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Techniques of Water-Resources Investigations
of the United States Geological Survey

Chapter A4

METHODS FOR COLLECTION AND ANALYSIS OF AQUATIC BIOLOGICAL AND MICROBIOLOGICAL SAMPLES

L.J. Britton and P.E. Greeson, Editors

This report supersedes TWRI 5A4, published in 1977, entitled "Methods for collection and analysis of aquatic biological and microbiological samples," edited by P.E. Greeson and others.

Revised 1987
Book 5

LABORATORY ANALYSIS

BENTHIC INVERTEBRATES

Introduction

The invertebrate animals inhabiting the bottom of lakes and streams and other water bodies perform essential consumer functions in aquatic ecosystems and serve as food for fish and other vertebrates including man. They are the most frequently used biological indicators of environmental quality. These organisms have the advantages of relatively large size, which facilitates identification; limited mobility, which restricts them to a particular environment; and a lifespan of months or years, which enables adaptation to conditions that have existed for a long period of time. Moreover, many benthic invertebrates inhabit specific types of environments that, if changed, result in changes in the composition of the benthic community (Hynes, 1970). In general, a varied benthic fauna, without excessively large numbers of any one group, is considered to be characteristic of good quality water. As conditions change (for example, in the presence of organic pollution), the number of species decreases, but the number of individuals of the remaining species may increase. Toxic pollutants may eliminate all benthic invertebrates. Thus, knowledge of the kinds and abundance of benthic invertebrates helps to indicate water-quality trends in the aquatic environment. The extensive literature about interpretation of benthic-invertebrate data and water quality has been reviewed by Hynes (1960, 1970), Warren (1971), Cairns and Dickson (1973), Hart and Fuller (1974), and Hellawell (1978).

Collection

Benthic invertebrates vary in size, and there is no clear distinction between the smallest benthic forms and the largest micro-organisms. Bottom-living invertebrates that are visible to the unaided eye commonly are included with the benthos. Because many early studies of the benthic invertebrates emphasized the quantity available for fishfood, the U.S. Standard No. 30 sieve (0.595-μm mesh openings), which retains most of the biomass, came into use (Davis, 1938; Welch, 1948). The No. 30 sieve also has been used in water-quality investigations, and the American Public Health Association and others (1985) states that the bottom-living invertebrates collected for study, termed "macroinvertebrates," are those which are retained on a No. 30 sieve.

The mesh openings of sampling nets and sieves ideally should be selected based on the needs of a particular study. If the mesh size is so large that the smaller invertebrates pass through the net, erroneous conclusions about life cycles or biomass result (Hynes, 1970). Mesh that is too fine clogs rapidly, resulting in loss of invertebrates by backwash. The results of sampling using a coarse and a fine net on the catch of different sizes of a particular benthic species are not easily predictable (Macan, 1963, p. 281). Jónasson (1955, 1958) reports that the diameter of the head determines whether or not a dipteran larva will pass through a given mesh. His data indicated a 640-percent increase in the number of invertebrates in lake samples as the sieve size decreased from 600 to 200 μm. Other investigators have reported similar results from various aquatic environments. Significant differences between retention of total individuals and total taxa in U.S. Standard No. 30 and No. 60 sieves were reported for reservoir silt substrates (Mason and others, 1975). Schwoerbel (1970) concluded that "***in quantitative studies of the bottom, especially in problems of population dynamics in which immature larvae are of importance, a mesh size of less than 200 μm must be used, and in other respects the mesh width must be carefully adapted to the size of the animals selected." In a study of stream benthic sampling, Mundie (1971) reported that the younger (hence smaller) stages of invertebrates tend to predominate in a natural community. He concluded that even a mesh of 116 μm could enable 50 percent of the fauna to pass through, if the community contained large numbers of chironomid larvae and mayfly and stonefly nymphs. Mundie estimated that a net of 200- to 250-μm mesh would enable 70 to 80 percent of the fauna to pass through, but it still would be adequate for many purposes, such as general faunistic surveys and the estimation of biomass.

For these reasons, the U.S. Geological Survey has adopted the U.S. Standard No. 70 sieve (210-μm mesh opening) for retaining benthic-invertebrates collected as part of its water-quality investigations. Nets are to be 210±2-μm mesh-opening nylon or polyester monofilament screen cloth that has 35- to 44-percent open area. For uses requiring more rapid filtration, large-capacity screen cloth, made of 209-μm nylon monofilament, that has 56-percent open area may be used. These mesh sizes are small enough to retain many of the immature stages of the benthic invertebrates and, yet, are practical to use in flowing water. Special studies may require the use of the No. 30 sieve or other mesh sizes appropriate to the objectives. The size of mesh used always should be reported.

The mud usually should be washed from the sample, and this often results in prolonged immersion of the hands in water. During cold weather, wearing long-gauntlet rubber gloves can make this more bearable. To wash mud from the samples, put small quantities into a No. 70 or other appropriate sieve and agitate it gently ensuring that the mesh is

submerged in the water. Washing samples by pouring water through the sieve must be done slowly to avoid forcing small invertebrates through the mesh.

Four methods for benthic-invertebrate sampling are described based on the type of sampling, and three methods for preparation of microscopic mounts needed for taxonomic identification of specific benthic groups are described. Recommended sampling equipment are listed in the "Apparatus" section for the first four methods. For additional information on benthic-invertebrate sampling methods, refer to Welch (1948), Hedgpeth (1957, p. 61-86), Macan (1958), Albrecht (1959), Barnes (1959), Needham and Needham (1962), Cummins (1962, 1966, 1975), Hynes (1964, 1970), Southwood (1966), Schwoerbel (1970), Edmondson and Winberg (1971), Holme and McIntyre (1971), Cairns and Dickson (1973), Weber (1973), Elliott and Tullett (1978), Hellawell (1978), Elliott and others (1980), Elliott and Drake (1981a,b), Cairns (1982), and American Public Health Association and others (1985).

Faunal surveys

Qualitative faunal surveys determine the taxa present and may estimate the relative abundance of each taxon at each site. Because collection of rare taxa at each site is important, sampling should include a large area of bottom and as many habitats as feasible. Use of several collection methods at each site can increase the total number of taxa in the samples (Allan, 1975; Slack and others, 1976). Moreover, evidence indicates that the larger the sample collected for qualitative analysis, the greater the number of taxa (Elliott and Drake, 1981b). A faunal survey of a large sampling area, such as a lake or river, usually precedes a quantitative investigation but may be an end in itself (Elliott, 1971a).

There is no universally accepted method for sampling benthic invertebrates. However, no habitat should be overlooked if the objective is to obtain a representative collection of the benthic invertebrates, and different habitats may require different collection methods. The success of the method will depend on the experience and skill of the collector. Sampling should include specimens from rocks, plant beds, logs and brush, clumps of decaying leaves, and deposits of mud, sand, and organic detritus. In streams, areas of fast current, slow current, backwater, near the banks, and in deeper parts should be sampled. Rocks may be lifted by hand and examined for invertebrates as the surface dries. Tufts of algae and moss should be collected and examined for animals. Invertebrates may be dislodged from floating vegetation or rooted plants using a dip net, or samples of the plants may be collected using grappling hooks or rakes, and then the invertebrates removed. Methods for collecting plants are described in the "Macrophytes" section. More elaborate methods for sampling invertebrates living in or on plants involve enclosing a unit volume of the vegetation and surrounding water in a bag or box from which the invertebrates subsequently are removed (Welch, 1948; Gerking, 1957). Additional information on sampling is given in the "References Cited" at the back of this section.

Two types of collection devices are described: those using netting to concentrate the invertebrates dislodged from the substrate and those involving removal of the substrate. However, any collection method, including quantitative or hand methods, may be used for qualitative collection of benthic invertebrates.

Dip or hand net

The dip, or hand net, is the most useful general implement for collecting benthic invertebrates in wadable water and invertebrates living among floating plants in deeper water. The net can be used in water containing large concentrations of suspended sediment and among plants or large boulders to depths of 1 m or more. Macan (1958) described a method of working slowly upstream lifting rocks and holding the net to catch invertebrates swept into it. Clinging invertebrates were dislodged from rocks by vigorously swirling the rocks in the mouth of the net. Alternatively, the net may be held against the bottom, and the area immediately upstream disturbed by the hands or feet, enabling the current to carry invertebrates into the net. In still water, the net can be scraped rapidly along the bottom to catch easily dislodged invertebrates, or it can be swept through plant beds, probed into piles of brush, or used as a scoop to sample mud, silt, and deposits of leaves or other detritus. Additional information about dip-net sampling is given in the "Numerical Assessment" subsection.

Empty the net frequently either into a shallow, white tray, if the sample is to be sorted onsite, or into a wide-mouth container for transporting to the laboratory. Label and preserve each sample.

Dredges

As described by Hynes (1970, p. 237), dredges are instruments that are pulled across or through the bottom sediment and grabs are instruments that bite into the bottom from above. Grabs are considered to be quantitative sampling devices and are described in the "Distribution and Abundance" subsection.

Qualitative samples of benthic invertebrates from deep or swift rivers usually are collected using a dredge (Elliott and Drake, 1981b) (figs. 21, 22). The design varies, but often, large rocks are excluded; whereas, the smaller particles and the benthic invertebrates are retained in a mesh bag. The dredges developed by Usinger and Needham (1956) and Fast (1968) are examples. Dredges are lowered from a boat or bridge or even thrown from a high bank then pulled upstream along the bottom so the leading edge digs into and disturbs the sediment. The current from the flow of the stream plus the forward motion of the dredge carries invertebrates into the net. In still or slowly moving water, dredges should be pulled by a powered boat to prevent loss of active benthic invertebrates.

Elliott and Drake (1981b) compared four light-weight dredges for river sampling. Because of the variability between sampling units in the same sample, there was a lack of precision in estimates of the mean number of individuals indicating that the dredges are not suitable for quantitative sampling. Considerable variation also existed in their effectiveness as qualitative samplers for estimating the total number of taxa per sample. The largest efficiencies for a small sample ($n=5$) were for the medium (greater than 57 percent) and large (greater than 76 percent) dredges (called Naturalist's dredges) similar in design to that shown in figure 22. The mouth of the medium dredge was 45×17 cm and for the large version was 59×20 cm. Greater penetration depth into the substratum (range in modal particle sizes was 1–2 mm, 64–128 mm, and 128–256 mm) accounted for the superior performance of the Naturalist's dredges compared to the other types tested.

After collection, empty the dredge into a shallow tray or bucket, if the collection is to be sorted onsite, or into a wide-mouth container for transporting to the laboratory. Label and preserve each collection.

