Nondestructive Quantitative Sampling for Freshwater Mussels in Variable Substrate Streams

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Abstract: Unionid mussels were sampled in the Big South Fork of the Cumberland River, Tennessee and Kentucky, from July to October 1988 with a chain grid of 10 1-m^2 quadrats. The chain grid was used to define 100-m^2 areas along the stream bed by repeatedly moving the 10-m^2 rectangle upstream. Within each 100-m^2 area, 30 systematically selected quadrats were sampled to estimate density and size class distribution of mussel populations. Sampling variance within grids reflected the patchiness of mussel distribution and increased with substratum heterogeneity; number of mussels encountered per quadrat ranged from 0 to 29. Among sites, densities ranged from 1 to 8 mussels/m^2. Across all sites, precision and estimates of species richness and density did not improve appreciably with sampling effort beyond 15 quadrats. Concurrent density estimates from quadrat and depletion sampling varied significantly among sites. As a percentage of quadrat estimates, depletion sampling consistently underestimated mean density from 8.5% to 87.5% across all sites. Depletion estimates were influenced by mussel size, substratum heterogeneity and observer experience. Also, depletion sampling underestimated smaller size classes (<75 mm) and overestimated larger size classes (>90 mm) by as much as 53% and 56%, respectively. Where substratum variability limits the use of rigid frame samplers and total substratum collection is not an option, the flexible chain grid provides a reliable means of obtaining precise estimates of mussel density and size class distribution.

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Distribution, abundance, and age structure often are used as baseline data for monitoring changes in mussel populations (Brice and Lewis 1979, Isom and Gooch 1986, Miller and Payne 1988). Obtaining reliable quantitative data in benthic studies is difficult (Dennison and Hay 1967), especially for freshwater mussels because of their contagious distribution in streams (Cummins 1962, Neves and Widlak 1987). Yet quantitative samples are required to obtain unbiased estimates of the relative abundance of species (Miller and Payne 1988). To accommodate the patchy distribution of aquatic macroinvertebrates, several investigators have suggested that sampling should occur within well-defined areas with a systematic or stratified-random sampling design (Cummins 1962, Wurtz 1959 in Isom and Gooch 1986, Isom and Gooch 1986). Systematic sampling can accommodate a wide range of environmental conditions (e.g., flow velocities, substratum characteristics), many of which influence distributional patterns of benthic fauna (Cummins 1962).

Several sample methods including brails (Coker 1918), grab samplers (Kraemer and Gordon 1981), visual searches along transects (Isom and Gooch 1986), and total substratum collections within rigid quadrats (Miller and Payne 1988) have been used to characterize mussel populations in large rivers or lakes. These methods are generally used in large rivers where the bottom sediment may be more uniform with presumably less sample variation. Many streams, however, have heterogeneous substrata. Throughout a stream, coarser material typically dominates in the headwaters and becomes less abundant downstream. In most streams, coarser materials characterize riffles and shoals, whereas finer material is deposited in pools. Sampling techniques that are used in large rivers or lakes may be impractical or ineffective in streams with highly variable substrata.

More recently, investigators have used several techniques specifically designed for coarse substrata. These included timed snorkeling collections (Isom and Gooch 1986), visual searches within rigid quadrats (Dennis 1984, Kovalak et al. 1986), and bucket samplers (Neves and Widlak 1987). The use of rigid-frame samplers in cobble and boulder-dominated streams probably compromises accuracy and precision. Distribution and size of these substrates frequently impede the placement of rigid samplers on the stream bottom. Total substratum collections within rigid samplers apparently yield the most accurate information (Kovalak et al. 1986, Neves and Widlak 1987, Miller and Payne 1988), but they are time consuming and, more importantly, may dramatically alter benthic habitat. When threatened or endangered species are present, collection of substratum constitutes a violation of the Endangered Species Act. Moreover, long-term studies requiring subsequent visits to the same site are not possible with repeated sampling of the same substratum because of disturbances associated with total substratum collections.

The purpose of this study was to describe and evaluate a quantitative sampling technique that can be used over a variety of substrata without appreciably altering habitats. Specific objectives were to compare the results of this tech-

nique with estimates from concurrent depletion sampling, to quantify the variability of the distribution and abundance of mussels within and among sites, and to determine whether this technique can provide estimates of density and size class distribution with sufficient precision to detect differences among mussel assemblages and population densities.

