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Two-phase sampling to estimate river-wide populations of freshwater mussels

RITA F. VILLELLA[1] AND DAVID R. SMITH[2]

Aquatic Ecology Laboratory, Leetown Science Center, Biological Resources Division, US Geological Survey, 11649 Leetown Road, Kearneysville, West Virginia 25430 USA

Abstract. Two-phase sampling provides a statistical framework for combining data from qualitative and quantitative sampling methods. It is a useful approach when the survey objective is a drainage-wide estimate of mussel density and the cost of qualitative sampling is small relative to the cost of quantitative sampling. This survey design has several advantages including: 1) no need for a priori classification of stream reaches into sampling strata, 2) allocation of sampling effort so that more time is spent sampling where mussels are at higher density and less time is spent sampling where mussels are not present, 3) the ability to evaluate the relationship between qualitative and quantitative estimates of density, and 4) efficient allocation of effort so that more stream reaches can be surveyed compared to quantitative-only sampling designs with similar effort. The survey design consists of sampling as many sites as possible during a qualitative 1st phase and sampling far fewer sites during a quantitative 2nd phase. In 1995, we used a 2-phase sampling design to estimate the distribution and abundance of freshwater mussels in riffle habitat in the Cacapon River, West Virginia. Our estimate of river-wide surface density of freshwater mussels in riffles was 0.59/m^2 (SE = 0.14). We used resampling simulation based on our data and determined that the most effective 2nd-phase sampling strategy was to sample a low to moderate proportion of low-density sites and a high proportion of high-density sites.

Key words: freshwater mussels, 2-phase sampling, optimal allocation, distribution and abundance, river-wide estimation.

Freshwater mussels are among the most threatened animals in North America as a result of habitat change and loss, loss of host species, and the expansion of non-native mollusk species (Williams et al. 1993). Therefore, sampling to estimate size, distribution, status, and population trends in freshwater mussel populations is of growing importance for conservation of biodiversity. Accurate estimates of population parameters (e.g., population density) are critical for effective conservation and management of freshwater mussels, but these parameters are difficult to estimate. Moreover, sampling attempts may yield few individual animals because of spatial clustering and the rarity of many mussel populations.

Selecting the appropriate sampling design requires clear definition of the survey goals and identification of the target population, evaluation of the resources available for sampling, and prior knowledge about site characteristics and the mussel population in the river (Strayer and Smith 2003). Freshwater mussel populations have patchy distributions at multiple spatial scales (Strayer 1983, Downing and Downing 1992, Vaughn et al. 1997), and many locations throughout the river should be sampled to assure an accurate assessment of species distribution and abundance. If just a few sites or only sites at access points (e.g., bridge crossings) are sampled, densities or richness may be severely under- or overestimated. When sampling a patchy population quantitatively, more sampling effort should be allocated in locations where the organism occurs than where it does not occur. However, if mussel distribution in a river is unknown, optimal allocation of sampling effort is challenging. Two-phase sampling can help resolve the sampling-effort problem because information on population distribution collected during phase-1 sampling is used to allocate sampling effort effectively in phase 2.

Many surveys of freshwater mussels have multiple objectives that call for both qualitative and quantitative sampling methods (Kovalak et al. 1986, Miller and Payne 1988, Vaughn et al. 1997, Smith et al. 2001). For example, qualitative information on mussel diversity and relative abundance often is needed at all sampling sites within a river, but quantitative information on

[1] E-mail addresses: rvillella@usgs.gov
[2] drsmith@usgs.gov

mussel density (e.g., density estimate and variance of that estimate) is needed for particular sites and the river as a whole. Precise information on mussel density at all sites is desirable, but the cost of conducting precise surveys can be prohibitive. Thus, allocation of sampling effort among and within sites becomes an important consideration because quantitative sampling is more costly than qualitative sampling (Kovalak et al. 1986). Two-phase sampling can help control sampling costs if a large initial set of sites is sampled using a quick and inexpensive method, and some fraction of the initial sites is resampled using a more costly method to gather more precise information.

Simple random sampling is robust only when the sample size is large enough to be representative of the entire population. A preferred approach is to stratify sampling to ensure a small but representative sample. In stratified sampling, the population of initial sample sites is partitioned into different strata on the basis of some auxiliary variable, and a subsample of sites is selected from each stratum for additional sampling. The 2-phase sampling design combines double sampling with stratification and is also referred to as double sampling for stratification (Cochran 1977, Thompson 1992, Strayer and Smith 2003). In phase 1 of 2-phase sampling, all sites within an initial random sample of reaches in a river are sampled using a qualitative sampling method for a rapid assessment of species presence and relative abundance. The results of phase-1 sampling and the principle of stratification are used to partition sites into strata so that sites within each stratum are as similar as possible. This strategy results in a more precise river-wide population estimate than simple random sampling (Thompson 1992). Moreover, stratified sampling of river reaches allows a great deal of flexibility because the proportion of reaches within a stratum that is sampled quantitatively and the quantitative method of subsampling a reach (e.g., adaptive or systematic sampling) can differ among strata. Thus, more sampling effort can be allocated to stream reaches where mussels are at higher densities or where species of interest (e.g., rare species) occur, and the flexibility of sampling method within sites can help minimize costs.