Numerical assessment

Relative or semiquantitative surveys are conducted to compare benthic communities or populations at a specific site for different sampling times or at different sites for the same sampling time. That is, the objective is to make within- or between-site comparisons. Accurate measurements of the total benthos are not obtained, nor are the estimates of relative abundance of each species in the samples necessarily reliable. Sampling effort is limited and, if using artificial substrates, may be restricted to a small area at each site. Because different sampling methods will produce different results, the

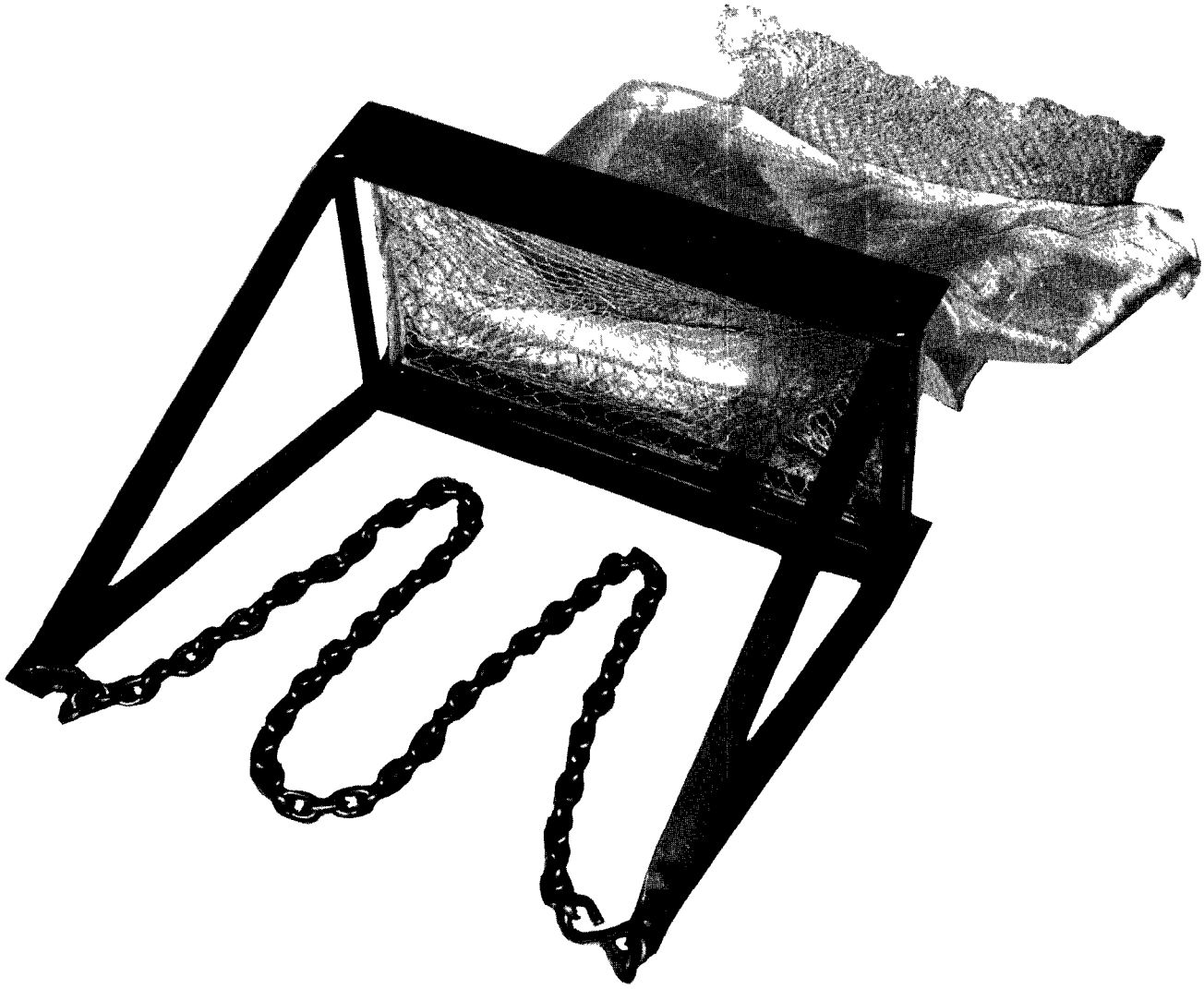


Figure 21.—Biological dredge. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)

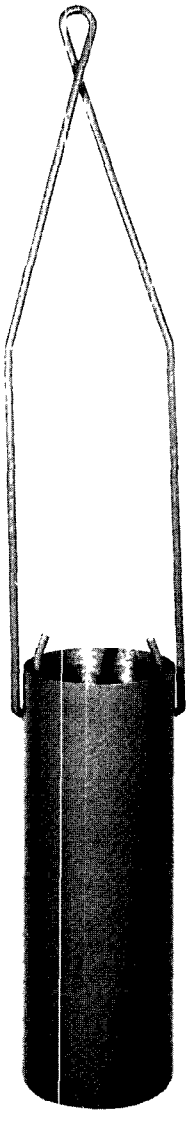


Figure 22.—Pipe dredge. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)

methods and sampling areas should be as uniform as possible throughout a study.

The statistical principles of benthic-invertebrate sampling are discussed by Elliott (1971a). The first requirement is a clear definition of the objectives of the study and the area to be sampled. The frequency of sampling may range from weekly, in detailed studies, to once a year, in general studies. When artificial substrates are used, sufficient time must be allowed for invertebrate colonization. Two sampling procedures using a dip net, one procedure involving collection of individual rocks, and three procedures using artificial substrates are described in the following subsections.

Dip or hand net

A dip, or hand, net is a mesh bag mounted on a metal rim that has an attached handle. It is a simple, effective sampling device for water less than 1 m deep and even may be effective in deeper water for sampling plant beds and other near-surface habitats. The dip net used in a standardized way will provide a numerical assessment of the differences between sampling sites in wadable water. Two general approaches are used, one in which the collector sweeps the net through the major aquatic habitats (Slack and others, 1976; Armitage and others, 1981) and one in which the net is held stationary while the substratum is disturbed with the feet (Hynes, 1961; Morgan and Egglishaw, 1965; Frost and others, 1971; Armitage and others, 1974). The latter method is restricted to streams. The collecting approach used and the effort expended will depend on the size and variability of the sampling area and on the study objectives. Using the moving-net method, the most abundant species may be sampled adequately within 5 or 10 minutes by an experienced biologist. In a river study, Armitage and others (1981) reported that a 3-minute dip-net sample collected about 62 percent of the families and 50 percent of the species that were collected during an 18-minute sample. Slack and others (1976) reported that a 45-minute dip-net sample contained the largest percentage of taxa (78 percent) and the second largest percentage of individuals (41 percent) in a comparison of three collecting methods. Generally, collecting continues for at least 30 minutes in streams as much as 15 m wide and continues for an additional 30 minutes for each 15-m increase in width. Macan (1958) described a method of working slowly upstream, lifting rocks, and holding the net to catch invertebrates swept into it; clinging invertebrates were dislodged from rocks by vigorously swirling the rocks in the mouth of the net. In still water, the net can be scraped rapidly along the bottom to catch easily dislodged invertebrates, or it can be swept through beds of attached or floating plants, probed into piles of brush, or used as a scoop to sample mud, silt, and deposits of leaves or other detritus. The collecting effort and technique must be kept as uniform as possible during a particular study. Empty the dip net frequently to avoid clogging the mesh, which can cause a backwash that would result in loss of sample.

A rapid and versatile method for sampling consists of holding the flat side of a D- or triangular-shaped dip net firmly against the streambed, facing upstream and disturbing the stream bottom for a definite distance (about 0.5 m) just upstream from the net by vigorously kicking three or four times into the bed in an upstream direction (Hynes, 1961; Morgan and Egglishaw, 1965). A proportion of the dislodged invertebrates and detritus will be carried into the net by the current; the kicks should be separated by several seconds to enable this to occur. The method can be used for a variety of substrates from sand to rocks that have a diameter of 45 to 60 cm in weedbeds, or on bedrock using the boot as a scraper. The method has been evaluated by Frost and others (1971) and Armitage and others (1974). The minimum procedure, modified from Morgan and Egglishaw (1965), is to take three (four-kick) samples in a reach of stream: one in

a riffle, one in a pool, and one in a position where conditions are intermediate between the other two sites. The minimum-procedure sites should not be near the banks and should be representative of the habitat; that is, select eroding areas in riffles and depositing areas in pools. Sampling may be increased or modified depending on the physical characteristics of the habitat and the study objectives, but it is important that the technique and net design be uniform throughout a study. Empty the dip net, after each series of kickings, into a shallow tray or bucket, if the collection is to be sorted onsite, or into a wide-mouth container for transporting to the laboratory. Label and preserve each collection.

Individual rocks

Because many benthic invertebrates from shallow streams or rocky shores of lakes live on or beneath rocks, a sampling method that involves lifting individual rocks and collecting the associated invertebrates was developed (Macan, 1958; Schwoerbel, 1970). The method consists of three procedures: selection of rocks, collection of rocks, and reporting of results. Because the number of benthic invertebrates per unit of rock area may vary with rock size (Lium, 1974), rocks of similar size should be collected for samples that are to be compared. In gravel-bed streams studied by Lium (1974), greatest invertebrate densities occurred on rocks between 45- and 90-mm mean diameter. As with other methods, the study objectives are decisive in selection of the sampling method and its application. Depending on the objectives, sampling may comprise 10, 20, or more individual rocks from a single habitat (for example, riffles) or from each of several habitats (for example, pools and riffles). Statistical techniques may be used to ensure random collection of rocks from each habitat.

The simplest collection procedure is to pick a rock at random, lift it gently off the substratum, quickly enclose the rock in a net of appropriate mesh size, and lift the net, rock, and associated invertebrates out of the water. This procedure is repeated until the desired number of rocks has been collected. A better method for rock collection is using the Lium sampler (fig. 23), which was designed to catch invertebrates that wash off a rock as it is lifted from the streambed. With the sampler opening facing upstream, approach the selected rock from the downstream side. Place the hood of the sampler over the rock, and press down to compress the flexible base against the streambed. The flexible base minimizes losses from around the edges of the sampler, and the hood minimizes outwash of invertebrates during rock removal. Invertebrates that are dislodged as the rock is lifted are carried by the current into the screen. Remove invertebrates trapped on the screen by inverting the sampler and washing them into a bucket. During each method of rock collection, scrub each rock thoroughly in a bucket of water using a soft-bristle brush to remove clinging invertebrates. Pour the contents of the bucket through a U.S. Standard No. 70 sieve. Empty the sieve into a shallow, white tray, if the sample is to be sorted onsite, or into a wide-mouth container for transporting to the laboratory. Label and preserve each collection.

If the results are to be reported as areal units, rock sizes must be determined. To report the population in terms of the projected area of rock, measure and record the two longest straight-line dimensions of each rock (A and B axes), in millimeters. To report the population in terms of total rock surface, measure each rock, in millimeters, across the B or intermediate axis (Leopold, 1970; Lium, 1974). The B axis, or breadth, is distinguished from the major axis (A, or length) and the minor axis (C, or width).

Artificial substrates

An artificial substrate is defined by Cairns (1982) "***as a device placed in an aquatic ecosystem to study colonization by indigenous organisms. Although the device may be unnatural in composition, location, or both, most of the biological processes that occur on it appear to be quite similar to those occurring on natural substrates." Many types of standardized, reproducible surfaces are used as collection devices for colonization by benthic invertebrates (Beak and others, 1973; Hellawell, 1978; Cairns, 1982). The uniform shape and texture of artificial substrates greatly simplifies sampling when correctly used. Standardized sampling is especially desirable when the results from different investigators or from different environments are to be compared.

Artificial substrates have been used to investigate various problems in benthic population and community ecology, including organism-substrate relations, community structure and distribution, and island colonization. Artificial substrates also have been widely used in marine fouling studies and for sampling benthic invertebrates in stream-quality programs. Generally, the objectives are: (1) To determine the

Figure 23.—Lium sampler.

composition of the resident benthic community, (2) to collect representative and reproducible samples of benthic invertebrates for areal or temporal comparisons, or (3) to determine rates of species or biomass accrual.

Selection of an artificial substrate sampler and its method of exposure are determined by study objectives and the nature of the environment. Rosenberg and Resh (1982) distinguish between representative artificial substrates (RAS) and standardized artificial substrates (SAS). RAS are samplers that closely resemble the natural substrate over, on, or within which they are placed, such as a basket filled with rocks similar in size distribution to the natural stream bottom. SAS are samplers that differ from the natural substrate of the habitat in which they are placed, such as a multiple-plate sampler. If the objective is to relate the quality of flowing water to the composition of the benthic community, off-bottom exposure may be preferred. Suspension of the samplers within the water column eliminates the effects of bottom conditions that can mask the effects of water composition that serves as a control on benthic community structure (Mason and others, 1973). If the objective is to sample the resident fauna or to evaluate the effects of sediment properties on invertebrate communities, bottom exposure is necessary (Voshell and Simmons, 1977). Before deciding on an artificial-substrate method, onsite tests should be made to compare the relative effectiveness of different samplers and exposures in the habitat to be studied.

