

Survey Protocol for Assessment of Endangered Freshwater Mussels in the Allegheny River, Pennsylvania



David R. Smith; Rita F. Vilella; David P. Lemarie

Journal of the North American Benthological Society, Vol. 20, No. 1. (Mar., 2001), pp. 118-132.

Stable URL:

<http://links.jstor.org/sici?sici=0887-3593%28200103%2920%3A1%3C118%3ASPFAOE%3E2.0.CO%3B2-I>

Journal of the North American Benthological Society is currently published by The North American Benthological Society.

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/about/terms.html>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/journals/nabs.html>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is an independent not-for-profit organization dedicated to creating and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact support@jstor.org.

Survey protocol for assessment of endangered freshwater mussels in the Allegheny River, Pennsylvania

DAVID R. SMITH,¹ RITA F. VILLELLA, AND DAVID P. LEMARIÉ

US Geological Survey, Biological Resources Division, Leetown Science Center,
1700 Leetown Road, Kearneysville, West Virginia 25430 USA

Abstract. The United States Endangered Species Act (ESA) requires a biological assessment of any activity that is authorized, funded, or carried out by a federal agency and likely to affect a federally listed endangered species or its critical habitat. We developed a standardized survey protocol for biological assessments of the effects of bridge replacements on 2 federally listed endangered freshwater mussels, *Epioblasma torulosa rangiana* and *Pleurobema clava*, found in the Allegheny River, Pennsylvania. The protocol combines qualitative sampling to determine species present with quantitative sampling to estimate density. Data on species present satisfy the minimum requirement of a biological assessment, whereas estimates of density are needed to assess the number of individuals that would die as a result of bridge replacement. Some excavation of substrate is necessary for unbiased population estimates because of species and sex-specific differences in detection at the substrate surface. We reduced the amount of excavation and cost of the survey by using a statistical sampling technique called double sampling, which uses counts from excavating a subset of quadrats to calibrate counts from searching the substrate surface of all quadrats. We applied the survey protocol to the Allegheny River at West Hickory where *E. t. rangiana* was the 3rd and *P. clava* was the 4th most abundant mussel at the site. Only 31% of *P. clava* and 52% of *E. t. rangiana* (80% of females, 45% of males) were detected at the substrate surface. We estimated that 9173 (95% CI: 6309–13,336) *E. t. rangiana* and 7010 (95% CI: 4462–11,013) *P. clava* lived within 50 m of the existing bridge and would be affected immediately by bridge construction. (Population estimates did not include mussels too small to be retained on a 6.35-mm-mesh sieve.) Application of the protocol is not limited to biological assessment under the ESA, but is appropriate where site-specific status of freshwater mussel populations is required.

Key words: freshwater mussels, population assessment, sampling design, *Epioblasma torulosa rangiana*, *Pleurobema clava*, Endangered Species Act.

The Allegheny River drainage supports some of the largest remaining populations of *Pleurobema clava* and *Epioblasma torulosa rangiana*, 2 freshwater mussel species listed as endangered by the US Fish and Wildlife Service (1994). The Pennsylvania Department of Transportation (PennDOT) plans to replace a series of older bridges along the Allegheny River within the species' current range. Bridge replacement will be undertaken with funds from the Federal Highway Administration, so biological assessments are required as stipulated under the Federal Endangered Species Act (ESA).

Biological assessment evaluates the potential effects of a federal activity on federally listed species to determine whether formal consultation is necessary (US Fish and Wildlife Service and National Marine Fisheries Service 1998). Formal consultation determines whether a proposed activity is likely to jeopardize the continued existence of a federally listed species or ad-

versely affect designated critical habitat (US Fish and Wildlife Service and National Marine Fisheries Service 1998). A biological assessment includes an on-site inspection to determine species present in the area to be affected by the federal activity and an analysis of the potential effects on the species and its habitat. A biological assessment is to be based on information that is reliable, credible, and represents the best scientific and commercial data available (US Fish and Wildlife Service and National Marine Fisheries Service 1998). However, the contents and methods of the biological assessment are left to the discretion of the Federal agency that is funding the activity.

We present a standardized survey protocol for the biological assessment of *E. t. rangiana* and *P. clava* at bridge replacement sites on the Allegheny River. The protocol combines qualitative sampling to determine species present with quantitative sampling to estimate density. Data on species present satisfy the minimum requirement of a biological assessment, whereas

¹ E-mail address: david_r.smith@usgs.gov

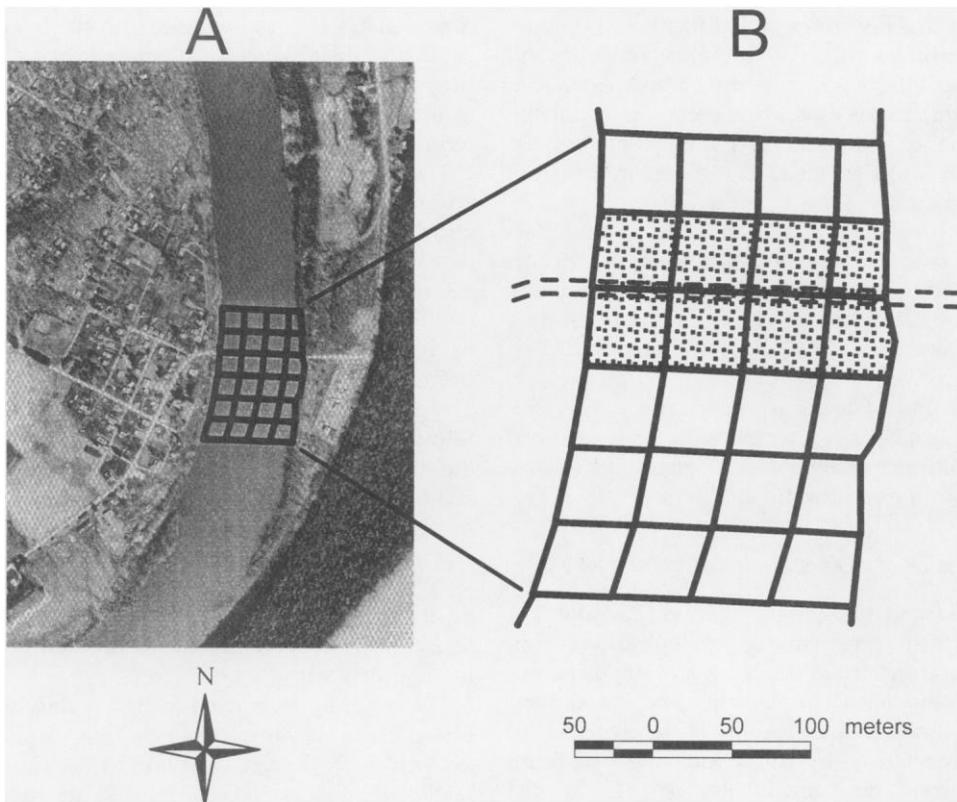


FIG. 1. Location of the study site. A.—Portion of a digital orthophoto quarter quadrangle showing West Hickory on the west bank of the Allegheny River. B.—Grid of 24 cells in 6 rows and 4 columns overlaid on a stretch of the Allegheny River and used for timed searches of freshwater mussels. The dashed lines show the position of the bridge. The small squares in the 4th and 5th rows from the bottom show location of quadrats in the direct-effects area; the remainder of the site is the indirect-effects area. Water flow is to the south.

estimates of abundance are needed to assess the number of individuals that may be negatively affected by bridge replacement. We applied the protocol to the Allegheny River at West Hickory, Pennsylvania, where bridge replacement was proposed. The protocol may be useful at other sites where an assessment of rare freshwater mussels is needed.

Methods

The bridge links Harmony Township to Hickory Township in Forest County (lat 41.574°N, long 79.411°W; Fig. 1). A previous survey (Aquatic Systems 1998) detected *P. clava* and *E. t. rangiana*, but did not estimate density or abundance. We designed a survey protocol as if the West Hickory site had not been surveyed previously because our protocol would be used at

other bridge sites along the Allegheny River where no surveys had been done.

Survey protocol

We wanted to determine species present, estimate population density, and estimate size structure to indicate recent recruitment. Effects of construction on mussels, which include mortality, displacement, and interference with growth or reproduction, can stem *directly* from the construction action or occur *indirectly*. An example of a direct effect would be burial under a causeway, and an example of an indirect effect would be change in habitat upstream of a causeway as a result of pooling. High mortality, displacement, or interference with growth or reproduction are certain as a result of direct effects, but their likelihood as a result of indirect

effects are uncertain and difficult to quantify. We partitioned the site into areas of direct and indirect effects and determined species present throughout the site. However, we estimated abundance and size distribution only for the area of direct effects to assess certain loss from construction.

We surveyed the site on 12 to 15 July 1999 when river flow was low and water clarity was high. High proportions of some mussels are at the substrate surface during summer (Amyot and Downing 1991, Balfour and Smock 1995).

There were 3 steps in the survey protocol: 1) delineation of areas of direct and indirect effects, 2) qualitative sampling in areas of direct and indirect effects, and 3) quantitative sampling in the area of direct effects.

Delineation of areas of direct and indirect effects

Road and bridge construction may alter the physical environment (e.g., by deposition of construction materials, scouring and deposition of river substrate, changes in flow, erosion of river bank, and increased turbidity), the chemical environment (e.g., by runoff carrying petroleum products), and animal behavior (e.g., by displacement of fish that host glochidia after habitat loss) (Trombulak and Frissell 1999). Severity and spatial extent of the effects will depend on construction practices, timing of construction activities, river flows, composition of substrate, and effectiveness of erosion controls.