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Methods

Study Site

This study was conducted from July to October 1988 within the Big South Fork National River and Recreation Area, Tennessee and Kentucky (Fig. 1). New River and Clear Fork tributaries join to form the Big South Fork, which flows northward to join the Cumberland River at Burnside, Kentucky. The Big South Fork drains more than 3,500 km^2 of the Cumberland Plateau, which is predominantly Pennsylvanian sandstone, siltstone, and shale (Harker et al. 1980). For most of its length, the Big South Fork has eroded through the sandstone to Mississippian limestone (Starnes and Bogan 1988).

Mean discharge in New River and Clear Fork averages 60 m^3/second and 36 m^3/second, respectively; Big South Fork averages 300 m^3/second (U.S. Geol. Surv. 1986). Stream discharge varies considerably, fluctuating annually between 20 m^3/second and 15,000 m^3/second after individual hydrological events. During the study period, stream discharge was less than normal, averaging only 25 m^3/second. At this time, water depth (<0.5m) and clarity (1–5 NTUs) greatly facilitated location and enumeration of mussels in riffles and shallow pools.

Sampling Procedures

Seven mussel sampling sites were selected along the Big South Fork and Clear Fork to compare the efficiency of depletion estimates with that of quadrat sampling (Fig. 1). These sites were chosen because preliminary sampling revealed that the sites supported densities of ≥ 1 mussel/m^2 in similar rocky or gravelly shoals. Stream width varied from 15 m in Clear Fork to >60 m in the Big South Fork. Sampling was deliberately located at the center of the shoal midstream or mid-channel.

At each site a continuous row of 10 1-m^2 quadrats made of 5.0-cm chain was laced perpendicular to the current within a shoal. The downstream, nearest

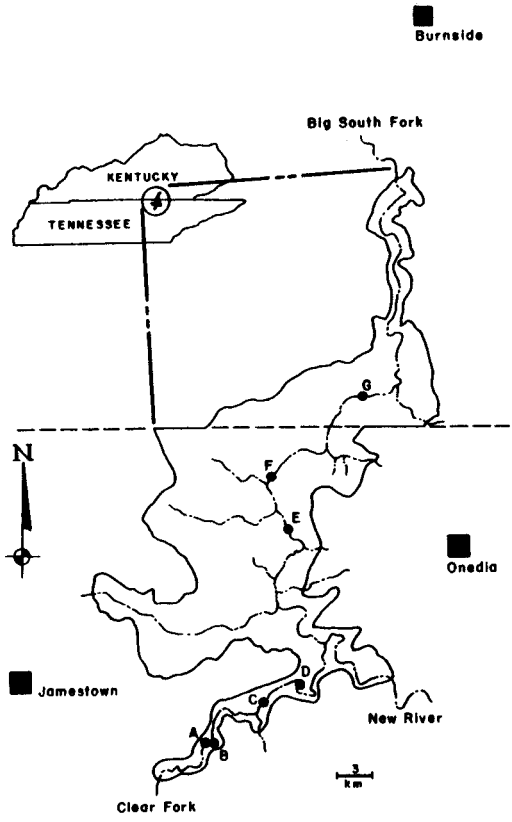

Figure 1. The Big South Fork National River and Recreation Area, Tennessee and Kentucky. (Unionid sampling locations are lettered and correspond to sites referenced in the text.)

shore corner of this chain grid was established as a benchmark by recording azimuth and distance from a landmark (Richardson 1989). After completing the sampling procedure in each row, the chain grid was flipped upstream and the procedure repeated until a 100-m^2 grid was sampled. Prior to any sampling, 30-m^2 were systematically selected within the grid to uniformly distribute the samples throughout the 100-m^2 area. The identical sampling design was applied to each site. This allowed the statistical comparison of current, depth, and substratum conditions that occurred within and among the total sample areas (grids).

Depletion estimates (Zippen 1958) were obtained by snorkeling along each row 3 times. During each pass, a visual search averaging about 30 seconds (20–40 seconds) was conducted within each 1-m^2 quadrat. A color-coded washer was placed near each mussel. After 3 passes, all marked mussels were gathered from each quadrat and length, height, and width were measured with

a vernier caliper (nearest 1 mm). Within each row, the 3 chosen quadrats were again visually searched using mask and snorkel. Surface rocks were then temporarily removed and each quadrat was searched while carefully sifting through the substratum with bare hands. Additional mussels were measured as before and then returned to their respective locations. This entire procedure was repeated for all 10 rows.