Colonization of artificial substrates, reported as biomass or numbers of individuals or species, normally increases rapidly at first then reaches a relatively stable or fluctuating equilibrium level (Rosenberg and Resh, 1982). Colonization rate and biomass vary seasonally, such as being slower in winter than in summer. For monitoring purposes, samplers should be retrieved during the equilibrium phase. The time required to reach equilibrium in 20 studies summarized by Rosenberg and Resh (1982) ranged from 3 to 49 days, but for most studies did not exceed 30 days. Until the colonization process is better understood, preliminary onsite tests should be made to determine optimum exposures for each study.

It is important to prevent losses of invertebrates during sampler retrieval. Many invertebrates leave artificial substrates as soon as they are disturbed. Rabini and Gibbs (1978) reported large losses of invertebrates from barbecue-basket samplers during removal by divers, and McDaniel (1974) reported some loss of invertebrates when retrieving multiple-plate samplers from deep water. Voshell and Simmons (1977) maintained that loss of invertebrates during sample collection and sampler retrieval was a factor contributing to variability among bottom samples in a reservoir. When retrieving a sampler from shallow water, approach from downstream and enclose the entire sampler in a net of appropriate mesh size to catch invertebrates that would be lost when the sampler is lifted from the water. Artificial substrates exposed in deep water should be designed to retain invertebrates that drop off the sampler during retrieval. When retrieved, empty or disassemble the sampler into a tub partially filled with water. Scrub all parts using a soft-bristle brush to remove clinging invertebrates. Pour the contents of the tub through a sieve of appropriate mesh size and add the invertebrates detached from the sampler during recovery. The sampler also may be placed into a container of preservative and transported to the laboratory for cleaning. Cleaned samplers may be reused unless there is reason to believe that contamination by toxicants or oils has occurred (Weber, 1973). Do not reuse rocks or hardboard plates that have been exposed to preservative.

Multiple-plate sampler

This sampler is a jumbo modification (Fullner, 1971) (fig. 24) and is the smallest and most adaptable of the artificial-substrate devices. These samplers are relatively inconspicuous by virtue of size and color, and the modest cost enables replication to further enhance the chances of recovery in small bodies of water where the samplers might be subject to vandalism. Attach multiple-plate samplers to floats, structures, weights, or rods driven into the streambed or lakebed. Install three samplers so they will remain submerged, and leave them to be colonized for the experimentally determined exposure period or for 4 to 5 weeks. Record the exposure time, which should be consistent among sites during a study.

The samplers may be installed in pools or riffles and on the bottom or suspended above it, but the macrohabitat should

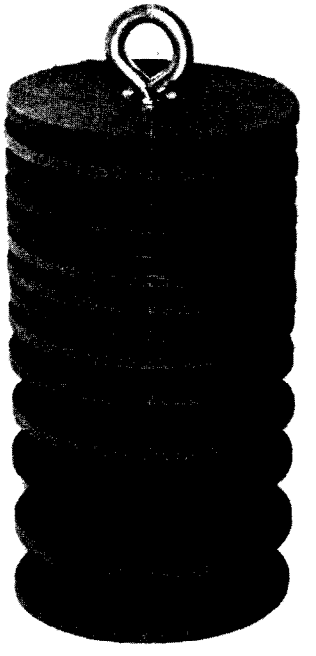


Figure 24.—Jumbo multiple-plate artificial-substrate sampler. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)

be as uniform as possible at all sites during a study. Usually samplers are installed on the bottom in riffles as much as 1 m deep. Make the collections as representative of the reach as possible by ensuring that the samplers are in eroding areas that are not close to the bank. In streams as much as a few meters in width, install the devices about midstream; in wider streams, install the devices about one-quarter of the total width from the nearest bank. In larger rivers or in lakes, the samplers usually are suspended from floats (fig. 25). When a float is used to suspend more than one sampler and the samples are to be kept separate, enclose each sampler in a retrieval net (fig. 26) to avoid loss of invertebrates when retrieving. It is necessary to reach into the water and gently pull a retrieval net over each sampler, securing the net by tightening the drawstring just above the top of the eyebolt that holds the sampler to the float rod. Enclose all multiple-plate samplers on the float before proceeding with substrate removal. When all the nets are in place, detach the samplers from the float. If only one sampler is used or if the results of multiple samplers are to be pooled, a dip net of appropriate size and mesh may be used to enclose the sampler(s) during recovery.

Barbecue-basket sampler

This sampler (fig. 27) is adapted for use in lakes and large rivers. Fill the basket with 30 rocks, 5 to 7.5 cm in diameter, and secure the sampler door using wire or small cable clamps. The rocks used to fill a series of samplers should be of the same general size, shape, and composition and should be cleaned by scrubbing with a brush before use.

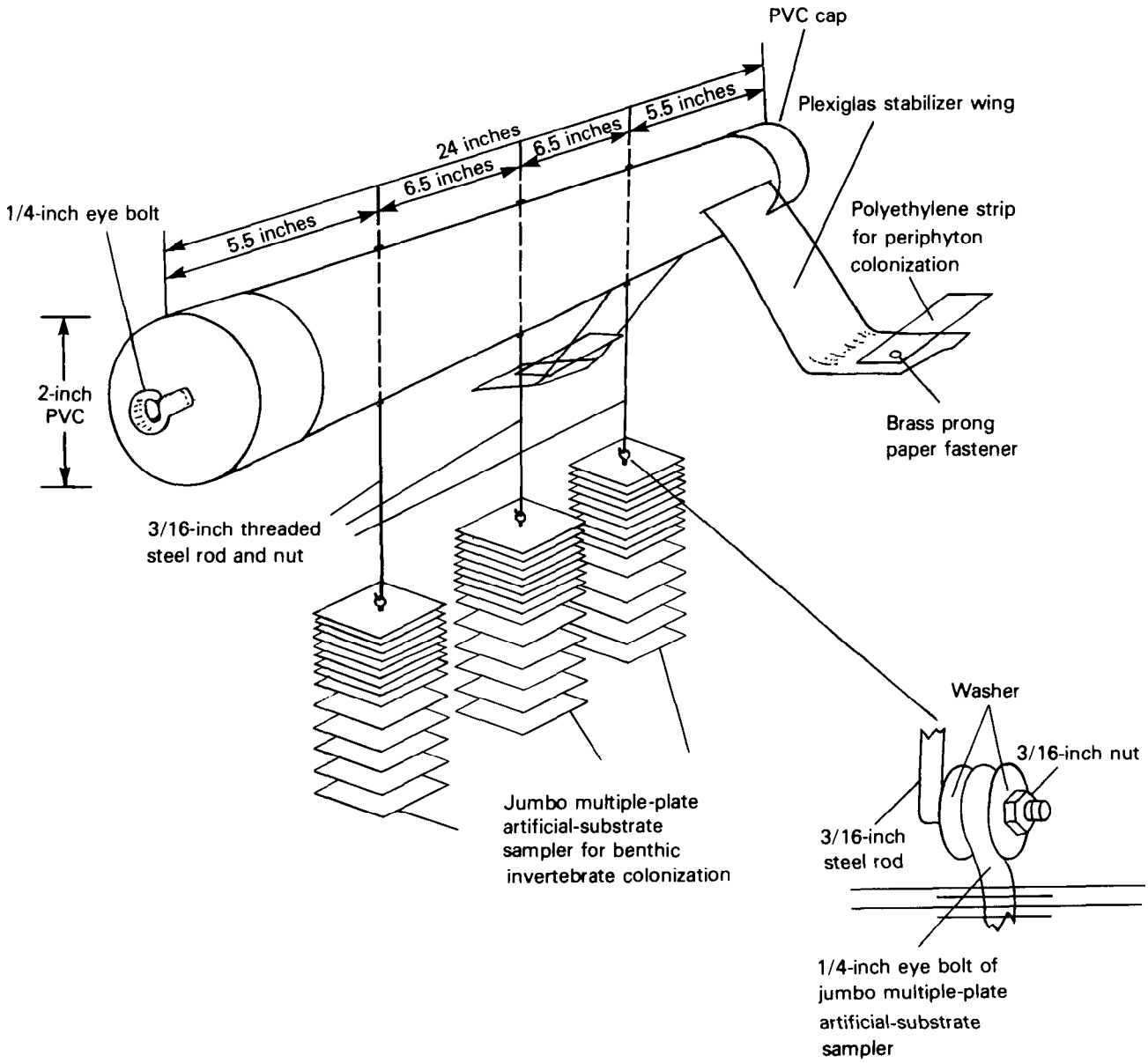


Figure 25.—Float for artificial substrates.

Angular limestone commonly is used in barbecue-basket samplers, although spheres of porcelain or concrete provide a more uniform substrate (Jacobi, 1971). Coniferous tree bark has been used as a lightweight substitute for rocks (Bergersen and Galat, 1975; Newlon and Rabe, 1977).

If possible, suspend three samplers at a depth of 0.3 m below the surface for the experimentally determined exposure period or for 4 to 5 weeks. In environments of variable depth, suspend the samplers from a float. Barbecue-basket samplers also may be installed on the bottom in shallow or deep water, but the macrohabitat, depth, and exposure period must be uniform throughout a given study. Samplers must be protected from loss of invertebrates during retrieval. Samplers exposed in deep water may be enclosed in a retrieval net and brought to the surface by divers, or a net can be mounted on a rectangular frame so the net collapses on the natural substrate during colonization, but lifts to enclose the basket during retrieval.

Collapsible-basket sampler

This sampler (fig. 28) is used if the objective is to compare sampler catches with the population of a surrounding rocky substrate. The basket can be loaded with materials simulating the natural bed on which it lies. This sampler is useful for lakes, shallow streams, or for deep, swift rivers. The sampler consists of a collapsible basket holding gravel or rocks and is surrounded by a nylon netting bag of appropriate mesh. A rim around the top helps retain the gravel. When lowered to the bottom, the basket collapses to form an area of gravel that is subsequently colonized. When raised

