

An Evaluation of Some Techniques for the Collection and Analysis of Benthic Samples with Special Emphasis on Lotic Waters

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ABSTRACT: A consideration of a large number of procedures for the collection and analysis of benthic samples, with particular emphasis on stream investigations and the importance of substrate particle size as a common denominator in benthic ecology, reveals that only certain techniques are suitable.

Although either systematic or stratified random samplings are appropriate for faunal surveys, the careful selection of sample sites in single-species studies can provide maximum information per unit sampling effort. In order to adequately describe the micro-distribution of benthic organisms, investigations must be conducted on a year-round basis. Only bottom samplers, such as the core-type, which retain the entire sediment sample for analysis are desirable. Measurements of current velocity should be made close to the substrate-water interface. The removal of the fauna by elutriation and hand sorting allows for further physical and chemical analyses. Physical analysis of stream sediments can be accomplished through the decantation of silt and clay followed by dry sieving of the coarser material. In addition, a new photographic technique for substrate analysis, described in detail, can provide information on the surface sediments. Indications of the organic content of sediments can be obtained by the dry combustion carbon train method or, when clay content is low, from loss of weight on ignition values. However, new techniques are called for, especially those directed toward the food habits of particular species. The Wentworth classification, modified to include a gravel category, should be followed, and the size classes converted to the *phi* scale in graphic presentations of sediment data.

Since Shelford (1914) became interested in the ecology of benthic macro-invertebrates, Needham (1928) conducted surveys of New York streams, and Shelford and Eddy (1929) formulated some fundamental approaches for the study of stream communities, a great many benthic investigations have appeared in the literature. The facts accumulated to date show that the orientation of aquatic invertebrates to various environmental parameters results in nonuniform distributions in which given animal groups are associated with measurable ranges of environmental conditions. Benthic ecologists have spent considerable effort measuring the most obvious parameters in the aquatic environment to determine their effects on the distribution of various groups of animals. Substrate, current velocity, and food materials have been shown to be of primary importance, although the way in which these interrelated parameters determine distribution remains to be completely delineated. Undoubtedly, some parameters are more critical than others, but it may be that all physical

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and chemical factors must be evaluated to achieve reasonable accuracy in the prediction of distributions.

The most extensively studied parameter, and in some cases the most important, is the physical nature of the substrate. The relationship between benthic invertebrate distribution and the nature of the substrate can be of a direct sort owing to the physical properties of the substrate, or it may be indirect. The indirect relationship is often due to the distribution of food materials which is directly dependent on substrate type. In either case, the measurement of the physical composition of the sediments should be an integral part of all benthic investigations. Physical analyses are especially needed as a common ground for comparison in stream studies where diverse sampling methods often render comparisons invalid when all that can be compared is the number of animals present. The results obtained by a number of investigators (Wene, 1940; Beaty and Hooper, 1958; Sanders, 1956, 1958, 1960; Wieser, 1959, 1960; Cummins, 1961; Eriksen, 1961) quite clearly support the general concept that substrate particle size can serve as a common denominator in benthic ecology.

Since the selection of the procedures to be followed in a benthic study is contingent upon a knowledge of methods previously utilized, discussions of the problems involved are of considerable value. However, the techniques that have been used in the past for the collection and analysis of benthic samples are so numerous and varied that any fairly comprehensive discussion must, of necessity, be quite lengthy. Nevertheless, a careful consideration of the limitations of the various procedures and their applicability to a particular investigation is absolutely essential to the success of any benthic study. This paper is not intended as a complete literature survey but rather as a discussion of the important kinds of techniques employed in benthic ecology. In the following discussion, particular emphasis has been placed upon investigations conducted in flowing waters because the sampling problems are particularly troublesome, and most stream workers have neglected the detailed measurement of environmental parameters. In addition, benthos is defined as the fauna (primarily macrofauna) living on or beneath the substrate; substrate is used in the broadest sense to include the sediments, both mineral and organic, and vegetation growing in or on the bottom.

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QUANTITATIVE SAMPLING PROCEDURES

A myriad of problems arise within the framework of the three basic questions of where, how, and with what frequency to take

samples. The answers depend upon the kind of information sought and the substrate types to be sampled, although time and equipment available usually impose strict limits on the decisions. As Allen (1959) has pointed out in an excellent paper on the distribution of stream bottom faunas, environmental irregularities must be sampled on a scale which corresponds to the size and habits of the particular animal or groups of animals under investigation.

SELECTION OF SAMPLE SITES

The majority of studies on the quantitative distribution of stream benthic organisms have been concerned with the total range of the macro-invertebrate fauna present, although some studies, such as Scott (1958), have dealt with one particular taxonomic group. Macan (1961) has reviewed a large number of studies of both types conducted on running waters. There have been, however, very few quantitative investigations oriented toward the local distribution of one or two species, although some life history studies have contained information on micro-distribution (Corbet, 1957).

The portion of the fauna under study should determine the areas to be sampled, number of samples taken, and sampling procedure. In investigations limited to one or two species, sampling of the particular micro-habitats selected by these species provides the most practical approach, whereas general quantitative faunistic studies require equal sampling effort in all habitats.

Naturally, considerable attention has been directed toward the selection of a sampling procedure that will yield a good approximation of the total environment from a small number of samples. Three statistical approaches have been utilized; the simple random sample, the stratified random sample, and the systematic sample (Cochran, 1953; Hansen *et al.*, 1953).

For the execution of the simple random sample, a grid is superimposed on the study area and the intercepts numbered. Then, either by drawing or using a table of random numbers, sample sites are determined. Macan (1961) has called this the biological approach, since factors typical to each species are sought by grouping the data obtained from random collections.

The stratified random sample, termed the physical approach by Macan, involves the selection of habitat types, such as riffle or pool, followed by the application of random sampling to each of these somewhat discrete areas. This was the approach employed by Gaufin *et al.* (1956) in sampling a riffle, pool, and marginal area with three appropriate samplers.

