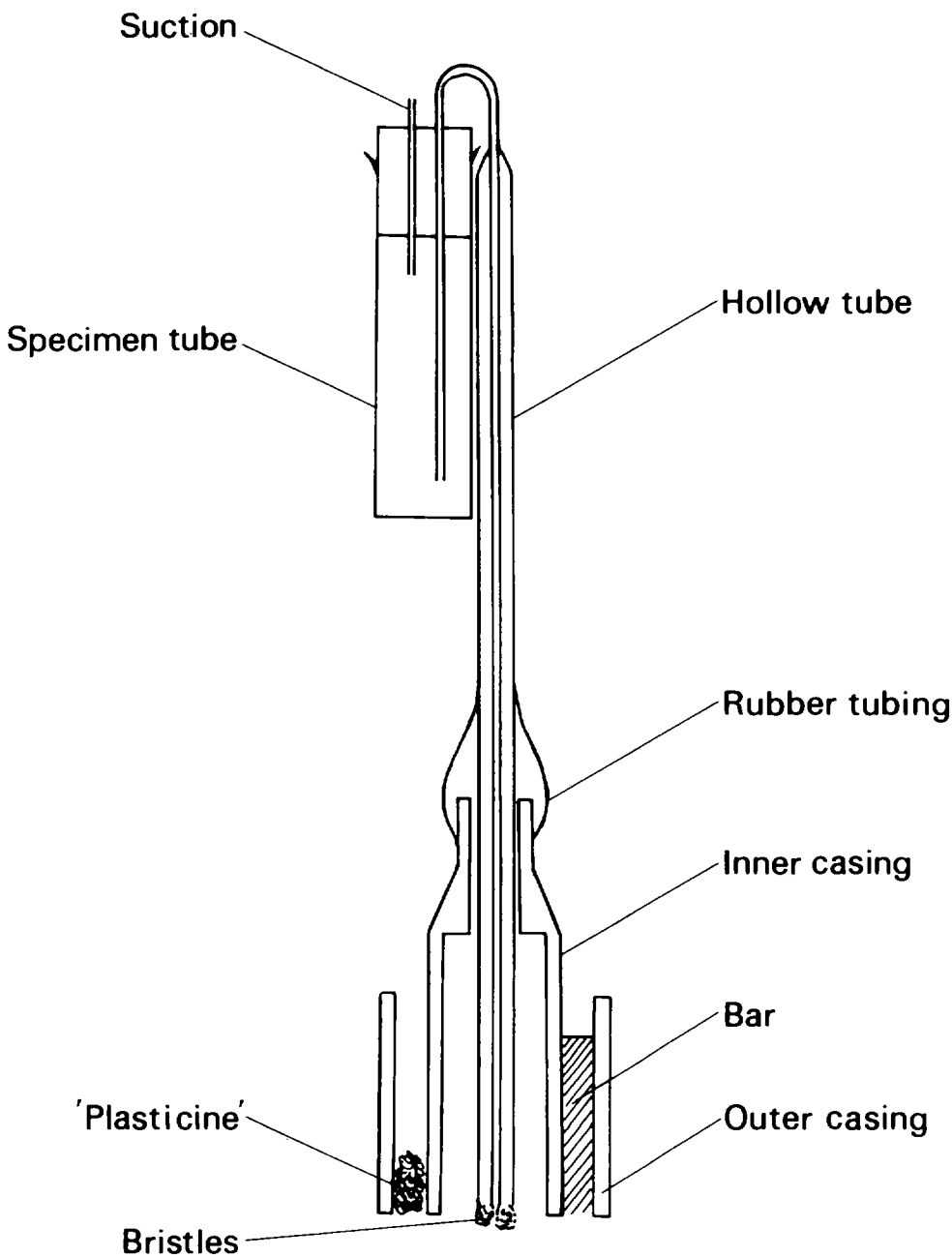


Figure 3 Periphyton sampler for submerged surfaces. After Douglas (1958) and Ertl (1971).