The 2-phase sampling design has been used as a cost-effective method for obtaining density estimates in wildlife surveys, resource inventories, and long-term surveys of monitoring plots (Thompson 1992). For example, phase 1 of a statewide inventory of forest populations might consist of collecting aerial photo or remote-sensing data on a large sampling grid, and phase 2 would consist of field visits to a subset of the sites sampled on the grid (Moisen and Edwards 1999). Photo-interpreted cover type and land ownership could be used to stratify sites before choosing sites for sampling in phase 2, resulting in unbiased estimates and improved precision of forest population totals.

No examples of this sample design have been published for freshwater mussels. We hoped to minimize costs of a river-wide mussel survey by using double sampling for stratification (Cochran 1977, Thompson 1992). Our objective was to evaluate the sampling design as a cost-effective method to estimate site-specific density of freshwater mussels and to obtain precise estimates of mussel population density over a large spatial scale, i.e., the length of a river. We applied the design to a freshwater mussel survey on the Cacapon River, evaluated the optimal sampling fraction, and described its efficiency and precision.

Methods

Application of 2-phase sampling

We applied 2-phase sampling on the Cacapon River, a tributary to the Potomac River in West Virginia (Fig. 1). The river lies within the Ridge and Valley physiographic province. Approximately 79% of the basin is forested, but agriculture and increasing development is a primary concern (Constantz et al. 1993). Unionid mussels are present but little information on their abundance and distribution is available.

We had no knowledge of the proportion of the riffles in the river that would have high or low mussel density, so we included many sites in the initial sample to ensure proper classification of sites into strata. We used 7.5 min topographic maps and marked the length of the Cacapon River into thirty-one 4-km sections. We selected one site at random within each 4-km stretch of river for sampling (Fig. 1), but we constrained sampling to riffles so that mussels could be collected without SCUBA. When we reached a randomly chosen site in the field, we sampled the riffle closest to that point. We de-

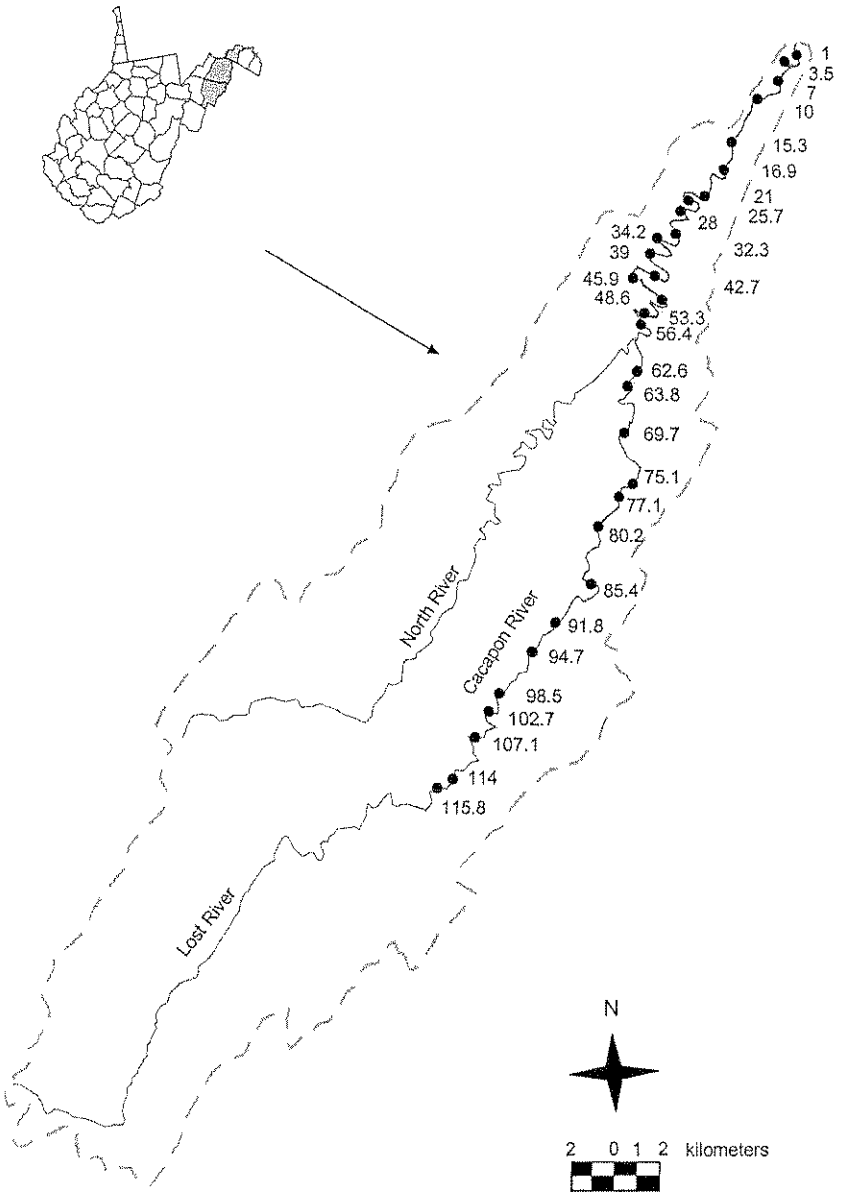

FIG. 1. Cacapon River basin showing the location of the basin in West Virginia and sample sites along the mainstem of the river. Sites are referenced by river kilometers (rkm) from the mouth of the river.

