Sampling stream invertebrates using electroshocking techniques: implications for basic and applied research

Brad W. Taylor, Angus R. McIntosh, and Barbara L. Peckarsky

Abstract: We present a new technique using electrofishing equipment to collect and quantitatively sample stream invertebrates. We used an electrofishing machine with a small anode to produce a localized field of pulsed direct current to induce invertebrate drift. We quickly obtained large numbers of live invertebrates for experiments by passing the anode over the stream bottom upstream of sampling nets. We compared the results of five techniques: (*i*) electroshocking inside a modified Hess sampler, (*ii*) repeated electroshocking over a large area to estimate population size by depletion, (*iii*) traditional Surber, (*iv*) Hess, and (*v*) individual stone sampling. Electroshocking techniques provided estimates of invertebrate density comparable with those of traditional sampling techniques. The electroshocking depletion method that sampled a large area provided higher measures of Ephemeroptera, Plecoptera, and Trichoptera richness. Hess and area-restricted electrobug methods had similar density and diversity estimates, whereas the Surber sampler provided low density estimates, especially for mobile taxa. Density estimates from individual stones were inflated, were biased for mayflies, and had low richness. Samples taken with the electroshocking method were processed 40% faster because these samples contained little detritus. Electroshocking techniques can provide accurate estimates of population size and diversity, minimize disturbance to benthic habitats, and reduce processing time.

Résumé : Nous présentons une nouvelle méthode de récolte et d'échantillonnage quantitatif des invertébrés des cours d'eau à l'aide d'un appareillage de pêche électrique. Nous utilisons un appareil avec une petite anode qui produit un champ restreint de courant direct à impulsions pour provoquer la dérive des invertébrés. Nous pouvons ainsi obtenir rapidement de grandes quantités d'invertébrés vivants pour usage expérimental en passant l'anode au-dessus du fond en amont de filets de récolte. Nous avons comparé l'efficacité de cinq techniques: (*i*) la pêche électrique dans un échantillonneur de Hess modifié, (*ii*) la pêche électrique répétée sur une grande surface pour estimer la population par retraits successifs, (*iii*) l'utilisation de l'échantillonneur de Surber classique, (*iv*) l'utilisation de l'échantillonneur de Hess et (*v*) la récolte manuelle sur des pierres individuelles. Les méthodes de pêche électrique fournissent des estimés de densité des invertébrés comparables à ceux des méthodes traditionnelles. La méthode d'estimation avec retraits par pêche électrique sur une grande surface fournit des estimations plus élevées des densités (richesse) des éphéméroptères, des plécoptères et des trichoptères. La méthode de Hess et la pêche électrique sur surface réduite donnent des estimations similaires de densité et de diversité, alors que la méthode de Surber fournit des estimations basses, particulièrement des taxons mobiles. Les estimations obtenues par récolte sur les pierres individuelles sont trop élevées; les éphéméroptères y sont surreprésentés et la richesse en espèces est basse. Le traitement des échantillons obtenus par pêche électrique est de 40% plus rapide, parce qu'ils contiennent peu de détritus. Les méthodes basées sur la pêche électrique peuvent donc fournir des estimations précises de la densité et de la diversité des populations, minimiser la perturbation des habitats benthiques et réduire le temps de manipulation des échantillons.

[Traduit par la Rédaction]

Introduction

Sampling benthic invertebrates is a challenging task that confronts many studies of running waters. Although numerous papers and reviews have examined sampling of stream and riverine benthic communities (Cummins 1962; Merritt et al. 1984; Peckarsky 1984), accurate and efficient collecting and processing of invertebrate samples remain difficult. Problems arise because no sampling device is suitable for all types of habitat (e.g., dependence on flow or sampler area).

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B.W. Taylor.[1,2] Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, CO 81224, U.S.A.
A.R. McIntosh. Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, CO 81224, U.S.A., and Department of Zoology, University of Canterbury, Private Bag 4800, Christchurch, New Zealand.
B.L. Peckarsky. Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, CO 81224, U.S.A., and Department of Entomology, Cornell University, Ithaca, NY 14853, U.S.A.

[1]Corresponding author (e-mail: btaylor@uwyo.edu).
[2]Present address: Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, U.S.A.

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In addition, the patchy distribution of invertebrates and attributes of the organisms (mobile or attached) can result in underestimates of population densities and (or) sampling bias. Moreover, benthic samples taken using current methods are usually labor intensive to process.

Traditional methods for sampling benthic invertebrates from streams rely on physically disturbing the substrate and collecting invertebrates in a net held downstream (Surber 1937; Hess 1941; Frost et al. 1971). Invertebrates are usually preserved along with detritus for picking and sorting in the laboratory or are sorted in the field if live specimens are desired. Since invertebrates are often associated with detritus (Cuffney and Wallace 1987) and interstitial spaces of the stream bottom (Reice 1980), these techniques have proven effective at sampling those habitats. However, the large amounts of detritus included in these samples make them time-consuming and difficult to process. Further, most traditional sampling devices only sample a small area of the substrate (Surber, 0.09 m^2; Hess, 0.09 m^2; D-net, undefined). Given the patchy distribution of invertebrates in streams (Downing 1979; Lancaster et al. 1991), a large number of samples must be taken to minimize the variance in measures of abundance and accurately estimate population size and (or) the diversity of a community (Needham and Usinger 1956; Allan 1982; Morin 1985). These estimates are often compromised by time and financial constraints that reduce the optimal number of samples that can be taken (Sheldon 1979; Ferraro et al. 1989).