A third, and very common procedure is systematic sampling or the transect method. Because the substrate characteristics of aquatic environments are determined, within limits, by water currents, the transect method is most likely to cut across the range of habitats present. In lentic waters, transects from shore to deep basins follow a line of decreasing particle size and increasing depth, with charac-

teristics such as vegetation growth and content of dissolved gases varying in relation to water depth. In lotic waters, the same general order of particle size range is found from central channel to shore. Since the deposition of fine particles along the shore of a stream is dependent on channel gradient and specific location within the channel, several complete transects are required to adequately sample a given area.

Random sampling is quite difficult and time consuming in the lentic habitat and even more so in lotic environments. The number of samples required to adequately sample all habitats may be very large even when the stratified modification is employed. However, in most cases one or more carefully placed transects or restricted stratified random sampling of carefully selected habitat types will provide much more information per unit sample effort. Furthermore, when the particular animals of interest are visible on the surface of the substrate, another approach is feasible. Samples can be taken at animal positions, thus yielding maximum information concerning the factors involved in distribution with a minimum number of samples. To eliminate all pretensions of such a technique it is probably best termed a biased sampling method, that is, biased toward the actual positions of visible individuals.

SAMPLING FREQUENCY

As pointed out above, single species studies employing an approach designed to sample known animal locations would minimize the number of samples required. So far, however, investigations of sampling efficiency as a function of sampling frequency have been concerned with the measure of total weight and total number of animals, and number of species in each sample. The number of samples required to adequately estimate each value is then determined. Such a study was conducted by Needham and Usinger (1956) who intensively sampled a short section of stream described as a riffle. On the basis of 100 samples, taken with a Surber sampler, the authors determined that 194 samples would be required to estimate, at the 95% confidence level, the total wet weight of animals per square foot; 73 samples would be required to estimate total numbers. However, they concluded that only two samples were required to obtain a representative faunal list. Although not pointed out by the authors, their study provides a striking example of the nonuniformity of a so-called "uniform" riffle.

Leonard (1939) intensively sampled two gravel riffles and a *Potamogeton* bed with a Surber-type sampler and concluded that a small number of samples would adequately describe the fauna in terms of total volume per unit area but not species present.

Gaufin *et al.* (1956) evaluated three sets of ten stratified random samples and determined that three samples recovered only 1/3 to 1/2 of the species found in all ten samples. It seems that their designa-

tion of habitats as riffle, pool, and marginal area was too gross to delimit the causal factors involved in the micro-distribution of many of the species encountered. Indeed, Harris (1957), in a paper concerning the further statistical analysis of the data, stressed the need for careful selection of sample sites.

In contrast to samples of benthic faunas taken at a particular time, seasonal variations must be considered. Not only will the abundance of each species vary in time according to its life cycle, but distributional patterns may be extremely divergent at different times of the year. For example, Morgan (1956) discovered that the larvae of a lake species of leptocerid caddisfly which he studied migrate shoreward from deep water and select pupation sites in shallow water. Benthic sampling programs must be conducted on a seasonal basis in order to obtain a true picture of either animal abundance or distributional patterns.

SAMPLING DEVICES

Quite often, a comparison of results from various benthic investigations is not possible because of the wide range of procedures employed. Much of the difficulty stems from the fact that a sampling device which is suitable for all types of habitat has yet to be developed. In fact, a review of the literature reveals that the number of samplers is nearly proportional to the number of investigations. Since a detailed review of bottom samplers is not the purpose of the present discussion, only certain general types will be considered.

Welch (1948) described a large number of benthic samplers suitable for quantitative limnological investigations, and Twenhofel and Tyler (1941) presented an exhaustive descriptive listing of sampling devices utilized in marine investigations. Longhurst (1959) has reviewed the sampling problem and discussed the suitability of some marine samplers. Macan (1958) described many samplers which can be utilized in stony streams and divided them into five basic types.

COLLECTION OF ANIMALS ONLY

Since all the sampling devices described by Macan (1958) do not retain the sediment for analysis, his general subdivision into five basic types has been followed here, with some modifications.

1. *Hand collection.*—The procedure involved the lifting of individual stones and washing the animals into a hand net. Britt (1955) has described the use of a weak alcohol and HCl solution as an aid for removing clinging forms from stones due to its effective reversal of their negative phototactic response. Macan (1958) has attempted to quantify hand collecting by working for a definite length of time, and Reynoldson (1958) has discussed some of the problems involved in such an approach. The procedure certainly can not be considered quantitative in any strict sense.

2. *Artificial substrate*.—Moon (1935, 1940) employed trays filled with washed substrates in investigations of Lake Windermere and the River Avon. The trays were sunk into the substrate and removed after a given length of time; the contained animals were counted. A similar method was followed by Wene and Wickliff (1940) in their study of a small Ohio stream. Britt (1955) lowered grooved concrete blocks onto gravel and pebble lake substrates. The blocks were raised, after one to four weeks, and the animals removed and counted. A similar technique was employed by Mundie (1956a) for sampling silt deposits on reservoir inclined rock surfaces. Cianficconi and Riatti (1957) utilized such a procedure in stream studies. These are actually experimental techniques and not true sampling devices and would be most valuable when used concomitantly with a standard sampling procedure.

3. *Boxes and cylinders*.—The general procedure involves the enclosure of an area of bottom and the removal of the animals contained, usually by dipping. Wilding (1940) described this type of sampler, and Macan (1958) has described the Neill sampler which is also of this type. Dendy (1944) employed a modification of the cylinder method in his study on the fate of stream animals in a lake. Jónasson (1948) utilized a box in his quantitative study of the benthos of the Danish River Susaa. Gerking (1957) sampled the fauna associated with the aquatic plants of the littoral zone, which he termed the phytomacrofauna, with a box designed to cut off the plants above the substrate. After removal of the plants, the fauna associated with the sediments was sampled with an Ekman dredge (see below).