Depletion estimates were derived from a software program to estimate population densities from depletion sampling (White et al. 1982). Mean density estimates were obtained by dividing the total number of mussels recorded by the total number of quadrats sampled. Mean density estimates with 95% confidence intervals were computed according to Elliot (1977). Elliot (1977) reported that the means of 30 samples approximated normality and allowed computation of confidence limits. Accordingly, we used simple linear regression to examine variation in sampling efficiency relative to substrate particle size and total length of mussels. Efficiency in this paper is defined as the relative agreement (%) between density estimates of depletion and quadrat sampling. Average surface particle size was estimated at each site using median diameters of 100 random surface particles (Wolman 1954).

To evaluate variation relative to sampling effort within and among sites, we compared the mean, variance, and standard error of mussel density estimates obtained from 5, 10, 15, 20, 25, and 30 quadrats. The set of 5 quadrats were randomly selected (without replacement) from the 30 1-m^2 quadrats that were systematically selected at each site. Preliminary sampling indicated that Site G supported the greatest density and species richness. Data from site G were therefore selected to compare the frequency distribution of size classes from depletion (3 pass removal; Zippen 1958) and quadrat sampling. A probability of <0.05 was accepted as indicating statistical significance.

Results and Discussion

Species richness and density of mussels relative to sampling effort at each site are summarized in Table 1. Quadrat estimates of species richness increased with sampling effort (Fig. 2*a*), whereas density estimates remained fairly uniform after 15 quadrats (Fig. 2*b*). The most notable example was site G, where despite a widely fluctuating variance, the density estimates remained statistically similar to 30 samples after only 15 quadrat samples (Table 1, Fig. 2*b*).

Generally, as the number of quadrats sampled within the grid increased, the number of species encountered also increased. However, an average of 75% (range 45%–100%) of the total species known to occur at any single site was recorded while sampling 10 or fewer quadrats. An increase in effort of 50% (additional set of 5 quadrats) only increased the total species count by 7%. Dennis (1984) reported that 20 m^2 of sampling effort were enough to assess species richness when 28–35 species were present at similar densities. Our results were consistent with her findings; for each additional set of 5 quadrats sampled

Table 1. Sampling effort (*N* quadrats) and estimates of species richness (S) and density (D) per m² of freshwater mussels within the Big South Fork of the Cumberland River, Tennessee and Kentucky, July to October 1988. (Variances are given in parentheses.)

N Quadrats	Sites													
	A		B		C		D		E		F		G	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S
5	0.6	1	2.8	1	3.0	5	2.0	4	2.4	7	3.2	5	5.0	8
	(0.8)		(2.2)		(6.5)		(4.0)		(2.3)		(4.7)		(13.5)	
10	0.4	1	3.8	3	2.4	6	1.4	5	1.4	7	3.1	5	8.7	12
	(0.5)		(15.1)		(4.5)		(2.7)		(2.3)		(2.8)		(29.8)	
15	2.3	3	4.0	3	2.2	6	1.4	5	1.4	9	2.9	9	7.9	12
	(25.1)		(17.0)		(3.6)		(2.8)		(1.7)		(2.6)		(23.1)	
20	2.8	4	3.1	4	2.5	6	1.2	5	1.4	10	3.2	10	7.9	13
	(23.9)		(15.2)		(5.2)		(2.5)		(1.4)		(2.8)		(21.4)	
25	2.5	4	3.2	4	2.4	6	1.4	5	1.2	10	3.5	11	7.8	14
	(19.4)		(13.0)		(5.2)		(3.4)		(1.3)		(5.0)		(19.8)	
30	2.1	4	2.7	4	2.4	6	1.6	6	1.2	11	3.4	11	8.4	15
	(16.8)		(11.1)		(5.5)		(4.1)		(1.1)		(4.8)		(32.6)	