The area of direct effects was where mussels were likely to die or be displaced during or shortly after construction activities. Preliminary engineering plans involve constructing a causeway, and dropping the existing bridge into the river and partly on the causeway before removal. Bottom width of the causeway will depend on its surface elevation and top width. However, the proposed bottom width of a causeway at a similar bridge replacement project (Kennerdell, Pennsylvania, ~82 km downstream from West Hickory) was as wide as 41 m. The project at Kennerdell is also on the Allegheny River and similar to the West Hickory bridge replacement in size and proposed construction methods. The exact location and area of disturbance of the causeway at West Hickory will depend on the final bridge design, but the causeway will likely be offset from the centerline of the existing bridge. Thus, we added a buffer and judged the

direct-effects area to be in the immediate vicinity of the disturbance (i.e., below existing and proposed bridges and causeways) extending 50 m upstream and 50 m downstream of the centerline of the existing bridge (Fig. 1).

The indirect-effects area was limited to likely scouring, sedimentation, and pooling from construction-related changes in river flow, and was 50 to 100 m upstream of the bridge and 50 to 200 m downstream of the bridge (Fig. 1), based on the hydrologic and hydraulic analysis for bridge replacement at Kennerdell (Parsons, Brinckerhoff, Quade, and Douglas, Inc. 1997). The report predicted that flow velocity would return to preconstruction levels across most of the river channel within 200 m downstream and 100 m upstream of the bridge. Thus, we determined that adverse effects at West Hickory would be contained within a similar 300 m stretch. The Allegheny River at West Hickory is, on average, 187.5 m wide so the study area was 56,250 m² (direct effects: 18,600 m², indirect effects: 37,650 m²).

The exact impacts from bridge replacement, especially in the indirect-effects area, cannot be known prior to construction, so we do not know whether our reasoning led us to adequately encompass the spatial extent of the impacts. Only through follow-up monitoring at multiple sites can the spatial and temporal scales of the impacts be measured and the protocol be refined.

Qualitative sampling in areas of direct and indirect effects

Qualitative sampling included a search for piles of shell material (i.e., shell middens) discarded by mussel predators and a timed search for live mussels. Both banks were searched for middens along the entire study area and near the bases of the bridge piers, and locations of middens were mapped. To determine relative abundance and species composition in the middens, we identified species and counted valve pairs.

We divided the survey area into smaller areas or *cells* to do the timed search (Fig. 1). We defined *effective sampling fraction* as the % of a cell that is searched thoroughly, and based cell dimension on this fraction. Effective sampling fraction can be calculated by:

$$\frac{\text{effective search rate} \left(\frac{\text{m}^2}{\text{min}} \right) \times \text{search time (min)}}{\text{cell area (m}^2\text{)}}$$

where *effective search rate* is the area that can be searched thoroughly per min, and *search time* is the sum of time searched by all observers in a cell. We assumed an effective search rate of 0.5 m²/min and selected a cell area of 2500 m² (cell dimensions = 50 m X 50 m) and a search time of 240 min/cell (e.g., 6 observers searching for 40 min each or 4 observers searching for 60 min each). This combination resulted in an effective sampling fraction of ~0.05. Effective sampling fraction can be used as a basis for standardizing and comparing qualitative searches. We established a marked grid in the river of 24 cells (4 columns and 6 rows) where 18 cells were 50 m wide and 6 cells (the 4th column) were of varying widths (Fig. 1). We typically deployed 3 teams of 4 observers allowing 3 cells to be surveyed simultaneously. Thus, each of the 4 observers spent a minimum of 60 min covering 1/4 of the cell. Search times were prorated for cells that were <50 X 50 m.

We snorkeled in wadeable water (<1.0–5 m deep) and used SCUBA in depths >1.0 to 1.5 m, depending on turbidity and river flow. We snorkeled beginning at the downstream end of the cell to avoid disturbing sediment and reducing visibility. With SCUBA, we began at the upstream end of the cell to minimize exertion and air usage. We assumed equal search efficiency for divers and snorkelers. However, if there was a known difference in effective search rate then search times could be adjusted so that effective sampling fractions would remain equal for dived and snorkeled cells. Observers recorded location, cell dimensions, species counts, and actual search time for each cell. Each observer fanned away fine sediment, removed loose, non-embedded material, and raked loose sediment with fingertips in an effort to detect mussels.

Quantitative sampling of the area of direct effects

We quantitatively sampled to estimate abundance and to assess uncertainty in that estimate. There are many statistically valid sampling designs from which to choose (Thompson 1992, Dorazio 1999), but we used a double sampling design with 0.25-m² quadrats, systematically placed with multiple random starts, and exca-

vation of a random subset of the quadrats (Smith et al. 2001). Systematic sampling is efficient for clustered and rare populations, provides good spatial coverage, and is easy to implement in field sampling (Murthy and Rao 1988, Thompson 1992, Christman 2000). Multiple random starts are important for variance estimation (Hedayat and Sinha 1991). Double sampling increases precision of density estimates for reduced cost by using total counts from a random subset of quadrats to calibrate surface counts from all quadrats.

Not all mussels can be observed on the substrate surface (Miller and Payne 1988, Amyot and Downing 1991, Balfour and Smock 1995, Smith et al. 2001), so we included excavation in the sampling protocol. Some excavation is required to eliminate observation bias, but it is usually inefficient to excavate all quadrats in a sample unless a low % (<40%) of mussels are detectable at the substrate surface (Smith et al. 2001). Use of a double sampling design reduces the amount of excavation, and therefore cost, required to achieve precise estimates (Smith et al. 2001). The 1st phase in the double sampling design includes a large sample (at least 100 in mid-Atlantic and northeastern US rivers: see below) of 0.25-m² quadrats within which only mussels on the substrate surface are counted. A representative subsample is selected from the 1st sample of quadrats for excavation; the size of the subsample depends on the expected proportion of mussels on the substrate surface. The excavated quadrats provide paired surface and total (= count below the surface + count at the surface) counts that are used to calibrate the surface counts for the entire sample. Calibration of the surface counts is done through a regression estimator, which is appropriate provided that the relation between surface and total counts is approximately linear (Hedayat and Sinha 1991). The linearity assumption can be examined with scatterplots. Formulae for estimating density and abundance for the recommended sampling design are presented in the Appendix.

For a double sampling survey with fixed total cost, Smith et al. (2001) found that the proportion of excavated quadrats that minimized variance of the density estimate depended on the expected % of mussels at the substrate surface. This relationship led to the following guidelines for determining the proportion of quadrats to excavate in the double sampling design (Smith

et al. 2001): if >60% of the mussels are likely to be detected at the surface then excavation of 25% of the quadrats will minimize variance; 50 to 60% = 33% of the quadrats, 40 to 50% = 50% of quadrats, and <40% = 100% of quadrats. We observed 50% of *P. clava* and 66% of *E. t. rangiana* at the substrate surface in August 1997 at Kennerdell, so we excavated 33% or every 3rd quadrat.

We placed quadrats in the site systematically after 3 random starts (Fig. 1) resulting in 3 systematic samples. Each systematic sample began at a randomly chosen location in the corner of the site (i.e., a random start) followed by a series of locations at equally spaced intervals. One concern with systematic sampling involves the possibility of finding the same number of mussels in all systematic samples. This event causes a variance estimate equal to 0, in which case we recommend an approximate variance formula (Appendix). Three random starts are small enough that implementing the systematic sample is still relatively easy, but large enough that finding equal numbers in all systematic samples is rare.

We selected intervals between systematically placed quadrats in the *across river* (d_1) and *up river* (d_2) directions. For each random start, we generated a pair of random numbers: from 0 to d_1 and from 0 to d_2 , which defined the starting location of the 3 systematic samples. We then placed quadrats at the preset intervals. This design is called an aligned systematic sample with multiple random starts (Bellhouse 1988).

Intervals between systematically placed quadrats depend on the size of the direct-effects area, sample size, quadrat size, and number of random starts. To find equal intervals, we used the following algorithm: let n' be sample size and $n'_i = n'/k$, where $i = 1, \dots, k$ is the number of quadrats in each of the k systematic samples (in our survey k was 3). Intervals are determined by $d = \sqrt{L \cdot W / (a \cdot n'_i)}$, where L and W are the length and width of the study site (m), and a is the quadrat area (m²). (Intervals will often need to be rounded.) The units for the interval d are quadrats; however, to calculate the interval directly in meters, use $d' = \sqrt{L \cdot W / n'_i}$. We anticipated the affected area to be 20,000 m² (100 m by 200 m), set sample size at 600 quadrats, and chose 3 random starts. Thus, there were 200 quadrats in each of the 3 systematic samples, and we separated quadrats by 10 m (or 20 quad-

rats) in the across-river and upstream-downstream directions.

Sample size is the total number of quadrats, both surface and excavated. In general, sample size depends on mussel density; the lower the density, the higher the sample size needed to achieve the desired precision. Because of the negligible effect of the finite population correction in mussel surveys, the study site area is not an important determinant of sample size, at least if the study site area is ≥ 500 m² and the sampling fraction is ≤ 0.35 (Smith et al. 2001). (Sampling fraction is the ratio of sample size to population size, i.e., number of quadrats sampled to total number of quadrats possible in the study site.) We considered 3 criteria to determine sample size: coefficient of variation (CV), margin of error/1000 m² (MOE), and probability of encountering a species given it is present at the site ($1 - \beta$). Margin of error is 2 SE for estimates of abundance/1000 m². (Margin of error is used commonly in reporting results of opinion polls, and we used it as a planning device, but do not recommend its use as a confidence interval [CI] width.) Formulae to calculate CV, MOE, and CI are presented in the Appendix. To calculate β , we used results of Green and Young (1993), which were adapted to the double sampling design (Appendix). Sample size calculations require prior knowledge of variances, which may be available from pilot surveys or surveys at similar sites. However, this limitation caused Smith et al. (2001) to fit a regression relationship between CV and density using available data, and we made use of this approximate relationship to guide sample size at West Hickory.