fined riffles as areas of shallow, fast-moving water where the surface of the water was rippled. We measured the average width of the riffle and set the length of the sampling area equal to 2× the average width of the site. If the riffle was <2× its width, we sampled the entire riffle. We restricted sampling to riffles, and we did not excavate below the substrate surface, so our inference was limited to riffle habitat and to those animals visible at the surface.

Sampling during the phase-1 rapid assessment consisted of a timed search throughout the site using viewing buckets for 1 person-hour (p-h) (e.g., 2 biologists for 30 min or 4 biologists for 15 min). We chose to stratify sample sites for phase-2 sampling based on number of mussels

found during the surface search at each site. We used the number of live mussels counted during the timed search to categorize the site as low (≤30/h) or high density (>30/h). We decided a priori on the distinction between low- and high-density sites, based on previous sampling experience on other Atlantic Slope rivers where we found that 30 mussels/h was the cutoff between low- (<1 mussel/m²) and high-density (≥1 mussel/m²) strata (RFV and DRS, personal observation).

Normally only a fraction of the phase-1 reaches would be sampled during phase 2, and this fraction would be allowed to vary among strata. Had we used heuristic notions of sampling allocation, the subset of reaches selected for phase-2 sampling would have been directly related to within-stratum variance and indirectly related to cost. However, we were interested in investigating this sampling allocation problem empirically, so we selected all of the 31 reaches for phase-2 sampling so that we could compute variance and cost for a range of sampling efforts.

The sampling method used during phase-2 sampling depended on density. We used either systematic or adaptive sampling to assess mussel populations quantitatively. For both methods, we placed 0.25-m² quadrats systematically throughout the site to ensure good spatial coverage. We began the systematic sample by placing the initial quadrat at a random start in the corner of the site. We placed the remaining quadrats at 3-m intervals in the across-river and 5-m intervals in the upriver direction, resulting in a grid of quadrats covering ~2% of the riffle. At all sites, we searched each quadrat thoroughly by snorkeling. We did not excavate, but we did search under large, nonembedded rocks that were easily moved. We handpicked all mussels visible at the substrate surface, identified them to species, measured their lengths, and placed them back in the substrate. At low-density sites, we used adaptive-cluster sampling. If we observed ≥1 mussel in the initial quadrat, we sampled the 4 adjacent quadrats (adaptive quadrats) as well. If we observed ≥1 mussel in any of those quadrats, we sampled its neighboring quadrats. We continued this procedure until no mussels were found in the adaptively sampled quadrats. Thus, the adaptive sample consisted of the mussels in a cluster of quadrats. Detailed description of adaptive-cluster sampling and formulae for estimating densities based on this design are presented in Thompson (1992) and Thompson and Seber (1996).

Statistical analyses

Two-phase sampling provides better estimates of density and abundance at a lower cost if the relationship between the 2 survey methods is linear (Thompson 1992). We used linear regression to evaluate the relationship between the surface-count data collected in phase 1 and the density estimates from phase 2. We multiplied the proportion of sites in each stratum by the within-stratum estimate of density and combined these values to arrive at a weighted estimate of basin-wide density. The estimated density of mussels at the surface in riffle habitat in the river is $\bar{y}_d = \Sigma w_h \bar{y}_h$ where w_h is the proportion of sample sites in each stratum from phase-1 sampling and $\bar{y}_h$ is the mean density estimate for each stratum resulting from phase-2 sampling. The variance of that estimate is

$$\text{var } \bar{y}_d = \left(\frac{N-1}{N}\right)\sum_{h=1}^{L}\left(\frac{n'_h - 1}{n' - 1} - \frac{n_h - 1}{N-1}\right)\frac{w_h s_h^2}{n_h} + \left[\frac{N-n'}{N(n'-1)}\right]\sum_{h=1}^{L} w_h(\bar{y}_h - \bar{y}_d)^2$$

where N is the total number of riffles, L is the number of strata, n'_h is the number of sites in stratum L, n_h is the subset of sites in a stratum sampled in phase-2 sampling, and n' is the total number of sampling sites in the phase-1 sample. We accounted for difference in the sizes of our sampling sites in each stratum using the ratio estimator

$$\bar{y}_h = \sum_{i=1}^{n_h} y_{h_i} \Big/ \sum_{i=1}^{n_h} x_{h_i}$$

where y_{h_i} is the number of mussels in quadrats at each site and x_{h_i} is the site area, and the variance of the estimate of each stratum is

