Chapter 5. Sampling the Stream Benthos

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1 Introduction

Numerous original papers and reviews have been published on sampling of stream or river benthos before and since Hynes’ (1971) review of this topic in the first edition of this manual. The most inclusive have been those of Macan (1958), Cummins (1962), Mason (1976), Mundie (1971), Resh (1979), Waters & Resh (1979), and Merritt et al. (1978). These studies discuss available samplers, problems unique to sampling the stream benthos, and recommendations for choosing the appropriate methodology. I do not intend to reiterate ideas reviewed in the above papers in great detail, but refer you to these reviews, and to other papers cited in this chapter, for more information. My objective is to comment on the available technology for sampling benthos in running waters, and to introduce another approach to choosing appropriate methodology.

Although 10 years have passed since Hynes’ (1971) chapter, no revolutionary break-throughs have occurred to rectify the largely unresolved problems of estimating secondary productivity in running waters. I hope to encourage those attempting to study this problem to avoid the existing, error-ridden techniques, and to apply creativity to the task of quantifying productivity of stream benthos.

This chapter is organized as follows: First, I review some traditional qualitative and quantitative methods for sampling stream benthos, contrast available techniques, and point out existing problems and needs. Next, I discuss experimental design for special problems requiring quantitative measurement of stream benthos. Finally, I conclude with a section on choosing the appropriate sampling methodology.

2 Review of traditional sampling techniques

The design of any sampling scheme and the choice of apparatus should depend largely upon the questions being asked. The procedure for measuring the density of invertebrates in a riffle will differ from that for estimating diversity. Estimating the production of each species requires a unique
approach, specific to the distribution, life history, and habits of that species. For example, measuring the production of surface-dwelling water striders (Gerridae) presents different problems to estimating production of interstitial stoneflies, or of net-spinning caddisflies. One must implement a sampling program that incorporates the special conditions that apply to each species and to the parameter being estimated.

In the past, this procedure has not generally been followed. Most measures of standing crop biomass or numbers, densities, diversity, and production of stream invertebrates have used a similar methodology: random samples with a standard stream sampler (Surber sampler or box sampler), or modifications of these (Merritt et al. 1978) (Fig. 5.1), and the number of samples taken has been based on practicality or avoidance of destruction of a small habitat (see reviews cited above). Although these samplers may be unreliable or inappropriate to the organisms or streams being studied, and unreasonable and destructive numbers of samples may be required to estimate benthic density, biomass, diversity or production, the same methods continue to appear in the literature (Resh 1979).

Several examples illustrate the potential imprecision of standard stream sampling techniques. These studies were designed to test the effectiveness of certain methods of estimating the standing crop of invertebrates in stream riffles. Needham & Usinger (1956) systematically took 100 samples in a 10 x 10 latin square design in a 'uniform' riffle in Prosser Creek, California. Five people took 10 Surber samples each over a two-day period. (Ideally,
replicate samples should be taken instantaneously.) These data allowed the authors to determine the variability among samples, and to calculate the number of samples necessary to estimate the benthic standing crop with 95% confidence intervals within 40% of the mean. Their estimates of 73 (numbers) and 194 samples (biomass) were later shown by Chutter (1972), to be too high. Chutter also showed that a sample size of 448 was necessary to estimate numbers of invertebrates within 5% of the mean.

This impractical and destructive number of samples is required to make reasonable population estimates due to the patchy distribution of benthic invertebrates in this seemingly 'uniform' riffle. The number of animals collected per sample ranged from two to 198. Radford & Hartland-Rowe (1971) and Frost et al. (1971) calculated that similarly impractical numbers of Surber samples would be necessary to obtain a reasonable estimate of standing crop in seemingly uniform riffles. Resh (1979) took 26 pairs of Surber samples side by side in an Indiana stream to estimate the patchiness of the net-spinning Trichopteran larvae, Cheumatopsyche pettiti (Hydropsychidae). If the distribution of these larvae was uniform, one would expect a positive correlation between the numbers of C. pettiti collected by the two investigators in adjacent samples. The result, however, showed that the distribution of these insects was contagious, requiring 24 samples to obtain estimates of standing crop within 40% of the mean.¹

Some of the variation in numbers of invertebrates per sample could be due to differences in sampling technique or efficiency of different people. Needham & Usinger (1956) showed that only four or five people were consistent in the range of numbers of animals obtained. Pollard (1981) implemented a standard traveling kick method (STKM) using a simple dip net to estimate standing crop of benthos in a Colorado stream. A net was held in place on the substrate, and the investigators moved downstream, vigorously kicking the substrate for a prescribed distance and time interval. Pollard compared the results of samples taken by different investigators, and between samples taken for 30 sec over a 3 m² of substrate to those for 15 sec over 1.5 m² of substrate. He found significant differences between samples taken by different investigators in depauperate reaches, and showed that samples taken for twice the time over twice the substrate area did not yield twice the standing crop, although fewer discrepancies occurred in faunal-rich reaches. Pollard concluded that there is an unknown relationship between length of kick, substrate area sampled, and faunal richness.