We determined sample size for *P. clava* because we anticipated it was the species of interest with the lower density and, therefore, the more difficult for which to estimate abundance. We found *P. clava* at a density ~ 0.10 /m² in the Allegheny River at Kennerdell. Thus, based on the relationship between CV and density (Smith et al. 2001), we predicted a sample size of 600 would result in CV $\cong 0.37$, MOE $\cong 75$, and $1 - \beta \cong 0.96$.

We recorded surface and buried mussels separately. We removed the surface animals, excavated quadrats to 10 cm or to hardpan, and sifted substrate through a 6.35-mm-mesh screen. Smaller mussels were not captured in our sampling, and are thus not included in our popu-

TABLE 1. Total density and abundance of mussels within the area of direct impact at the West Hickory bridge site on the Allegheny River, July 1999.

Species	Relative abundance (%)	Density (no./m ²)	SE	95% CI	Abundance (no./18,600 m ²)	SE	95% CI
All		2.810	0.2261	2.400–3.290	52,266	4204.63	44,642–61,192
<i>Actinonaias ligamentina</i>	28.72	0.807	0.1101	0.618–1.055	15,019	2048.06	11,497–19,621
<i>Alasmidonta marginata</i>	0.25	0.007	0.0124	0.0002–0.218	132	231.12	4–4054
<i>Elliptio dilatata</i>	29.25	0.822	0.1306	0.603–1.123	15,300	2428.87	11,209–20,885
<i>Epioblasma torulosa rangiana</i>	17.54	0.493	0.0942	0.339–0.717	9173	1751.43	6309–13,336
<i>Fusconaia subrotunda</i>	0.25	0.007	0.0124	0.0002–0.218	132	231.12	4–4054
<i>Lampsilis cardium</i>	0.78	0.022	0.0218	0.003–0.155	407	406.06	57–2879
<i>L. fasciola</i>	0.75	0.021	0.0123	0.007–0.066	397	228.43	128–1226
<i>L. siliquoidea</i>	0.78	0.022	0.0218	0.003–0.155	407	406.06	57–2879
<i>Lasmigona costata</i>	0.75	0.021	0.0124	0.007–0.067	397	231.12	127–1243
<i>Ligumia recta</i>	1.57	0.044	0.0308	0.011–0.174	813	572.67	204–3233
<i>Pleurobema clava</i>	13.42	0.377	0.0869	0.240–0.592	7010	1615.66	4462–11,013
<i>P. sintoxia</i>	2.06	0.058	0.0332	0.019–0.178	1079	617.58	352–3313
<i>Ptychobranchus fasciolaris</i>	1.53	0.043	0.0214	0.016–0.114	794	398.10	297–2121
<i>Strophitus undulatus</i>	2.35	0.066	0.0376	0.021–0.202	1220	699.44	396–3753

lation estimates. All mussels were replaced in the substrate.

Data analysis

We estimated density and abundance using a regression estimator (Appendix). We used kriging, a statistical technique for spatial prediction, to map the distribution of mussels at the surface based on the sample of quadrats (Thompson 1992). We used GS⁺™ version 3.1 (Gamma Design Software, Plainwell, Michigan) to generate the spatial predictions and ArcView® GIS version 3.1 (Environmental Systems Research Institute Inc., Redlands, California) to map the predictions generated through kriging.

Results

Qualitative sampling involved 12 biologists searching ~100 person h over 1.5 d. Sixteen biologists (12 observers and 4 data recorders) participated in the quantitative sampling over 2 d. Approximately 80% of sampling was conducted while snorkeling and 20% while SCUBA diving.

Qualitative sampling in areas of direct and indirect effects

Recent shell material was found in 11 mid-dens, which included 1392 shells from 15 spe-

cies. We found 17 species during the timed search of the direct- and indirect-effects areas; live mussels occurred in all 24 cells. The dominant species were *Actinonaias ligamentina* and *Elliptio dilatata*. *Epioblasma t. rangiana* and *P. clava* were encountered frequently. The remaining species were present in lower numbers. *Epioblasma t. rangiana* was present in all 24 cells. *Pleurobema clava* was present in 22 cells and was not detected in cells that were 50 to 100 m from the right bank and within 50 m of the bridge. In general, few individuals and species were found 50 to 100 m from the right bank in the deep, fast current (Fig. 1). We found *Fusconaia subrotunda* only in the river channel upstream of the bridge.

Quantitative sampling of the area of direct effects

We sampled 562 quadrats and excavated 183 of those in the direct-effects area. Estimates of total density, including surface and buried mussels are shown in Table 1. The CVs were 19% for *E. t. rangiana* and 23% for *P. clava*.

We found a wide range of sizes, including some small individuals, which indicated that the 2 federally endangered species reproduced recently at the study site (Fig. 2). *Epioblasma t. rangiana* ranged from 12.5 mm to 67.9 mm, and 25% were \leq 28.3 mm. The smallest sexually di-

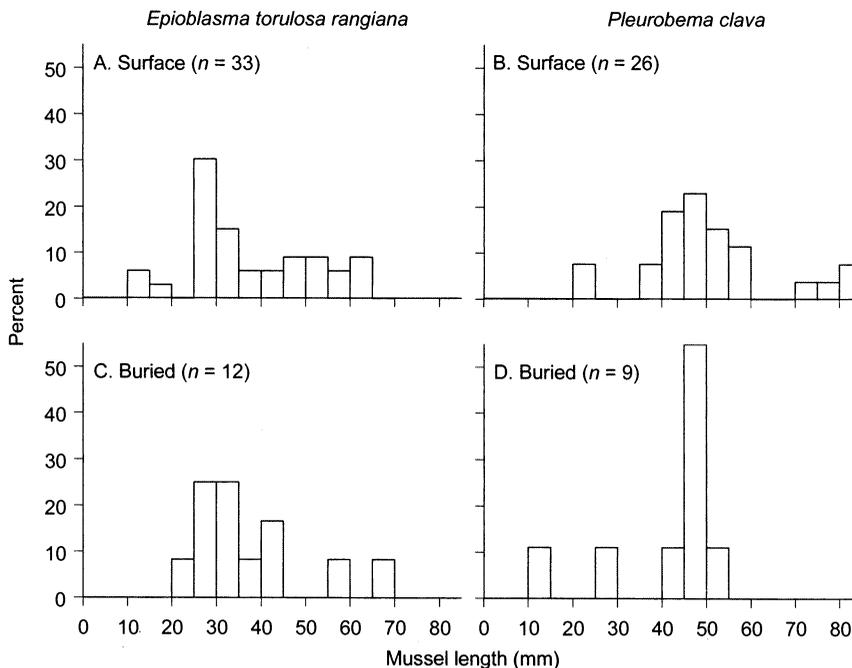


FIG. 2. Size distributions for surface (A) and buried (C) *Epioblasma torulosa rangiana* and surface (B) and buried (D) *Pleurobema clava* in the Allegheny River at West Hickory, Pennsylvania, July 1999. Size distributions are shown separately for those observed at the substrate surface and by excavation of substrate to 10 cm. Numbers of mussels are shown in parentheses; those at the substrate surface include mussels found in quadrats that were not excavated.

morphic *E. t. rangiana* was 28.2 mm, so we classified *E. t. rangiana* <28 mm as juvenile or indeterminate sex. *Pleurobema clava*, which is not sexually dimorphic, ranged from 13.2 mm to 81.9 mm, and 25% were ≤ 42.6 mm.

Spatial distributions of the 2 federally endangered species at the substrate surface within the area of direct effects are predicted in Fig. 3. The distributions show spatial clustering and incomplete overlap between species. We thought the excavated quadrats alone did not comprise a sufficient sample for spatial prediction.

Detectability of mussels

Detection of mussels at the surface varied across species and was low for some species. For example, only 31% of *P. clava* and 52% of *E. t. rangiana* were detected at the surface. In contrast, >70% of *A. ligamentina* was observed at the surface. In addition to species differences, detection at the surface was sex-specific for *E. t. rangiana*: 80% of females, but only 45% of males were detected at the surface.

Species detectability differed among observation methods and caused bias in relative abundances (Table 2). Ranked abundance varied less than relative abundance. The 2 federally listed species were 3rd, 4th, or 5th most abundant and *A. ligamentina* and *E. dilatata* were usually 1st or 2nd. *Epioblasma t. rangiana* was overrepresented in the middens (ranked 2nd). The ranked abundances of several other species changed dramatically among observation methods. Because it was relatively difficult to detect, *Strophitus undulatus*, which tied for 10th based on surface counts and had a similar ranking in the midden search, was actually the 5th most abundant species. Because of its high detectability, *Ligumia recta* was 3rd based on the timed search, but was actually the 7th most abundant species.