$$s_h^2 = \frac{1}{n_h - 1}\sum_{i=1}^{n_h}(y_{h_i} - \bar{y}_h x_{h_i})^2.$$

We evaluated the efficiency of the 2-phase sampling design analytically and by simulations (Cochran 1977). We calculated the fraction of reaches in each stratum that should be selected to minimize both cost and variance of the pop-

ulation estimate using formula 12.21 from Cochran (1977):

$$v_h = s_h \left[\frac{c'}{c_h} \left(s^2 - \sum w_h s_h^2 \right) \right]^{1/2}$$

where v_h is the optimal sample size for phase-2 sampling in each stratum, s^2 is the population variance, c' is the typical cost to sample qualitatively at all sites (phase 1), and c_h is the typical cost to sample quantitatively at each site within the stratum (phase 2).

An obvious question in 2-phase sampling is how to divide available resources between phase-1 and phase-2 sampling. We resampled the Cacapon River data to simulate 2-phase sampling and examine the effect of sampling allocation on variance and cost. The simulation began by taking a bootstrap sample from the 31 sites (i.e., randomly selecting sites with replacement). The selected sites represented phase-1 sampling (i.e., the n' sites selected for qualitative sampling). Each selected site was assigned to a stratum based on mussels/p-h that had been observed at the site, and a proportion of the sites within each stratum was selected randomly for quantitative (phase-2) sampling. The proportion (n_h/n'_h) ranged from 0.1 to 0.9. Observations from the sites selected for phase-1 and phase-2 sampling were used to compute variance and cost. Total cost (C*) was defined in units of p-h to complete the survey, excluding travel between sites. C* was calculated as

$$C^* = c'n' + \sum c_h n_h.$$

Cost for phase-1 sampling (c') was 1 p-h. Stratum-specific cost for phase-2 sampling (c_h) was 2 p-h for low-density and 2.5 p-h for high-density sites. Bootstrap sampling was repeated 1000 times; variance and cost were averaged across 1000 replicates for each combination of n_h/n'_h. Minimizing variance for fixed cost or cost for fixed variance is equivalent to minimizing their product (Cochran 1977). Therefore, we evaluated the effect of sample allocation on the product of cost and variance.

Results

Phase-1 sampling

We found unionids at 28 of the 31 sites (Table 1). We placed 24 of the 31 sites in the low-density stratum and 7 in the high-density stratum.

TABLE 1. Number of species and individual mussels found during phase-1 rapid assessment at 31 sites on the Capacon River. All sites were searched for 1 person-hour (p-h). High density sites had >30 mussels/p-h and low-density had ≤30 mussels/p-h. Site name is based on the river kilometer (RKM) starting at the mouth and ascending upriver (Fig. 1).

Site (RKM)	Site area (m²)	No. of speccies	No. of mussels
1.0	1218	1	127
3.5	850	4	19
7	337	3	15
10	1769	1	5
15.3	911	1	1
16.9	1697	2	7
21	2005	2	19
25.7	1383	1	15
28	1501	1	1
32.2	1983	1	1
34.2	1709	1	4
39	1478	1	5
42.7	1580	2	53
45.9	1006	1	4
48.6	621	1	5
53.3	511	2	73
56.4	2177	1	1
62.6	749	0	0
63.8	738	0	0
69.7	1290	2	4
75.1	1626	3	42
77.1	2243	3	84
80.2	502	1	22
85.4	3020	3	74
91.8	650	1	4
94.7	1470	2	14
98.5	620	1	4
102.7	199	2	163
107.1	340	2	30
114	720	1	1
115.8	399	0	0

The high-density sites were at river km (RKM) 1.0, 42.7, 53.3, 75.1, 77.1, 85.4, and 102.7 (Table 1, Fig. 1). We found highest counts at RKM 1.0 and 102.7. At the 7 high-density sites, we found 616 animals, 4 species, and 87.5 animals/p-h. At the low-density sites, we found 181 animals, 4 species, and 7.74 animals/p-h. The Cacapon River mussel fauna included 6 species: *Elliptio complanata, E. fisheriana, Lampsilis, Alasmidonta varicosa, Lasmigona subviridis,* and *Strophitus undulatus.* We identified all *Lampsilis* as *L. cariosa,* but some question exists regarding which species currently is found in the Cacapon and other

TABLE 2. Location, surface density, abundance (N), standard error (SE), and coefficient of variation (CV) for mussels sampled at 31 riffle sites surveyed during phase-2 sampling on the Cacapon River between June and August 1995. Site name is based on the river kilometer (RKM) starting at the mouth and ascending upriver (Fig 1). Initial sample size is the number of quadrats systematically placed within each site. At high-density sites, all (and only) initial quadrats were searched. At low-density sites, final sample size is the number of quadrats actually searched using adaptive sampling (see text for details) and is a count of all network quadrats plus edge units. CV for density and abundance are the same. – = CV could not be calculated.