Another popular methodology has been the use of artificial substrate samplers, such as basket samplers and multiplate samplers (Fig. 5.2). These may be useful for collecting selected taxa that prefer such introduced habitats;

¹. Editor's Note: see Section 5.2 for a treatment of sample size and precision.
however, caution must be exercised when using artificial substrates to avoid obtaining biased samples. For example, I attempted to estimate the composition of the benthic invertebrate community in Otter Creek, Wisconsin, during the summer of 1975 using various different sampling techniques, including Surber samplers, Dendy samplers, drift net samples, and mesh cages filled with natural stream substrates (Fig. 5.3). I compared estimates of percent composition of the four major orders between the different methods (Fig. 5.4). Surbers and drift nets obtained similar numbers of Ephemeroptera, but drift nets captured more Plecoptera and Trichoptera, and fewer Diptera than Surber samplers. Cage samples contained a slightly higher percentage of Ephemeroptera, more Plecoptera, fewer Diptera, and the same proportion of Trichoptera as Surbers. Dendy samplers gave the most divergent picture of percent composition at the ordinal level, with much higher estimates of Plecoptera, and smaller proportions of Trichoptera and Diptera (Peckarsky 1979b). Had I used Dendy samples to estimate the standing crop or production of benthos in Otter Creek, I could have made a serious error.

Many authors (e.g. Hilsenhoff 1969: Brooks 1972: Rosenberg & Resh 1982) have summarized the potential disadvantages of the use of artificial substrate samplers to characterize stream benthos:

1. They differentially attract some taxa, as illustrated above.
2. The time required for colonization to reach equilibrium may be impractically long.
3. Organisms may be lost upon retrieval unless precautions are taken.
4. Passers-by may tamper with these devices.

Given knowledge of these possible problems, artificial substrate samplers can
Fig. 5.3 Colonization cage, first generation designed by S.I. Dodson (from Peckarsky 1979a).

Fig. 5.4 Percent composition (by order) of samples taken with Surber samplers, drift nets, cages, and Dendy samplers in Otter Creek, Wisconsin.
be implemented if they are an efficient means of collecting certain taxa. Extrapolation of results to estimates of absolute benthic density or production may be erroneous. However, they can be effective in comparison of the production of a taxon from site to site.

Core samples have been used to measure the vertical distribution of hyporheic benthos by several investigators (Bishop 1973; Coleman & Hynes 1970; Hynes 1974; O'Conner 1974; Williams & Hynes 1974; Godbout & Hynes 1983). Historically, these studies identified a habitat that had been overlooked by surface sampling techniques, and suggested that estimates of standing crops based on surface samples might be serious underestimates. These techniques may be appropriate in certain substrates (loose gravel or sand), but impractical in cobble, rubble, and substrates where hardpan exists a few centimeters below the surface. Furthermore, criticisms of surface samples as underestimates may only apply to penetrable substrates. Caution must be exercised in retrieval of core samples, as in other artificial substrate samplers, so that animals are not lost.

Resh (1979) presents a tabular summary (see Appendix, Section 5.1) of factors that affect various benthic sampling devices and may result in sampling bias. Even more useful, he includes possible remedies to the problems of each sampler. This table is extremely important to all investigators who must choose between the available devices within the limitations of each particular study. Use of the information given in Resh (1979) will ensure more responsible implementation of traditional benthic sampling techniques.

A different number of samples and type of sampling program may be appropriate to estimates of standing crop, production, and species composition. Needham & Usinger (1956) showed that fewer samples were required to estimate diversity than standing crop, but that samples needed to be taken over a wider range of substrate types. Two to three samples were enough to collect the most common Ephemeroptera, Plecoptera, Trichoptera, and Diptera. Nelson & Scott (1962) found that 96% of the taxa recovered in 12 Surber samples were included in the first four samples. Random samples are most likely to give unbiased estimates of standing crop or production of the entire benthic community. However, stratified random sampling, concentrating more effort in habitats rich in fauna (riffles versus pools), or systematic sampling, such as transects, may be more efficient at gathering information about specific taxa given information about their life histories and distribution (Resh 1979; Tanner 1978; see also Chapter 8).