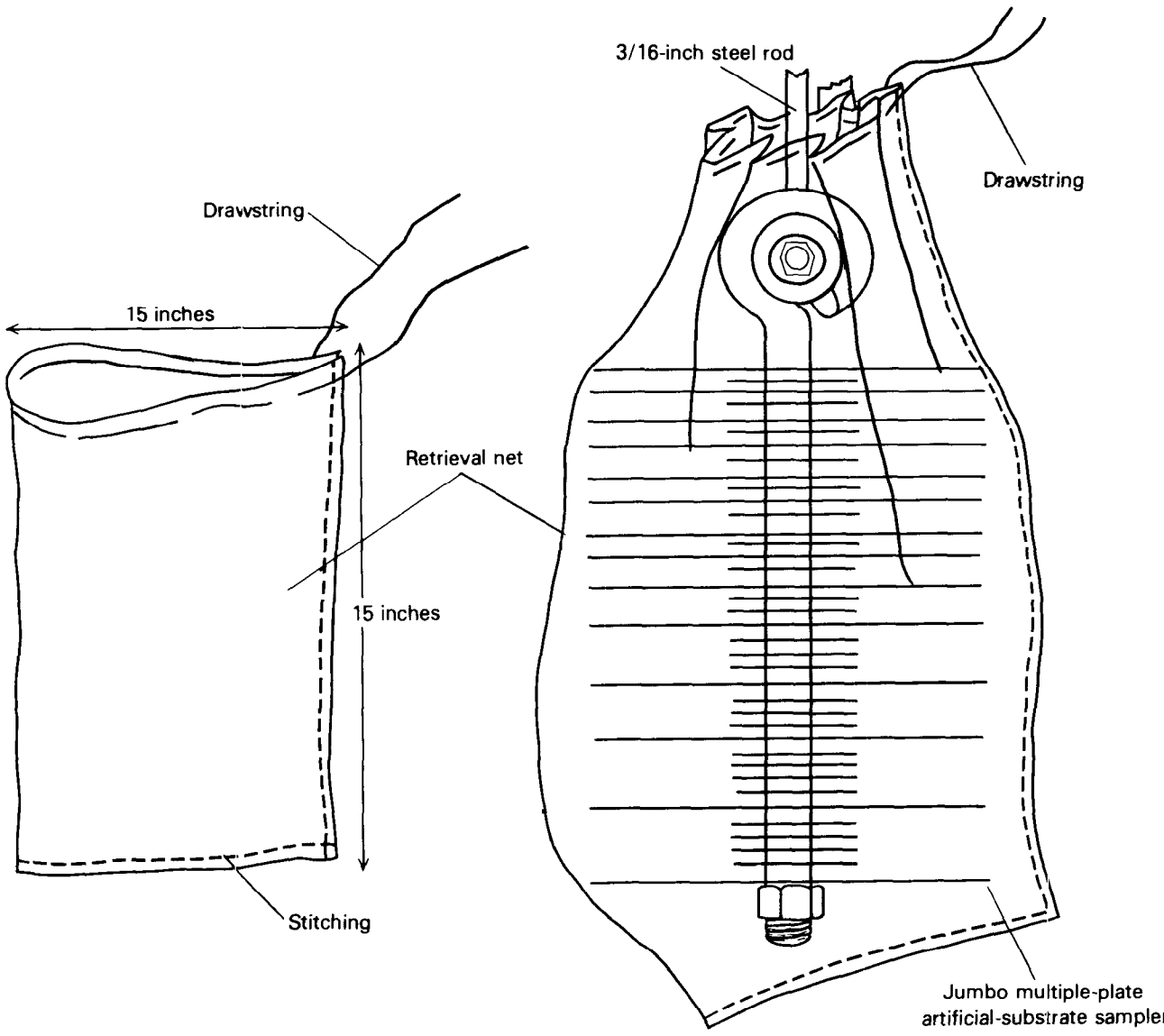


Figure 26.—Retrieval net.

off the bottom, the basket extends to its original hemispherical shape, and the surrounding net bag prevents loss of invertebrates during retrieval.

Expose the samplers in uniform macrohabitats at all sites during a study. If possible, install three samplers in a riffle in shallow streams. Make the collections as representative of the reach as possible by ensuring that the samplers are not close to the bank. In streams as much as a few meters in width, install the devices about midstream; in larger streams, install the devices about one-quarter of the total width from the nearest bank. Currents occasionally hinder the collapse of the sampler, but this can be overcome by connecting a strong rubberband to one side of the basket rim, extending it under the bottom of the wire basket, and attaching it to the other side of the rim (Bull, 1968). The samplers are stable on the bottom at velocities as much as 0.9 m/s, but recovery often is easier if a line or light chain connects the sampler to an inconspicuous anchorage. At velocities greater than 0.9 m/s, the samplers should be anchored.

Distribution and abundance

Absolute quantitative surveys are used to determine the numbers or biomass per unit area of streambed or lakebed and indicate changes in space and time. This type of sampling requires the greatest effort and, in many environments, the objectives cannot be achieved. Because all methods are somewhat selective, comparisons of the benthic invertebrates between sites or sampling dates should be based on uniform sampling methods.

The statistical principles of benthic-invertebrate sampling are discussed by Elliott (1971a). The first requirement is a clear definition of the objectives of the study and the area to be sampled.

When a knowledge of numbers or biomass per unit area is required, the major considerations are: (1) The size of the sampling units, (2) the number of sampling units in each sample, and (3) the location of sampling units in the sampling area. In general, the smaller the sampling units used, the more accurate and representative will be the results. Practical factors, such as particle size, will set a lower limit to the sampling-unit dimensions. Large numbers of sampling units in the total sample (greater than 50) are preferable, but usually impractical because of the labor involved in collection and analysis. The size of small samples can be calculated with a specified degree of precision (Elliott, 1971a, p. 128-131). The sampling units usually are randomly located in the sampling area, and all the available sites in the area must have an equal chance for selection. Stratified random sampling is preferable to simple random sampling.

A complete and accurate estimate of the numbers of all species in a large area of bottom often is impossible. Therefore, "***most quantitative investigations are restricted to a study of a small number of species in a large area, or a larger number of species in a small area***" (Elliott, 1971a, p. 127). This means that if the study objective is to compare the number and abundance of species at several sites or on

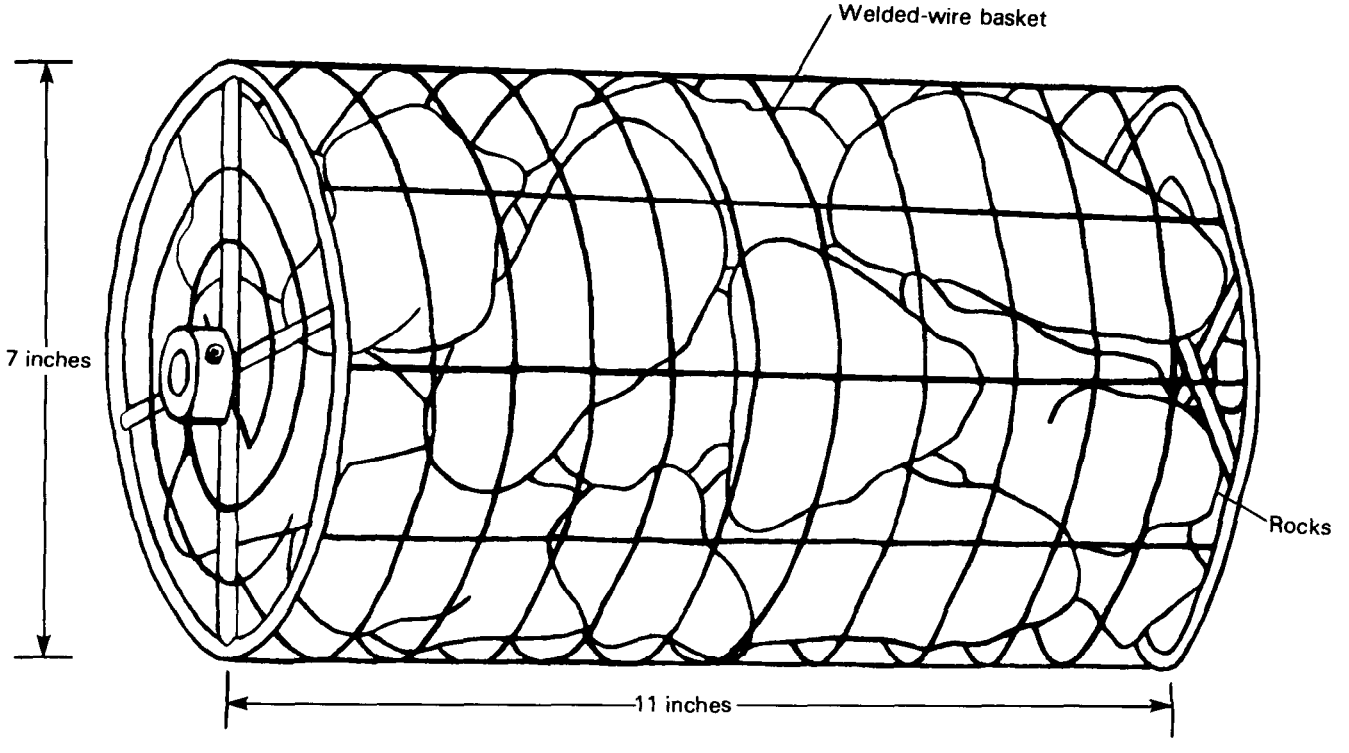


Figure 27.—Barbecue-basket artificial-substrate sampler.

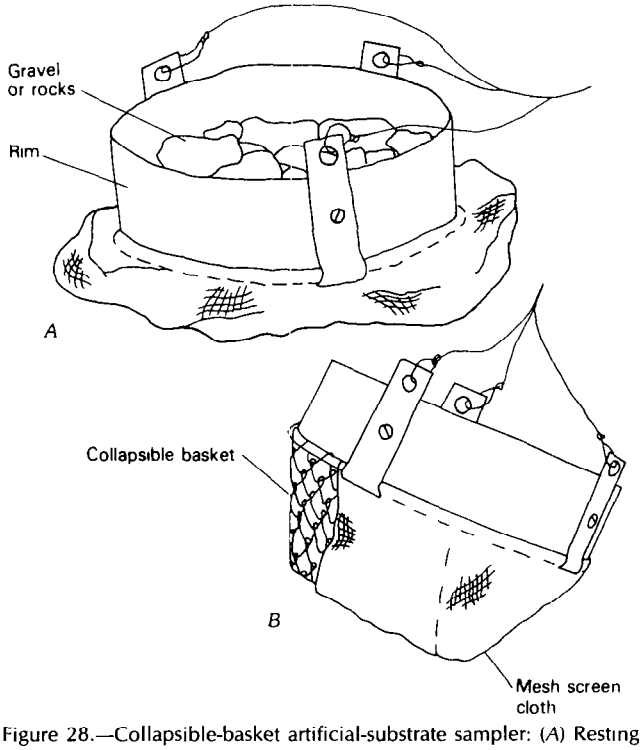


Figure 28.—Collapsible-basket artificial-substrate sampler: (*A*) Resting on streambed. (*B*) Being retrieved. (Redrawn from Bull, 1968.)

different sampling dates, numbers or biomass per unit area may be needed only for a particular type of homogeneous substrate. However, the area of the substrate sampled must be clearly defined.

The literature about the quantitative study of benthic invertebrates in flowing water was reviewed by Hynes (1970) who concluded that quantitative data about the benthic invertebrates are extremely difficult to obtain and are, at best, very rough estimates. Nevertheless, if three or more samples are collected, a general idea of the abundance of the more common species can be obtained. Sampling in a long transect line, which parallels some obvious environmental gradient, such as from shallow to deep water, provides a greater probability that most species will be collected at least once (Elliott, 1971a, p. 127).

Sampling frequency must be based on study objectives. Waters (1969a) and Cummins (1975) emphasized that sampling for the estimation of benthic invertebrate production should be done during the period of maximum change in growth and survivorship. For populations having typical survivorship and maximum mortality during the early instars and having approximately exponential growth curves, initial sampling should be at short intervals and later sampling at decreased frequency. For a complete faunal study, short-interval sampling, weekly, or less, should be done during periods when most of the species are in early age classes. In the temperate zone, this period generally is late spring and late fall (Cummins, 1975).

Quantitative studies require the collection from the sampling unit of all benthic invertebrates within the selected size range. The area of the sampling unit is defined by the area of the sampling device, but the depth to which sampling should extend into the sediments remains a problem. The vertical distribution of invertebrates in soft sediments (Lenz, 1931; Cole, 1953; Ford, 1962; Brinkhurst and others, 1969) and in coarse sediment (Coleman and Hynes, 1970; Mundie, 1971; Bishop, 1973) has been studied. As a guide to the depth of sampling, Cummins (1975) proposed measuring the oxygen profile in the sediment to determine the depth of the oxygenated zone (Ericksen, 1963) or sampling at least to a depth at which the sediment seems anaerobic; 0.01 to 0.1 m in fine, homogeneous sediment and 0.1 to 0.3 m in coarse, heterogeneous sediment.