4. *Stationary nets*.—Undoubtedly the most widely used sampling device for investigations of stream benthos is the Surber-type square-foot sampler. The animals in a one square-foot area, delineated by a frame, are washed into a net on the downstream side of the frame (Moffett, 1936; Surber, 1937). Modifications of the general type were made by Leonard (1939) for work in slow water and Hess (1941) for investigations in fast waters. In both cases, the addition of a screen enclosure prevented animals from moving into or out of the square foot area. Ide (1940) determined quantitative benthos on the basis of emergent adults collected in stationary screen traps which he placed in riffle areas. Brundin (1949), Jónasson (1954), and Judd (1957) used emergence traps for the collection of aquatic insects, and Mundie (1956b) has reviewed the sources of error and the limitations of emergence traps. Guyer and Hutson (1955) found that catches from funnel and tent-type traps compared favorably, but that trapping and bottom sampling gave different results. Gledhill (1960) showed that emergence traps on two streams did not catch all the species of aquatic insects known to be present and pointed out that quantitative information obtained from such traps is not consistent due to the varying emergence habits of different species. It is apparent that emergence traps cannot be used as truly quantitative samplers for bottom fauna.

The use of stationary nets to capture organisms carried as stream drift has been reviewed by Waters (1961). Of course, only certain faunal elements occur in stream drift.

5. *Push or drag nets*.—These samplers take the form of various types of scoops or shovels equipped with nets which retain the animals and larger substrate materials. Allen (1951) used such a scoop in his survey of the Horokiwi Stream. It required two operators, one to push while the other pulled the apparatus a specific distance through the substrate. Macan's (1958) sampler was built onto a shovel handle and could be operated by one person. Kamler and Riedel (1960) sampled stream substrates with a frame and bag, built somewhat like a Surber sampler, which was pushed along the bottom the length of the frame. The coarse sediments collected in the bag were roughly sorted into size classes and the number of particles counted. Some workers have sampled with various types of tow nets, on metal frames or runners, in deep water where they are pulled along like a trawl. Such a device was described by Usinger and Needham (1956) for sampling coarse substrates in water too deep to wade.

The primary objection to all the sampling methods described above is their failure to retain the entire sediment sample for physical analysis, although the cylinder or box method could be modified so that the sediment and animals were lifted from the stream.

COLLECTION OF SUBSTRATE AND ASSOCIATED ANIMALS

Because sediment analysis has been neglected in stream investigations, with the exception of a study by Wene (1940), any discussion of sampling devices, suitable for the collection of substrate and animals, is limited to dredges or grabs and coring devices originally developed for marine and lake studies. Although a large number of dredges have been employed by oceanographers (Twenhofel and Tyler, 1941; Longhurst, 1959), only two dredges have been used extensively by limnologists—the Ekman and Petersen dredges; both have been described and figured by Welch (1948). Both devices are best suited for fairly soft sediments which are relatively free of aquatic vegetation, although the latter can be used in gravel. Although Wene (1940) used the Petersen dredge to sample stream midges, he collected sediment for particle size analysis with a hand trowel. Kellen (1954) described a bottom sampler, somewhat similar to the Ekman dredge, which was operated manually and designed for work in shallow water, and Allan (1952) has constructed a hand-operated dredge for sampling river beds composed of gravel or finer sediments. Ford and Hall (1958) designed a dredge for quantitative sampling in stream muds which takes a core-like sample by virtue of its closing mechanism consisting of two horizontally sliding steel plates. Lauff (1957) and Lauff *et al.* (1961b) used the orange-peel dredge for physical studies in Grand Traverse Bay on Lake Michigan and the Straits of Mackinac,

and Hensen (1954) employed it in his study of the profundal bottom fauna of Cayuga Lake. This device was first modified for studies of sediments by Sumner *et al.* (1914). Birkett (1958) compared the efficiencies of the Petersen and several types of van Veen dredges on the basis of animal density per unit volume of sample and found that the dredges collected only 70% or less of their expected sample volume. He also pointed out that sample depth is extremely critical because of intra- as well as inter-specific burrowing differences.

The majority of the dredges mentioned above can be applied to stream investigations only with difficulty since they are virtually useless in sediments more coarse than fine gravel.

As Longhurst (1959) has pointed out, a corer should be the best sampling device, but a satisfactory design has yet to be developed. The primary difficulty for studies of animal distribution is the small sample size taken by most coring devices so that reliable results are obtained only from a large number of samples (Lackey, 1961). This is especially true of piston corers of the type employed in lake studies (Mortimer, 1942; Livingstone, 1955; Vallentyne, 1955; Brown, 1956). These corers would be suitable for stream sampling, but are severely limited in diameter by the weight of sediments which must be held in the tube by the piston.

In a study of the micro-distribution of two species of limnephilid caddisfly larvae, the author utilized a $4\frac{1}{8}$ inch diameter cylinder for the collection of quantitative samples of stream sediments. Once the cylinder had been pushed into the substrate to a desired depth and a metal disc placed in the upper end to prevent the silts and clays from washing out, a steel plate was pushed under the sampler; the substrate and contained animals were lifted from the stream (Cummins, 1961). A similar technique was used by Kamler and Riedel (1960) during a portion of their studies on the bottom fauna of Tatra streams in Poland.

A promising approach, which not only permits the sampling of fairly coarse sediments (up to fine pebbles) but also the study of vertical distribution of animals in the sample, has been described by Efford (1960). The Efford sampler, of the general type built by Shapiro (1958) for lake studies, utilizes liquid oxygen to freeze the core sample *in situ*; after the sample has been removed from the stream bottom, the sediment core is sectioned with a hacksaw.

Cole (1955) has discussed some types of suction bulb samplers utilized in the collection of microbenthic fauna from very fine flocculent lake sediments.

PHOTOGRAPHIC ANALYSIS

A technique which has not been exploited as a true sampling method is photographic analysis. Photographs of the ocean floor (Emery, 1952; Hunkins *et al.*, 1960) and lake bottoms (Ohle, 1960)

have contributed information on the nature of the surface sediments and the animals present. As Odum (1959) has indicated, the resolution is sufficient so that some taxonomic determinations have been made from such pictures. Television, accompanied by appropriate photographic records, also shows great promise as a technique for the observation of the bottom in deeper water (Barnes, 1958, 1959). Allen (1959; personal communications) has applied a photographic technique to his studies on New Zealand streams. He employs a special camera stand equipped with an intricate system of lights above and below the water surface. The purpose of the photograph is for