			Density			Abundance	
Site (RKM)	Initial sample size	Final sample size	Mean (no./m²)	SE	CV	N	SE
1.0	121	121	2.81	0.5026	0.18	3422	1103
3.5	67	107	0.33	0.1404	0.42	282	127.4
7.0	42	67	0.48	0.1991	0.41	162	67.1
10.0	125	145	0.19	0.0884	0.46	340	179.6
15.3	69	77	0.12	0.0806	0.70	106	73.4
16.9	137	264	0.59	0.1453	0.25	996	314.5
21.0	112	157	0.33	0.1142	0.35	660	298.6
25.7	119	148	0.18	0.0877	0.49	246	136.9
28.0	121	125	0.03	0.0327	0.99	50	49.1
32.2	94	94	0	0	–	0	0
34.2	119	178	0.34	0.1013	0.30	573	220.1
39.0	90	111	0.18	0.0867	0.49	264	176.8
42.7	112	112	1.18	0.0547	0.20	1862	442.4
45.9	70	74	0.06	0.0566	0.99	57	56.9
48.6	62	76	0.20	0.1085	0.56	121	67.4
53.3	52	52	2.38	0.6970	0.29	1219	317.2
56.4	149	164	0.11	0.0523	0.49	233	157.6
62.6	52	52	0	0	–	0	0
63.8	53	53	0	0	–	0	0
69.7	112	112	0	0	–	0	0
75.1	115	115	0.63	0.1682	0.27	1018	310.2
77.1	116	116	1.48	0.1257	0.25	3326	873.2
80.2	38	71	0.63	0.2850	0.46	328	143
85.4	175	175	0.53	0.1122	0.21	1588	383.2
91.8	62	73	0.19	0.1086	0.56	126	70.6
94.7	95	314	0.71	0.2031	0.29	1119	319.9
98.5	48	52	0.08	0.0825	0.99	52	51.1
102.7	12	12	3.33	1.1802	0.35	663	147.8
107.1	38	146	0.57	0.2226	0.39	193	75.8
114.0	55	55	0	0	–	0	0
115.8	36	36	0	0	–	0	0

tributaries of the Potomac River basin (Ortmann 1913). *Elliptio complanata* was the most common species and was found at all 28 sites where mussels were detected. We found *E. fisheriana* at 10 sites, and *L. cariosa* at 7 sites. The remaining species were rare (2 sites).

Phase-2 sampling

Stratum-specific time spent sampling (c_h) averaged 2.0 and 2.5 p-h for low- and high-density strata, respectively. The area of low-density sites ranged from 337 to 2177 m² (mean = 1121 m², SE = 577.05), and the area of high-density sites ranged from 199 to 3020 m² (mean = 1485 m², SE = 969.52) (Table 1). Depending on size of the sample area, we sampled 12 to 175 quadrats in high-density sites and 36 to 314 quadrats, including adaptive quadrats, in low-density sites (Table 2). We found mussels at 25 of the 31 sites. *Elliptio complanata* was the most common species and we found it at 24 sites. We found *E. fisheriana* at 12 sites, and *L. cariosa* at 8 sites. The remaining species were rare.

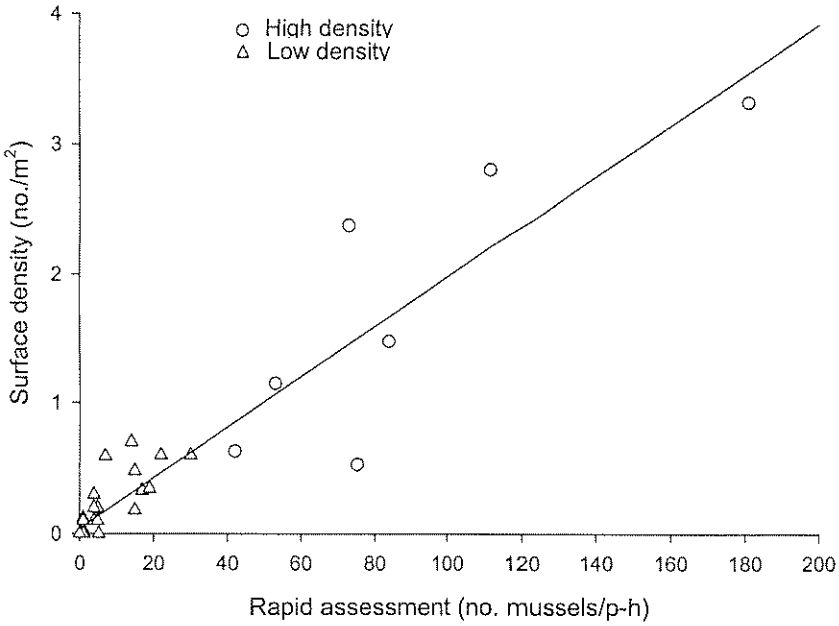

FIG. 2. Linear regression of qualitative assessment of count data from phase-1 sampling and quantitative surface-density estimates from phase-2 sampling. Low-density sites had ≤30 mussels/person-hour (p-h) and high-density sites had >30 mussels/p-h in the qualitative assessment.