Figure 4 Underwater cutter for removing weeds. (After Knudson, 1957).

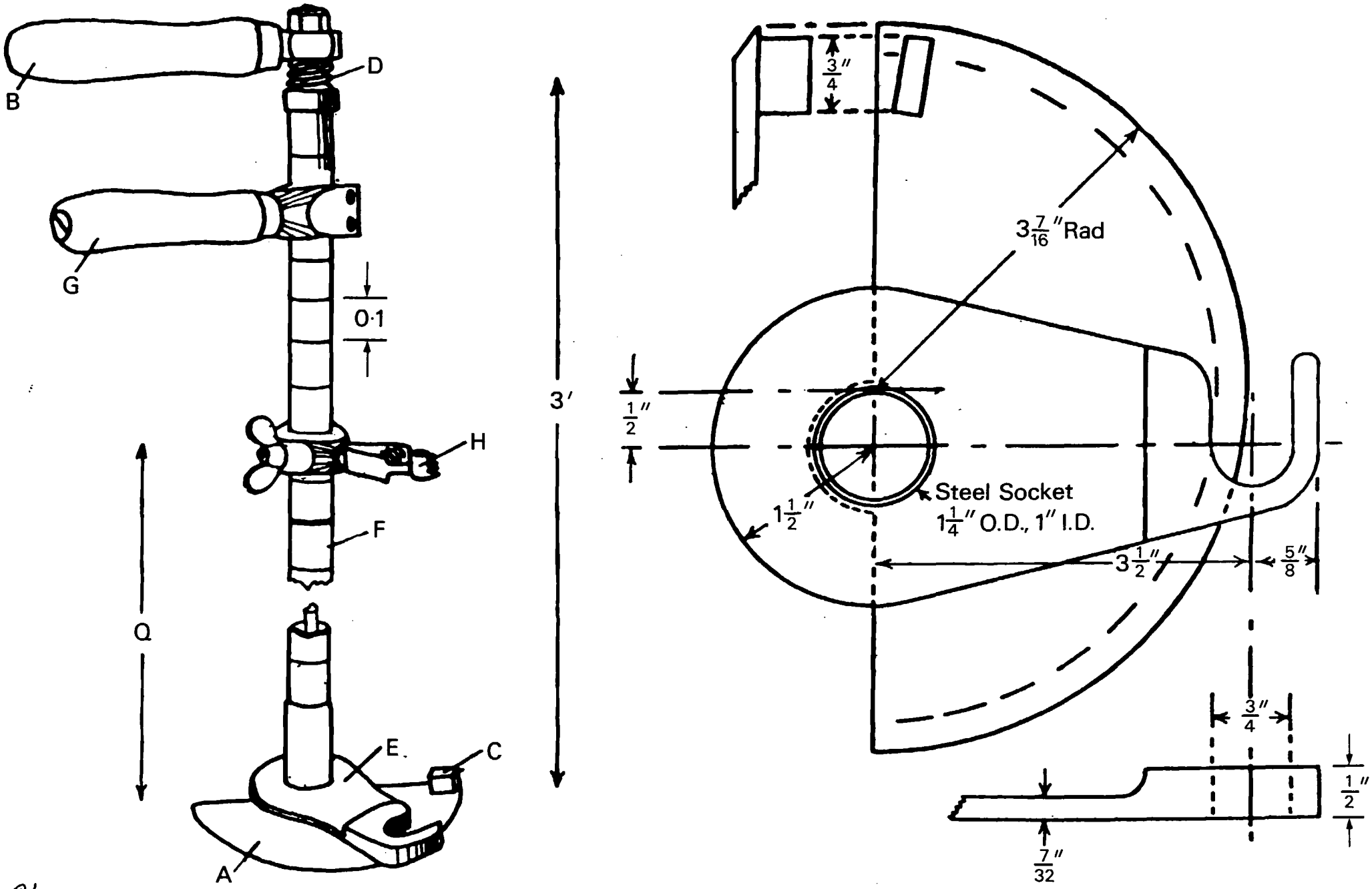
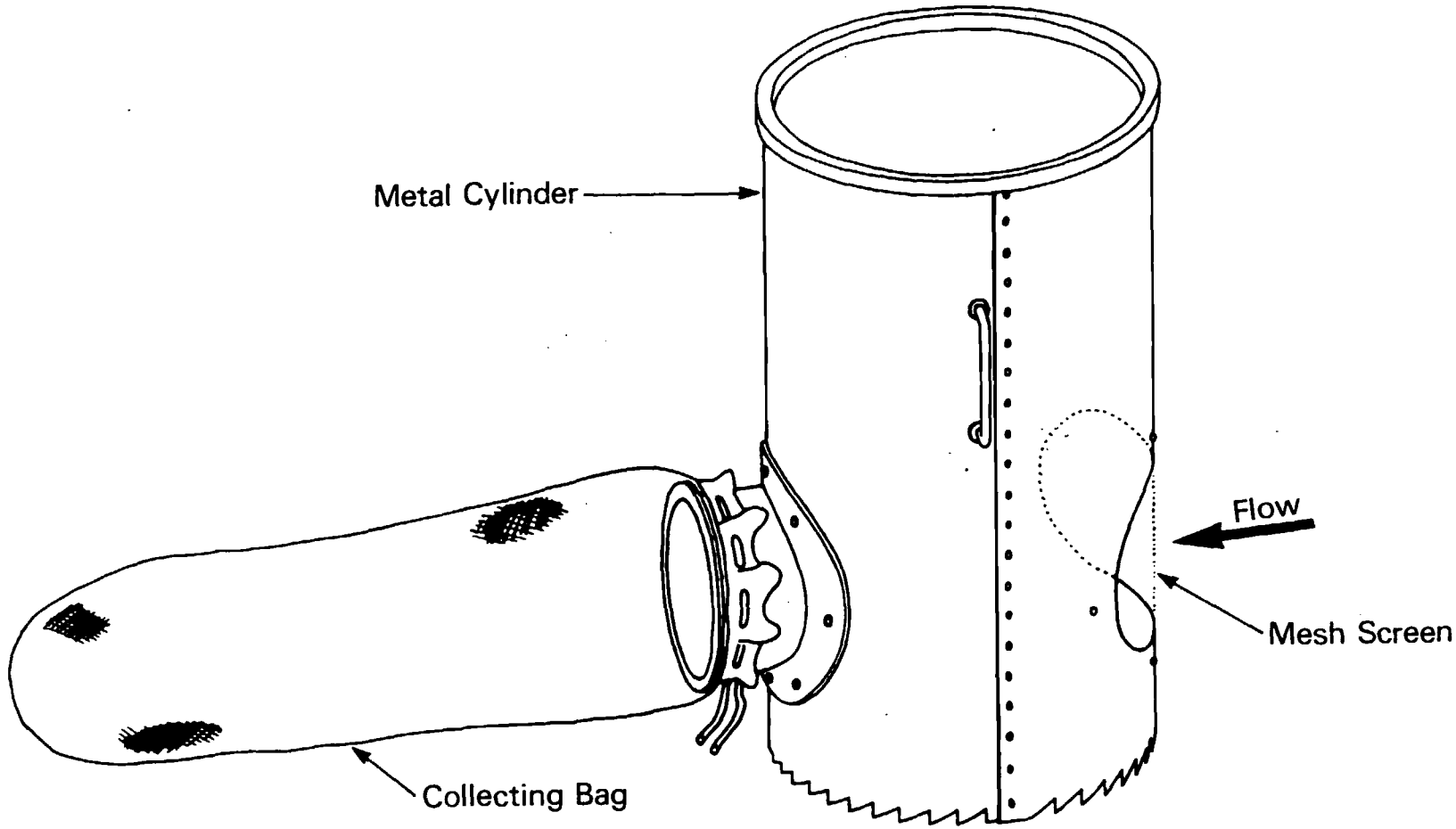


Figure 5 Aston Cylinder Sampler for *Cladophora*. (After Quantitative Sampling for Benthic Macroinvertebrates 1981 in this series.)