I am not aware of any simple solutions to the impracticality associated with obtaining good estimates of benthic standing crop or production in streams. At the very least, we should be aware of the potential error in our calculations of production or standing crop, given the limitations of sampling patchily distributed organisms. Merritt et al. (1978) describe methods for
analyzing stream bottom samples to arrive at estimates of standing crop biomass and numbers (cf. their Fig. 3.1, p. 14). Gillespie & Benke (1979), Benke (1979), Krueger & Martin (1980), and Menzie (1980) have contributed to techniques for calculating production of stream insects. Krueger & Martin (1980) emphasize the importance of carrying out separate calculations on species with different voltinisms, maximum sizes, growth rates, and trophic levels. Cushman et al. (1978) present a comparison of the removal-summation, instantaneous growth, and size-frequency methods of calculating production, based on a computer simulation considering such variables as sampling interval and growth rate. In addition, Whittaker (1972) and Peet (1974) have defined the problems and appropriateness of various diversity indexes for use in ecological studies. Kaesler & Herricks (1979) apply the concept of diversity indexes to problems of environmental impact assessment.

These studies provide sound advice based on the mathematical constraints of various available measures of production and diversity. For example, Cushman et al. (1978) showed that the removal-summation technique for calculating production was the most robust over different simulated growth curves and mortality, as well as over different sampling intervals. Peet (1974) showed that indexes of species richness (S = number of species) are affected by arbitrary choice of sample size (N). One can actually predict the rate of increase of S with increasing N. Peet also notes that different types of heterogeneity indexes that take both richness and the distribution of individuals within each species (evenness or equitability) into account are sensitive to different changes. The commonly used Shannon type index is more responsive to changes in rare species, whereas the Simpson type index is more sensitive to changes in common species. All heterogeneity indexes give ranges which are comparable qualitatively but not statistically, due to peculiarities in the underlying distribution of the index values. They also assume an infinite community of known proportions. Peet suggests that one who uses any heterogeneity index should develop a 'response curve', that is, graph the components of the index summed over all species. This will show how the changes in the sample composition affect the index.

Other types of heterogeneity indexes have been adapted from information theory (Brillouin type) (see Kaesler & Herricks 1979). These indexes are not dependent upon sample size, but are very cumbersome to calculate. Cuba (1981) developed an index that separates the influences of S and evenness, and gives values that indicate how close the observed sample is to a theoretical sample of equivalent S and N where numbers of individuals are evenly distributed among species. This index always gives higher values with larger S, which is not always true of other heterogeneity indexes. Equitability indexes are also reviewed by Peet (1974). Most require a knowledge of the number of species in the sampling universe, and increase with species richness. Alatalo
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(1981) suggests that Hill's ratio is the least ambiguous, is not dependent on sample size, and does not require a knowledge of $S$. It is, however, quite new and has not been fully explored.

No matter how sophisticated the manipulation of data, however, standing crop biomass, production, or diversity estimates are unreliable unless they have been calculated from appropriately gathered data. The precision of each of these measures is dependent upon adequate sample sizes, generally in excess of the actual number of samples taken (see Appendix, Section 5.2). The most elegant calculations cannot overcome the shortcomings of a poor sampling program.

In summary, stream biologists are faced with the frustrating problem of lacking adequate methods to determine accurately how many organisms inhabit lotic ecosystems. What can we do to resolve this dilemma? Rather than abandoning the challenge altogether, or lowering our standards of desired levels of precision, I suggest that a problem-oriented approach may improve the precision with which data are gathered, and allow us to answer some of these difficult questions.

3 Experimental design for special problems

Below are case histories as examples of studies in which creativity has been applied to quantitative problem solving in investigations of stream benthos. The studies discussed cover a broad range of questions that may be of interest to stream ecologists, such as density or production estimates, life histories, benthic distributions, dispersal, stream invertebrate behavior, and biological interactions among benthic invertebrates.

3.1 Benthic density: production studies

In a study of the leech fauna of Lake Esrom, Denmark, Dall (1979) introduced a technique that can be applied to measuring the density of stream-dwelling species on relatively homogeneous mineral substrates. Using a circular iron ring with a diameter of 15.3 cm (185 cm$^2$ area), Dall estimated the surface area of the stones in randomly selected samples taken at two-week intervals. Unembedded stones (>2.5 x 2.5 x 1.0 cm) were collected and measured. Buried stones, gravel, and sand were considered as a separate fraction. Sampling was terminated when 100 stones, an average of 13.8 sample units, had been collected. Leeches and stones were also collected from a 1 m$^2$ horizontal surface of the substrate.

Dall calculated the surface area of the unembedded stones by the formula

$$S = \pi/3(LW + LH + WH)$$  \hspace{1cm} (5.1)
measuring length (L), width (W), and height (H) of each stone to the nearest 0.5 cm. He discussed the biological importance of this parameter as a measure of available habitat for negatively phototactic animals such as leeches (and most stream invertebrates). In addition, he was able to deduce the mean stone surface area per m² of bottom (2.77) from the 1 m² samples. This factor is a more accurate representation of actual habitable space for stone-dwelling organisms than is horizontal surface area of the substrate, and should be considered when estimating the density of these organisms.