To advance sampling of benthic invertebrates, a method is required that removes invertebrates effectively from the substrata while minimizing the amount of debris collected and that allows a larger area to be sampled, integrating more habitats and reducing variance among samples. Electrical current (electroshocking) to stun and capture freshwater fish has been used for many decades (Cowx 1983; Hayes and Baird 1996), but the application of this technique to sample benthic invertebrates is relatively new. Recently, studies have explored the use of electroshocking to sample decapods (Penczak and Rodriguez 1990; Fièvet et al. 1996; Rabeni et al. 1997) and in association with freeze-core sampling of hypoheic stream fauna (Bretschko 1990). Electroshocking has also been used to collect large numbers of mayfly larvae for experiments (McIntosh and Townsend 1994). Other studies have examined the indirect effects of electroshocking for fish on invertebrate density and drift in a stream reach (Elliot and Bagenal 1972; Fowles 1975; Bisson 1976). In all of these studies, electroshocking induced drift of invertebrate taxa with no physical disturbance to the stream bottom. Thus, we hypothesized that a technique using electroshocking would be effective for making quantitative estimates of invertebrate abundance with improved sample processing efficiency because the amount of detritus collected would be reduced.

Here, we describe new methods for quantitatively sampling invertebrates from streams that use standard electrofishing equipment to induce invertebrates to drift into capture nets. Two methods were developed: one requires electroshocking inside a 0.09-m^2 modified Hess sampler (area-restricted electrobug) and the other involves repeated shocking to deplete invertebrate populations from a large area (2–4 m^2) delineated by upstream and downstream nets (depletion by electroshocking). First, we compared differences in density estimates, efficiency, and size selectivity between the electroshocking technique and other methods (e.g., Surber, Hess, and individual stones). To determine the effects of electricity on invertebrates, we compared the drift behavior, growth rate, and mortality of a common mayfly, *Baetis bicaudatus*, collected using electroshocking with those of individuals collected with a D-net. Next, we compared estimates of biotic indices used in biomonitoring programs between electroshocking techniques and traditional sampling methods. Finally, we compared the processing time (picking and sorting) for samples collected with the electrobug technique and the traditional Surber technique.

Materials and methods

We sampled invertebrates from the East River and tributaries in the vicinity of the Rocky Mountain Biological Laboratory, a high-altitude (2945 m) field station located in Gunnison County, Colorado, U.S.A. Streams at this elevation are high-gradient, cobble-bottom streams, ranging in conductivity from 126 to 270 $\mu S \cdot cm^{-1}$, with mean summer temperatures of 5–13°C. Streams in the East River watershed range in size from first-order streams with an average width of 1.7 m and summer discharge of 2.8 $L \cdot s^{-1}$ to third-order streams with average width and discharge of 9.3 m and 770 $L \cdot s^{-1}$, respectively (Peckarsky et al. 2000, 2001). Invertebrate communities in these streams are dominated by Ephemeroptera (mayflies), Plecoptera (stoneflies), Diptera (true flies), and Trichoptera (caddisflies) (Peckarsky 1991).

Sampling procedures

Five quantitative sampling procedures were used: (*i*) electroshocking within a Hess-type sampler (area-restricted electrobug sampling), (*ii*) depletion electrobug (sampling using electroshocking equipment over a large area), (*iii*) Surber sampling, (*iv*) Hess sampling, and (*v*) individual stone sampling. For both electroshocking techniques, we used a Smith–Root model 15-C backpack-style electroshocker to produce a pulsed direct current (DC) through the water. We replaced the standard 28-cm-diameter electrode (anode) with a 15-cm electrode (Smith–Root part No. APA83-6), which produced a stronger current over a smaller area. Effective voltage requirements were similar to those used for electrofishing brook trout (*Salvelinus fontinalis*) in these streams, ranging from 500 to 700 V DC depending on the conductivity. Although the Smith–Root electroshocker has a variety of frequencies and pulse widths, we found that the standard setting recommended for salmonids was satisfactory (60–80 $pulses \cdot s^{-1}$ and a pulse width of 6 ms).

In 1997, we compared five area-restricted electrobug samples and five Surber samples among three different streams in the East River watershed. In 1999, we compared five area-restricted electrobug, three depletion electrobug, five Surber, five Hess, and five individual stone samples from only one stream. Because the electroshocking methods induced drift of invertebrates, we took Surber, individual stones, and Hess samples before the electroshocking samples. Samples were stained with Rose Bengal and preserved in 95% ethanol. We classified substrate size (Wentworth 1922) for 10 randomly selected substrate particles in each sample area as a potential covariate with invertebrate density.