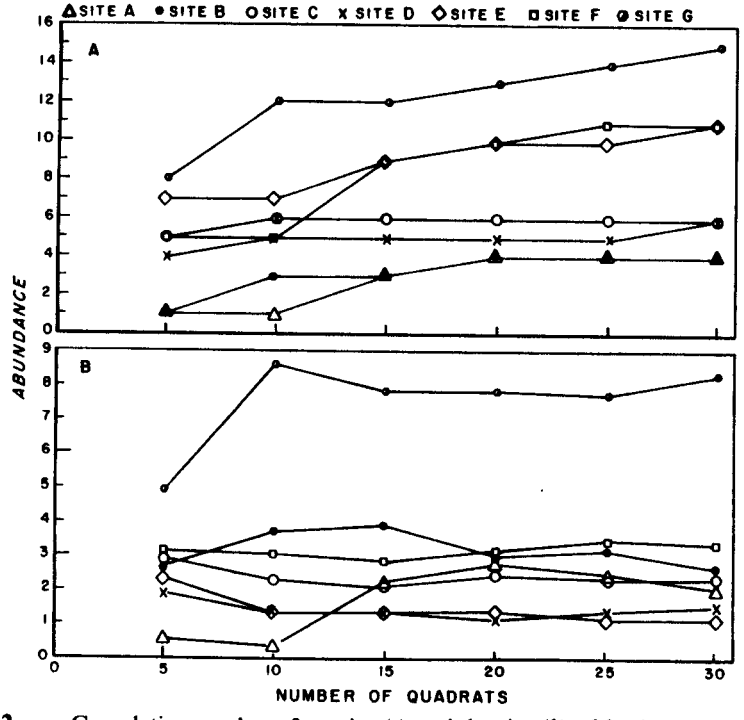

Figure 2. Cumulative number of species (*a*) and density (*b*) of freshwater mussels relative to number of quadrats samples taken at each site from the Big South Fork of the Cumberland River at Annie Branch (site G), McCreary County, Kentucky, August 1988.

beyond 15 m^2, the average species richness estimate across all sites increased an average of only 6.7% (range 3.8%–10.7%).

Quadrat density estimates (Table 1) ranged from 1.2 mussels/m^2 (Site E) to 8.4 mussels/m^2 (Site G). Corresponding estimates from depletion sampling were consistently lower, ranging from 0.2 mussels/m^2 to 2.0 mussels/m^2 (Fig. 3). Depletion estimate efficiency (i.e., [depletion estimate/quadrat estimate] × 100%) varied considerably among the 7 sites (Fig. 3), ranging from 8.5% at site A to 87.5% at site E. Observer experience, water clarity, variability of surface particle size, and total length of mussels may have influenced efficiency of depletion estimates. Although the relationship was weak, particle size accounted for about 33% of the variation in depletion sampling efficiency ($Y = 58.810 + -0.150 \log x$, $r^2 = 0.343$, $P = 0.13$). Lower efficiency occurred among sites with a predominance of larger (>375 mm) surface particles. Total length of mussels, however, was a highly significant independent variable, explaining 75% of the variation ($Y = -100.31 + 1.803 \log x$, $r^2 = 0.751$, $P = 0.005$) in sampling efficiency. Depletion estimates were more similar to quadrat estimates at sites

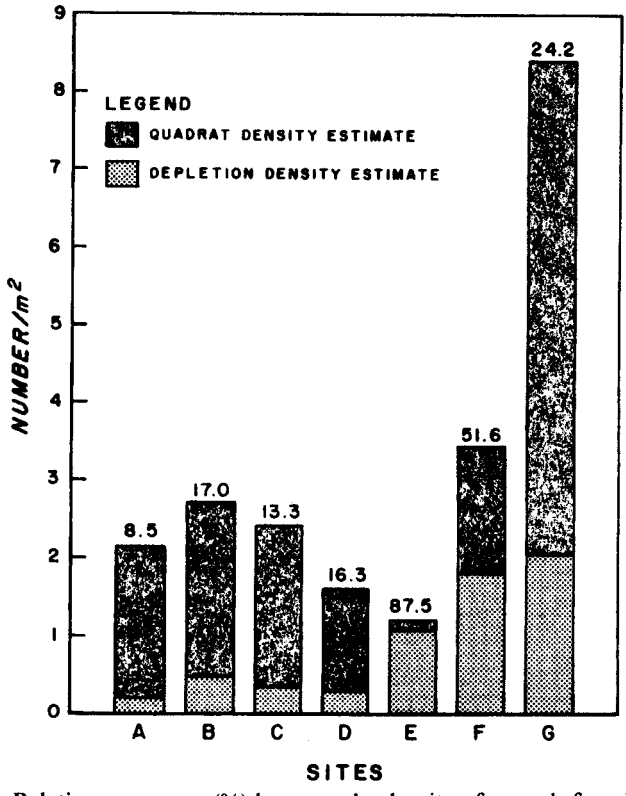

Figure 3. Relative agreement (%) between the density of mussels found in 30 1-m^2 samples during depeltion and quadrat sampling at seven locations in the Big South Fork of the Cumberland River and Clear Fork, Tennessee and Kentucky.

where large mussels (>91 mm) represented a greater proportion of the population.