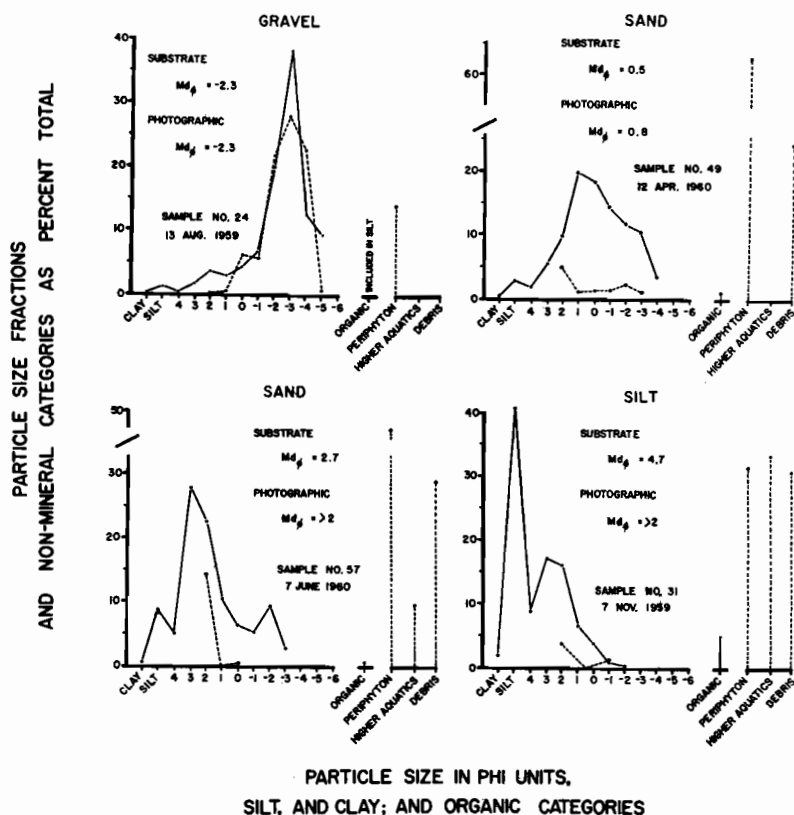


Fig. 1.—Comparison of physical and photographic analyses of stream substrates. Weight analysis of particle size fractions and organic material, solid lines; photographic analysis of particle size fractions and organic categories (periphyton, higher aquatic plants, and debris), broken lines. Data from Cummins (1961).

the accurate observation of an area which has been sampled with a Surber-type frame and net.

One advantage of a photographic technique lies in the fact that the degree of magnification can be adjusted to any particular level of environmental irregularity. By using the appropriate extension with either a telephoto lens or an underwater camera box, detailed close-up pictures can be obtained. In fact, using a series of magnifications, more than one "sample" can be taken at a particular site. Subsequently, the animals can be removed with a Surber-type sampler or other device, and numbers determined.

The photographic technique is best suited for the analysis of the distribution of "sprawling" forms which move about on top of the substrate; the picture will include a record of animal positions. The validity of the technique with respect to burrowing forms (including negatively phototactic clinging forms) is dependent upon the relationship between the surface substrate layer and either the nature of the subsurface materials or the behavior of the burrowing forms. It may well be that these animals, after selecting burrowing sites from surface features, move very little in a horizontal plane; such was definitely shown to be true for burrowing larvae of the caddisfly *Pycnopsyche lepida* (Cummins, 1961). In fact, those burrowing animals which can be generally classed as filter feeders are closely dependent upon the nature of the surrounding surface substrate. When studying benthic macro-invertebrates associated with vegetation and debris ("climbers"), depth of field problems can be surmounted by shooting underwater pictures horizontally.

Figure 1 presents a comparison of physical and photograph analyses of four sediment samples taken in a small southern Michigan stream (Cummins, 1961); the particle sizes and median diameters are given in ϕ units (Table I). The substrate samples were taken with the cylinder described above (Cummins, 1961) and analyzed by dry sieving after aqueous removal of silts and clays (see discussion below). The organic category includes periphyton, higher aquatic plants, and debris (primarily leaves and sticks of terrestrial origin). This category of the physical analyses represents dry weights of organic material which was hand-picked from the substrate samples. Since the photographic procedure has been described in detail elsewhere (Cummins, 1961), only the essentials of the method will be considered here. Analysis involved the projection of a color transparency of the stream bottom, taken prior to sampling with the cylinder, onto a 442-square grid (squares were 25 mm on a side). Once the color slide was centered within the grid and the projector properly focused, eight sharp-pointed dividers, marked according to particle sizes, were adjusted to the scale of the photograph by setting each divider to the proper millimeter opening as measured on the projected image of a scale which had been placed on the stream bottom within the photographic field. Mineral particle-size categories corresponded to sieve sizes used in the physical analysis, although all particles smaller

than $\frac{1}{2}$ mm were lumped into one size class. The dividers were set to the lower limit of each size range and any particle which fell within one and two divider intervals was counted in that particular size class. To render the measurements comparable to sieve analysis, the least diameter of particles was measured. Each square on the grid was examined and the particle size or other category which was judged to be most abundant by area was recorded.

The photographic method can at least provide more certain documentation for the phenotypic descriptions of bottom type which usually accompany stream collections. The agreement of results obtained by sieve analysis and photographic analysis suggest the merit of the technique as a quantitative method; information provided about the surface substrate layer, vegetation, and organic debris can be of significant value.

COMPACTION OF THE SUBSTRATE AND INTERSTITIAL WATER FLOW

Measurements of the compaction of the substrate in terms of the resistance to penetration (Chapman, 1949; Carruthers, 1954) can provide useful information concerning the burrowing suitability of various sediments, particularly as compaction relates to the physical nature of the substrate and the action of water currents. A closely related technique is the determination of interstitial water flow (Pollard, 1955; Terhune, 1958).

DETERMINATION OF CURRENT VELOCITY

Inman (1949) discussed three factors which must be considered in studying the relationship between current velocity and sediment character. The first of these is roughness velocity or the current velocity required to convert laminar flow into turbulent flow over a given particle size. This turns out to be a linear relationship—the more coarse the sediment, the slower the current that will cause turbulence. The second factor is the settling velocity which is a constant relationship, given by Stokes' Law, for particles smaller than fine sand of 0.18 mm diameter. Particles of larger diameter fall more slowly than Stokes' Law predicts because of turbulence. The third factor is threshold velocity or the velocity necessary to move a particle of a given size along the bottom.