We constructed an area-restricted Hess (or Neil) type sampler with the same dimensions (30.5 × 30.5 cm) and area (0.09 m^2) as a Surber sampler but with clear plastic (Lexan®) sides and 280-µm Nitex® mesh on the upstream end. A U-shaped aluminum slot on the downstream end enabled a Wildco® 30 × 25 cm drift net (202 μm) to be inserted and removed from the sampler.

To sample invertebrates using the area-restricted electrobug sampler, we randomly selected five locations in each of three streams in 1997 and one stream in 1999. Holding the sampler in place while wearing insulated rubber gloves, we electroshocked close to, but did not disturb, the substrate within the sample area for 90 s. We created an electrical gradient from the area being sampled to the capture net by placing the anode inside the sampler and the cathode immediately downstream of the sampler. In 1999, we determined the efficiency of this sampler in one stream by removing the drift net after sampling, inserting a new drift net, and then disturbing the substrate by hand for another 90 s.

In 1999, for depletion sampling by electroshocking, we placed 2-m-wide × 1-m-high block nets (280-μm Nitex® mesh) at the upstream and downstream ends of each stream to isolate a large area (2–4 m^2) for sampling. The top and bottom edges of nets were pleated to create a bag shape, and lead weights were attached to the bottom. Nets were anchored in place with wooden poles fitted through sleeves on both sides, and three passes with the electroshocking unit were made. We stood on the streambank while shocking and did not disturb the substrate with the electrode. The cathode was placed below the downstream net to establish an electrical gradient from the anode to the cathode. Three width measurements and the length of the sample area were used to determine the area sampled. The size of 20 substrate particles within the sample area was also recorded (Wentworth 1922).

The efficiency was estimated using the equations derived by Zippin (1956, 1958). As an empirical test of the maximum likelihood functions used to determine the probability of capture during one pass, we also took five Hess samples from the stream reach after three passes had been made with the electroshocker to determine the number of invertebrates remaining after three passes.

In 1997 and 1999, we randomly selected five locations in each stream and disturbed the substrate by hand within the frame of a standard Surber sampler (0.09 m^2, 202-μm mesh) for 90 s. In 1999, we determined the efficiency of the Surber sampler in one stream by taking another Surber sample from the exact same location.

In 1999, we used the Hess sampler without electricity (Fig. 1) to sample invertebrates in one stream by physically disturbing the substrate by hand within the sampler area for 90 s. In 1999, we determined the efficiency of the Hess sampler in one stream by removing the drift net after the first sample, inserting a new net, and disturbing the substratum again for 90 s.

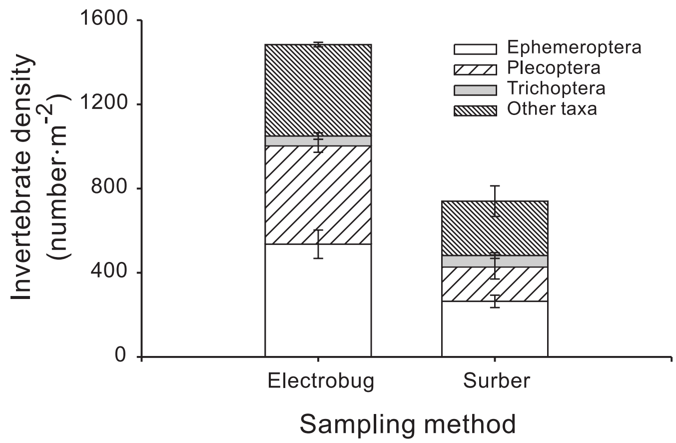
In 1999, invertebrate densities from individual stones were estimated in one stream by randomly selecting five stones (10–15 cm), placing a net (250 μm) directly downstream, and quickly lifting the stone into the sample net. Stone area was estimated by tracing the outside perimeter of each rock onto a clear acetate sheet. We used a digitizer to determine the two-dimensional surface area of each stone based on the perimeter tracing.

In 1999, to determine if the electroshocking technique was size selective, we measured head capsule widths of 120 mayfly (*Baetis bicaudatus*, Ephemeroptera: Baetidae) larvae (the most common invertebrate) from five different Surber and five area-restricted electrobug samples from one stream.

To determine if processing time differed between samples collected using the area-restricted electroshocking technique and Surber sampler, samples were emptied into picking trays in the laboratory and the time to sort and identify taxa was recorded. The same person processed all samples. The time to process a sample was divided by the total number of invertebrates in each sample to obtain the number of minutes to process 100 individuals.

To determine if there were any detrimental effects of electroshocking on invertebrates, we compared survival, growth, development, and drift behavior of *B. bicaudatus* collected using electroshocking and conventional kick sampling techniques. Larvae were collected from the East River by physically disturbing the substrate by hand and capturing *Baetis* with a D-net (500-μm mesh size) and with the electroshocker and D-net held downstream to collect invertebrates using electricity. We reared and observed *Baetis* in circular flow-through tanks (Peckarsky and Cowan 1991) with 15 individuals (stage III, summer generation, n = 10 tanks per treatment). Treatments were randomly assigned to tanks, and rocks with algae were placed in the tanks to provide food resources. We determined the initial size of a subsample of *Baetis* collected by drying individuals at 60°C for 24 h and then weighing them on a Cahn C-31® microbalance. Final size of all individuals was obtained similarly. Mortality was determined by counting the number of *Baetis* remaining after 7 days, corrected for the number that emerged, which was observed daily. To examine the possible effects of electroshocking on drift behavior, we counted the number drifting in each tank for 60 s during the night (20:00 mountain daylight savings time) on the evening after they were collected.