The relationship between density estimates from quantitative sampling and qualitative sampling was linear ($y = 0.075 + 0.019x$, $R^2 = 0.87$, $p < 0.001$) for both low- and high-density sites (Fig. 2). We considered the relationship between qualitative and quantitative sampling for the low- and high-density sites separately; both relationships were linear with similar slopes ($y = 0.062 + 0.021x$, $R^2 = 0.61$, $p < 0.001$ for low-density vs $y = 0.036 + 0.019x$, $R^2 = 0.68$, $p < 0.02$ for high-density sites).

Estimated surface density of mussels ranged from 0.53/m² (SE = 0.1122) to 3.33/m² (SE = 1.1802) in the high-density sites with highest densities found at RKM 102.7, 53.3, and 1.0 (Table 2). We did not find mussels at 6 of the low-density sites. At the remaining 18 low-density sites, estimated surface density ranged from 0.03/m² (SE = 0.0327) to 0.71/m² (SE = 0.2023).

Mean density of mussels was 0.22/m² (SE = 0.21) for the low-density stratum and 1.76/m² (SE = 1.08) for the high-density stratum. Median CV was 46% and 25% for low- and high-density strata, respectively. The river-wide estimated density of mussels in riffles was 0.59/m² with variance = 0.02 (CV = 26%). Based on formulae in Cochran (1977), the optimal proportions of qualitative sites that should have been sampled quantitatively were 12% and 48% of the low- and high-density strata, respectively. In theory, this allocation of stratum-specific quantitative sampling would have minimized both variance and costs.

The resampling simulation results indicated that variance and cost responded in opposite directions to increased sampling effort. Thus, variance × cost was relatively insensitive to effort. Nevertheless, variance × cost was minimized when 20 to 40% of low-density sites and ≥60% of high-density sites were selected for quantitative (phase 2) sampling (Fig. 3). Within those survey designs, cost to complete the survey ranged from 52 to 67 p-h, and CV ranged from 28% to 34%. To decrease the CV, >31 sites would have had to be selected for phase-1 sampling; however, the proportion of sites selected for phase-2 sampling could have remained low to moderate for low-density and high for high-density sites. Of course, total cost would have increased had more sites been selected. For example, to achieve a CV of 25% for a Cacapon River-wide density estimate of mussels in riffles, we would have had to conduct a rapid assessment at 47 reaches and then sample a random

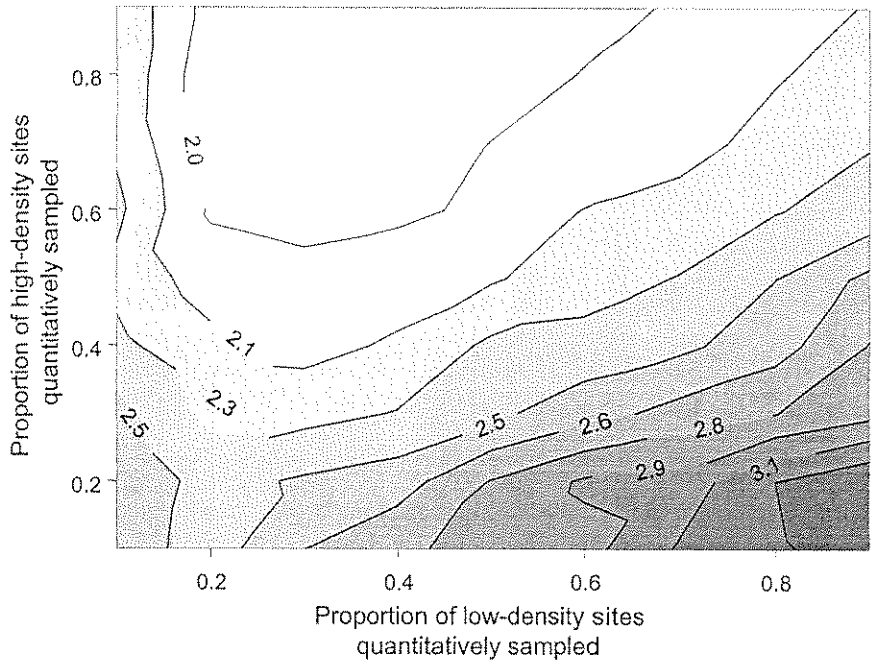

FIG. 3. Variance and cost in person-hours (p-h) as a function of the stratum-specific proportion of sites sampled quantitatively using a 2-phase sampling design. Contours show the product of variance and cost. Efficient allocations are found where the product is minimized. Results are based on a resampling of the data collected from the Capacon River in 1995.

20% of low-density reaches and 70% of high-density reaches quantitatively. This survey design would have required ~65 p-h of sampling, excluding travel or setup time. In contrast, had we sampled 100% of high-density reaches quantitatively, a CV of 25% would have been achieved by conducting a rapid assessment at a random sample of 37 reaches. This survey design would have required slightly more time to complete (<1 h more of sampling time) compared to the previous design. We actually sampled 100% of the low- and high-density sites quantitatively. Our survey design required ~98 p-h, excluding travel or setup time, with ~30% of the time spent sampling all 31 sites qualitatively.