Brinkhurst (1967) listed the following theoretical specifications for a quantitative sampler:

1. Depth of penetration. Invertebrates are found deep in the sediment, and a true measurement of total standing crop or proportional representation of species requires that the sampler collect sediment from the surface to a depth of at least 20 cm.
2. Bite. The bite of a sampler should be deep enough so all depths are sampled equally in any one attempt. The bite characteristics should enable accurate estimation of the surface area that was sampled.
3. Closing mechanism. Complete closure is required, or some of the sample will be lost. The closing mechanism should be powerful enough to shear through twigs and other obstructions.
4. Internal pressure. The descent of a sampler should not cause a pressure wave that will disturb the topmost sediment or give a directional signal to invertebrates capable of retreating from the sample area.

Although a corer that is completely open during descent satisfies many of the theoretical requirements in still water, no sampler available satisfies all requirements, especially for rocky sediment and flowing water. One problem is that any solid object, such as a corer or box, lowered into a stream deflects the current downward and scours the bottom where the sample is to be collected (Macan, 1958). The devices listed in the following sections are those most commonly used or those that seem to be best suited to the work of the U.S. Geological Survey.

Box, drum, or stream-bottom fauna sampler

The box, drum, or stream-bottom fauna sampler (fig. 29), depending on its design, is used by pushing the bottom edge downward to seal a compressible edge or by rotating a cylinder back and forth into the substratum. In the latter design, teeth dig into the bed, and a flange of metal and foam rubber or plastic also isolates the enclosed area. In flowing water, mesh panels in the sides of the sampler decrease scour as it approaches the bottom. To remove the invertebrates from the sample area, begin by placing the large rocks into a bucket of water. Thoroughly disturb the remaining sediment by digging and stirring as deeply as possible using a

garden trowel or fork, then stir the water vigorously using a small dip net to strain suspended material from the liquid. Some samplers have an attached bag net into which suspended invertebrates are carried by the current. Others require repeated sweeps. Empty the dip net into the bucket and continue the process until no additional invertebrates are collected. More sediment from the enclosed area may need to be removed as digging and stirring proceed. Remove the large rocks from the bucket and discard after scrubbing using a soft-bristle brush. Pour the contents of the bucket through a U.S. Standard No. 70 sieve. Transfer the concentrated sample to a shallow, white tray, if the sample is to be sorted onsite, or into a wide-mouth container for transporting to the laboratory. Label and preserve each collection.

Surber sampler

Press the bottom edge of the Surber sampler (fig. 30), or one of the modified samplers, firmly against the substrate to isolate the enclosed area as completely as possible. These samplers depend on the current to carry invertebrates into an attached net bag. Slack (1955) enclosed the sides and front of a Surber sampler with wire mesh and, in slowly moving water, used a rectangular fabric-covered paddle to produce a flow sufficient to sweep benthic invertebrates into the net. To remove the invertebrates from the area enclosed by the sampler, lift the larger rocks and scrub them into the mouth of the net. Thoroughly disturb the remaining sediment by repeatedly digging and stirring as deeply as possible, allowing the current to sweep the invertebrates and lighter detritus into the bag net. It is important, but difficult in practice, to avoid contamination of the sample by material from outside of the enclosed area. Empty the contents of the bag net into a shallow, white tray, if the sample is to be sorted onsite, or into a wide-mouth container for transporting to the laboratory. Label and preserve each collection.

Ekman grab

The preferred sampler for mud, silt, or fine sand is the Ekman grab (fig. 31). In shallow water, the sampler is operated manually, usually mounted on a pole. The Ekman grab can be used in this way to sample fairly hard sediment because the operator can force the sampler shut by exerting additional pressure on the upper edge of each jaw. In deep water, the sampler is lowered to the bottom, allowed to settle into the sediment, and then closed by dropping a messenger down the line.

In a tank and onsite comparison of seven grabs, Elliott and Drake (1981a) reported that the pole-operated Ekman grab

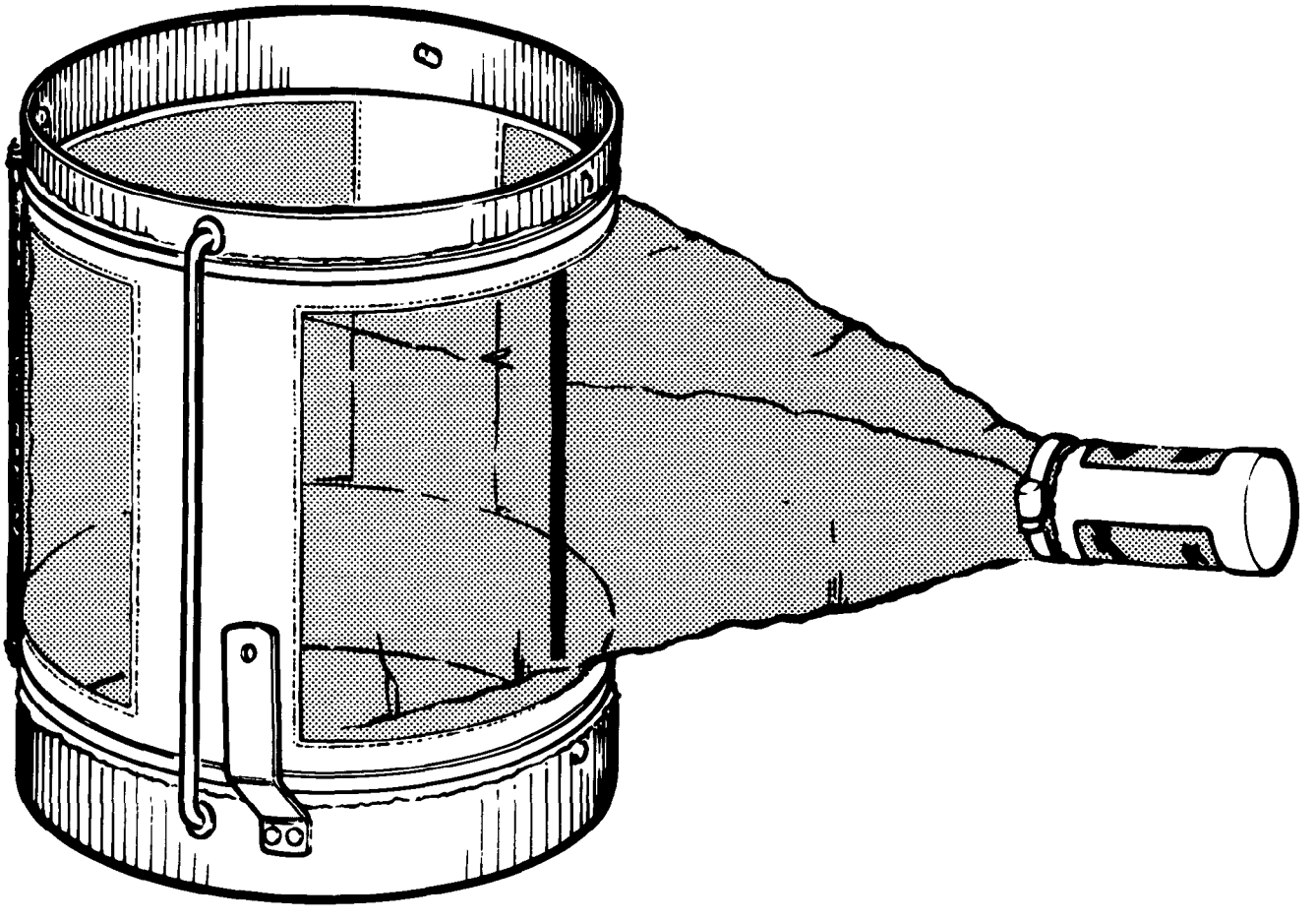


Figure 29.—Box, drum, or stream-bottom fauna sampler. (Sketch courtesy of Kahl Scientific Instrument Corp., El Cajon, Calif.)

performed well on a predominantly muddy bottom (particle size 0.004–0.06 mm) where the mean depth of penetration into the bottom was greater than 5 cm. In fine gravel of modal size (2–4 mm), efficiencies in terms of numbers per square meter were 54 percent, and the depth of penetration was less than 5 cm. The grab did not perform satisfactorily on a predominantly gravel bottom that had some rocks larger than 16 mm.

At the water surface, the sampler jaws are opened and the contents emptied into a tub, a large sieve, or a wide-mouth container for transporting to the laboratory. Label and preserve each collection.

Ponar and Van Veen grabs

Ponar and Van Veen grabs (figs. 32, 33) are heavy samplers that should be operated using a winch. They generally are used for deep-water sampling in gravel, hard sand, and clay, as well as in soft sediment. These instruments close on contact with the bottom; but, to operate effectively, they must bite vertically. This requirement poses little problem in lakes, but in river work, bottom sampling is especially difficult. When used from a drifting boat, the grab sometimes can be lowered nearly to the bottom, then dropped suddenly so it makes contact in an upright position.

In a tank and onsite comparison of seven grabs, Elliott and Drake (1981a) reported that the Ponar performed well on a predominantly muddy bottom (particle size 0.004–0.06 mm) where the mean depth of penetration into the mud was greater than 5 cm. In fine gravel of modal size (2–4 mm), and where the mean depth of penetration was greater than 5 cm, efficiencies in terms of numbers per square meter were 94 percent for the unweighted Ponar and 93 percent for the weighted Ponar. The only grab to operate adequately on a gravel bottom that had some rocks greater than 16 mm was the weighted Ponar.

In a tank and onsite comparison of seven grabs, Elliott and Drake (1981a) reported that the Van Veen grab had an efficiency of 71 percent in terms of numbers per square meter on a fine-gravel bottom (modal size 2–4 mm). The mean depth of penetration was greater than 5 cm. However, the Ekman and Ponar grabs performed better than the Van Veen grab on a predominantly muddy bottom.

Empty the sampler into a tub, and if mud is present, wash it from the sample. Pour the contents of the tub through a U.S. Standard No. 70 sieve. Transfer the concentrated sample to a shallow, white tray, if the sample is to be sorted onsite, or into a wide-mouth container for transporting to the laboratory. Label and preserve each collection.