When the three velocity regimes are plotted against particle size on a log log scale, they intersect at a grain size of 0.18 mm and a velocity of about 2 cm/sec. Therefore, environments approaching these two characteristics would be fairly stable with very little sediment transport. Sanders (1958) has found a significant correlation between sediments with a median diameter approaching 0.18 mm and abundance of marine benthic filter-feeders. All three parameters, roughness, settling, and threshold velocities, have their impact on the sediments and benthic animals. For example, a current velocity of 4 cm/sec would be sufficient to move particles in the coarse silt through

coarse sand range, to carry all particles smaller than medium sand in suspension, and to create turbulence over very fine sand and larger particle sizes. Although the turbulence (roughness velocity) and shifting of the substrate (threshold velocity) are undoubtedly of importance in animal distribution, no method has been devised to quantify them directly. Current velocity (primarily settling velocity) can be quantitatively determined, but few workers have measured it at the stream bottom where it influences animal distribution.

Float methods and devices for measuring current velocity at the surface provide little or no useful information about the micro-distribution of benthic animals. Measuring devices based on the general plan of the Pitot tube (described by Welch, 1948) yield results that are too gross, even though their design would permit measurement at the stream bottom.

Both the Leupold Stevens midget current meter (Leupold & Stevens Instruments, Inc., Portland 13, Oregon) and the pigmy current meter (Corbett, 1955) are instruments that permit measurements to be made that are suitable for micro-distributional studies, although the latter meter provides more reproducible results. Allen (1959, and personal communications) has designed a current meter that is also suitable for such studies.

Several deficiencies in the sampling procedures employed by freshwater benthic ecologists are abundantly clear. In almost all cases there is no basis on which to compare the results obtained by different investigators, even though there is overwhelming evidence that consideration of the physical nature of the substrate would prove most fruitful in analyses of benthic faunas. Therefore, it seems that all benthic studies should include a physical analysis of the sediments rather than noncomparable verbal descriptions of the substrate. Furthermore, sample size should be adjusted so that important environmental irregularities remain discrete, and a sampling frequency should be selected to yield an adequate distributional picture on a seasonal basis. Stream investigations should include current measurements near the substrate-water interface where the benthic animals reside.

LABORATORY ANALYSIS OF QUANTITATIVE BOTTOM SAMPLES

PRETREATMENT

Once the quantitative sample has been collected, it must be treated in accordance with the nature of the substrate materials and the types of analyses to be made. If the objective of the investigation is the study of the benthic fauna, these animals should be removed in the field or the samples preserved for sorting in the laboratory. Although there are advantages to sorting bottom samples while the organisms are alive, it is usually more convenient to preserve the samples and analyze them in the laboratory. However, if analysis of the total organic matter in the sediments is to be made, the sample should either not be preserved or a carefully measured amount of

preservative of known organic content utilized. If neither of these procedures is practicable, then an animal-free subsample should be set aside for organic analysis. In some cases it may be feasible to freeze the samples until laboratory analysis can be accomplished.

Most workers have intended that organic analysis should measure the total amount of dead organic material available as food for detrital feeders. However, it would seem that living aquatic vegetation should also be included in measurements of total organic matter, since many benthic forms are herbivores, either exclusively or in addition to being detrital feeders. Nevertheless, if the intention is to measure only dead organic matter, then living aquatic plants must be removed from the portion of the sample to be analyzed.

In studies concerned with the physical nature of sediments containing marl concretions or large silt aggregations, care should be taken to maintain them in their natural state because benthic animals probably respond to the whole aggregation rather than its components. Such samples should be stored and analyzed wet.

FAUNAL ANALYSIS

The most thorough, but also most time consuming, procedure for removing animals from benthic samples is hand sorting. If sediment analyses are to be made, preliminary washing in the field cannot be done, and hand sorting is particularly tedious, even when the samples are picked in the field while the animals are still alive.

Jónasson (1955; 1958) discussed aspects of sieving but with particular regard to mesh size used in the preliminary sorting of samples in the field. Anderson (1959) has reviewed most of the flotation techniques that have been used in faunal analysis and described his own procedure. Flotation methods employ a solution (such as a sugar or calcium chloride solution) of a density which will float benthic animals but not mineral or heavier organic substrate materials. The technique does not aid in the removal of mollusks or case-bearing larvae, and cannot be utilized on samples destined for physical or organic analysis. It is of limited use for samples containing appreciable amounts of vegetation and debris.

Elutriation and decantation techniques have also been used in faunal analysis. For example, Moon (1935) separated benthic forms from substrate materials in a trough fitted with baffles. Water flowing through the system deposited animals and sediment in different portions of the trough. Allen (1951) used a similar technique but employed a straight trough. Lauff *et al.* (1961a) have described a special tube for sorting bottom fauna samples. A sample placed in the tube, which has been filled with water, is agitated with compressed air, and the animals put into suspension are decanted off.

Elutriation and/or hand picking appear to be the only methods of animal removal suitable for studies involving sediment analyses.

ORGANIC ANALYSIS

The organic content of a sediment sample is usually estimated by one of three general procedures: 1) loss of weight on ignition of the sample; 2) collection of CO_2 evolved following dry combustion of the sample; and 3) determination of organic carbon or nitrogen. Morgans (1956) has reviewed several techniques and presented a discussion which is of special value for ecologists.

Both the loss of weight on ignition and carbon combustion train methods must be preceded by the removal of inorganic carbonates with acid. This is especially important in the case of samples containing large amounts of mollusk shell fragments or marl deposits. Values obtained by loss of weight on ignition are not reliable estimates of organic content due to the loss of constitutional water from silts and clays which accompanies ignition, although a fairly constant relationship exists between sediment texture and water content (Sherman, 1951), and some workers (Hansen, 1959; Frey, 1960) have concluded that the ignition technique is the most reliable method when clay content is low. Buscemi (1961) has realistically termed his loss of weight on ignition values "total volatile solids." Colloidal water driven off in the dry combustion method is removed in an absorption tube prior to absorptive collection of the evolved CO_2 . Piper (1947) has reviewed the carbon combustion train technique in detail. Although the recovery of carbon from dry combustion is assumed to be 100%, I found this not to be the case when known amounts of sucrose and sand were mixed and combusted; sample size, nature of the organic matter, and length of time of combustion are all extremely critical. Carbon values obtained by dry combustion are usually converted to total organic matter by the application of an appropriate factor (see below). Since the carbon combustion train technique is laborious and requires specialized apparatus, it has not been widely used by ecologists.