Discussion

Advantages of 2-phase sampling

Several goals are common to all surveys. The 1st is an unbiased estimate of the variable of interest. Unbiased estimates allow the biologist to make inferences about population parameters such as relative abundance, absolute abundance, or density. The 2nd is a precise estimate and an assessment of the accuracy of the estimate. The 3rd is a cost-effective and convenient sampling design (Thompson 1992). The basic steps of survey design are to set clear objectives, determine the target population, choose the sample size, and select a method for conducting the survey. Often the decision about sample size is determined by the time and budget available to conduct the survey and the desired level of precision.

Our objective was to obtain precise estimates of mussel density at the levels of individual sites and the entire Cacapon River using a sampling design that would reduce survey costs. Two-phase sampling was an efficient sample design for estimating mussel density, especially for rare species. Mussel populations in riffles in the Cacapon River were patchy with few and scattered high-density areas separated by many areas where mussels were scarce or absent (Table 2). Phase-1 sampling provided good spatial coverage, and phase-2 sampling reduced quantitative

sampling costs by focusing sampling effort where mussels were abundant. Concentrating survey effort where mussels were more numerous increased the precision of the population density estimate (Strayer and Smith 2003).

Our sampling design had several advantages. We were able to obtain separate unbiased estimates of density for each stratum (because the intensive sampling sites were chosen at random). This ability provided useful information about the mussel population in the river and enabled us to learn more about mussel–habitat relationships and to identify areas of high abundance throughout the river. The method yielded unbiased estimates of population density and, therefore, unbiased estimates of trends in mussel density both within strata and for the river.

In general, the 2-phase sampling design is flexible and can be implemented at multiple scales to select sample sites within a river or basin or to select sample units within a stream reach. The cost of sampling should be lower because not all sites must be revisited during the 2nd phase of sampling. A time cost is associated with establishing the frame of sampling (i.e., assigning sites to strata), but the 2-phase design has the advantage that a great deal of prior knowledge of the whole river is not needed before stratifying sites. The design flexibility can be used to reduce total survey time. For example, the 1st phase of sampling does not have to be completed at all the sample sites before initiating the 2nd sampling phase. The decision to conduct a quantitative survey at a site can be made upon completion of the qualitative assessment of the site. This flexibility can further reduce survey costs by reducing travel time. On the other hand, all qualitative sampling could be completed during one year and all quantitative sampling could be completed during the next. For these reasons, we believe the 2-phase sampling method warrants consideration in large-scale mussel surveys.

Survey techniques

We used timed search to count mussels at the surface to categorize each site. Timed search is generally efficient (less costly) and more effective (more species/level of effort) than quantitative sampling for detecting the presence of rare mussel species (Miller and Payne 1993, Strayer et al. 1997, Vaughn et al. 1997, Obermeyer 1998) because of the spatially clustered distribution of mussels (Kovalak et al. 1986, Downing and Downing 1992). Timed search was effective for partitioning sites into density strata because our objective was to estimate density (Fig. 2). However, how sites are stratified should depend on the survey objective. For example, sites could be stratified based on species richness or, if the objective were to describe distribution and density of certain rare species, sites could be stratified on the basis of the presence of rare species.

Our main objective in using adaptive-cluster sampling was to take advantage of the characteristic distribution pattern of mussels (i.e., rare and clustered) within the low-density sites. Adaptive-cluster sampling did increase the number of individual mussels found and it improved detection of species other than *E. complanata* (Table 3); however, it did not increase precision over conventional quadrat sampling (Smith et al. 2003). Two of the 6 species were only found in adaptively placed quadrats (*A. varicosa* and *S. undulatus*). We anticipated that embedding adaptive-cluster sampling in a 2-phase sampling design and restricting its application to low-density sites would limit the final sample size. Final sample size at most sites was $\leq 1.5\times$ the initial sample size. Adaptive-cluster sampling could have been used at high-density sites, but some measure to control the final sample size (e.g., setting the criterion for initiating adaptive sampling >1 mussel in the initial quadrat) would have been needed to keep the number of adaptive quadrats $\leq 1.5\times$ the initial sample size (Smith et al. 2003). Varying the criterion among sites has unknown consequences on efficiency, and additional research would be required before this approach could be recommended (Smith et al. 2003). However, adaptive-cluster sampling was effective for detecting mussels at low densities and for locating rare species, and additional application of the method at known low-density sites in other basins is warranted.

Estimated density of mussels in riffles in the Cacapon River was low. Adaptive-cluster sampling in the low-density sites did not affect the estimate of the mean population size or the precision of that estimate (river-wide estimate: 0.59/m^2, variance = 0.02, CV = 26%; adaptive-sampling estimate: 0.56/m^2, variance = 0.03, CV = 28%). Had we included excavation in our

TABLE 3. Species and number of individuals observed in the initial and adaptively placed quadrats during phase-2 sampling on the Capacon River. Adaptive sampling was inplemented within the low-density sites if >1 mussel was found in an initial quadrat (see text for details).