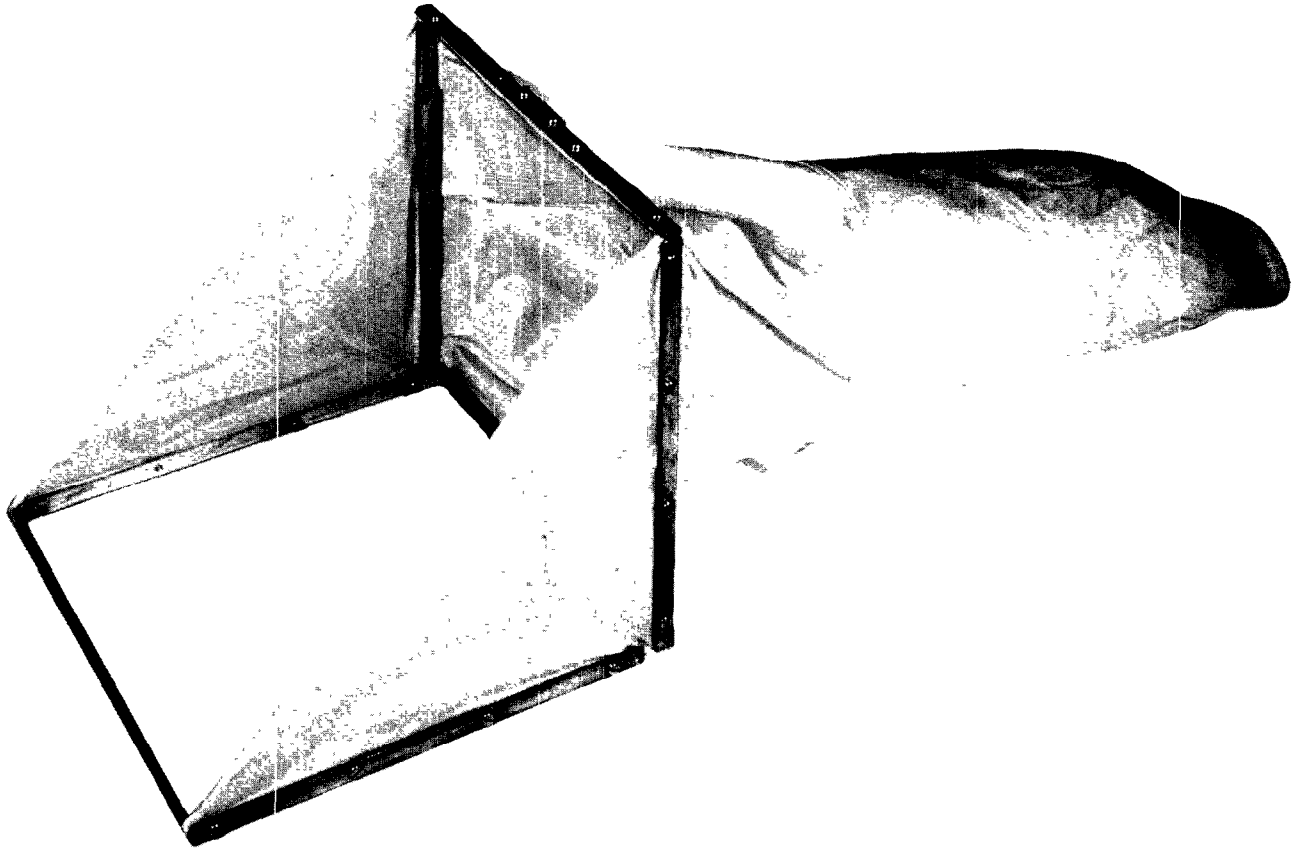


Figure 30.—Surber sampler. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)

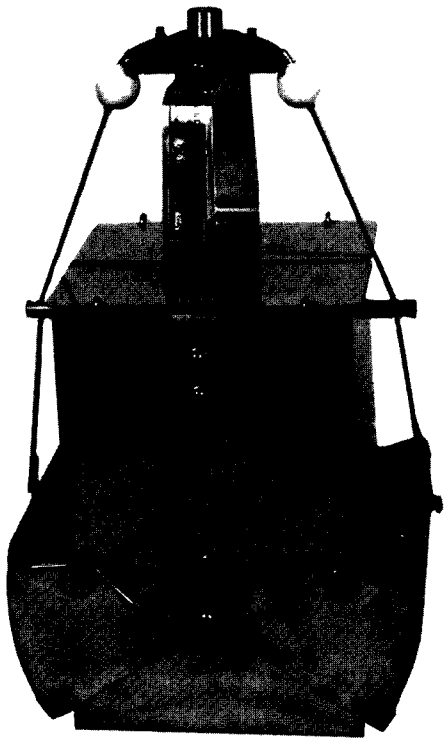


Figure 31.—Ekman grab, tall design. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)

Corers

These devices are used when an undisturbed sample of sediment is required. They are suitable especially for clay, silt, or sand bottom, and are used more widely in lakes than in streams. Hand corers designed for manual operation can be used in shallow water as much as several meters in depth. Deeper water requires devices such as the K.B.-type or Phleger corer (fig. 34), which depend on gravity to drive them into the sediment. All corers have been designed to retain the sample as the instrument is withdrawn from the sediment and returned to the surface. Follow the manufacturer's instructions for operating corers. Depending on the study objectives, sections of the core can be extruded and preserved separately, or the entire core may be retained in the tube. Intact cores are best preserved by freezing, but the sample can be sieved, labeled, and preserved.

Invertebrate drift

Studies have indicated that many kinds of benthic invertebrates become entrained in streamflow and that the resulting downstream drift of invertebrates is a regular feature of running water (Waters, 1969b, 1972; Müller, 1974). Because drifting invertebrates come from a variety of habitats, drift samples contain a relatively large variety of taxa (Waters, 1961; Larimore, 1974; Slack and others, 1976). The rate of invertebrate drift is affected by many factors, including light intensity, time of day, season of the year, stream discharge, and weather. The relation of invertebrate drift to

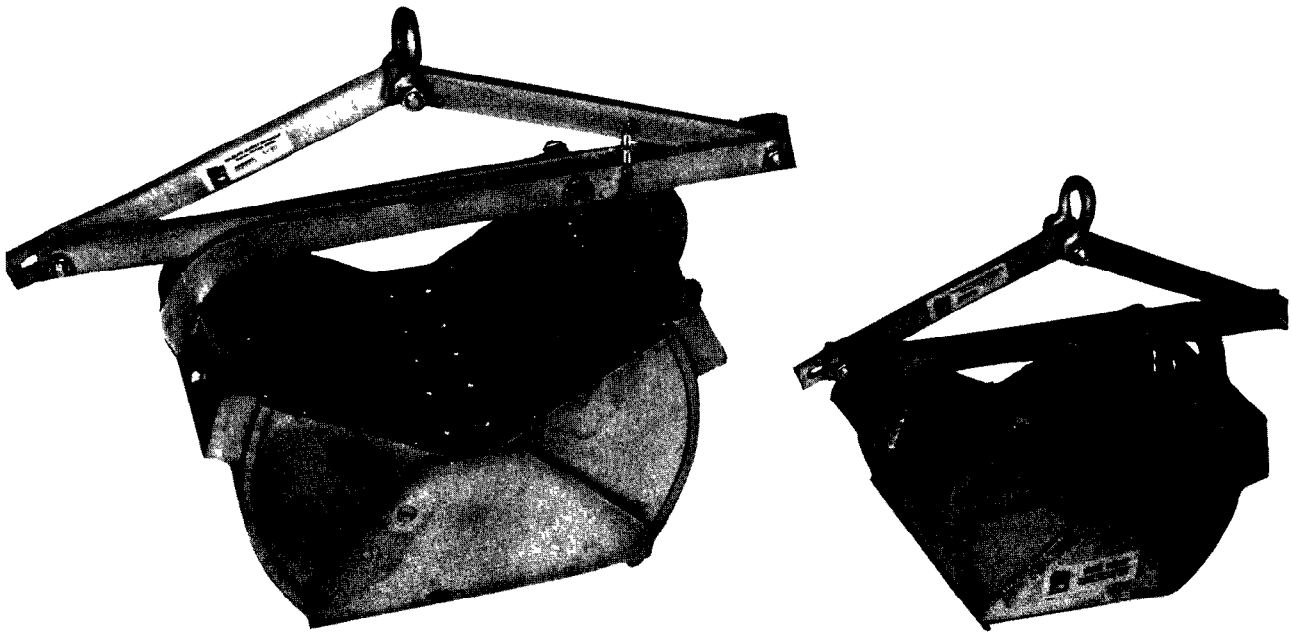


Figure 32.—Ponar grab. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)

water quality has been reported by Coutant (1964), Besch (1966), Wojtalik and Waters (1970), Wilson and Bright (1973), and Larimore (1974). Collections should be made upstream from any artificial disturbance of the streambed or banks. The distance that invertebrates drift varies with different species and with environmental conditions. Estimates of drift distances range from less than 1 m to more than 100 m (Hemsen, 1956; Waters, 1965; McLay, 1970), although McLay (1970) and Elliott (1971b) reported an exponential upstream decrease in the number of benthic invertebrates in the drift. Drift collections for impact assessment should be made; however, the fact that clean-water invertebrate species can be carried into stressed areas where they cannot survive needs to be emphasized.

Methods and equipment for collecting invertebrate drift are described by Elliott (1970). Drift samplers vary from simple nets to elaborate battery-powered devices capable of automatically collecting up to eight timed samples. A simple net of 210±2-μm or other appropriate mesh size on a square or rectangular frame is sufficient for making invertebrate drift collections (fig. 35). In shallow water, anchor the net with the opening upstream by driving steel rods into the streambed. Two types of deep-water exposures are shown in figure 36. Study objectives will determine the location, type, and duration of net exposure. Nets anchored downstream from riffles will catch more invertebrates than those downstream from pools, and the greater the volume of flow through the net, the larger the collection. The vertical position of drift nets in the water column is determined by water depth and study objectives. In water as much as 1 m deep, a mid-depth position commonly is used for a single drift net. Nets may be stacked, one above the other, to sample the entire water column from surface to bottom (Waters, 1969a). If the net opening is in contact with the stream bottom, nondrifting invertebrates may be collected. If the net opening extends above the water surface, the collection will include maximum numbers of floating adults, pupae, exuviae, and terrestrial species. If only aquatic invertebrates and life stages are of interest, the top of the net should be under water. In deep rivers, the net(s) may be near the stream bottom or near the surface, but the technique should be uniform if comparable collections are required. Because drift rates are faster at night than during the day, drift data are needed for at least 24 hours and collection periods commonly are 30 minutes, or 1-, 2-, or 3-hours, although collecting sometimes can last as much as 8 hours using properly designed nets. At the end of the collecting period, empty each net into a separate shallow, white tray, if the collection is to be sorted onsite, or into a wide-mouth container for transporting to the laboratory. Label and preserve each collection. Invertebrate drift can be collected as an adjunct to a faunal survey to determine drift density or to determine drift rate. Collection methods will vary depending on the study objectives.

Figure 33.—Van Veen grab. (Photograph courtesy of Kahl Scientific Instrument Corp., El Cajon, Calif.)

Drift density

The nets, location, and exposure periods described in the preceding section are suitable for determination of invertebrate drift density (the quantity of invertebrates per unit volume of water) when the volume of water passing through the net during the collection period is known. Water volume can be determined from an average of the speed of the current measured in the mouth of the net at the beginning and the end of the collection period, multiplied by the area of the net opening and the length of the exposure period. A digital flowmeter mounted in the net opening can be used to determine the cumulative volume of water passing through the drift net. Drift density usually is assumed to be fairly uniform in the cross section at a given time (Waters, 1972), and results from a single drift net are assumed to be adequate. This can be checked by collecting, using two or more nets exposed simultaneously at different points in the cross section.

Drift rate

The drift-density procedures also are suitable for determination of invertebrate drift rate (the quantity of invertebrates passing a given point per unit of time). Drift rate can be calculated from drift density if stream discharge is known.

When drift density and discharge values are available for a 24-hour period, the total daily drift rate per instantaneous discharge or per total daily discharge can be calculated.

Sample preparation

Samples for which only biomass will be determined need to be frozen, preferably freeze-dried, as soon as possible after collection. Samples for taxonomic determination need to be preserved in alcohol or formaldehyde. (Use of alcohol for preserving samples for biomass determinations will result in small values because of extraction of alcohol-soluble substances from the invertebrates.) To ensure adequate preservation of benthic-invertebrate collections, fill containers no more than one-half full with the sample so a volume of preservative can be added at least equal to the volume of organic material, including detritus. Preserve the invertebrates or the unsorted samples in 70-percent ethyl alcohol, 70-percent isopropyl alcohol, or 4-percent formaldehyde solution. If formaldehyde is used, replace with alcohol prior to identification and enumeration. Containers should be filled to the top to avoid excessive sloshing and damage to delicate specimens. If unsorted samples are to be stored for more than a few weeks, the preservative should be drained after 1 week and replaced with fresh preservative.

Label samples indicating the location, habitat, date and time of collection (local standard time) for drift collections, name of collector, and sample preparation (type of preservative, mesh size of sieves or nets, or other treatment). Soft black pencil may be used onsite, but use a water-proof carbon ink for permanent labels. Place labels inside the sample containers so they are visible from the outside, or place duplicate labels inside and outside the containers. Secure jar lids using tape to prevent loosening and subsequent loss of preservative by evaporation. This is especially important if samples are to be shipped or stored for more than a few weeks.