Estimated sex ratio for *E. t. rangiana* depended on observation method because adult females were more likely to be on the surface and more visible. Based on timed searches, 60% of the *E. t. rangiana* population was adult female, the rest being adult male or juvenile. However, based on surface counts using quadrats, 30% were adult

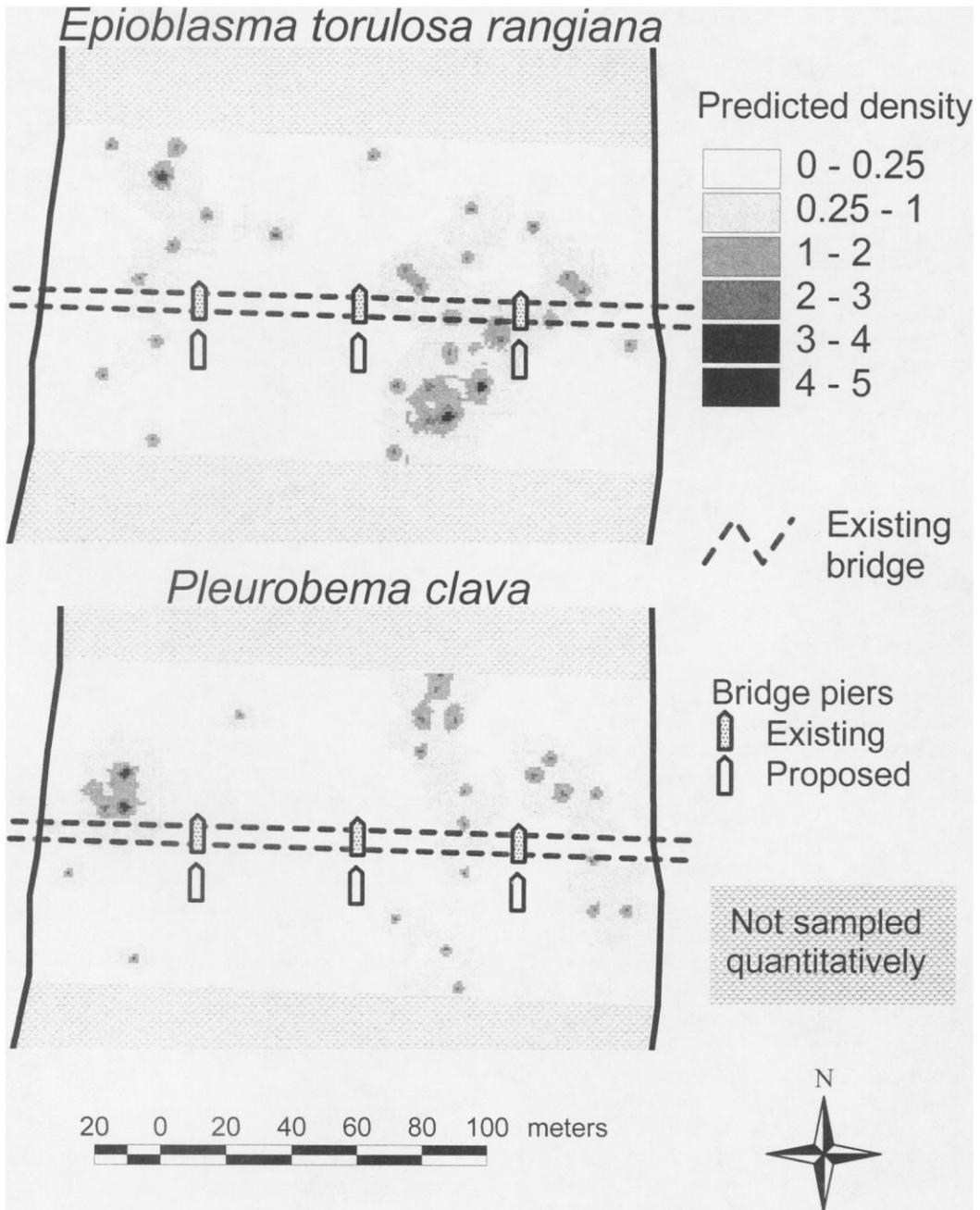


FIG. 3. Predicted spatial distribution of *Epioblasma torulosa rangiana* and *Pleurobema clava* at the substrate surface in the Allegheny River at West Hickory, Pennsylvania, July 1999. Density (no./m²) was predicted using kriging from a systematic sample of 562 0.25-m² quadrats over an area of 18,600 m².

TABLE 2. Relative and ranked abundance based on excavated quadrats, surface counts in quadrats, timed searches, and a midden search that were conducted within 50 m of the existing bridge at West Hickory on the Allegheny River, July 1999. – = species not detected.

Species	Relative abundance (%)				Ranked abundance			
	Excavated quadrats	Surface counts	Timed searches	Midden search	Excavated quadrats	Surface counts	Timed searches	Midden search
<i>Actinonaias ligamentina</i>	28.72	35.16	54.56	11.21	2	1	1	4
<i>Alasmidonta marginata</i>	0.25	0.46	0.04	0.07	13.5	12.5	16	15
<i>Amblema plicata</i>	–	–	0.01	–	–	–	17	–
<i>Elliptio dilatata</i>	29.25	26.03	21.62	35.56	1	2	2	1
<i>Epioblasma torulosa rangiana</i>	17.54	15.07	5.21	23.92	3	3	4	2
<i>Fusconaia subrotunda</i>	0.25	0.46	0.12	0.22	13.5	12.5	14	13
<i>Lampsilis cardium</i>	0.78	0.91	0.61	0.29	9.5	10	11	11.5
<i>L. fasciola</i>	0.75	1.37	0.57	0.93	11.5	7.5	12	7
<i>L. ovata</i>	–	–	0.08	0.36	–	–	15	9.5
<i>L. siliquoidea</i>	0.78	–	1.83	0.14	9.5	–	7	14
<i>Lasmigona costata</i>	0.75	1.37	0.65	0.29	11.5	7.5	9.5	11.5
<i>Ligumia recta</i>	1.57	2.74	6.39	0.79	7	5.5	3	8
<i>Pleurobema clava</i>	13.42	11.87	4.32	18.46	4	4	5	3
<i>P. sintoxia</i>	2.06	0.91	0.65	2.23	6	10	9.5	5
<i>Ptychobranchus fasciolaris</i>	1.53	2.74	2.24	1.36	8	5.5	6	6
<i>Strophitus undulatus</i>	2.35	0.91	0.94	0.36	5	10	8	9.5
<i>Villosa fabilis</i>	–	–	0.16	–	–	–	13	–

females, and based on surface and buried mussels in excavated quadrats, 20% of the *E. t. rangiana* population was adult female.

Sample size for future surveys

We calculated sample sizes using variance observed for *E. t. rangiana* and *P. clava* at 9 sites on the Allegheny River and French Creek where we implemented the double sampling design. Density at these sites, which included West Hickory, ranged from 0.08 to 1.5/m²; 75% of the species–site combinations were <1.1/m² with a median of 0.64/m². The relationship between CV and density given sample size was strong, negative, and linear for density on a transformed scale of 1/√density ($R^2 = 0.86$). We used this relationship to calculate CV and MOE (Table 3); these values can be used for planning future surveys of *E. t. rangiana* and *P. clava* in the Allegheny River drainage. For example, if a species of interest was expected to occur at 0.10/m², then a sample size of 500 would assure a CV = 0.41, MOE = 83/1000 m², and a 93% chance that at least 1 individual would be detected.

Discussion

It is US public policy that biological assessments “should always use the best available scientific and commercial data to make findings regarding . . . effects of a proposed action on the species or critical habitat” (US Fish and Wildlife Service and National Marine Fisheries Service 1998:xxi). This statement also applies to freshwater mussels, so statistically valid site-specific surveys are needed to correctly determine potential impacts on federally listed species.

Survey protocol for freshwater mussels

We combined qualitative and quantitative sampling approaches in our protocol because neither method alone was sufficient to meet all objectives. The timed search is generally efficient (less costly) at detecting the presence of rare species (Miller and Payne 1993, Strayer et al. 1997, Vaughn et al. 1997). Timed searches, however, are inappropriate for determining relative abundance of species and may actually provide misleading information by overestimating the abundance of some species and under-

TABLE 3. Calculations of sample size based on a double sampling design and data from surveys of *Epioblasma torulosa rangiana* and *Pleurobema clava* at 9 sites in the Allegheny River. Coefficient of variation (CV), margin of error/1000 m² (MOE), and probability of encountering an individual (1 - β) vary by density (no./m²) and number of quadrats sampled. MOE is 2*SE for the estimate of no./1000 m².

Den- sity	Sample size (no. of quadrats)																	
	200			300			400			500			600			700		
	CV	MOE	1 - β	CV	MOE	1 - β	CV	MOE	1 - β	CV	MOE	1 - β	CV	MOE	1 - β	CV	MOE	1 - β
0.05	0.92	92	0.46	0.75	75	0.58	0.65	65	0.68	0.58	58	0.75	0.53	53	0.80	0.49	49	0.85
0.10	0.65	131	0.68	0.53	106	0.80	0.46	92	0.88	0.41	83	0.93	0.37	75	0.96	0.35	70	0.97
0.15	0.53	160	0.80	0.43	129	0.91	0.38	113	0.96	0.34	101	0.98	0.31	93	0.99	0.29	86	0.99
0.20	0.46	186	0.88	0.37	149	0.96	0.33	131	0.98	0.29	117	0.99	0.27	107	0.99	0.25	99	0.99
0.25	0.42	207	0.93	0.33	167	0.98	0.29	147	0.99	0.26	132	0.99	0.24	120	0.99	0.22	111	0.99
0.30	0.38	228	0.96	0.31	183	0.99	0.27	161	0.99	0.24	144	0.99	0.22	132	0.99	0.20	122	0.99
0.40	0.33	264	0.98	0.26	211	0.99	0.23	186	0.99	0.21	167	0.99	0.19	152	0.99	0.18	141	0.99
0.50	0.30	296	0.99	0.24	236	0.99	0.21	209	0.99	0.19	187	0.99	0.17	171	0.99	0.16	158	0.99
0.60	0.27	325	0.99	0.22	259	0.99	0.19	230	0.99	0.17	206	0.99	0.16	188	0.99	0.14	174	0.99

estimating the abundance of others (Miller and Payne 1993, Vaughn et al. 1997, Smith et al. 2001). The combination of surface counts and excavation in the double sampling design allows increased spatial coverage while estimating mussel densities free from the biases of detectability, which affect qualitative methods. The regression estimator used with the double sampling design is based on an approximate linear relationship between surface and total counts, and this assumption should be verified during analysis. The double sampling design balances the cost and benefits of excavation, but does add complexity to data analysis (Appendix). We believe the complexity of analysis is more than compensated for by greater spatial coverage for fixed cost compared to a survey with 100% excavation. However, if all quadrats are excavated, then the design becomes a straightforward systematic sampling design, the simplicity of which may be preferable.