The determination of total organic content from the nitrogen content of a sediment sample is based upon the average percentage of nitrogen in protein and the average amount of protein in organic matter. Of course, these two proportions will vary significantly with the nature of the substances composing the conglomeration that is total organic matter. The amount of organic matter is usually considered to be about 18 times the nitrogen content (Trask, 1939) as based on the carbon-to-nitrogen ratio. However, as Sverdrup *et al.* (1942) indicated, values for the carbon-to-nitrogen ratio in organic matter vary from 5.5 to 20 and more in some instances. Metson (1956) has described a semimicro-Kjeldahl method for the analysis of nitrogen in soil samples (see also Niederl and Niederl, 1942).

The procedures most frequently employed by ecologists for the estimation of organic matter concern the determination of organic carbon. Two methods, the Schollenberger-Allison method (Schollenberger, 1927, 1931, 1945; Allison, 1935) and the Walkley-Black

(1934) method have received particular attention. Both procedures involve the partial oxidation of organic matter by chromic acid followed by a titration for the unused acid. Walkley (1935, 1947) has critically reviewed the latter technique, and modified forms of both methods are given in detail by Metson (1956). Although the results of the Walkley-Black method are more variable due to the lower, less constant temperatures of oxidation, it is the most popular with ecologists because of the ease and rapidity with which it is completed.

Two factors are applied to the carbon values obtained by wet combustion techniques to convert the results to total organic matter. A correction must be made to allow for the incomplete recovery of carbon by wet oxidation techniques. For example, carbon recovery is 60% to 90% complete by the Walkley-Black method depending upon the nature of the soil (Walkley, 1947), but recovery is usually assumed to be about 75% complete. Factors ranging from 1.67 to 2.0 have been utilized for the conversion of carbon values obtained by various techniques to total organic matter (Morgans, 1956), but the most widely used figure is 1.8 as proposed by Trask (1939). Since carbon recovery itself varies significantly according to the nature of the substrate analyzed, rather than compound the error, results are best left in terms of carbon (Bader, 1954).

After considering various techniques and the results obtained by many workers, Morgans (1956) concluded that the Walkley-Black method was suitable for benthic studies. Nevertheless, the dry combustion method is the technique best designed to achieve reliable results in the analysis of organic carbon in aquatic sediments. However, the great variability of results obtained by any of the standard procedures of organic analysis renders them of very limited use for the comparison of micro-habitats where important subtle differences lie outside the accuracy of the techniques. The time required to obtain such tentative results may not be warranted in many benthic investigations. In many cases food studies on particular organisms would yield more valuable information, with specific attention being directed toward food materials identified in gut analyses.

PHYSICAL ANALYSIS

A discussion of techniques of physical analyses oriented for ecologists was given by Morgans (1956), and standard works on methods have been presented by Krumbein and Pettijohn (1938), Wright (1939), and Twenhofel and Tyler (1941). A historical treatment is given by Krumbein (1932a).

VISUAL MEASUREMENTS

As Krumbein and Sloss (1951) have indicated, various techniques are of special advantage in the analysis of different size classes of sediments. Certainly boulders, cobbles, and coarse pebbles should be measured individually, because of the large weights involved. At

the other end of the scale, sands, silts, and clays can be analyzed microscopically by measuring the particles in a small fraction of the sample. No matter what method is employed in sediment analysis, it is desirable that representative size fractions be examined microscopically to estimate contamination and efficiency of the technique.

In investigations of coarse stream substrates, individual boulders, cobbles, pebbles, and pieces of gravel were measured by Kamler and Riedel (1960), and Scott (1958) measured three dimensions of the large stones with which caddisfly larvae were associated. Furthermore, Scott and Rushforth (1959) and Scott (1960) have suggested that the area of stream bottom covered by stones be determined within a sample plot, presumably by measuring the stones, since this parameter, designated as C_v , is of considerable importance as a measure of available habitat niches and, therefore, of animal density. Then, the ratio of covered bottom to the sample area, C_{v_r} or the cover fraction, can be utilized as an index of benthic macro-invertebrate population density.

METHODS BASED ON SETTLING VELOCITY

A simple and convenient method for analysis of fine sediments (*phi* 4 and finer) is the pipette method, first described by Robinson (1922) as a simplified modification of earlier German techniques. The detailed procedure given by Krumbein (1932b) was modified by Rittenhouse (1933). The method is based on the density of a soil suspension at a fixed depth in a liter graduate after a given length of time. The sediment material, withdrawn with a pipette from the fixed depth at various time intervals, is dried and weighed. These weights are adjusted by the ratio of pipette volume to total suspension volume to determine the total weight of sediments grouped by settling velocity. This was the sediment analysis procedure followed by Wene (1940) in a study on stream midges collected in sand and mud bottom areas.

Another technique for the analysis of very fine sands, silts, and clays is the hydrometer method, first described by Bouyoucos (1927). It is similar to the pipette method, but the density of the soil suspension is measured with a hydrometer at various time intervals. Since this method, like the one above, depends on the individual settling of particles, the sediments first must be dispersed with a deflocculating agent and by mechanical agitation. Numerous modifications have been made in the technique (Bouyoucos, 1936; Day, 1950; Lambe, 1951).

The pipette and hydrometer methods for the analysis of silts have their greatest application in the study of deep-water lake and marine sediments. In most streams, silt and clay constitute small percentages of the substrate and do not warrant special treatment. Nevertheless, from the standpoint of the distribution of benthic organisms, the amounts of these two size fractions may be quite critical and small

variations of great importance. It is doubtful that particle size percentages within the silt and clay fractions have an important impact on the micro-distribution of stream macro-invertebrates, and it appears that silts and clays should be removed by decantation and treated collectively as size classes in the physical analysis of stream beds. Once the silts have settled out the clays can be removed by centrifugation (Cummins, 1961).