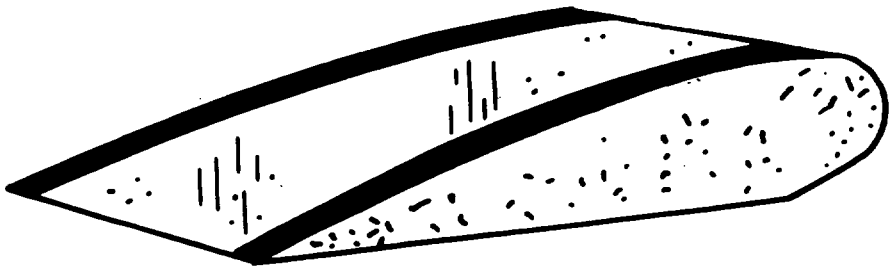
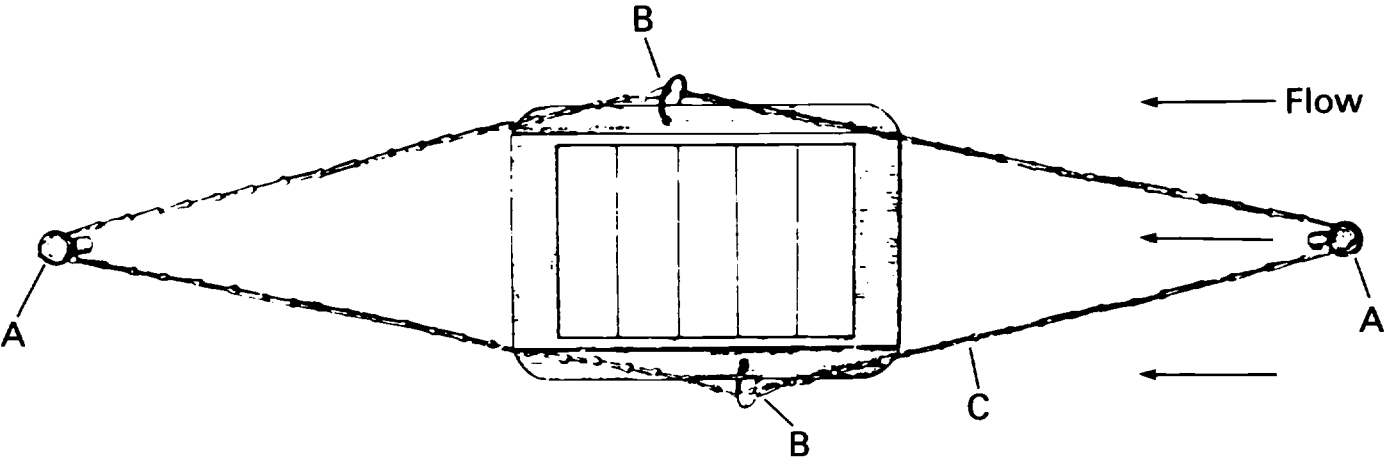
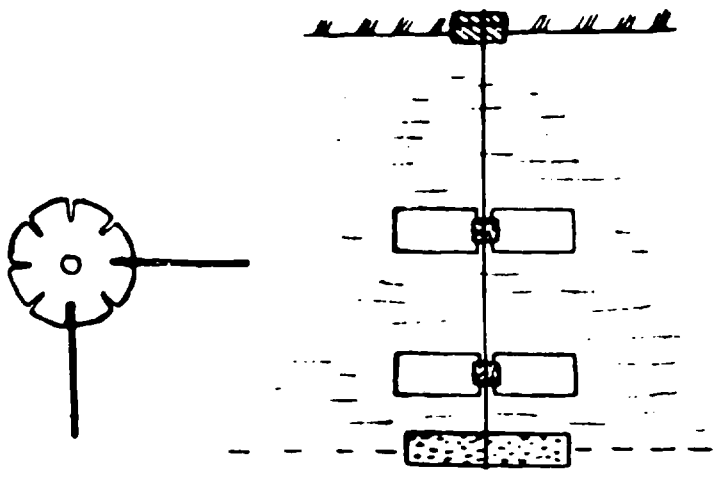


Figure 6 Apparatus used for securing artificial substrata.