The size-class distribution of mussels obtained with each technique differed substantially (X^2 = 46.6, df = 9, P < 0.0001). Comparison of the frequency distributions recorded from Site G revealed that depletion sampling underestimated smaller size classes (<75 mm) by 53% and overestimated larger size classes (>91 mm) by 56% when compared to quadrat sampling (Fig. 4). Most of this difference can be attributed to under representation of a single species; only 3 of the 23 *Pegias fabula* (Lea) (length <30 mm) were encountered during depletion sampling. Miller and Payne (1988) stressed the importance of total substratum collection to obtain all size and age classes. We located siphoning individuals as small as 15 mm in length in clear water by carefully searching the substratum. For most species, sampling procedures that can include individuals in the 15 mm size class should be adequate for quantifying most third year (and many second-year) juvenile mussels (Neves and Widlak 1987).

A more difficult task is to estimate mussel density with sufficient precision to discriminate differences among habitats or other environmental conditions.

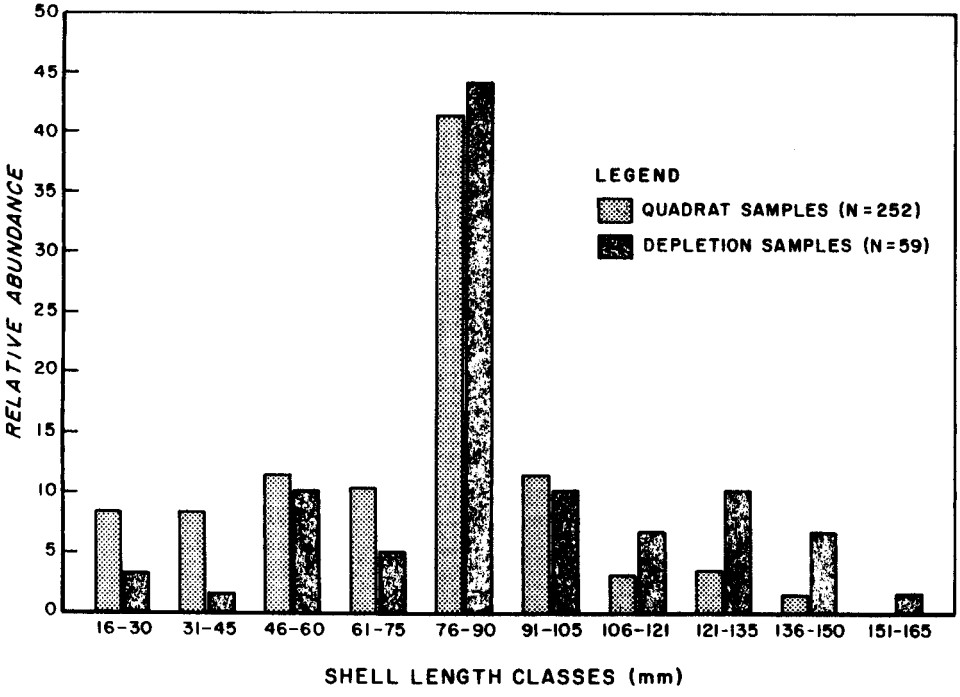

Figure 4. Frequency distribution of size classes of freshwater mussels recorded within 30 1-m² quadrats during 3 pass removal and quadrat sampling at Annie Branch (site G), Big South Fork of the Cumberland River, McCreary County, Kentucky (includes 15 species).

For benthic studies in general, "most of the procedures currently in use produce at best semi-quantitative data" (Dennison and Hay 1967, p. 706). Miller and Payne (1988) estimated that 40 0.25-m^2 quadrats would adequately sample habitats that supported relatively dense populations, whereas 200 0.25-m^2 quadrats were required for low-density populations. Our density estimates at all sites remained essentially unchanged whether we sampled 15 or 30 1-m^2 quadrats (Table 1), suggesting that sampling of the total area was sufficient to capture existing variation in 100-m^2 sampling areas with moderate densities (1–8 mussels/m^2). This is somewhat surprising because our sites supported much lower densities than the lowest density population (10 mussels/m^2) reported by Miller and Payne (1988). Both the number of quadrats and total area sampled in this study were well below their recommended sampling effort.