Fig. 1. Invertebrate density (mean ± 1 SE) for the area-restricted electrobug and Surber sampling techniques. Means are for three streams sampled in 1997.



Statistical analyses

Comparisons between area-restricted electrobug and Surber samples taken in 1997 were performed using a mixed-model analysis of variance (MANOVA) with stream as a random effect and sampling method as a fixed effect tested using the interaction term sampling method × stream as the denominator for sampling method. We tested effects of sampling method on multiple independent response variables using a MANOVA and individual ANOVA when the MANOVA was significant. Benthic densities were natural log transformed to normalize the data. Substrate size did not differ among sampling methods (ANOVA, F = 1.02, P > 0.05) and therefore was not included in analyses. Size–frequency distributions of *Baetis* were compared using a Kolmogorov–Smirnov two-sample test.

The patchy distribution and subsequent high variability in density estimates are one of the main problems associated with sampling stream invertebrates. Although we took samples randomly, it was also not possible to sample the exact same areas with each method. As a result, the statistical differences or similarities among methods may have been due to spatial differences in habitats sampled. We addressed this issue by performing a Levene (1960) test to assess homogeneity of variance among sampling methods.

To calculate mean densities, standard error of the mean, and efficiency for the depletion sampling technique, we used the maximum likelihood model developed by Zippin (1956, 1958). If a taxon was captured in only one out of the three passes, the Zippin model was not applicable and densities were determined by assuming that density equaled the number caught in one pass. When the number of invertebrates caught in previous passes was less than that in subsequent passes, density was estimated by multiplying the total number caught by the density estimate for the taxonomic group with the highest variance divided by the total number caught for that group. The difference in sample processing time between Surber and area-restricted electrobug samples was analyzed using Student's *t* test. Differences in survival, growth rate, development, and drift behavior of *Baetis* collected with and without electroshocking were analyzed using MANOVA after arcsine square root transforming the percent survival and emergence data and square root transforming the drift data. All statistical analyses were performed using SAS (SAS Institute Inc. 1989).

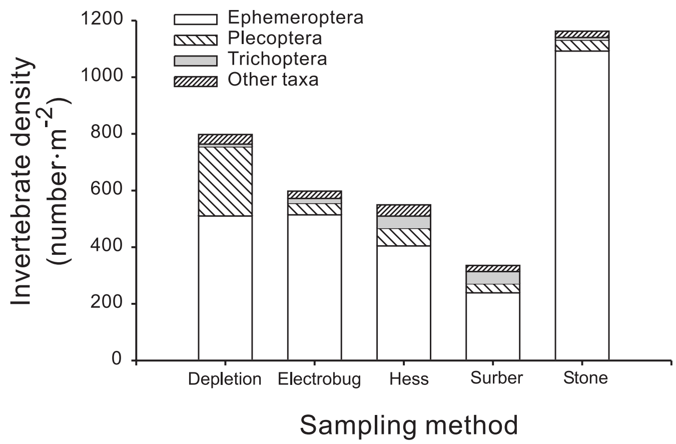
The number of individuals in a sample increases the probability of encounter for a given species; we standardized for abundance levels before comparing richness among techniques (Sanders 1968; Hurlbert 1971; Gotelli and Graves 1996). Each sampling technique was standardized to a common number of individuals, that is, we "rarefied" samples to a common abundance level (Hurlbert 1971; Simberloff 1972) for both total taxa richness and richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT richness; Lenat 1983) using EcoSim (Gotelli and Entsminger 1999). EcoSim uses Monte Carlo methods similar to rarefaction to estimate the expected richness for a given number of individuals (abundance level) drawn randomly from a sample (Gotelli and Graves 1996). We combined the replicates for each method within a stream and repeated the randomizations 100 times, which generated an average expected richness and confidence limits. Confidence intervals were used to compare differences among sampling techniques.

Results

There were significant differences in densities of invertebrates sampled by the area-restricted electrobug and Surber sampling techniques among the three streams sampled in 1997 (MANOVA, Wilk's λ, $F = 27.20$, $P = 0.0349$) (Fig. 1). These differences were attributed to low density estimates for Ephemeroptera ($F = 48.06$, $P = 0.0202$) and Plecoptera ($F = 18.22$, $P = 0.0409$) for the Surber method. There were no significant differences in density estimates for Trichoptera ($F = 1.33$, $P = 0.3681$) or "other taxa" ($F = 2.39$, $P = 0.2621$) between techniques. Differences between area-restricted electrobug and Surber sampling methods were not a function of variation in performance among streams, as indicated by the nonsignificant interaction between sampling method and stream (MANOVA, Wilk's λ, $F = 1.36$, $P = 0.2406$). In addition, the Levene (1960) test for homogeneity of variances among sampling methods within each stream was not significant ($P > 0.05$ for all taxonomic groups), indicating that the variances were equal among sampling techniques.