This study exemplifies a carefully conceived scheme by which the author obtained valuable information regarding the growth and production of leeches. Not only did he recheck his primary sampling technique with the square meter sampling, but he incorporated additional systematic sampling designed to collect rarer leech species. Dall thereby accounted for the biology and distribution of the taxa being studied, and double-checked the adequacy of his primary sampling technique to provide improved estimates of growth and production of leeches. This study can be used as a model, and modified to obtain similar information on lotic inhabitants with known biological characteristics on similar substrates.

3.2 Life history studies

Resh (1979) pointed out the need for consideration of life history information in the design of studies of benthic production. Conversely, if one wishes to determine life history information such as growth rates, mortality, ontogenetic movement patterns or dispersal, and timing of hatching, diapause, pupation or emergence, specific techniques must be employed to ensure proper interpretation of data. Resh (1979) described the distribution of *Cheumatopsyche pettiti* (Trichoptera; Hydropsychidae) in Rock Creek, Carroll Co., Indiana. All five instars of *C. pettiti* coexist in single riffles, but different instars construct filtering nets with different mesh sizes, requiring different optimal current regimens.

Resh emphasized that sampling design must include the entire range of habitats for all five instars to accurately assess population parameters such as growth and migration. Resh (1975) suggested systematic transect sampling as a technique that maximizes information per unit sampling effort in single-species studies, and enhances the collection of early instars. The procedure is deliberately biased to increase the range of habitat types sampled. Mesh size of sampling nets must also be carefully chosen to avoid overlooking the smallest instars.

The number of samples necessary to estimate population sizes may vary throughout the life cycle of a species. Sampling at regular intervals may not be appropriate for obtaining adequate information; it may be necessary to
sample more frequently during periods when the population is changing more rapidly. Unfortunately, a priori information from which a sampling program can be designed is not always available. Ideally, an investigator should obtain as much relevant information on the biology of a species as possible, either from preliminary samples or from the literature, before designing the sampling program. If this is not possible, Resh (1979) suggested short-interval sampling when populations are in early age classes, and a gradual increase in the length of sampling intervals as the growth rate declines. Another practical solution is to begin a sampling program with short intervals, and then to lengthen intervals if changes in the populations are slow enough to sample less frequently without losing a great deal of information.

3.3 Distributional studies

As we have already seen, complications necessarily arise when populations that are not dispersed evenly in space are to be sampled. Greater numbers of samples are required to accurately estimate population densities as distributions diverge from random, and as sample means become smaller (Elliott 1977; Resh 1979). Since aggregated spatial patterns are so common for stream benthos (see studies summarized in Section 2), techniques must be designed to meet the challenge of measuring these distributions. For example, Downing (1979) described an index of aggregation based on the exponent of the power relationship between density and variance as a means by which to predict the degree of patchiness. He then showed how to plan a sampling program based on this index, and how to transform data for analysis.

Peckarsky (1979a, b) developed a technique by which the factors mediating distributions of stream benthos could be determined as an alternative to multiple regressions of concurrent samples of biotic and abiotic variables. Cages constructed of stainless steel screen and filled with natural substrate were buried in the substrate to allow colonization by invertebrates (Fig. 5.3). These cages were placed along transects to determine the spatial patterns of stream benthos relative to distance from the stream bank, and were stacked vertically to measure the vertical distribution of invertebrates (Fig. 5.5). Substrate type was also manipulated to test the effect of substrate size and heterogeneity on the distribution of benthos.

This technique is very useful since the screen cages contain 'natural' substrate, do not present the obstruction problems of some artificial substrate samplers, mentioned by Hynes (1971) and discussed in Section 2, are relatively light-weight and easily manageable, and allow manipulation of a number of different habitat variables. The experimental technique is more incisive than a correlative sample survey approach for determining factors mediating distributions, since differences between experimental and control treatments
Fig. 5.5 Cross-section of stream showing vertical arrangement of cages. This technique is useful in measuring vertical stratification of benthic distribution in the substrate.

can be interpreted with less ambiguity. However, the cages are themselves an artefact, and may be partially responsible for the observed results (Hulberg & Oliver 1980).

Recently, stream ecologists have introduced association techniques, such as ordination (Culp & Davies 1980), that can handle large sets of data, but, if poorly designed, these can produce ambiguous data subject to a variety of interpretations. Individuals implementing ordination techniques should realize that they reduce information in order to extract it, and thus simplify complex situations. They are also an a posteriori analytical tool, and are less adapted to show causal relationships, but they can be a powerful method for identifying associations among variables under natural conditions. Again, caution should be used in interpreting data based on methods with the limitations discussed in Section 2.