	Initial quadrats		Adaptive quadrats	
Site	Species	Number	Species	Number
3.5	*Elliptio complanata*	6	*E. complanata*	12
			Lampsilis cariosa	4
7.0	*E. complanata*	5	*E. complanata*	3
10.0	*E. complanata*	5	*Alasmidonta varicosa*	1
15.3	*E. complanata*	2		
16.9	*E. complanata*	8	*E. complanata*	16
	E. fisheriana	12	*E. fisheriana*	24
			A. varicosa	1
21	*E. complanata*	4	*E. complanata*	2
	E. fisheriana	4	*E. fisheriana*	6
25.7	*E. complanata*	2	*E. complanata*	7
	E. fisheriana	2	*E. fisheriana*	5
			A. varicosa	1
28	*E. complanata*	1		
34.2	*E. complanata*	9	*E. complanata*	7
			A. varicosa	1
			Strophitus undulatus	1
39	*E. complanata*	3	*E. complanata*	2
	E. fisheriana	1		
45.9	*E. fisheriana*	1	*E. fisheriana*	1
48.6	*E. complanata*	3	*E. complanata*	2
56.4	*E. complanata*	2		
80.2	*E. complanata*	6	*E. complanata*	8
91.8	*E. complanata*	3		
94.7	*E. complanata*	17	*E. complanata*	145
	L. cariosa	1	*L. cariosa*	8
			E. fisheriana	1
			A. varicosa	2
98.5	*E. complanata*	1		
107.1	*E. complanata*	3	*E. complanata*	92
			L. cariosa	1

sampling methods, more animals would have been counted, and the estimated total density of mussels in riffle habitat would have been higher. The proportion of the population below the surface could be incorporated into quadrat sampling using double sampling (i.e., sample all quadrats for mussels on the surface and excavate a subset of those quadrats to find mussels below the surface) to reduce both the cost of excavation and disturbance (Smith et al. 2000).

Optimizing sampling schemes

The optimal sampling scheme in 2-phase sampling allocates larger sample sizes to the more variable strata (high-density sites) and smaller sample sizes to the less variable (but possibly more difficult to sample) strata (low-density sites). Stratum-specific sampling allocations can deviate from optimal allocation when specific objectives warrant. For example, a higher fraction of high-density reaches (even up to 100%) could be selected for quantitative sampling if high-density reaches have special significance. One caveat is that ≥2 reaches must be sampled per stratum so that stratum-specific variance can be computed. If no estimate of density is obtained in one of the strata (e.g., a low-density stratum), then density cannot be estimated for the basin as a whole.

Two-phase sampling is a useful approach when the survey objective is a river-wide or ba-

sin-wide estimate of density or abundance and the cost of qualitative sampling is small relative to quantitative sampling. For our study, it provided a cost-effective method for obtaining essentially unbiased estimates of mussel density in a long river where many of the sites were remote and travel was difficult. We probably could have improved the sampling design by delineating sites so they were more similar in size (to simplify analyses), adding habitats other than riffles, and using double sampling to include some level of excavation in phase 2 sampling. We are currently implementing this modified approach on the upper Allegheny River, a more productive and species-rich system than the Cacapon River.

Two-phase sampling could be used as an effective sample design for monitoring natural populations of all types, not just freshwater mussels. Benthic invertebrates, like freshwater mussels, have clustered distributions (Allan 1984). If the objective were to evaluate the diversity and abundance of benthic invertebrates in a stream, 2-phase sampling could be used as described in our survey of mussels in the Cacapon River. If the objective were to evaluate the relationship of habitat variables with invertebrate abundance or richness, the results of 1st-phase sampling could be used to select a subset of sites in the low- and high-density strata or species-rich vs species-poor strata for intensive habitat description in phase 2. This sampling design also could be useful for biomonitoring projects. For example, water-quality assessment could be done at a number of sites in phase 1 and the results used to stratify sites along a gradient of good-to-poor water quality. In phase 2, a high % of good water-quality and a low % of poor water-quality sites could be subsampled to determine the presence or abundance of indicator species. The phase-2 sampling could be repeated regularly as part of the biomonitoring program.

Biologists need efficient methods to estimate population parameters and to document change in those parameters. Documenting change requires sampling at larger spatial and temporal scales than have been previously evaluated (Silsbee and Peterson 1993). Often sites are selected for monitoring because historical records indicate that mussels were locally abundant or the sites are accessible, or both. Sites selected in this way are a biased sample from the population of interest. Palmer (1993) showed that selecting sites based on local abundance may give the appearance that the species is increasing or declining even though abundance fluctuates randomly around a stable long-term average. Addressing this problem requires the use of efficient probability-based sampling designs. Devising a sampling design that minimizes the costs associated with large surveys, provides a representative sample, and yields precise estimates of mussel densities in rivers is not easy. However, 2-phase sampling seemed to achieve these goals in our study.

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