There were significant differences among the area-restricted electrobug, depletion electrobug, Surber, Hess, and individual stone sampling methods for estimates of total invertebrate density within one stream in 1999 (MANOVA, Wilk's λ, $F = 4.05$, $P < 0.0001$). Samples taken from individual stones had the highest density estimates with the greatest variability (Fig. 2). The traditional Surber technique gave the lowest density estimates, but this difference was only significantly different from the stone and depletion sampling methods. Examination of individual ANOVAs for each taxonomic group revealed that the high-density estimate for individual stone samples was driven by high estimates of Ephemeroptera ($F = 9.42$, $P < 0.0003$). Area-restricted electrobug and Hess sampling methods produced intermediate density estimates and were not significantly different from any of the other sampling methods (Tukey's, $P > 0.05$). Ephemeroptera were the most abundant group across all sampling methods. However, Surber samples gave the lowest density estimates for Ephemeroptera compared with all other sampling methods ($P < 0.05$). The Plecoptera were the second most common group (Fig. 2), and densities were statistically different among the five methods ($F = 2.98$, $P < 0.0474$) because of their abundance in depletion samples (Tukey's, $P < 0.05$). Densities of Trichoptera were significantly different among methods ($F = 4.46$, $P = 0.0112$), with Hess and Surber containing more than individual stone samples (Tukey's, $P < 0.05$). There was no statistical difference in densities of "other taxa" among the five sampling methods ($F = 1.05$, $P = 0.4089$).

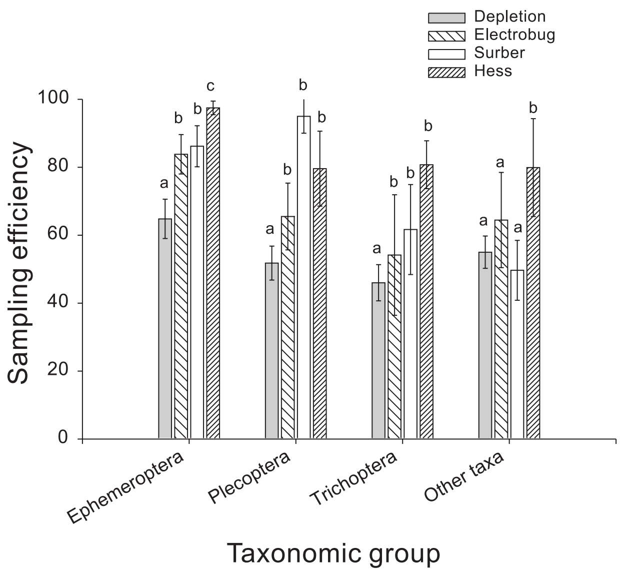
Fig. 2. Invertebrate density (mean ± 1 SE) for the electroshocking depletion, area-restricted electrobug, Hess, Surber, and individual stone sampling techniques. Data are from one stream sampled in 1999. Error bars are omitted for clarity.



The Levene (1960) test for homogeneity of variance indicated that the variances were equal for total invertebrate density ($F = 0.94$, $P = 0.4634$) among the five sampling methods. Examination of the homogeneity of variance for the individual taxonomic groups revealed that for Ephemeroptera and Trichoptera, variances were equal among sampling methods ($P > 0.05$) but unequal for Plecoptera ($F = 11.05$, $P = 0.0001$) and the "other taxa" group ($F = 8.93$, $P = 0.0004$).

The Hess sampling was the most efficient technique (MANOVA, Wilk's λ, $F = 7.86$, $P < 0.0001$) with an average of 86% of total individuals obtained in the first sample. The Surber technique was similar in efficiency to the Hess but was not significantly different from the area-restricted electrobug technique ($P > 0.05$). The Hess sampler was more efficient for most groups (Fig. 3). The probability of capture for an invertebrate on a single pass (or efficiency) for the depletion sampling technique was 73% on average. However, 92.5 ± 6.1% (mean ± 1 SE) of the invertebrates were removed after three passes with the electroshocker, determined from Hess samples taken after shocking. Surprisingly, efficiency estimates for the depletion were low for the "other taxa" group but only significantly different from those of the Hess. The

Fig. 3. Sampling efficiency (mean ± 1 SE) for the electroshocking depletion, area-restricted electrobug, Surber, and Hess sampling techniques. Means with different letters are significantly different (adjusted least squares means, $P < 0.05$). Data are from one stream sampled in 1999.



Levene (1960) test for homogeneity of variances in efficiency among the five sampling methods was not significant for overall efficiency or for the individual groups ($P > 0.10$), indicating that the variability in efficiency among replicate samples was similar for each method.

Size distribution of *Baetis* did not differ between samples collected using the Surber and those collected using the electroshocking technique (Kolmogorov–Smirov asymptope (KSa) = 0.9337, P = 0.3479, n = 75 for females; KSa = 0.6573, P = 0.7805, n = 45 for males).