3.4 Dispersal, colonization studies

A modification of the cages described above can be used to determine the direction and distance of dispersal of stream invertebrates within the substrate (Fig. 5.6). Mesh baffles on two sides of the cage allow colonization of benthos from a maximum of two directions. Cages can be attached end-to-end and arranged in a longitudinal pattern with respect to the current velocity (Fig. 5.7). Direction of migration can be controlled by the use of restrictive mesh size baffles on the upstream or downstream end of the colonization cage. Other types of apparatus applied to this question are described by Bishop & Hynes (1969b), Elliott (1971a), Hughes (1970), Hultin et al. (1969), and Keller (1975). They include boxes, tapered mesh bags, and artificial troughs.
Fig. 5.6 Second generation colonization cage; lids held in place with neoprene fasteners.

Fig. 5.7 Schematic representation of an experiment to measure the directional migration of stream benthos in the substrate; cages held together with neoprene fasteners.
Effective colonization devices allow analysis of the colonization pattern of individual species and factors affecting the rate of colonization. Devices can be removed after different periods of time to test the effect of duration of availability of 'new habitat' on the migration of invertebrates. Cages buried with different initial benthic densities allow patterns of density-dependent and density-independent colonization to be measured and equilibrium densities to be predicted (Peckarsky 1979a, 1981). The effect of experimentally introduced detritus on habitat choice can also be measured using colonization cages (Peckarsky 1980a). This technique has also been shown to be an effective indicator of environmental disturbance (Peckarsky & Cook 1981). Baffles of intermediate mesh sizes can be constructed such that large predators, such as Perlidae (Plecoptera), may be confined within the cages, while small prey species (Ephemeroptera, other Plecoptera, some Trichoptera and Diptera) are given access to the cage habitat. In this way, theories concerning the avoidance of predators and effects of predators on prey colonization can be tested (Peckarsky & Dodson 1980a).

An area of considerable interest to stream ecologists has been the study of invertebrate drift. Drift is usually sampled by placing nets in the stream to 'trap' organisms as they travel downstream in the current (Fig. 5.8) (Extensive reviews by Waters 1972; Müller 1974; Wiley & Kohler, in press). This drift net technique can suffer from potential backwash, clogging, inappropriate mesh size, and other logistical problems (see Resh 1979; Section 5.1). A drift net essentially reveals the number of animals that drifted past a certain point in the stream over a specified time period. It does not, however, tell you where the individuals came from, how far they would have drifted had they not been trapped in the net, why they released hold of the substrate, or why they would have settled. Elliott (1971b), Keller (1975), and Müller (1974) review methods for determining drift distances. Hypotheses on causal factors of drift behavior from studies of a correlative nature are reviewed extensively by Waters (1972) and Müller (1974). Here, I describe a few controlled experiments designed to determine some of the causal variables of invertebrate drift.

Ciborowski, Corkum, and associates have conducted refined experiments within a laboratory system to generate data on settling distances, and on the effects of certain variables, such as food, current, photoperiod, substrate, and predators, on drift behavior of some species of stream insects (Ciborowski 1979; Ciborowski & Corkum 1980; Ciborowski et al. 1977; Corkum 1978a, b; Corkum & Clifford 1980; Corkum & Pointing 1979; Corkum et al. 1977). They manipulated these variables and tested the drift responses of certain animals in the laboratory in a plexiglass elliptical channel powered by an Archimedes screw (Ciborowski et al. 1977). Studies of this type and similar work in laboratory streams of other designs by Wiley & Kohler (1980), Walton (1980a, b; Walton et al. 1977), and Hildebrand (1974) have provided
more easily interpretable information than field drift net studies on the factors mediating drift behavior. However, the artificiality of laboratory conditions must be taken into account when extrapolating the results to explain field phenomena.

Another technique for measuring effects of exogenous variables on drift is that employed by Peckarsky (1980b). Mayflies were observed in the field within streams in plexiglass observation boxes with screen ends (Fig. 5.9). Drifting behavior was quantified as a response to encounters with large stonefly predators. *Baetis* (Ephemeroptera, Baetidae) species, commonly trapped in drift nets, were found to enter the water column primarily to avoid the large predators. Wiley and Kohler (1981) also filmed drift events as a response to foraging invertebrate predators in the field. This direct observational approach provided definitive support for the importance of predator avoidance as a causal factor of invertebrate drift in some species.

Finally, a question of considerable importance to those interested in stream production is whether the numbers drifting from substrates cause significant reduction in benthic standing crop. Estimates of the proportion of...
the benthos in the drift are quite variable across species, in different streams, and depending upon the procedure used in calculation. Some examples are 0.0002–0.004% of the benthos in the drift at any given time (Bishop & Hynes 1969a), 0.00059 m$^{-2}$ sec$^{-1}$ (Elliott 1971a), and a mean drift (100 m$^{-3}$ discharge): benthos ratio (0.1 m$^{-2}$) of 0.24–5.31 (Lehmkuhl & Anderson 1972). Waters (1966) showed that drift has no denuding effect on upstream reaches and suggested that drift removes animals in excess of the carrying capacity of the stream, a hypothesis that has received little empirical support (Dimond 1967; Pearson & Kramer 1971). Hildebrand (1974), Kroger (1972), Reisen & Prins (1972), and others present data that do not support this hypothesis. Recently, Waters (1981) demonstrated that production of *Gammarus pseudolinneaus* is sufficient to compensate for the observed drift.