A method for the detailed analysis of sands, often used in conjunction with hydrometer analysis, is the "rapid sands technique" first described by Emery (1938). In general, the method involves the measurement of the amount of sand grains settling to the bottom of a long column of water, at empirically determined time intervals. Since the technique is designed for the analysis of sands only, fine gravel is removed by wet sieving and most of the silt and clay by decantation. Modifications and refinements of the technique have been presented by Poole and Butcher (1951), by the Subcommittee on Sedimentation of the Inter-Agency Committee on Water Resources (1957), and by Whitney (1960).

The primary objection to this technique is that only a small fraction of the sample (5 gm or less) can be analyzed. While splitting of a sample introduces only small errors in well sorted sediments, it is not advisable in the analysis of stream substrates. The rapid sands method might be employed for a more detailed analysis of the sands separated by sieving. However, since the great majority of stream sediments contain a significant portion of particles larger than 1 mm, the most satisfactory approach is to sieve the sands along with the coarser materials.

SIEVE ANALYSIS

Undoubtedly the most widely used method of sediment analysis is sieving. The lower limit of sieve analysis is most frequently set at 0.062 mm or the division between very fine sand and silt on the Wentworth Scale (Wentworth, 1922; Table I), and cobbles larger than 16 mm are usually measured individually. Sieving separates particles on the basis of their least cross sectional area and provides reproducible results only if the procedure is strictly standardized. A mechanical shaking device is essential to achieve this purpose and each sample should be about the same size and sieved for the same length of time. The details of sieve analysis were reviewed by Krumbein and Pettijohn (1938), and Morgans (1956) has discussed some important features of the method.

Dry sieving of the entire substrate sample is unsatisfactory because aggregations of silt and clay particles appear in all the finer size fractions, rendering them too heavy and the silt fraction too light. Water must be used to disassociate these silt aggregations. However, wet sieving is equally unsatisfactory, since the water interferes with passage of particles through the fine sieves. Therefore, dry sieving

must be preceded by decantation or elutriation for silt and clay removal. The remaining sediment, very fine sand and coarser, can then be dry sieved with highly reproducible results.

Large particles of organic matter, such as pieces of aquatic vegetation and debris (sticks and dead leaves primarily of terrestrial origin), present a special problem in physical analysis of substrate samples. Since size does not bear a constant relation to settling velocity, some large fragments may be decanted prior to sieving. But, many benthic organisms may respond to the size of these organic fragments in the same general way as they do to mineral particles of similar size. In this regard, it may be important to include organic fragments in dry sieving. If analysis of size fractions is made on a weight basis, dry sieving of organic material not removed by decantation or elutriation would involve only very minute weights for most stream samples. In some cases the majority of the aquatic vegetation and debris can be removed by hand sorting, prior to physical analysis, and weighed as a separate unit designated as vegetation or organic matter. Because of the subordinate position given organic material by this procedure, the more detailed analysis provided by the photographic technique mentioned previously would supply important supplemental information.

On the basis of the applicability of the various methods discussed, particle size analysis of stream sediments is best accomplished by the wet removal of silt and clay followed by dry sieving of the remainder (Cummins, 1961; Eriksen, 1961).

PRESENTATION OF RESULTS

The results of physical sediment analysis should be presented in a manner that is of maximum value in elucidating animal-substrate relationships. Almost without exception, benthic ecologists have measured the weights of each size fraction and reported these as a percent of the total sample weight. In final form, the data are usually presented graphically as particle size plotted against either percent frequency or a cumulation of percent frequency. In order that such results can be compared, it is essential that a standard grade scale be adopted by all benthic ecologists. The Wentworth Scale, which has been most widely used, divides the sediments into size classes that have been shown to be of ecological significance. However, the Wentworth system needs to be modified to include the widely used descriptive term "gravel." From a survey of the literature, it seems that gravel should be used in place of the granule plus the finer half of the pebble classification on the Wentworth Scale (Table I).

The use of the *phi* (ϕ) scale (Table I) developed by Krumbein (1936) simplifies the statistical treatment of sediment data. The *phi* scale converts the geometric Wentworth classification, in which each size category is twice the preceding one, into an arithmetic one with equal class intervals. Since *phi* is defined as the negative log to the

TABLE I.—Substrate particle size terminology and categories

Modified classification (Cummins, 1961)	Wentworth classification		Designation of size class in mm		Phi scale	Sieve sizes	
	Name	Particle size range in mm	Decimal	Fraction		Opening in mm	U.S. Tyler sieve no.
Boulder	Boulder	> 256	256		-8		
Cobble	Cobble	64 - 256	64		-6, -7		
Pebble	Pebble	32 - 64	32		-5		
		16 - 32	16		-4		
Gravel*	Pebble	8 - 16	8		-3	7.93	0.312
		4 - 8	4		-2	4	0.157
		2 - 4	2		-1	2	0.0787
Very coarse sand	Very coarse sand	1 - 2	1		0	1	0.0394
		0.5 - 1	0.5	1/2	1	0.5	0.0197
Coarse sand	Coarse sand	0.25 - 0.5	0.25	1/4	2	0.25	0.0098
		0.125 - 0.25	0.125	1/8	3	0.125	0.0049
Medium sand	Medium sand	0.0625 - 0.125	0.0625	1/16	4	0.0625	0.0024
		0.0039 - 0.0625	0.0039	1/256	5, 6, 7, 8		
Fine sand	Fine sand	< 0.0039	< 0.0039	1/256	9		
Very fine sand	Very fine sand						
Silt	Silt						
Clay	Clay						

* The elimination of the granule and incorporation of part of the pebble category into a gravel category was warranted because of the absence of the term granule and frequent use of the term gravel in the literature.