Sample processing time was 40% faster for samples taken with the area-restricted electrobug technique than for Surber samples because electrobug samples contained less sediment and detritus. It took 15 min to pick and identify 100 invertebrates from samples taken using area-restricted electroshocking compared with 25 min for the Surber method (Student's t test: time, t_{28} = 2.52, P = 0.0176; detritus, t_{28} = –7.41, P < 0.0001) (Table 1).

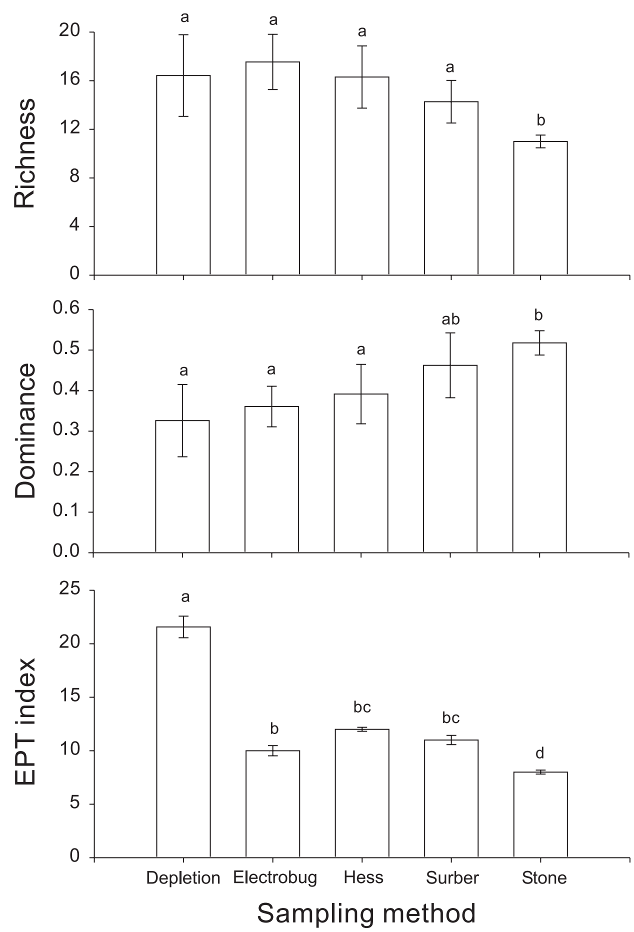
The depletion, area-restricted electrobug, Hess, and Surber methods all had similar estimates of taxa richness, whereas the individual stone sampling method had low taxa richness (Fig. 4). Stone samples were composed mostly of Ephemeroptera taxa, resulting in higher percent dominance scores. The depletion sampling method had significantly higher EPT richness than the other methods; the three area-restricted samplers had similar EPT richness, and individual stone samples had the lowest EPT richness (EPT richness determined by rarefaction). Both electroshocking techniques sampled taxa that commonly attach to rocks (e.g., Blephericeridae larvae).

There were no effects of electroshocking on survival, growth rate, development, or drift behavior of *Baetis* (MANOVA, Wilk's λ, F = 1.163, P = 0.3683) (Table 2). Retrospective power analyses indicated a low chance of type II error in this analysis for growth rate (power = 94%), mortality (power = 99%), drift (power = 85%), and emergence (power = 61%).

Table 1. Time to process 100 invertebrates and amount of detritus (inorganic and organic material) for the area-restricted electrobug and Surber sampling techniques. Values are means (±1 SE).

Sampling method	Time (min·100 invertebrates^{-1})	Detritus (g·m dry mass^{-2})
Electrobug	15.7 (1.50)	8.79 (1.89)
Surber	25.2 (2.80)	273 (35.7)

Fig. 4. Rarefaction diversity indices (taxa richness, percent dominance of the most common taxon, and EPT richness index) (mean ± 95% confidence interval) for samples taken using the electroshocking depletion, area-restricted electrobug, Hess, Surber, and individual stone sampling techniques. All samples were rarefied to an abundance level of 250.



Discussion

This study provides a new and efficient method for sampling invertebrates that has broad application to stream ecology. Processing benthic invertebrate samples from streams is a laborious task that often constrains observational, experimental, and biomonitoring studies. Investigators are frequently forced to take fewer than the optimal number of

Table 2. Mean (±1 SE) percent mortality, growth rate, number drifting, and percent emerged for *Baetis* collected using electroshocking and kick sampling.

Response variable	Electroshocking	Kick sampling
Percent mortality	2.1 (1.10)	1.00 (0.07)
Growth rate ($mg \cdot day^{-1}$)	0.1 (0.02)	0.06 (0.02)
Number drifting (min^{-1})	3.0 (0.44)	2.90 (0.50)
Percent emerged	5.4 (1.64)	4.00 (1.75)

samples because of financial constraints and (or) because of the long delay involved in sample processing. The new methods that we present provide estimates of invertebrate density, species richness, and biotic indices comparable with those of traditional methods and take significantly less processing time and cause less disturbance to the stream bottom. We have also used this method to save precious field time when collecting large numbers of live specimens for experiments (Peckarsky and McIntosh 1998; McIntosh and Peckarsky 1999). In addition, it has enabled us to collect and count samples of live invertebrates for density estimates in the field, alleviating the delay between field sampling and laboratory processing.