### 3.5 Behavioral studies

Stream ecologists may be interested in quantifying behavior of benthic invertebrates to obtain information related to their distribution and abundance. This information can be used to help plan sampling programs or interpret distributional data. Predator avoidance responses such as crawling and posturing were measured for mayflies that did not drift or swim from encounters with stonefly predators in plexiglass observation boxes (Peckarsky 1980b). The mechanisms by which prey detect predators were also determined using this technique. Predators were presented visually (within test tubes), chemically (within screen tubes), and free (tactilely), and prey responses were
recorded. The effect of predator presence on prey activity was also quantified. Pilot experiments have been conducted with a slightly modified observation box (Fig. 5.10) that improves the versatility and convenience of this observation technique. Questions concerning predator search behavior, feeding rates, effects of competitors on activity and distribution, effects of alarm substances on prey behavior, and many others can be answered using this approach.

Fig. 5.10 Observation box—second generation: substrate with longitudinal depressions, sliding plexiglass plate on top.

Others have implemented direct observational techniques to monitor stream insect behavior. Hart & Resh (1980) quantified the foraging patterns of a diurnal, pool-dwelling caddisfly, *Dicosmoecus gilvipes* in a California stream by SCUBA. Mackay (1977) and Gallepp (1974) observed the behavior of caddisfly larvae in laboratory stream systems. Wiley & Kohler (1980) designed a simple laboratory technique by which to measure positioning changes of mayfly nymphs in relation to oxygen and current regimens. In addition, they implemented an elegant system whereby behavior of stream benthos was filmed *in situ* using a super-8 movie camera taking individual frames on an automatic timer (Wiley & Kohler 1981). The use of this type of
film technology has much potential in the study of the behavior of stream benthos.

In summary, knowledge of the behavior of stream invertebrates can be incorporated into the design of effective programs to investigate secondary production.

### 3.6 Studies on biological interactions

A few investigators have begun to examine biological interactions such as predation and competition, which may be influential in determining production as well as the community structure of stream benthos. Hildrew and Townsend (1976, 1977) and Townsend and Hildrew (1978, 1979a, b) used primarily the traditional sample-survey approach, which, again, limits our interpretative ability to associations among predator-prey and competitor distributions. The experimental design employed by Peckarsky & Dodson (1980a, b) allows controlled manipulations of predator, prey, or competitor densities within cages (Figs. 5.3, 5.6); experiments were conducted to measure the causal role of such biological factors in determining distributions or colonization of stream benthos.

### 4 Choosing the appropriate methodology: summary and conclusions

Reviews by Hynes (1971), Resh (1979), and others discuss general rules for the choice of an appropriate sampling scheme. I have mentioned many of these in Section 2. Here I present a list of guidelines that can be useful to a stream ecologist in designing an effective sampling program.

First, identify the objectives of your research. What are the questions? Are you measuring diversity, production, standing crop numbers or biomass, dispersal? Each of these requires unique treatment. Second, decide upon a desired level of precision. Do you wish to estimate density within 5% of the mean or 40% of the mean? This decision will affect the choice of number of samples. Third, and very critical, gather as much information as possible on the biology of the taxa in question. The size of the animals will dictate proper mesh sizes; the life history, behavior, and distribution of different instars will be important in determining the scheduling of sampling intervals and the choice of sampling design (random, stratified, etc.). The habits and preferred habitat of the group will also be important in choice of sampling design. The dispersion pattern (even versus clumped) will be important in choosing sample sizes and design. Finally, the density of animals should be taken into account when choosing the size of a sample unit and the number of replicate samples. Preliminary samples should be taken to obtain as much of this information as possible.
Whatever methodology is chosen, a responsible investigator should understand the strengths and weaknesses of each technique so that data can be interpreted appropriately. For example, sample-survey designs can be subject to large error due to variability among samples if one attempts to use these data to extrapolate to absolute numbers, biomass, or production. Use of Surber samplers to compare estimates of benthic invertebrates in different riffles or in the same riffle over time, or above and below a disturbance, can be quite informative if the procedure for obtaining samples is standardized (same investigator, same volume of substrate sampled for the same time period, using the same sorting technique). Association techniques may generate testable hypotheses from large data sets, but causal relationships are least ambiguously elucidated through controlled experimentation.