Our decision to use 0.25-m² quadrats as the sampling unit was guided by sampling efficiency. We considered the best sampling unit as one that results in the most reliable (least variable) estimate of population density or abundance. It is clear from the literature that for clustered populations, such as freshwater mussels (Downing and Downing 1992), the smaller the sampling unit the more reliable the estimate of population size or density (see Elliott 1977 and citations therein). However, there is a limit to this recommendation; a unit can be so small that errors in deciding whether an organism is inside the unit can exceed reductions in sampling variance. In our opinion, 0.25-m² quadrats are small enough to benefit from the reduced variance, but not so small that boundary errors dominate.

Our protocol can be adapted to meet specific conditions. Logistics, especially, will be determined on a case-by-case basis. For example, boundaries of the site will depend on construction methods, and how boundaries are marked will depend on the configuration of the site. Some may find our quantitative sampling protocol too complicated or costly; however, we stress that reliable and credible estimates of abundance of rare mussels require a substantial effort. Given that the survey at West Hickory took 3.5 d to complete, we believe the protocol is practical and provides a useful framework and starting point.

Stratification would improve the protocol in

certain cases. For example, cells could be stratified into high- and low-density strata, depending on results from the timed search, and sampling effort could be allocated directly proportional to variance. Alternatively, cells could be stratified into wadeable and deep-water strata, and sampling effort could be allocated inversely proportional to cost to account for the higher cost of SCUBA diving. Formulae to estimate density or abundance must be adjusted accordingly when stratification or other complexity is incorporated into the protocol.

The regression relationship between density and CV reported by Smith et al. (2001) is based on the double sampling design and surveys at 14 sites of multispecies assemblages, with little data from sites with densities <1/m². Nevertheless, the predictions of CV from this relationship were within a couple percentage points of the predictions of CV using data exclusively from *E. t. rangiana* and *P. clava* where most densities were <1/m². The closeness of these 2 independent sets of predictions gives us some confidence in recommending the use of the sample size calculations in Table 3 to determine sample size for species other than *E. t. rangiana* and *P. clava*.

Protocol application and interpretation of results

Application of our protocol in the Allegheny River at the West Hickory bridge site revealed an extensive freshwater mussel bed, which included the presence of 2 federally listed species. The abundance of *P. clava* relative to its known distribution and abundance (US Fish and Wildlife Service 1994) made West Hickory a significant site for this species. The predicted spatial distribution of mussels within the area of direct effects showed spatially clustered populations, which is typical for freshwater mussel populations (Kovalak et al. 1986, Downing and Downing 1992). If mussels need to be relocated from the direct-effects area, managers can use the predicted spatial distribution to plan the extent of and allocate effort for relocation.

Reliable and credible site-specific population estimates are just the beginning of an assessment. There remains the problem of interpreting site-specific effects in the context of river-wide (or range-wide) population viability. Suppose that all of the mussels in the direct-effects area die as a result of bridge construction. In that

case, we predict that 9173 (95% CI: 6309–13,336) *E. t. rangiana* and 7010 (95% CI: 4462–11,013) *P. clava* will be lost. How will that amount of mortality affect the viability of populations in the Allegheny River and of the species throughout their ranges? The Allegheny River is presumed to support a sparse and discontinuous distribution of *P. clava* and a more uniform distribution of *E. t. rangiana*; these 2 species persist in few other river systems (US Fish and Wildlife Service 1994). Most of the Allegheny River has not been disturbed by bridge construction and should support comparable mussel populations to those at the few bridge sites where quantitative surveys have been conducted. However, the necessary quantitative surveys have not been done throughout the Allegheny River to support this conclusion/assumption. In the face of this uncertainty, the precautionary principle (Buhl-Mortensen and Welin 1998) requires that potentially damaging impacts be avoided to significant populations of *P. clava* and *E. t. rangiana* in the Allegheny River. Ultimately, the issue returns to availability of best scientific and commercial data, or rather the lack of such information.

Acknowledgements

Funding for this project was provided by Pennsylvania Department of Transportation and US Geological Survey Leetown Science Center through Memorandum of Agreement 440093. We thank Gary Ayers, Glen Black, Marc Blouin, Jeff Cole, Angela Dunn, Scott Fincham, Greg Kennedy, Jennifer Lambert-Newman, Bill Lellis, Meade McCoy, Glenn Nelson, John Swift, Dave Weller, and Priscilla Young for help collecting data. Marc Blouin advised on SCUBA diving practices and mussel sampling. The manuscript was improved by helpful comments from Susi von Oettingen, Bill Lellis, Tom Proch, Bob Anderson, Heidi Dunn, David Strayer, David Rosenberg, Bob Dorazio, and an anonymous referee.

Literature Cited

- AMYOT, J.-P., AND J. A. DOWNING. 1991. Endo- and epibenthic distribution of the unionid mollusc *Elliptio complanata*. *Journal of the North American Benthological Society* 10:280–285.
- AQUATIC SYSTEMS. 1998. Mussel survey report: West Hickory bridge site. Prepared for Pennsylvania Department of Transportation District 1–0. McLaren/Hart, Inc. Pittsburgh, Pennsylvania. (Available from: PennDOT District 1–0, 255 Elm Street, Oil City, Pennsylvania 16301 USA.)
- BALFOUR, D. L., AND L. A. SMOCK. 1995. Distribution, age structure, and movements of the freshwater mussel *Elliptio complanata* (Mollusca:Unionidae) in a headwater stream. *Journal of Freshwater Ecology* 10:255–268.
- BELLHOUSE, D. R. 1988. Systematic sampling. Pages 125–145 in P. R. Krishnaiah and C. R. Rao (editors). *Handbook of statistics*, Vol. 6. Elsevier Science Publishers, Amsterdam, The Netherlands.
- BUHL-MORTENSEN, L., AND S. WELIN. 1998. The ethics of doing policy relevant science: the precautionary principle and the significance of non-significant results. *Science and Engineering Ethics* 4:401–412.
- CHRISTMAN, M. C. 2000. A review of quadrat-based sampling of rare, geographically clustered populations. *Journal of Agricultural, Biological, and Environmental Statistics* 5:168–201.
- DORAZIO, R. M. 1999. Design-based and model-based inference in surveys of freshwater mollusks. *Journal of the North American Benthological Society* 18:118–131.
- DOWNING, J. A., AND W. L. DOWNING. 1992. Spatial aggregation, precision, and power in surveys of freshwater mussel populations. *Canadian Journal of Fisheries and Aquatic Sciences* 49:985–991.
- ELLIOTT, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Scientific Publication No. 25. Freshwater Biological Association, Cumbria, UK.
- GREEN, R. H., AND R. C. YOUNG. 1993. Sampling to detect rare species. *Ecological Applications* 3:351–356.
- HEDAYAT, A. S., AND B. K. SINHA. 1991. Design and inference in finite population sampling. John Wiley and Sons, New York.
- KOVALAK, W. P., S. D. DENNIS, AND J. M. BATES. 1986. Sampling effort required to find rare species of freshwater mussels. Pages 34–45 in B. G. Isom (editor). *Rationale for sampling and interpretation of ecological data in the assessment of freshwater ecosystems*. ASTM STP 84. American Society for Testing and Materials, Philadelphia.
- MILLER, A. C., AND B. S. PAYNE. 1988. The need for quantitative sampling to characterize size demography and density of freshwater mussel communities. *American Malacological Bulletin* 6:49–54.
- MILLER, A. C., AND B. S. PAYNE. 1993. Qualitative versus quantitative sampling to evaluate population and community characteristics at a large-river mussel bed. *American Midland Naturalist* 130:133–145.
- MURTHY, M. N., AND T. J. RAO. 1988. Systematic sampling with illustrative examples. Pages 147–185 in

P. R. Krishnaiah and C. R. Rao (editors). Handbook of statistics, Vol. 6. Elsevier Science Publishers, Amsterdam, The Netherlands.

Parsons, Brinckerhoff, Quade, and Douglas, Inc. 1997. Preliminary hydrologic and hydraulic analysis report: replacement of the Kennerdell bridge over the Allegheny River (S.R. 3008, section B00). Prepared for Pennsylvania Department of Transportation District 1-0. Parsons, Brinckerhoff, Quade, and Douglas, Inc., Baltimore, Maryland. (Available from: PennDOT District 1-0, 255 Elm Street, Oil City, Pennsylvania 16301 USA.)

SMITH, D. R., R. F. VILLELLA, D. P. LEMARIE, AND S. VON OETTINGEN. 2001. How much excavation is needed to monitor freshwater mussels? In P.D. Johnson and R. S. Butler (editors). Proceedings of the first symposium of the Freshwater Mollusk Conservation Society. Ohio Biological Survey, Columbus, Ohio (in press).

STRAYER, D. L., S. CLAYPOOL, AND S. J. SPRAGUE. 1997. Assessing Unionid populations with quadrats and timed searches. Pages 163-169 in K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and management of freshwater mussels II: initiatives for the future. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

THOMPSON, S. K. 1992. Sampling. John Wiley and Sons, New York.

TROMBULAK, S. C., AND C. A. FRISSELL. 1999. Review of ecological effects of roads on terrestrial and aquatic communities. Conservation Biology 14:18-30.

US Fish and Wildlife Service. 1994. Clubshell (*Pleurobema clava*) and northern riffleshell (*Epioblasma torulosa rangiana*) recovery plan. US Fish and Wildlife Service, Hadley, Massachusetts. (Available from: US Fish and Wildlife Service Regional Office, 300 Westgate Center Drive, Hadley, Massachusetts 01035-9589 USA.)