** Methods unique to the study by Cummins (1961).

base 2 of the particle size diameter in millimeters, the divisions of the Wentworth classification are whole integers on the ϕ scale. Results can be plotted on regular graph paper as cumulative percent curves and various statistical measures made directly. One or more of these measures are frequently used to replace the curve, and serve as a convenient mathematical description of a particular sediment sample. The one most commonly used is the median diameter in ϕ units ($Md\phi$); 50% of the sample, by weight, lies above and below this grain size. Other measures, such as the ϕ values at the 25% (first quartile or $Q1\phi$) and 75% (third quartile or $Q3\phi$) levels and curve shape (kurtosis and skewness) can also be made. Inman (1952) has summarized the various mathematical descriptions of cumulative sediment curves.

General treatments of the graphical presentation and statistical analysis of sediment data are given by Krumbein and Pettijohn (1938), Krumbein (1939), Twenhofel and Tyler (1941), Krumbein and Sloss (1951), Inman (1952), and Morgans (1956).

Although weight is the measurement usually made, the volume of the various particle size fractions or the number of particles in each size category is probably of more direct ecological significance. Morgans (1956) has shown that percent frequencies of size fractions determined by volume and by weight do not differ significantly. Weight determinations are more desirable because additional manipulation is required for volume analyses and the results are less accurate.

The number of particles in a certain size range may be of fundamental importance to benthic animals that construct cases and those that feed from the surfaces of particles handled individually. Complete analysis of a sorted sediment sample in terms of the number of particles in each size class would involve prohibitive amounts of time. However, there is a fairly constant relationship between number of particles and weight within a given size fraction. A few determinations can be utilized to convert the weights of each fraction to numbers of particles with reasonable accuracy. A size-frequency curve plotted on the basis of particle numbers is nearly a mirror image of the resulting curve from the same data plotted as weight. Whereas, weight analysis probably overemphasizes the large particles, number analysis overweights the importance of the fine size classes. For this reason, if number analyses are made, they should be limited to the range of ecological significance for the particular behavior patterns of the organisms under study.

Weight analysis of sieved size classes is undoubtedly the most desirable method, because of its greater accuracy and wider use as well as its relatively constant relationship to both volume and number analyses.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

In addition to the experimental procedures described above, in the section concerning the provision of a substrate for colonization,

laboratory studies on the behavior of benthic animals should be an integral part of all investigations. The detailed analysis of localized distributions can be accomplished only when data from field and laboratory experiments are combined. For example, the actual importance of a single parameter, such as substrate particle size, organic content of the sediments, or current velocity, can be evaluated only under controlled experimental conditions. However, the experimental approach is not to be viewed as a replacement for field sampling, but rather as a tool for the refined evaluation of the complex relationships observed in the natural environment.

Marine ecologists have been responsible for the majority of such studies (e.g., Wieser, 1956; Williams, 1958; Wilson, 1958) although Shelford's (1914) experiments with riffle and pool animals stand as important examples in freshwater ecology. More recently, Lauff and Cummins (in preparation) have utilized a model stream for experiments of substrate selection by stream macro-invertebrates. Their procedure involved the use of eight aluminum trays filled with stream sediments which had been sorted by sieving into size categories according to the Wentworth classification. The trays were arranged in various orders in the model stream and the substrate selection of ten macro-invertebrates was determined under controlled conditions of current, temperature, and light.

In addition, Cummins (1961) and Eriksen (1961) combined field data and experimental information to provide detailed distributional pictures for some freshwater invertebrates.

PROBLEM AREAS OF BENTHIC RESEARCH

From the preceding discussion, several problem areas of benthic research are apparent, particularly those concerning studies of lotic communities. In the first place, the information accumulated to date warrants the adoption of physical sediment analysis as a standard procedure in benthic ecology. Most work needs to be done by stream workers who have heretofore neglected such measurements. The photographic technique, described above, can be utilized to supply valuable information on substrate type. Secondly, determinations of current velocity should be confined to the substrate water interface, with particular emphasis on turbulence. A method for the quantitative measurement of turbulence is needed, perhaps through some sort of vector analysis. Finally, the whole problem of organic analysis calls for severe revision. Many of the values which have appeared in the literature are open to question and their meaning in terms of the trophic relationships of benthic animals is quite obscure. Certainly new techniques are called for, especially those oriented toward the study of the particular food materials of specific organisms. In this case also, the photographic method, previously described, can provide useful supplemental field data.

RECOMMENDATIONS

Based on the preceding review, a series of procedural recommendations for benthic investigations, particularly in lotic waters, is presented below.

1. Systematic sampling (the transect method) or stratified random sampling (selection of habitat types) are the most desirable sampling techniques for faunal survey. Studies limited to one or two species can provide maximum information per unit sampling effort, particularly if sampling can be confined to known animal positions. Sampling should be year-round and of sufficient frequency and duration to obtain a consistent picture of animal distribution.

2. Sampling devices should retain the sediments for analysis; dredges are suitable for most lake and marine investigations. However, it seems that the most desirable type of sampler for all habitats would be some sort of coring device. A cylinder is described which can be employed in streams and a core sampler which permits the sample to be frozen *in situ* is reviewed. A photographic technique is also described which can be used either in conjunction with samplers that do not retain the sediments or to provide supplemental information about the substrate surface.

3. Measurements of current velocity should be made as close to the substrate-water interface as possible.

4. Field samples destined for chemical analyses should be frozen if possible, otherwise special treatment of subsamples is necessary. Preliminary faunal sorting in the field is desirable; in the laboratory faunal removal can be accomplished by elutriation and further hand sorting.

5. Organic analysis is best accomplished by dry combustion train carbon determinations or, for samples with little silt and no clay, loss of weight on ignition values can be reported as total volatile solids. Conversion of carbon values to total organic matter is not advisable. A more fruitful approach concerning organic material is the study of the food relationships of particular species through a coordination of field and laboratory procedures with gut analyses of the animals in question.

6. Physical analysis of stream sediments can be readily accomplished through silt and clay removal by elutriation, and silt clay separation by centrifugation, followed by dry sieving of the coarser particles. The sediments should be separated into size fractions according to the Wentworth classification and the dry weight of each determined. In the case of fine and well sorted lake or marine sediments, the rapid sands and hydrometer methods are suitable.

7. The results of physical analyses should be presented as size-frequency plots. Frequency should be expressed as percent or cumulative percent. The Wentworth size classification should be followed (as modified in Table I) with the size classes graphed according to the *phi* scale; *phi* median diameters should accompany each plot.

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