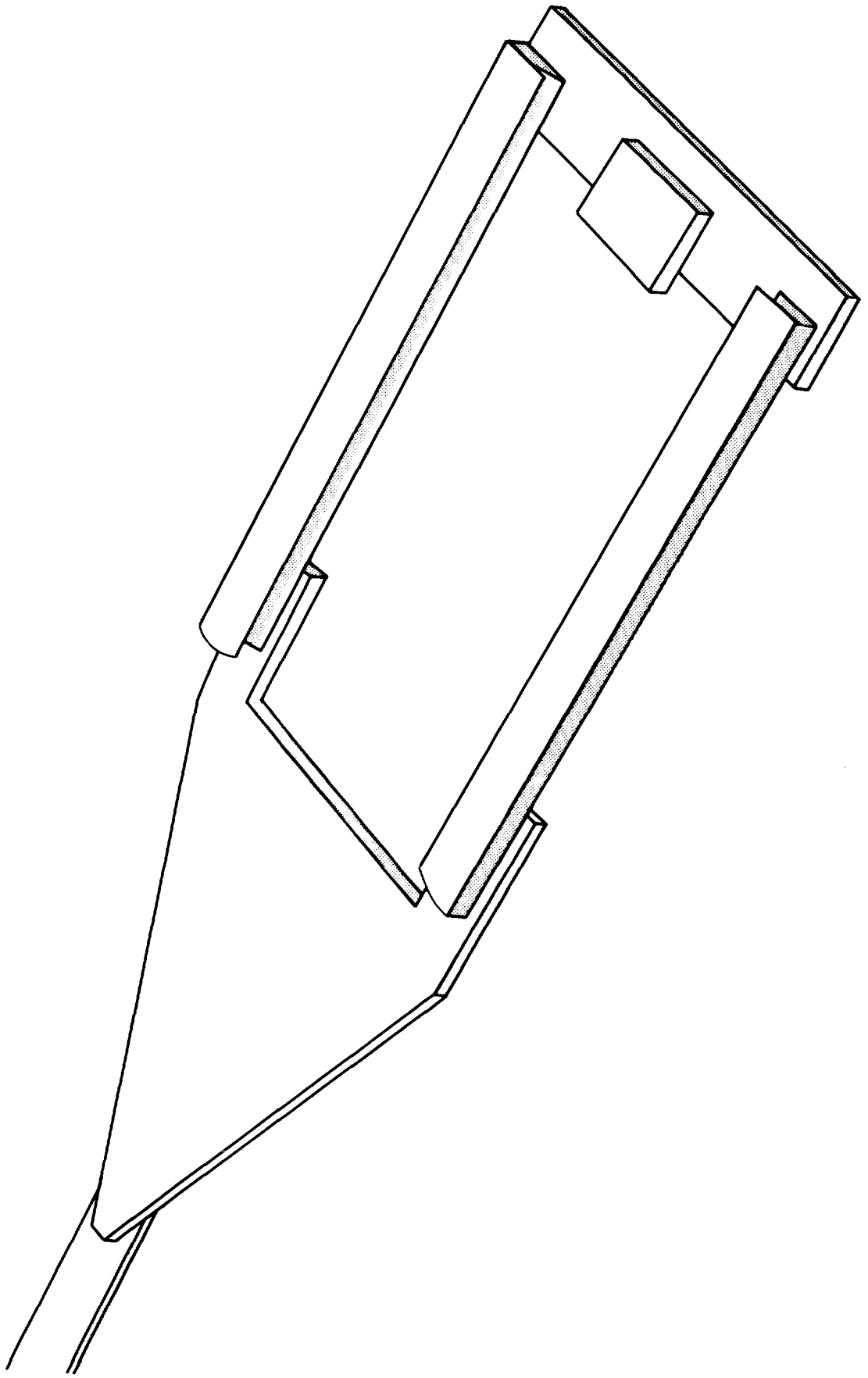
A Concrete block (16 x 22 cm) used to reduce turbulence. The plastic foil is affixed by means of tape (Klasvik, 1974).