US Fish and Wildlife Service and National Marine Fisheries Service. 1998. Endangered species consultation handbook: procedures for conducting consultation and conference activities under Section 7 of the Endangered Species Act. US Government Printing Office, Washington, DC. (Available from: <http://endangered.fws.gov/consultations/s7hndbk/s7hndbk.htm> or GPO Main Bookstore, 701 N. Capital Street NW, Washington, DC 20401 USA.)

VAUGHN, C. V., C. M. TAYLOR, AND K. J. EBERHARD. 1997. A comparison of the effectiveness of timed searches vs quadrat sampling in mussel surveys. Pages 157-162 in K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and management of freshwater mussels II: initiatives for the future. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Appendix. Formulae for estimating density and calculating sample size.

Systematic sampling formulae

These formulae apply when estimating surface density or total density if all quadrats are excavated. The underlying sampling design is systematic sampling with multiple random starts. Let M denote the number of possible systematic samples in the site to be sampled, and let m denote the number of random starts:

$$M = \frac{A}{a} \frac{m}{\sum_{i=1}^m n_i}$$

where A is the area of the site, a is the area of the sampling unit (e.g., 0.25-m² quadrat), and n_i is the number of quadrats in the i^{th} systematic sample. Let x_i be the sum of the counts for all quadrats in the i^{th} systematic sample. An unbiased estimator of population abundance is:

$$\hat{T} = \frac{M}{m} \sum_{i=1}^m x_i.$$

An estimate of variance for \hat{T} is:

$$\widehat{\text{var}}(\hat{T}) = \frac{M(M - m)}{m} \frac{\sum_{i=1}^m (x_i - \bar{x})^2}{m - 1}$$

where $\bar{x} = (1/m) \sum_{i=1}^m x_i$. An estimate of density (no./m²) is calculated by $\hat{\mu} = \hat{T}/A$. An estimate of variance for $\hat{\mu}$ is $\widehat{\text{var}}(\hat{\mu}) = (1/A)^2 \widehat{\text{var}}(\hat{T})$.

It is sometimes possible that the survey results in $x_i > 0$ and equal for all m systematic samples. If so, the estimate of variance will equal 0, and we suggest estimating variance assuming that the $n = \sum_{i=1}^m n_i$ quadrats were selected by simple random sampling rather than by systematic sampling with multiple starts. This solution is conservative in the sense that variance for \hat{T} will tend to be overestimated (P.S. Pooler, US Geological Survey, Kearneysville, West Virginia, and D.R. Smith, unpublished data). The formulae for \hat{T} , $\hat{\mu}$, and $\widehat{\text{var}}(\hat{\mu})$ are unchanged. However, the estimate of variance for \hat{T} becomes:

$$\widehat{\text{var}}(\hat{T}_{\text{SRS}}) = \frac{N(N - n)}{n} \frac{\sum_{i=1}^m \sum_{j=1}^{n_i} (x_{ij} - \bar{x})^2}{n - 1}$$

where N is the number of possible quadrats in

the site (i.e., A/a), and x_{ij} is the surface count for the j^{th} quadrat in the i^{th} systematic sample.

Regression estimator for incorporating excavated quadrats in estimates of abundance

These formulae apply when estimating total density if a representative portion of the quadrats in the sample is excavated (Smith et al. 2001). The underlying sampling design is double sampling. A simple linear regression model is fit to the data from the excavated quadrats. In this regression, the total count is the response variable (y) and the surface count is the explanatory variable (x). Provided that the relationship between surface and total counts is approximately linear, the regression model can be used to calibrate surface counts and estimate density. This assumption was met for a variety of species at West Hickory and elsewhere (Smith et al. 2001); however, the assumption should be verified routinely during analysis. Formulae for estimating population density ($\hat{\mu}_{lr}$) using the regression estimator under the double sampling design are (Hedayat and Sinha 1991, Thompson 1992):

$$\hat{\mu}_{lr} = a^{-1}[\bar{y}_2 - \hat{\beta}_1(\bar{x}_2 - \bar{x}_1)]$$

with variance

$$\widehat{\text{var}}(\hat{\mu}_{lr}) = a^{-2} \left\{ \left(\frac{N - n'}{N} \right) \frac{s^2}{n'} + \left[\frac{n' - n}{n'n(n-2)} \right] \sum_{i=1}^n (y_i - \hat{\beta}_0 - \hat{\beta}_1 x_i)^2 \right\}$$

where a is the quadrat area, \bar{y}_2 is the mean total count from the excavated subsample, \bar{x}_1 and \bar{x}_2 are the mean surface counts from the 1st sample and excavated subsample, $\hat{\beta}_0$ and $\hat{\beta}_1$ are estimates of the regression parameters, s^2 is the variance of total counts in the excavated subsample, N is the total number of quadrats at a site (i.e., A/a), n' is the sample size of the 1st sample, and n is the number of excavated quadrats. The variance s^2 can be estimated from the systematic sample of excavated quadrats by (Thompson 1992):

$$s^2 = \frac{M(\bar{n}_i - 1)s_w^2 + (M - 1)\bar{n}_i s_b^2}{M\bar{n}_i - 1}$$

where M is the number of systematic samples in the population, n_i is the number of quadrats in the i^{th} systematic sample (\bar{n}_i is the mean of

the n_i), $s_w^2 = \sum_{i=1}^m \sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)^2 / [m(\bar{n}_i - 1)]$, $s_b^2 = \sum_{i=1}^m (x_i - \bar{x})^2 / (m - 1)$ and $\bar{x}_i = \sum_{j=1}^{n_i} x_{ij} / n_i$. An estimate of population abundance is simply $\hat{T}_{lr} = A \times \hat{\mu}_{lr}$ with an estimate of its variance $\widehat{\text{var}}(\hat{T}_{lr}) = A^2 \widehat{\text{var}}(\hat{\mu}_{lr})$.

Calculation of confidence intervals (CI)

Based on simulations of sampling mussel populations (P. S. Pooler and D. R. Smith, unpublished data), the sampling distributions for the estimators of population total and density are not normally distributed and tend to be skewed right. A simple logarithmic transformation of the estimates usually results in CIs with coverage close to nominal. For example, we calculated approximate 95% CIs for population abundance by:

$$\exp\left(\log(\hat{T}) \pm 1.96 \cdot \sqrt{\frac{\text{var}(\hat{T})}{\hat{T}^2}}\right).$$

Sample size calculations

By removing the finite population correction, the variance of density estimate (i.e., $\text{var}[\hat{\mu}_{lr}]$) under the double sampling design can be written:

$$\text{var}(\hat{\mu}_{lr}) \leq \left[\frac{(s^2 - s_{lr}^2)f_2 + s_{lr}^2}{n'a^2 f_2} \right]$$

where n' is the sample size or number of quadrats, s^2 is the variance of total counts among quadrats, f_2 is the fraction of the sample size that is excavated ($f_2 = n/n'$), s_{lr}^2 is the mean square error from the regression ($s_{lr}^2 = \sum_{i=1}^n (y_i - \hat{\beta}_0 - \hat{\beta}_1 x_i)^2 / (n - 2)$), and a is the quadrat area. We have found that the above inequality is very close to an equality at least for study site areas >500 m² and sampling fractions <0.35. Thus, we use this simpler, albeit approximate, variance formula to calculate sample size.

To achieve a desired CV, say CV_{or} , the sample size formula is:

$$n' = \left(\frac{1}{CV_{or}} \right)^2 \left[\frac{(s^2 - s_{lr}^2)f_2 + s_{lr}^2}{a^2 f_2} \right].$$

If the objective is to achieve a desired margin of error / 1000 m² (say MOE_0), the sample size formula is:

$$n' = \left(\frac{2000}{MOE_0} \right)^2 \left[\frac{(s^2 - s_{lr}^2)f_2 + s_{lr}^2}{a^2 f_2} \right].$$

Based on Green and Young (1993), if the objective is to control the probability of failing to detect a species in n' quadrats, the sample size formula is:

$$n' = \frac{-4 \ln(\beta_0)}{\mu[(1 - f_2)\lambda + f_2]}$$

where β_0 is the acceptable risk of not detecting the species and λ is the proportion of the species at the substrate surface.

Received: 24 May 2000

Accepted: 23 October 2000

How much excavation is needed to monitor freshwater mussels?

David R. Smith^{1,3}, Rita F. Vilella¹, David P. Lemarié¹, and Susanna von Oettingen²

¹ USGS Biological Resources Division, Leetown Science Center, Aquatic Ecology Laboratory, Kearneysville, WV 25430; ² US Fish and Wildlife Service, New England Field Office, 22 Bridge St., Concord, NH 03301

ABSTRACT: To examine variation in detecting freshwater mussels at the substrate surface, we counted mussels on and below the substrate surface by excavating 907 0.25 m² quadrats to a depth of 10 cm at wadeable sites in 14 streams in 9 states. Probability of detection on the surface was related to water depth, coverage of rooted vegetation, substrate type, and mussel species and length ($\chi^2 = 174.3$, $df=25$, $P<0.0001$). These factors interacted to cause large differences in the probability of detection among habitat types and species. As a result, when detection differed among sites for the same mussel species (e.g., percent at the surface of *Alasmidonta heterodon* was 22% at one site and 64% at another), surface density was an unreliable surrogate for comparing true density. When detection differed markedly among species within a site (e.g., at French Creek, 25% of *Villosa fabalis* was detected at the surface compared to 71% of *Actinonaias ligamentina*), surface counts did not accurately measure relative abundance. Our results indicate that some amount of excavation is necessary for rigorous comparison of density across sites, time, habitat, or taxa. We considered application of the double sampling design to weigh the costs and benefits of excavation and determine the proportion of quadrats to excavate that minimizes variance of population estimates for a fixed cost of sampling. We found that the optimal proportion to excavate depends on the percent of mussels detected at the substrate surface. If >60% are likely to be detected at the surface then excavation of 25% (or 1 of 4) of the quadrats will minimize variance. Similarly, 50 - 60% detection at the surface leads to excavating 33% of the quadrats; 40 - 50% detection at the surface leads to excavating 50%; and <40% detection at the surface leads to excavating 100%. The double sample design could be useful for monitoring low-density populations. For example, at a site where mussel density is 0.2 m⁻², sample size of 400 would result in an estimate with CV of 0.36 and power ≥ 0.80 to detect declines to 0.02 m⁻² over 5 y or 0.07 m⁻² over 10 y.