There are at least 2 possible explanations for what appears to be substantial disagreement over required sampling intensity. First, the estimate of sampling effort reported by Miller and Payne (1988) may not be directly comparable to our results because we used different sampling units or sampled different specific locations in the stream. Although 10 1-m^2 quadrats incorporate the same total area as 40 0.25-ms^2 quadrats, the sampling effort is probably not equivalent in circumstances where organisms exhibit aggregated distributions (Green 1979). Varying the size of the sampling unit (i.e., 0.25-m^2 versus 1.0-m^2 quadrat) alone can significantly influence sampling error associated with density estimates obtained from the same aggregated population (Green 1979). The computed variance of mean density estimates obtained with a 1-m^2 sampling unit varied from 3 to 30 times greater than that obtained from the same population with 0.25-m^2 quadrats (Green 1979). To achieve comparable precision, one would presumably have to increase the number of quadrats by a corresponding multiple (3X–30X for this example).

An alternate explanation is that 15 1-m^2 quadrats may yield mean density estimates that are comparable to those obtained with twice the effort; but these estimates may be imprecise and thus afford little statistical power in testing hypotheses related to distribution and relative abundance of freshwater mussels. In this study, sampling error fluctuated dramatically and unpredictably relative to sampling effort at all sites (Table 1). Variance and the range in sampling error also varied greatly among sites. Within each grid, much of the variability was probably related to the degree of aggregation in mussel distribution (Cummins 1962, Neves and Widlak 1987), which was influenced further by variation in habitat features.

When sampling errors were compared (Bartlett's test for homogeneity of variances; Zar 1984), the variance associated with estimates for more than 5 quadrats were not homogeneous among sites (Table 1). Increasing sampling effort did little to mitigate the apparent differences in spatial distribution patterns among sites (Table 1). Mussel distributions were more patchy and the number of individuals encountered per quadrat was more varied (0–29) at sites with more heterogeneous substrata (e.g., Site G). Site G also had the highest density

and species richness and the greatest range in sampling error relative to sampling effort (13.5–32.6). Fifteen 1 m^2-quadrats yielded a 95% confidence interval that was within ±28% of the mean (7.9±2.2); an additional 15 quadrats only reduced this confidence interval to ±21% (Fig. 2*b*). In contrast, sites supporting low densities (e.g., 1.2 mussels/m^2) typically showed less variability in the number of individuals encountered per quadrat (0–4) and the corresponding 95% confidence limits were within 8% and 6% of the mean for 15 and 30 quadrats, respectively.

Unlike Miller and Payne (1988), our results suggest that streams with variable substrata, having moderate mussel diversity and densities (15 species, >8 mussels/m^2), require as much sampling effort as sites with lower densities to accommodate existing variation. Differences in sampling unit and in range of densities investigated, however, preclude any direct comparisons.

Despite a widely fluctuating variance, relatively precise density estimates in streams with variable substrata may still be possible as evidenced by a consistent decrease in standard error (Table 1) with increasing sample sizes beyond 15 quadrats. Moreover, the precision of our estimates with 20 quadrats was comparable to estimates derived with 30 quadrats. With 20 quadrats, differences in density ($\alpha = 0.05$) of ≤1 mussel/m^2 could be detected in comparisons between areas sampled in four of the seven sites. The remaining sites yielded estimates with sufficient precision to detect differences of 1.5–2.0 mussels/m^2. Corresponding precision for estimates derived from 30 quadrats were similar, with a sufficient increase in precision at 1 additional site to discriminate differences of 1 mussel/m^2 in this study.

Finally, the chain grid was a lightweight (4.5 kg) sampler that was easily deployed by a single individual. More importantly, this technique did not require significant disturbance of the habitat and thus facilitates repeated estimates at the same location while presumably minimizing adverse effects to rare species. However, one needs to be cautious about generalizing the results of our study to other circumstances, especially in streams with a variety of macrohabitats. Further investigations are needed to determine whether this procedure will have broad application across a wide range of streams with variable substrates.

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