Without disturbing the stream bottom, the electroshocking techniques provided estimates of the invertebrate density comparable with those of traditional techniques. The Surber sampler, which is partly enclosed on the sides but open on the upstream end, consistently underestimated densities of mobile taxa such as mayflies, which are strong swimmers (e.g., *Baetis*). When Surber samplers are placed in fast-flowing streams, the fine nets may create enough resistance to form eddies or back-flow, which may enable highly mobile taxa to escape. We suspect that the differences in density estimates of the depletion method compared with the various other methods reflected actual differences in habitat sampled because the depletion technique integrated over a broad array of habitats. For instance, depletion sampling would effectively sample invertebrates in bryophytes along the undercut streambank that are not easily sampled by the quadrant or stone techniques. Also, the depletion method was the most effective in capturing riffle-beetle larvae (Elmidae) because it sampled woody debris where they are commonly found. The probability of being captured in one sampling bout (efficiency) was similar among various methods. However, efficiency for stonefly taxa using the electroshocking techniques was low (65%) because stoneflies seldom enter the drift unless the substrate is physically disturbed. On the other hand, Ephemeroptera taxa such as *Baetis* were easily induced to drift and had a high probability of capture in the first pass (depletion: P = 92%; electrobug: efficiency = 96%). The low efficiency for the "other taxa" group was the result of a poor depletion curve for Oligochaeta and Chironomidae, which did not exhibit a sharp decline after only three passes. Estimating population size using removal methods is only appropriate if a large proportion of the population can be collected (Zippin 1956; Cowx 1983). The depletion method removed >90% of the invertebrates after three passes with the electroshocker. Similarly, Frost et al. (1971) found that disturbing the substrate two times reduced total invertebrate density by 60% and an additional disturbance decreased density 30% more. Despite the low density estimates for the Surber sampler, it had a high apparent efficiency, which we suspect is an artifact of highly mobile taxa leaving the sample area and thus not being captured in the second sample.

An alternative method to area-restricted samplers that has become increasingly used is sampling individual stones (Peckarsky 1991; Scrimgeour et al. 1993), which can reduce processing time because less area of stream bottom is sampled, resulting in fewer invertebrates to sort and identify. However, this method is biased toward taxa associated with large stones and underestimates those associated with interstitial spaces, fine sediments, leaf packs, or woody debris. Moreover, to express estimates of invertebrate density on a per square metre scale by this method requires knowing the availability of comparable substratum and the relationship between different substrate sizes and invertebrate density. Simply extrapolating from an individual stone to 1 m^2 of stream bottom assumes that the stream is homogeneous, which is seldom true, and may result in overestimates of population size. Thus, individual stone sampling will overestimate total invertebrate density and underestimate species richness. Based on comparisons of the five sampling techniques that were rarefied to standardize for number of individuals (Sanders 1968; Gotelli and Entsminger 1999), as expected, the individual stone samples had the lowest taxa and EPT richness and the highest dominance. Thus, individual stone sampling was taxon specific, relevant at only small scales, and resulted in population overestimates when extrapolated to larger scales.

Taxa and EPT richness

Knowledge of the number of different taxa in a community is often central to both basic and applied studies. Typically, multiple samples are taken from a community to estimate richness, and as more samples are taken and more individuals are included, richness increases to an asymptote indicating the maximum richness. This phenomenon, or collector's curve (Colwell and Coddington 1994), also applies to comparisons of different sampling techniques, in that each technique may collect different numbers of invertebrates due to the effectiveness of the technique as well as the area sampled. A larger sampling area usually includes more types of habitats and thus more species (Arrhenius 1921; Slocomb and Dickson 1978). However, it is impossible to simultaneously control for sample area and invertebrate abundance (James and Wamer 1982). In this study, differences in sample area are viewed as an attribute of each sampling method. The use of rarefaction was especially important in this study because it enabled accurate comparisons of richness for different sampling methods independent of abundance. Differences in abundance can also be problematic in applied or basic research when comparisons among sites or treatments differ in number of individuals (Vinson and Hawkins 1996; McCabe and Gotelli 2000).

The high taxa and EPT richness for the depletion sampling technique could be attributed to sampling a larger area and thus more habitat types, or the two additional passes may have increased the capture of rare taxa (Pusey et al. 1998). Whatever the mechanism, this method yielded relatively high richness values and is a useful procedure for obtaining an accurate measure of the taxa richness of a community.