Sample sorting

A requirement of all benthic-invertebrate methods is to separate the invertebrates from sediment and detritus in the samples. The following general apparatus, reagents, and procedures for sample sorting apply to all methods in this section.

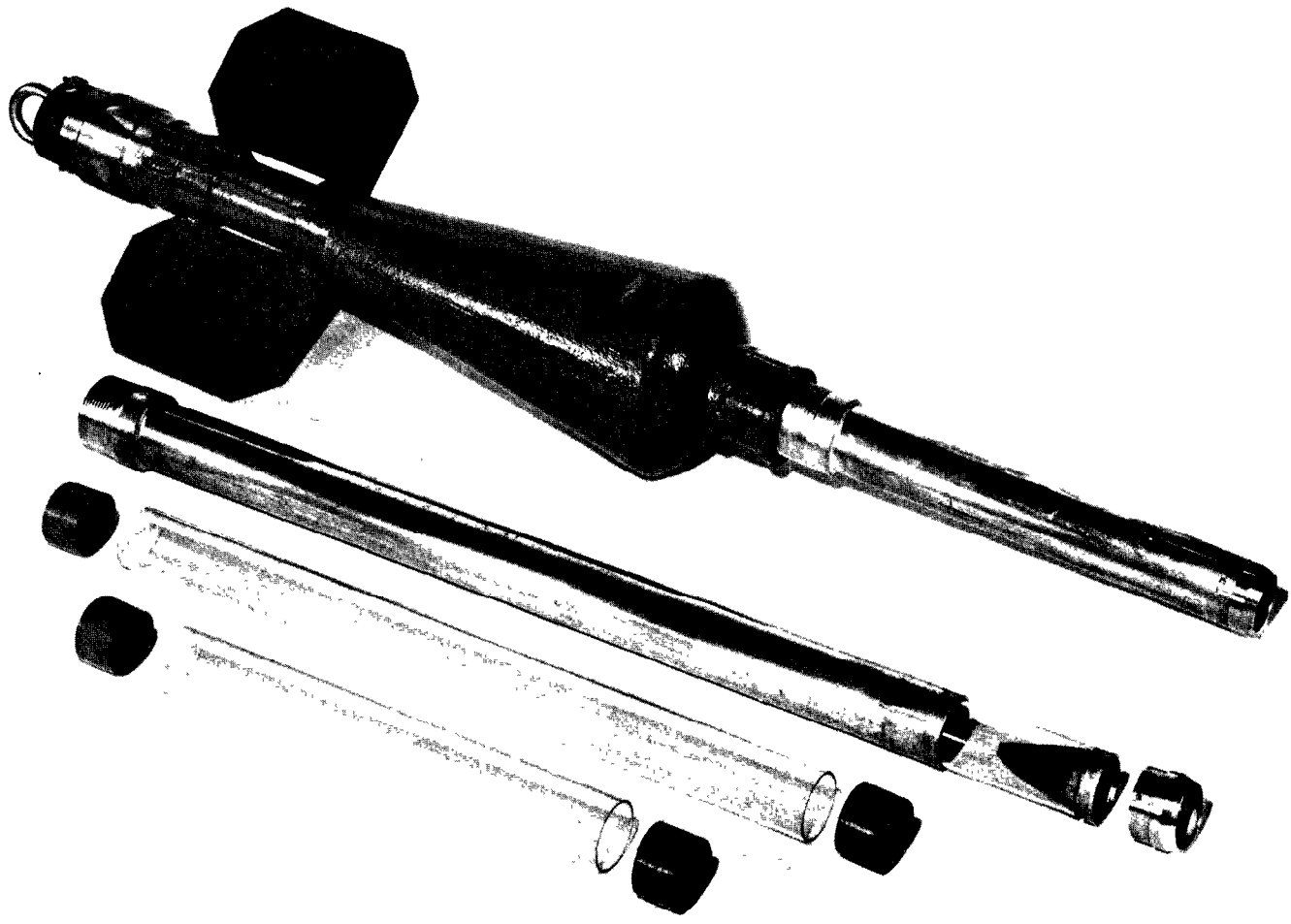


Figure 34.—Phleger corer. (Photograph courtesy of Kahl Scientific Instrument Corp., El Cajon, Calif.)

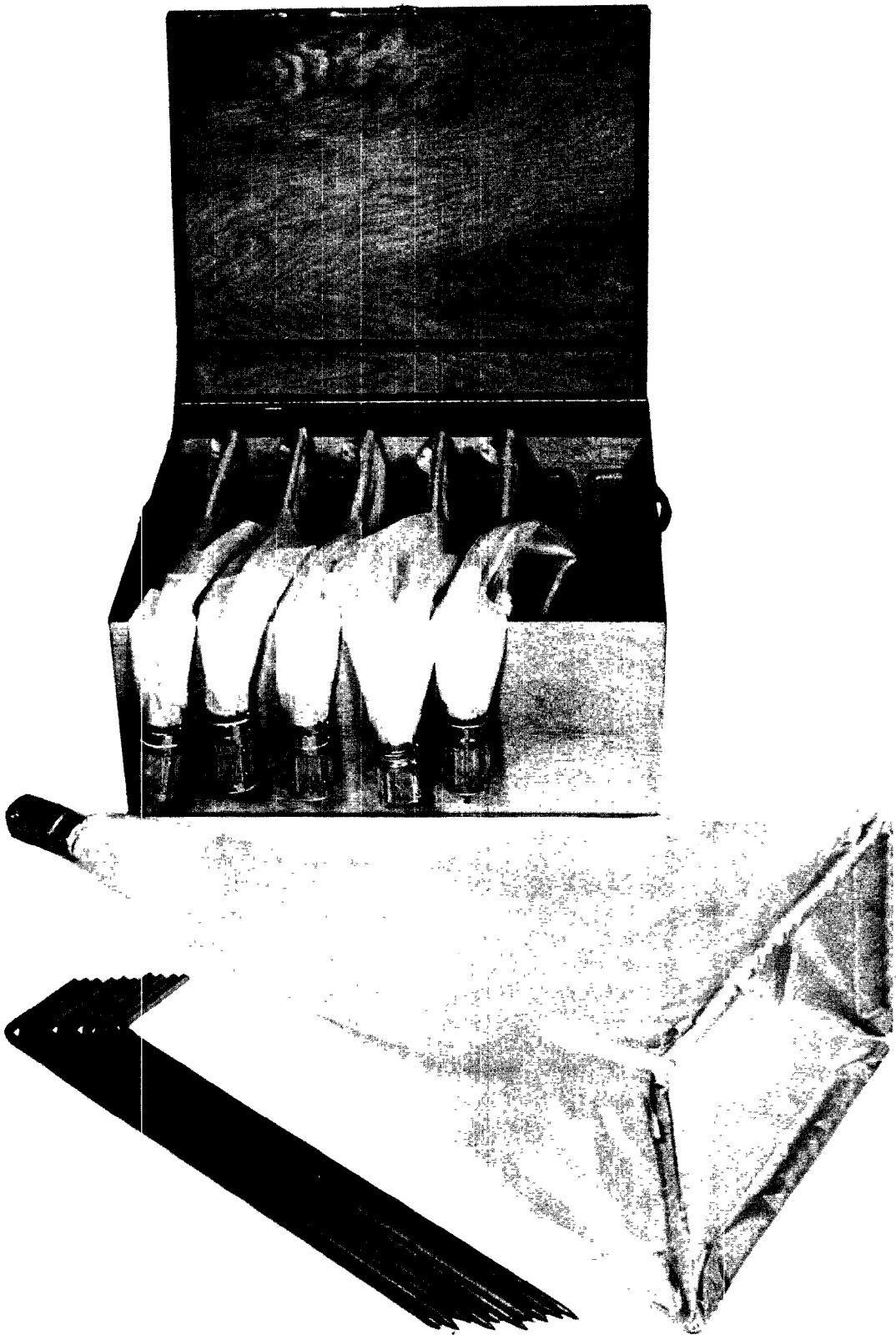
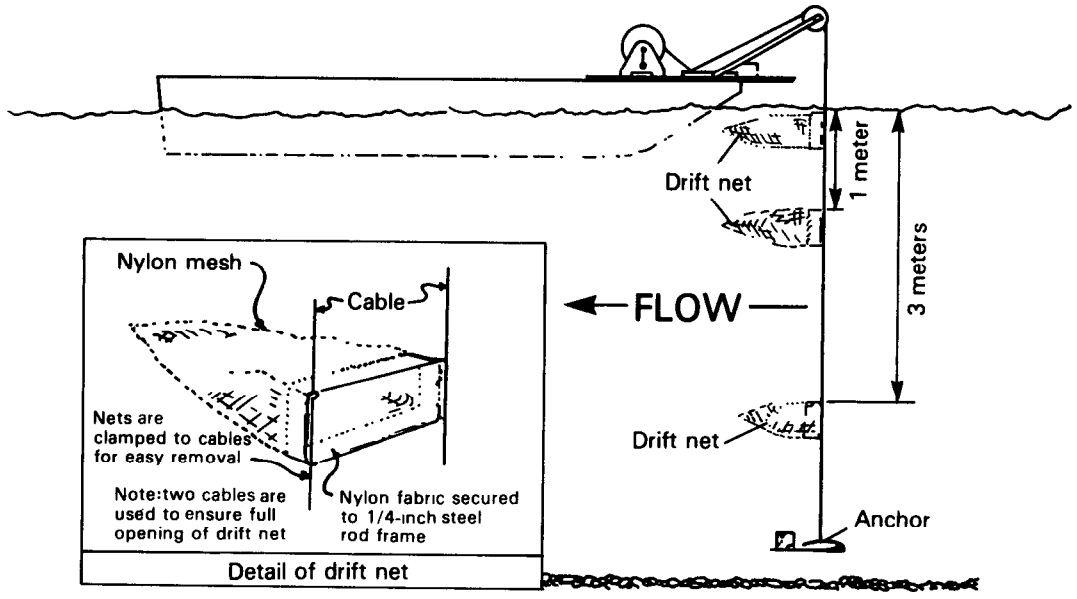


Figure 35.—Stream drift nets. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)



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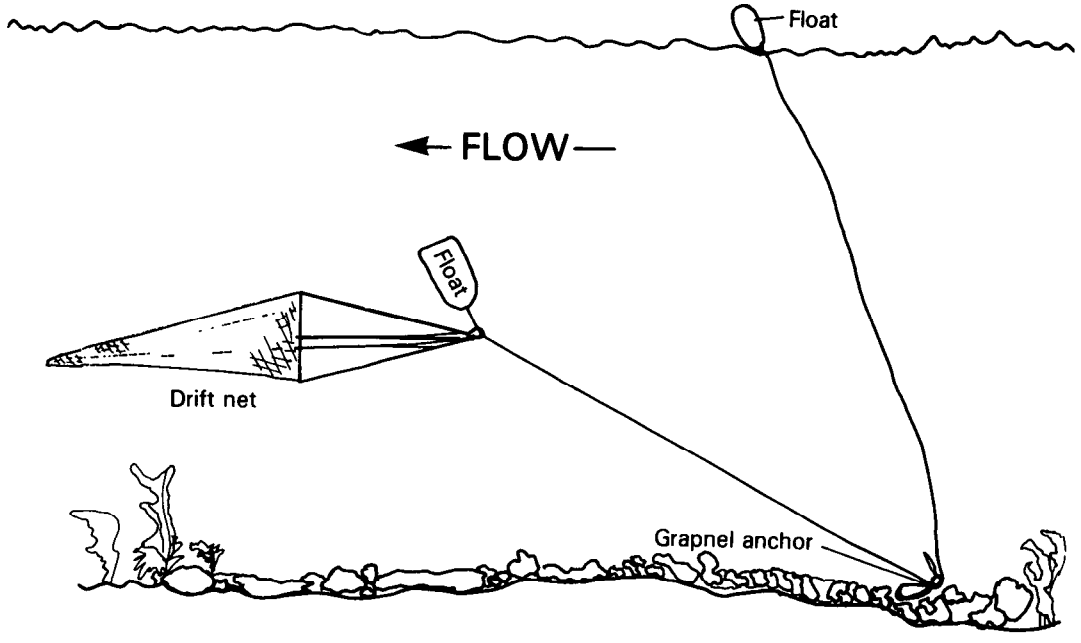

B

Figure 36.—Methods of exposing drift nets in deep rivers: (*A*) From an anchored boat (from Ferreira and Hoffman, 1978). (*B*) Float-supported net (from J.L. Barker, U.S. Geological Survey, written commun., 1982).