Keywords: freshwater mussels, population assessment, monitoring, sampling design, excavation, costs and benefits, precision, uncertainty, statistical power, sample size

Excavation is the process of removing and sifting through stream substrates to collect and count mussels, and it is one possible technique in a protocol for sampling freshwater mussel populations (Miller and Payne 1988). Biologists use excavation because not all mussels are detected at the substrate surface, and because detection at the surface can change across sites or with season. If detection changes from one site (or season) to another then comparisons based on counts of mussels at the surface will not provide an accurate comparison of population density.

Biologists have expressed differing opinions on the need for excavation in mussel surveys. Miller and Payne (1988) equate excavation with accurate or “quantitative” sampling, and use the term “semi-quantitative” for counting mussels by tactile searches of the substrate surface. They state that tactile searches underestimate densities of smaller individuals and should be limited to assessments of distribution or relative abundance. In contrast, Strayer and Ralley (1993) did not excavate in their study of habitat use. They based their analysis on visual searches for

mussels at the substrate surface, although they did lift non-embedded stones to find mussels.

Several recent studies specifically addressed distribution of mussels at and below the substrate surface (*i.e.*, vertical distribution). Amyot and Downing (1991) examined vertical distribution of *Elliptio complanata* (Rafinesque, 1820) in a sand-bottomed lake in Québec, Canada, and reported that the proportion of mussels found at the substrate surface varied seasonally. Percent at the surface in early summer (*i.e.*, >96%) and declined in the autumn months (*i.e.*, <40%). They also found that the percent below the substrate surface was related to mussel length with smaller mussels being more likely to be buried. Balfour and Smock (1995) studied populations of *E. complanata* in a sand-bottomed stream in Virginia, USA. Their results were qualitatively similar to those of Amyot and Downing (1991); they found significant seasonal and length-related variation in the proportion of mussels found at the substrate surface. Richardson and Yokley (1996) surveyed sites on the Apalachicola River, Florida, USA for evidence that *Amblyma neislerii* (Lea, 1858) or *Glebulula rotunda* (Lamarck, 1819) had experienced

³ For correspondence contact D.R. Smith
(Email: david_r_smith@usgs.gov)

recent recruitment. Previous surveyors of these sites applied only visual or tactile searches of the substrate surface and failed to find evidence of reproduction or recruitment. However, Richardson and Yokley (1996), who included excavation in their survey, found juveniles and concluded that excavation is necessary to assess recruitment.

Excavation, however, has its costs. Mussels and their habitat are disturbed when substrate is removed and sifted. Although we found no documentation in the literature, we hypothesized that excavation could cause an increase in mortality, especially for small, thin-shelled, or juvenile mussels. It is also possible that excavation interferes with reproduction, an effect that would most likely occur if the survey coincided with periods of reproductive activity.

Relative to surface counts excavation is time-consuming. The amount of time available to conduct a survey is always limited. More quadrats could be sampled (and more of the site covered) if excavation is not applied. Thus, more excavation means less spatial coverage. Because mussels tend to cluster, a sample with less spatial coverage results in a population estimate that is less precise especially at low population densities.

We considered the application of the double sampling design (Thompson 1992) to minimize the amount of

excavation required to achieve accurate and precise population estimates. Typically, the design stipulates that samples are taken in two phases. During the first phase, an inexpensive (but inaccurate) sampling method is applied to a large, random sample. Subsequently, during the second phase, an expensive (but more accurate) sampling method is applied to a random subset of the first-phase sample. The second-phase sample is used to model the relationship between the two methods, and then the model is used to calibrate the response for the remainder of the first-phase sample.

Our objectives were to: 1) examine variation in detection at the surface, 2) evaluate the application of double sampling to sampling freshwater mussels, 3) determine the amount of excavation that would minimize variance of population estimates for fixed cost, and 4) calculate sample size required to achieve desired levels of precision and power to detect population change. We used a case study approach to identify factors that affect detection and used data from the case study to evaluate the application of double sampling. After determining the optimal amount of excavation, we conducted a series of sample size calculations to examine the magnitude of population change likely to be detected when monitoring mussels over multiple years.

Table 1. Locations of the 14 sites surveyed during June-September 1997 and the number of 0.25 m² quadrats that were excavated in wadeable water.

Major drainage	Site	State	Watershed	Excavated quadrats ^a
Atlantic Slope	Ashuelot River	NH	Connecticut River	65
	Cacapon River	WV	Potomac River	61
	Connecticut River	NH/VT	Connecticut River	49
	Little River	NC	Neuse River	41
	Neversink River	NY	Delaware River	62
	Norwich Creek	MD	Choptank River	40
	Piscataquog River	NH	Merrimack River	100
	St. George River	ME	St. George River	99
	West River	VT	Connecticut River	69
	Farmington River (W. Br.)	MA	Connecticut River	50
Interior Basin	Allegheny River	PA	Allegheny River	118
	French Creek	PA	Allegheny River	28
	Little Tennessee	NC	Tennessee River	24
St. Lawrence River	Poultney River	NY/VT	Lake Champlain	101

^a In addition, surface counts alone were conducted on an approximately equal number of quadrats.

Methods

Factors affecting detection: a case study

During June through September 1997, we surveyed sites in 14 streams: 10 systems were in the Atlantic Slope, 3 were Interior Basin Drainages, and 1 was in the St. Lawrence River drainage (Table 1). At each site 0.25 m² quadrats were systematically placed. Positions along a bank were selected at equal intervals after a random start, and quadrats were placed at equal intervals across the stream after a random start from each bank position. Mussels at the substrate surface were collected, counted, identified to species, measured along their longest axis, and re-embedded in the substrate. Searches at the substrate surface were conducted while snorkeling, or through a glass-bottomed bucket, in wadeable water (<1.5 m). Observation was visual or tactile depending on turbidity. As part of the search, fine sediment was fanned away, non-embedded material was lifted and loose sediment was raked with fingertips in an effort to detect mussels at the surface. We excavated every other quadrat (50% of all quadrats) after the surface count was completed. Excavation consisted of removal of substrate to a depth of approximately 10 cm and sifting substrate through a mesh screen with openings of 6.4 mm. Altogether 907 quadrats were excavated at the 14 sites in wadeable waters. We recorded time to complete the surface count and excavation separately.

To test the hypothesis that excavation increases mortality of mussels when compared to surface counts, we placed mussels in plastic cages that resembled “milk crates” (30.5 cm x 35.6 cm x 25.4 cm) in the stream for at least 7 wks at 3 sites. We monitored mussel survival as a function of removal during a surface count or during excavation. The cages contained sediment, were wrapped in plastic mesh screen with openings of 3.2 mm, and were anchored to the stream bottom.

We recorded turbidity (LaMotte model 2008 turbidity meter) and temperature for each day of sampling. At each quadrat we recorded the observer, macrohabitat (riffle, run, pool), substrate size using the Wentworth scale (Gordon *et al.* 1992), depth, and percent rooted vegetation. Because the same observers did not visit all sites nor survey all habitat types within a site, observer effects could have been confounded by habitat effects. We used logistic regression (see below) to test whether detection differed among observers within each site prior to modeling detection across sites. For those sites where an observer effect was apparent we conditioned the test on habitat. Because sample sizes were often small (*i.e.*, expected values <5 for >20% of observer, habitat combinations),

we used exact methods for these latter tests (Mehta and Hilton 1993).

We used logistic regression (Hosmer and Lemeshow 1989) to model and test whether the probability of detection at the substrate surface was related to habitat, observer, or mussel length. In this analysis the response variable was whether a mussel was detected at the surface or not, and habitat, observer, and mussel length were the explanatory variables. We compared models using likelihood ratio statistics to test for effects of explanatory variables and their interactions. Akaike’s Information Criteria (AIC; Burnham and Anderson 1998) was used to select the model which best explained detection at the surface. Extra-binomial variation was accounted for by William’s method (Williams 1982). To assess adequacy of the model and look for systematic lack of fit we plotted a series of diagnostic statistics (Hosmer and Lemeshow 1989).

We determined the correct scale for the relationship between response and explanatory variables by plotting the response on the logit scale against each explanatory variable (Hosmer and Lemeshow 1989). This procedure provided some evidence of nonlinearity for depth and length, thus quadratic terms were added to the model for these variables. Although cubic terms were included, model fit was not improved so we present results only for models including quadratic terms. To aid interpretation of interactions, we converted depth and percent vegetation from continuous to ordinal. The categories for depth were <0.25 m, 0.25 to 0.75 m, and 0.75 to 1.5 m. The categories for percent rooted vegetation were 0%, 0 to 33%, and >33%.

To assess the usefulness of surface counts for determining relative abundance, we ranked species density within each site using both surface and total counts and looked for discrepancies between the two lists of relative abundance. When the lists of ranks differed we tested for statistical significance by ordering the species according to ranked total counts and testing for a shift in ranks using the Wilcoxon test.

The optimal proportion of quadrats to excavate

We determined the optimal amount of excavation in the context of a double sampling design (Thompson 1992). In the first phase of the double sampling design, mussels are counted on the surface in a large random sample of quadrats. In the second phase, a representative subset of the first-phase sample is selected, and these quadrats are excavated. The second-phase sample is sometimes referred to as a calibration sample (Luo *et al.* 1998).

Surface counts and total counts (total count = count below the surface + count at the surface) from the calibration sample are used to calibrate the surface counts for the entire sample.