Electroshocking may be taxon and size specific. We examined the body size distributions of one mayfly taxon, *B. bicaudatus*, and found no evidence for size selectivity with the electroshocking apparatus used and under the environmental conditions of the sampling sites. Electricity has been shown to be effective for inducing mayflies to drift (Elliot and Bagenal 1972; Brown et al. 2000). Within the range of streams in this study, the environmental conditions (e.g., conductivity), and the electroshocking apparatus used, there was little evidence that the electroshocking technique was taxon specific. However, other studies that have investigated the effects of electricity on invertebrates (Bisson 1976; Mesick and Tash 1980; Brown et al. 2000) indicate that the effectiveness can vary according to environmental conditions and electroshocking apparatus.

Advantages of electrobug techniques

Physically disturbing the substrate to sample invertebrates has been the universal standard, but these methods have three major problems. First, large amounts of inorganic and organic debris are included in these samples, which makes sorting difficult and often requires staining to assist in separating organisms from debris. Subsampling has been used to decrease sample processing time but can reduce the accuracy of measurements and loss of rare taxa (Barbour and Gerritsen 1996; Courtemanch 1996; Vinson and Hawkins 1996). Second, physical disturbance of the substratum can slow the recolonization of areas sampled. Traditional techniques are also destructive of resources (algae or organic matter). Third, it is only practical to sample a small area by disturbing the substrate.

The electrobug technique provides solutions for all of these problems. Foremost, the use of electroshocking enables comparable estimates of the invertebrate community to be made with no physical disturbance to the stream bottom. Less detritus in samples makes extracting invertebrates much easier. In addition, repeated sampling of a site is possible without long-term physical disturbance. Furthermore, the electrical current produced from the electroshocking unit is unlikely to affect the algal community or detrital particles (Pringle and Blake 1994; Brown et al. 2000).

Because invertebrate distributions are highly aggregated, devices that sample a small area relative to what is available require more samples to increase the probability of sampling each aggregation and thus decrease the variance (Needham and Usinger 1956; Allan 1982; Morin 1985). The need for high replication to reduce sample variance below 20% was discussed by Allan (1982) and was proposed as one possible problem in detecting differences among locations. The electroshocking depletion technique samples a large area, integrating many habitats and aggregations into one sample. Large numbers of small sampling units have been considered more cost-effective and precise than a few large-area samples (Pringle 1984). However, large-area sampling (e.g., depletion) may be more precise if the mean density (which influences variability) and the number of samples required to provide acceptable variance are known in advance. Morin (1985) has shown that the number of samples necessary to obtain an average standard error of 20% of the mean decreases with increasing sample size and invertebrate density.

Caveats, improvements, and opportunities for innovation

Using electricity for sampling may have negative effects on nontarget organisms such as amphibians but not invertebrates (also see Mesick and Tash 1980). The electroshocking technique requires hauling an electrofishing machine around and two people to sample. Although the cost of an electroshocking machine is not trivial, it may be offset by financial savings in processing time, the increase in turnaround time of samples, and its application for other purposes (i.e., electrofishing). The large-area depletion method will not be practical for large rivers, but in small streams, it may be the most accurate method for detection of differences in the mean abundance of invertebrates when large-scale comparisons are made.

The application of this method may be limited if stream physical characteristics differ from those of Rocky Mountain streams. In terms of physical characteristics, the electroshocking technique, like many stream sampling methods, relies on water velocity to carry invertebrates dislodged from the substrate into a collection net held downstream. As a result, electroshocking may not work well in low-gradient, slow-flowing streams. The type of substrate, be it rocky cobble bottom, fine sediment, or bedrock, will probably have little influence on the effectiveness of the electroshocking method, provided taxa respond and the substrate is not conductive.

We have obviously only tested our method in Rocky Mountain streams where the dominant taxa were mayflies (Ephemeroptera). At our field site, chironomids are not abundant (comprising ~30% of the “other taxa” group). Interestingly, we did capture many invertebrates that we did not expect to, including free-living caddisflies (Rhyacophilidae), net-spinning caddisflies (Hydropsychidae), net-winged midges (Blephariceridae), and chironomids (Chironomidae). This method will not work well for collecting invertebrates with heavy cases or shells, such as snails or stone-cased caddisflies, because they do not drift easily.

The electroshocking techniques have broad application for sampling, collecting, and manipulating invertebrates for experiments. Electrical devices have also been developed to exclude freshwater shrimp (Pringle and Blake 1994) or sample marine arthropods (Phillips and Scolaro 1980). Studies in New Zealand, Venezuelan, and North American streams have used an electroshocker and a collection net held downstream to collect large numbers of invertebrates (Penczak and Rodriguez 1990; McIntosh and Townsend 1994; Fièvet et al. 1996) for surveys of species richness (Rabeni et al. 1997) or for experiments in which large numbers of invertebrates were needed (Peckarsky and McIntosh 1998; McIntosh and Peckarsky 1999). Although the electroshocking technique may not work well for all taxa or in every stream type, modifications can be made to apply this technique for a variety uses and across many systems. For example, the depletion electrobug method may enable sampling of habitats that are not accessible using traditional methods (e.g., bedrock streams or debris dams). Furthermore, our ability to remove ~90% of the invertebrates from a large area of stream may provide opportunities for innovations involving large-scale manipulations of invertebrates in natural streams using electricity (B.W. Taylor, unpublished data).

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