In conclusion, I strongly encourage stream ecologists to design new and original apparatus, and to implement unique sampling schemes with considerable forethought so that hypotheses concerning productivity of stream benthos can be rigorously tested. It is hoped that the case studies presented in Section 3 can serve as examples that can be modified into individualized experimental designs appropriate to questions involving particular species. We must remember that the most sophisticated data analyses cannot override weaknesses in experimental design.

5.1 Appendix 1

The following table lists selected examples of factors that affect benthic sampling devices and may result in sampling bias (Table 1 from Resh 1979).
### Appendix 1, (contd)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Samplers affected</th>
<th>Problems created</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Backwash created in sampler by water not being able to pass through net</td>
<td>Netted and kick samplers</td>
<td>4–30% loss of benthos around sides of sampler (Badcock 1949; Macan 1958; Mundie 1971; Frost et al. 1971)</td>
<td>Increase the net's surface area and/or decrease size of net opening (Macan 1958); use enclosed double netted sampler (Mundie 1971); alternatively use a hand-operated Ekman grab or cylinder box sampler (Hynes 1971)</td>
</tr>
<tr>
<td>Disruption of substrate surface by shockwave when sampler strikes bottom</td>
<td>Corer and Grab samplers</td>
<td>Loss of small organisms and surface dwellers (Flannagan 1970; Howmiller 1971; Milbrink &amp; Wiederholm 1973)</td>
<td>Modify Ekman grab by removing screens and incorporating heavier materials in design (Burton &amp; Flannagan 1973); alternatively use a pneumatic grab (Murray &amp; Charles 1975), a box sampler (Jónasson &amp; Olausson 1966; Farris &amp; Crezee 1976), or a modified corer (Brinkhurst et al. 1969; Kajak 1963, 1971)</td>
</tr>
<tr>
<td>Disturbance of biota</td>
<td>Surber sampler and Allan grab</td>
<td>Underestimation of biota due to disruption when sampler is set in place (Surber: Kroger 1972; Allan grab: Kajak 1971)</td>
<td>Modify Allan grab by adding screened openings on top</td>
</tr>
<tr>
<td></td>
<td>Shovel sampler</td>
<td>Loss of motile organisms (Macan 1958; Hynes 1970)</td>
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### 5.1 Appendix 1, (contd)