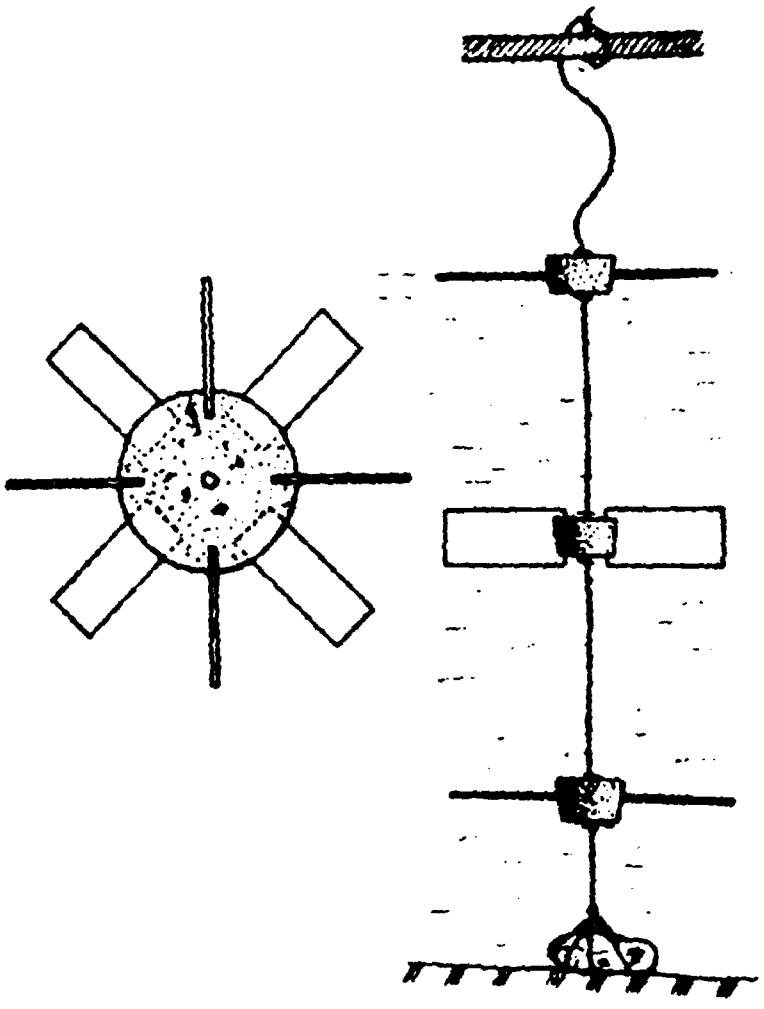
B Frame anchored onto river bed (Butcher, 1931). To hold five slides each 7.6 x 2.6 cm.
a. Iron stakes, b. Spring, c. Brass chain.