Apparatus

A.1 *Dishes*, glass, petri, or Syracuse watchglasses.

A.2 *Forceps* that have fine or rounded points. Forceps that have fine points are useful for handling small invertebrates. Forceps that have rounded points are less likely to tear netting or puncture the mesh of sieves or other sampling equipment.

A.3 *Hydrometer*, plain form, range 1.000 to 1.220.

A.4 *Ink, waterproof.*

A.5 *Labels, waterproof*, or labels may be cut from sheets of plastic paper.

A.6 *Microscope*, stereoscopic variable power, 7× to 30×, and *microscope illuminator.*

A.7 *Pipet*, wide-bore.

A.8 *Scoops, fine-mesh*, made in various sizes and shapes, as needed, from pieces of brass or stainless-steel wire mesh attached to a handle. A convenient handle for the scoops is an X-Acto knife handle, or equivalent.

A.9 *Sieves, U.S. Standard*, 20-cm diameter, and mesh size appropriate to the study objectives. The No. 70 sieve (210-μm mesh opening) has been adopted for retaining benthic invertebrates collected as part of the water-quality programs of the U.S. Geological Survey. Sieves that have smaller or larger mesh may be more suitable for some studies. The No. 18 sieve (1,000-μm mesh opening) is useful for removing large rocks and sticks from samples. Stainless-steel mesh is recommended for all sieves because of its greater durability compared to brass.

A.10 *Subsampler jar* (Hynes, 1970, p. 244). Divide the bottom of a screw-topped jar into equal quadrants about 2 cm deep by embedding thin cardboard or plastic in paraffin.

A.11 *Tape, plastic*, or *paraffin* for sealing jar and vial lids.

A.12 *Trays, white enamel.* Useful sizes are 30×19×5 cm and 42×26×6 cm.

A.13 *Vials* that have poly seal screw lids. Convenient sizes are 7.5-, 15-, and 22-mL capacity.

Reagents

R.1 *Rose Bengal biological stain.*

R.2 *Sucrose solution*, specific gravity 1.12, for density separation of invertebrates from the debris in benthic samples. Dissolve 360 g granulated sugar per liter of water.

Procedure

P.1 If the study objectives require determination only of the most abundant benthic invertebrates, sorting often can be completed onsite. Wash the sample gently in a sieve of appropriate mesh size to remove mud and fine detritus. Pick the invertebrates directly from the sampled material; or, to enhance visibility of small invertebrates, cover the sample with water in a white enamel tray and stir repeatedly while removing the invertebrates using forceps or scoops.

P.2 Generally, sorting must be done in the laboratory. Pour small quantities of the sample into a shallow dish, covering the material with water, and scan the dish under low-power magnification (7× to 10×). Remove the invertebrates from the debris using forceps, fine-mesh scoops, or wide-bore pipets.

The sorting process is very time consuming for many types of collections. The optional steps described in the following paragraphs may be used to speed the work when the study objectives require complete analysis.

P.3 *Density separation (optional).* This step consists of treating the sample with a solution of such density that most of the invertebrates will float, and most of the unwanted detritus will sink. The recommended method employs a sucrose solution that has a specific gravity of 1.12 (Anderson, 1959; Lackey and May, 1971).

Drain the sample in a No. 70 or other appropriate sieve, discard the liquid, and transfer the residue to a white enamel tray. Flood the material in the tray with the sugar solution, and stir so the material is evenly spread over the bottom. Remove invertebrates quickly from the surface of the liquid using forceps, fine-mesh scoops, or wide-bore pipets. After removing all visible invertebrates, stir the material and remove any other invertebrates that appear. Pour the sugar solution through the sieve and cover the residue in the tray with water. Examine as described in P.2 looking carefully for oligochaete worms, for aquatic mites, and for heavier invertebrates, such as mollusks and caddisfly larvae. After this examination, pour the water through the sieve and repeat the sucrose treatment. Few invertebrates should be found but, if large numbers are seen, soak the sample in water and again treat with the sugar solution. Reuse the sugar solution by adjusting the specific gravity to 1.12 as determined using a hydrometer. However, the solution spoils rapidly and should not be stored for more than a few days.

P.4 *Differential staining (optional).* Separation of invertebrates, especially transparent forms, from detritus in the samples is facilitated by staining them red using 200 mg/L of Rose Bengal added to the preservative solution. Expose the invertebrates to the stain for at least 24 hours before examination. Prolonged contact with the stain may result in uptake of the red color by algae and plant detritus. If necessary to restore natural coloration for identification, remove the stain from the invertebrates by placing them in 95-percent ethyl alcohol (Mason and Yevich, 1967). A counterstaining technique in which Rose Bengal or Lugol's solution is counterstained with chlorazol black may be used to provide a definite color contrast between invertebrates and detritus (Williams and Williams, 1974).

P.5 *Subsampling (optional).* Some benthic samples are so large, or contain such large numbers of invertebrates, that sorting or counting the entire sample is impractical. Remove the larger invertebrates and pieces of detritus from the entire sample. Transfer the remainder of the sample to a screw-topped subsampler jar and add 70-percent alcohol to a depth of 10 to 12 cm. Close the jar and invert several times to mix thoroughly, then wait until the invertebrates have settled. Remove the contents of any two opposite quadrants using a wide-bore pipet to obtain one-half of the original sample. Repeat the process on one-half of the sample if further subsampling is required before sorting and counting.

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Faunal survey (qualitative method)

(B-5001-85)

Parameter and Code: Not applicable

1. Applications

The method is applicable to all water.

2. Summary of method

Benthic invertebrates are collected by hand, dip net, dredge, or any other procedure appropriate to the environmental conditions and to the objectives of the study. The sampling equipment described in the following methods may be used to ensure that all habitats are sampled. Unsorted samples, usually containing varying quantities of sand, gravel, and plant detritus, are preserved onsite. In the laboratory, the benthic invertebrates are sorted from the extraneous material, identified, and counted. Results are reported as numbers of different kinds of benthic invertebrates (taxa) and the relative abundance of each taxon at different sites or times.

3. Interferences

Physical factors, such as stream velocity and depth of water, may interfere with sample collection. Most samples contain relatively large quantities of sediment and plant debris from which the benthic invertebrates must be sorted.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Biological dredge* (fig. 21). The design depends on environmental conditions and study requirements.

4.2 *Dip or hand nets* are made in various shapes and sizes, are sturdy in design, and have a flat side for pressing the net closely against the streambed. Commercial nets are available in various materials and mesh sizes. The desired material and mesh opening should be specified when ordering. Dip nets for general use in the U.S. Geological Survey should have bags of 210±2-μm mesh-opening nylon or polyester monofilament screen cloth, unless otherwise indicated by the study objectives.

4.3 *Forceps*, that have fine or rounded points. Forceps that have fine points are useful for handling small invertebrates. Forceps that have rounded points are less likely to tear netting or puncture the mesh of sieves or other sampling equipment. Forceps are less likely to be lost onsite if marked with bright paint or colored tape.

4.4 *Gloves, waterproof*, Trapper's, shoulder length.

4.5 *Ink*, waterproof.

4.6 *Labels, waterproof*, or labels may be cut from sheets of plastic paper.

4.7 *Microscope*, stereoscopic variable power, 7× to 30×, and *microscope illuminator*. A compound microscope of at least 200× magnification also is useful for taxonomic work.

4.8 *Pipe dredge* (fig. 22). This simple device, or a modification, is useful for collection of benthic invertebrates in swift, rocky rivers. Commercial dredges weigh 25 kg, but smaller and lighter versions can be made for special purposes. For collecting benthos, the dredge may be constructed without a bottom and with a sturdy mesh bag secured over the rear opening by a hose clamp.

4.9 *Sample containers*, plastic or glass, and plastic lids, for transporting unsorted samples to the laboratory. Wide-mouth jars of 120-, 240-, and 475-mL capacity are useful sizes. Sealable plastic bags also may be used for temporary storage of benthic-invertebrate samples.

4.10 *Sieves, U.S. Standard*, 20-cm diameter, and mesh size appropriate to the study objectives. The No. 70 sieve (210-μm mesh opening) has been selected for retaining benthic invertebrates collected as part of the water-quality programs of the U.S. Geological Survey. Sieves that have smaller or larger mesh may be more suitable for some studies. The No. 18 sieve (1,000-μm mesh opening) is useful for removing large rocks and sticks from samples. Stainless-steel mesh is recommended for all sieves because of its greater durability compared to brass.

4.11 *Tape, plastic*, or *paraffin* for sealing jar and vial lids.

4.12 *Vials*, that have plastic poly seal screw lids. Convenient sizes are 7.5-, 15-, and 22-mL capacity.

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Distilled or deionized water.*

5.2 *Glycerin.*

5.3 *Preservative solutions*. Invertebrate samples may be preserved in 70-percent ethyl alcohol, 70-percent isopropyl alcohol, or 4-percent formaldehyde. A mixture of 70-percent ethyl alcohol and 5-percent glycerin is preferred for permanent storage. Prepare as follows:

5.3.1 *Ethyl alcohol.* Dilute 70 mL 95-percent alcohol to 95 mL using distilled water.

5.3.2 *Ethyl alcohol and 5-percent glycerin.* Dilute 70 mL 95-percent alcohol to 100 mL using 25 mL distilled water and 5 mL glycerin.

5.3.3 *Isopropyl alcohol.* Dilute 70 mL concentrated isopropyl alcohol to 100 mL using distilled water.

5.3.4 *Formaldehyde.* Dilute 10 mL 37- to 40-percent aqueous formaldehyde solution (formalin) to 100 mL using distilled water.

6. Analysis

Identify and count the benthic invertebrates in the sample according to taxonomic categories. The degree of identification required (species level is desirable) varies depending on the objectives of the study. A stereoscopic microscope is required; and, for some groups, dissections or microscopic mounts are needed to observe key characteristics. Appropriate reference books (Part 3, "Selected Taxonomic References" section of this report) should be available. The different categories of invertebrates can be placed in separate vials of 70-percent ethyl or 70-percent isopropyl alcohol, and can be labeled with the name of the invertebrate and the identification number, date, and origin of the sample. Add a few drops of glycerin or use the ethyl alcohol-glycerin preservative, and seal vial caps if the specimens are to be stored.

7. Calculations

7.1 When only part of the total sample is sorted or counted, project the results from the subsample to the number of specimens in the total sample:

$$\begin{array}{l}\text{Total number of benthic invertebrates of a particular taxon in sample}\end{array} = \frac{\text{Number of benthic invertebrates of the taxon in subsample}}{\text{Fraction of total sample in subsample}}.$$

7.2 Percent composition in sample

$$= \frac{\text{Number of benthic invertebrates of a particular taxon}}{\text{Total number of individuals of all taxa}} \times 100.$$

8. Reporting of results

Report the number of taxa present, the percent composition of each taxon in the sample, and the type of sampling method(s) used.

9. Precision

No numerical precision data are available.

10. Sources of information

None.