<table>
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<tr>
<th>Factor</th>
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<th>Problems created</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable depth of penetration into substrate by sampler</td>
<td>Grabs</td>
<td>Inconsistent volume of sediment sampled; loss due to overfilling</td>
<td>Leave 5-cm space above substrate (Flannagan 1970); alternatively use a corer whenever possible (Kajak 1963; Gale 1971)</td>
</tr>
<tr>
<td></td>
<td>Surber</td>
<td>Failure to consider stream hyporheic zone</td>
<td>Two stage sampling, surface and hyporheic</td>
</tr>
<tr>
<td>Variable area sampled</td>
<td>Shovel sampler</td>
<td>Area sampled laterally is variable</td>
<td>Finer mesh, or preferably a double bag sampler (Macan 1958)</td>
</tr>
<tr>
<td>Sampler mesh size too coarse</td>
<td>Netted samplers</td>
<td>Early instars, small and slender organisms missed</td>
<td>Coarser mesh as in double bag samplers (McMan 1958; Frost <em>et al.</em> 1971; Mundie 1971; Zelt &amp; Clifford 1972; Barber &amp; Kevern 1974)</td>
</tr>
<tr>
<td>Sampler mesh too fine</td>
<td>Netted samplers</td>
<td>May cause backwash (see above)</td>
<td>Smaller samples (Elliott 1977; Voshell &amp; Simmons 1977)</td>
</tr>
<tr>
<td>Sampler dimension too large</td>
<td>All samplers</td>
<td>Increase sorting time: may not detect population aggregations</td>
<td>When density &gt; several hundred/m$^2$ use corer, when &lt; use Ekman grab (Kajak 1963); alternatively use multiple corer (Brinkhurst <em>et al.</em> 1969; Flannagan 1970; Hakala 1971)</td>
</tr>
<tr>
<td></td>
<td>Grab samplers and</td>
<td>Inefficient cost/sample ratio</td>
<td>Use nested sampler to determine optimal sampler dimension</td>
</tr>
<tr>
<td></td>
<td>corers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampler dimension too small</td>
<td>All samplers</td>
<td>May not detect aggregations: variability increased due to edge effect</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator inconsistency</td>
<td>All samplers</td>
<td>Systematic error in population estimates (Needham &amp; Usinger 1956)</td>
<td>Single operator; or correction factor for each operator (Chutter 1972)</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------</td>
<td>---------------------------------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Water depth limitations in lotic environments</td>
<td>Surber and Hcss samplers</td>
<td>Surber sampler limited to &lt;30 cm depth (Macan 1958; Albrecht 1959; Chutter 1972)</td>
<td>From 0-5 to 4 m use an airlift sampler (Mackey 1972; Pearson et al. 1973); 0-4 to 10 m deep, SCUBA and dome suction sampler (Gale &amp; Thompson 1975) or modified Hess sampler (Rabeni &amp; Gibbs 1978); or use a modified Allan hand operated grab (Allan 1952; Kajak 1971) or bottombasket samplers (e.g. Crossman &amp; Cairns 1974; Rabeni &amp; Gibbs 1978)</td>
</tr>
<tr>
<td>Substrate—stony</td>
<td>Grab samplers, corers</td>
<td>Grabs may not close (Kajak 1971); cylinder sampler cannot penetrate (Ulfstrand 1968)</td>
<td>Substitute airlift or dome suction sampler and artificial substrate as above</td>
</tr>
<tr>
<td>Substrate—mixed</td>
<td>Grab samplers, corers</td>
<td>Differential penetration</td>
<td>Flannagan (1970) recommends specific samplers for different substrate types; stratified sampling (Scherba &amp; Gallucci 1976)</td>
</tr>
<tr>
<td>Current too slow</td>
<td>Surber and kick samplers</td>
<td>Organisms do not drift into net</td>
<td>Enclosed sampler such as modified Surber or Hess sampler (Leonard 1939; Waters &amp; Knapp 1961)</td>
</tr>
<tr>
<td>Current too fast</td>
<td>Netted samplers</td>
<td>Backwash, resulting in a loss of organisms</td>
<td>Substitute a modified sampler with controlled flow (Mundie 1971)</td>
</tr>
<tr>
<td>Current fluctuations</td>
<td>All samplers</td>
<td>Rapid change in flow may scour study area</td>
<td></td>
</tr>
<tr>
<td>Factor</td>
<td>Samplers affected</td>
<td>Problems created</td>
<td>Remedy</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Low air temperatures</td>
<td>Netted samplers</td>
<td>Samples freeze in net before organisms are removed</td>
<td>Catch bottle or a zippered net (Waters &amp; Knapp 1961; Lane 1974)</td>
</tr>
<tr>
<td>Sampling in vegetation</td>
<td>All samplers</td>
<td>Loss of organisms during removal; inability to close sampler</td>
<td>Use samplers described by Welch (1948), Macan (1949), Gerking (1957), Gillespie and Brown (1966), Mackie &amp; Qadri (1971), Minto (1977), or artificial vegetation (Glime &amp; Clemons 1972; Higler 1977; Macan 1977a)</td>
</tr>
<tr>
<td>Sampling in open water</td>
<td>Lotic: drift samplers; lentic: all samplers</td>
<td>Lotic: net clogging and changes in current and flow pattern affect estimation of water volume sampled (Pearson &amp; Kramer 1969); lentic: sampling a consistent volume of water; scattering of organisms (Legner et al. 1975)</td>
<td>Lotic: use Parshall flume drift net (Hales &amp; Gaufin 1969) or waterwheel drift sampler (Pearson &amp; Kramer 1969); lentic: use column samplers (e.g. Legner et al. 1975; Enfield &amp; Pritchard 1977; Henrickson &amp; Oscarson 1978) or pull-up trap (Higler &amp; Kolipinski 1967)</td>
</tr>
<tr>
<td>Habitat small</td>
<td>All samplers</td>
<td>Sampling destroys habitat (Chutter 1972; Mason 1976)</td>
<td>Smaller sampler dimension; artificial substrates (Glime &amp; Clemons 1972; Macan 1976, 1977a, b)</td>
</tr>
<tr>
<td>Water chemistry</td>
<td>All samplers</td>
<td>Presence of springs, man-made outfalls, and other conditions may influence microhabitat distribution of biota</td>
<td>Reconnaissance</td>
</tr>
</tbody>
</table>
5.2 Appendix 2

Figure reproduced from Resh (1979, Fig. 7) showing the required number of samples for estimating the mean density (number m⁻²) of stream benthos samples with 95% confidence limits ± 20% and ± 40% of the mean, based on the general formula of Elliott (1977; see also Chapter 8, Section 2.1.3). Data were collected using a Surber sampler and are from Needham & Usinger (1956, Table 3) and Chutter & Noble (1966, Table 1). Each point represents a taxon. These figures can be used by reading values from the relationship: one must take 'Y' samples if the density is 'X'. See similar treatment for lake benthos in Chapter 4.
6 References


Chapter 5

Sampling the Stream Benthos


Peckarsky B.L. (1979b) *Experimental manipulations involving the determinants of the spatial distribution of benthic invertebrates within the substrate of stony streams.* Dissertation. University of Wisconsin, Madison.


Sampling the Stream Benthos


Ulfstrand S. (1968) Benthic animal communities in Lapland streams. *Oikos (Suppl.)*, 10, 1–120.