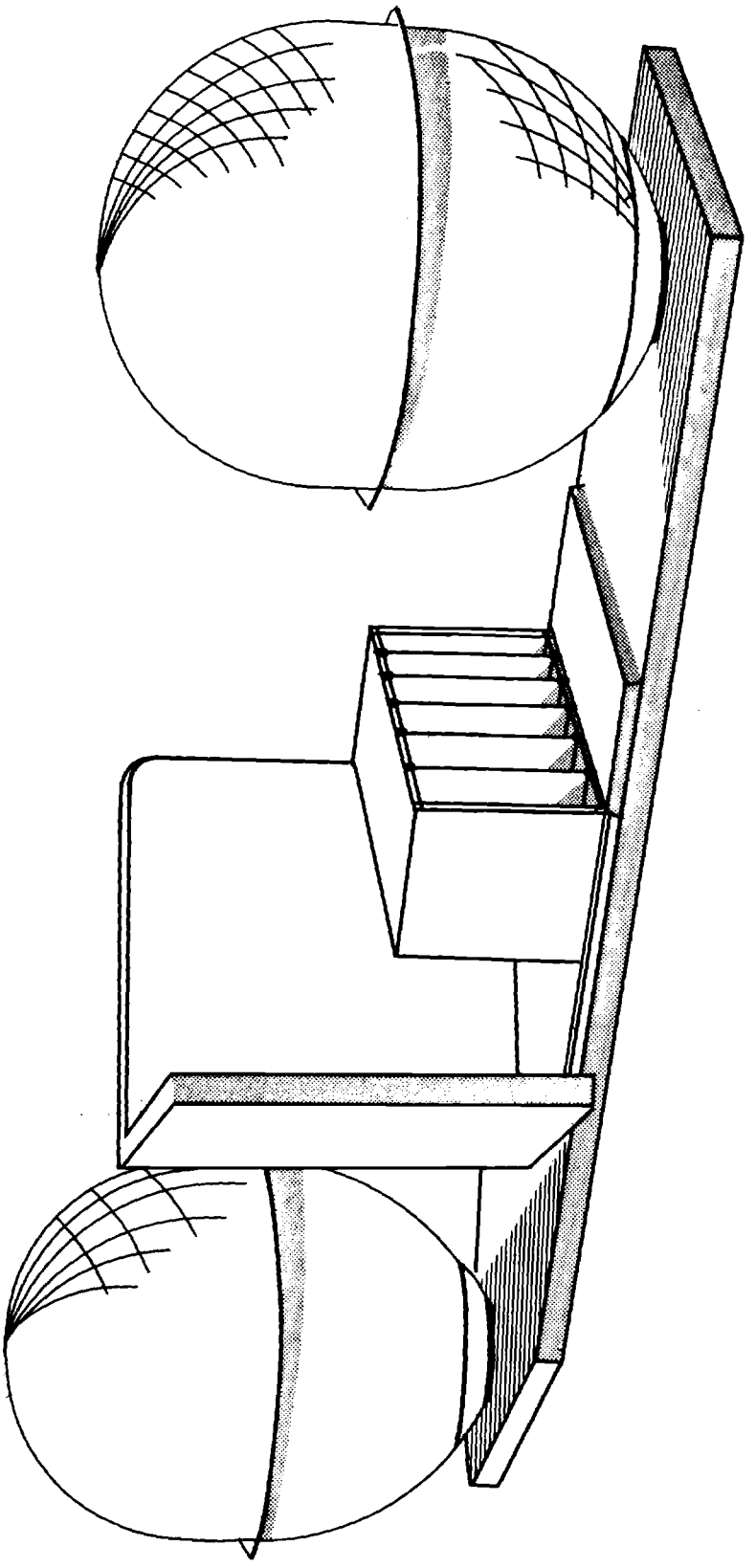
C Apparatus for immersion in standing water bodies (Kusnetsov, 1952, in Sladeckova, 1962). Pieces of rubber, attached to a rope, hold the glass slides (each 7.6 x 2.6 cm). The rope is anchored to the bottom and suspended from a buoy (after Sladeckova, 1962).



D Frame anchored to river bed (Friedrich, 1973).



E Apparatus for immersion in standing water bodies (Sladeckova, 1958) modified from 6C (after Sladeckova, 1962).



F Catherwood diatometer for immersion in rivers (Patrick, Hohn & Wallace, 1954). Immersion depth may be adjusted by float buoyancy.

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