The ratio estimator and the regression estimator are two common estimators available under the double sampling design (Thompson 1992). In the estimators, the total count is the response variable (y) and the surface count is the explanatory variable (x). The ratio estimator is based on the assumption that if $x=0$, then $y=0$. However, the regression estimator does not require that assumption; it allows $x=0$, but $y>0$. Thus, we recommend the regression estimator because it allows for the probable event that mussels are found during excavation even though none are detected on the surface. A regression model (*e.g.*, a simple linear regression model) is fit to the data from the second-phase sample. We present formulae for the regression estimator under double sampling in the Appendix.

To determine the optimal proportion of quadrats to excavate, we found the proportion that minimized variance of the population density estimate for a fixed total cost. We considered 3 costs: time to set up and move around the site (c^*), time to count mussels on the surface of a quadrat (c'), and time to excavate a quadrat (c). We set total cost to be $C = c^* + c'n' + cn$, where n' was the sample size for the first phase of sampling, and n was the sample size for the second phase. If C is fixed, then variance of the population estimate is a function of the proportion of the first-phase sample that is excavated (*i.e.*, the “proportion to excavate” or n/n'). We wanted to find the proportion that resulted in the smallest variance given that total cost was fixed. We found this “optimal proportion to excavate” by Thompson (1992)

$$\tilde{f}_2 = \frac{n}{n'} = \sqrt{\frac{c'}{c} \left[\frac{s^2}{s_{lr}^2} - 1 \right]^{-1}}, \quad (1)$$

where s^2 was the variance in total counts among excavated quadrats and s_{lr}^2 was the mean square error from the regression between surface and total counts. Using the relationship between s^2 , s_{lr}^2 , and an adjusted version of R^2 (Ryan 1997), we wrote an equivalent formula for the optimal proportion to excavate as

$$\tilde{f}_2 = \frac{n}{n'} = \sqrt{\frac{c'}{c} \left[\frac{1 - R_{adjusted}^2}{R_{adjusted}^2} \right]}. \quad (2)$$

We used Eqn. 1 to calculate the optimal proportion to excavate for the 14 sites in our study. We combined all species at a site for these analyses. For each site we fit a simple linear regression of total count against surface count and computed average times to complete a surface count (c') and an excavation (c).

We calculated variance and coefficient of variation (CV) for a range of sample sizes (*i.e.*, sample size = the total number of quadrats in the first phase of sampling = n'). We made the simplifying assumption that n' was a negligible fraction of the possible number of quadrats at a site (N), which is a reasonable assumption for mussel surveys where area sampled tends to be <5% of the area at the site. This is a conservative assumption in that our sample size calculations will overestimate sample size needed to achieve a desired precision especially for small sites (*e.g.*, <1000 m²). From this assumption we derived a simplified version of the variance

$$\text{var}(\hat{\mu}) \cong \frac{s^2}{a^2 n'} \left(R_{adjusted}^2 + \frac{1 - R_{adjusted}^2}{\tilde{f}_2} \right), \quad (3)$$

where \tilde{f}_2 is the optimal proportion to excavate, which we found using Eqn. 1. In addition to using Eqn. 1 to calculate the optimal proportion to excavate, we examined the functional form of the relationship between variance and proportion excavated by plotting the variance from Eqn. 3 for $R_{adjusted}^2$ of 0.4, 0.6, and 0.8. For these latter calculations the $s^2/(a^2 n')$ term in Eqn. 3 was constant and did not affect the form of the variance curve.

We conducted a power analysis to determine how sensitive the survey design was to changes in density if the survey were to be repeated annually for 5 or 10 y (or biannually for 10 or 20 y, for example). We used the program TRENDS (Gerrodette 1987, Thompson *et al.* 1998) to calculate the minimum change in density that would be detected in surveys of 5 and 10 y with probability > 0.80 for 1 tailed t -tests where $\alpha = 0.10$. To generalize the power analysis, we determined the relationship between CV and density given sample size. For a range of densities and sample sizes we used the relationship to predict CV, which was then entered into program TRENDS to compute minimum detectable change in density for power ≥ 0.80 .

Table 2. Species found in excavated quadrats at 14 sites that were sampled June-September 1997.

Species	Sites	Count
<i>Actinonaias ligamentina</i>	Allegheny, French Creek	118
<i>Alasmidonta heterodon</i>	Ashuelot, Connecticut, Neversink	65
<i>Alasmidonta marginata</i>	Allegheny, French Creek	14
<i>Alasmidonta undulata</i>	Ashuelot, Connecticut, Farmington, St. George, West	62
<i>Alasmidonta varicosa</i>	Cacapon, Neversink, Piscataquog, St. George, West	31
<i>Alasmidonta viridis</i>	Little Tennessee	1
<i>Amblema plicata</i>	French Creek	3
<i>Anodonta implicata</i>	Neversink	1
<i>Elliptio complanata</i> ^a	Cacapon, Connecticut, Neversink, Norwich Creek, Piscataquog, Poultney, St. George, West	510
<i>Elliptio dilatata</i>	Allegheny, French Creek, Little Tennessee	97
<i>Elliptio fisheriana</i>	Norwich Creek	20
<i>Epioblasma torulosa rangiana</i>	Allegheny, French Creek	12
<i>Fusconaia subrotunda</i>	French Creek	1
<i>Lampsilis cardium</i>	French Creek	1
<i>Lampsilis cariosa</i>	Cacapon, St. George	2
<i>Lampsilis fasciola</i>	Allegheny, French Creek, Little Tennessee	7
<i>Lampsilis ovata</i>	Allegheny, Poultney	5
<i>Lampsilis radiata</i>	Poultney	14
<i>Lasmigona costata</i>	Allegheny, French Creek, Poultney	12
<i>Lasmigona subviridis</i>	Little	20
<i>Leptodea fragilis</i>	Poultney	6
<i>Ligumia recta</i>	Allegheny	3
<i>Pleurobema clava</i>	Allegheny	2
<i>Pleurobema sintoxia</i>	French Creek	1
<i>Potamilus alatus</i>	Poultney	4
<i>Ptychobranhus fasciolaris</i>	French Creek	1
<i>Pyganodon cataracta</i>	Ashuelot, Farmington	3
<i>Pyganodon grandis</i>	Poultney	1
<i>Quadrula cylindrica</i>	French Creek	1
<i>Strophitus undulatus</i>	Ashuelot, Farmington, Neversink, Poultney, West	18
<i>Villosa fabalis</i>	Allegheny, French Creek	109

^a *E. complanata* was found, but not counted at Ashuelot and West Branch of the Farmington Rivers.

Results

Factors affecting detection: a case study

We found 31 species in excavated quadrats (Table 2). The most widespread was *E. complanata*, which we found at all of the Atlantic Slope sites (however, counts of *E. complanata* were not recorded at Ashuelot or West Branch of the Farmington Rivers due to its very high abundance). Because we wanted to examine detection across a wide range of conditions, we focused the analysis of detection and habitat on *E. complanata*.

Preliminary analyses led us to drop some of the explanatory variables prior to performing logistic regression analysis. We excluded temperature in the

model because our surveys occurred during summer months and temperature varied little (*i.e.*, 16-23 °C). We dropped turbidity because of its relationship to substrate size. Fine sediments such as organic debris and silt were found only where turbidity was high (≥ 6 NTU); and coarse material such as gravel, cobble, and boulder was found only where turbidity was medium or low (< 6 NTU). Macrohabitat was dropped because it was related to depth. (Riffles were limited to depths < 0.65 m; pools and runs were found in a wide range of depths.) To represent habitat in the model we retained depth, percent vegetation, and substrate size.

We found significant observer effects within 3 of the 8 sites where *E. complanata* was recorded. However, when comparisons among observers were made

conditional on habitat, observer effects were limited to 1 or 2 substrate types per site. At the Cacapon River site there was an observer effect among 4 observers in sand ($\chi^2 = 12.34$, $df=2$, $P=0.002$) and small cobble ($\chi^2 = 7.72$, $df=3$, $P=0.043$), but not in large cobble ($\chi^2 = 3.18$, $df=3$, $p = 0.52$) and boulder ($\chi^2=4.13$, $df=3$, $P=0.29$). At the Little River site there was an observer effect among 6 observers in silt ($\chi^2=40.3$, $df=5$, $P<0.001$), but not in organic debris ($\chi^2=5.7$, $df=4$, $P=0.24$), clay ($\chi^2 = 0.15$, $df=2$, $P=1.00$), or sand ($\chi^2 = 3.7$, $df=5$, $P=0.62$). At the Neversink River site there was an observer effect among 6 observers in large cobble ($\chi^2=14.9$, $df=5$, $P=0.063$), but not in small cobble ($\chi^2 = 3.8$, $df=5$, $P=0.70$). We did not want to diminish the importance of observer effects, but wanted to examine effects of habitat and mussel length on detectability. Thus, we chose to pool data across observers and concentrate on modeling detection as a function of habitat and length of the mussel. We feel confident that this approach did not compromise analysis because regression diagnostics showed that lack of fit in the best fitting model was not related to observers.

We found that the probability of detection was significantly related to depth, percent vegetation, substrate size, and mussel length ($\chi^2 = 174.3$, $df=25$, $P < 0.0001$). The best fitting model included complex interactions between the explanatory variables. The effect of mussel length appeared to be strongest in silt and sand (Fig. 1), but weak in gravel where the effect depended on percent rooted vegetation. We found no length effect in small cobble ($\chi^2=0.259$, $df=1$, $P=0.611$). In 4 of the 8 substrate types, the greater the percent of rooted vegetation the lower the probability of detection (Fig. 1). However, in gravel and small cobble the data suggests the opposite effect—the greater percent vegetation the more likely that a mussel would be detected. We found no apparent relationship between vegetation and detection in substrates of sand or organic debris. Consistently among substrates, there was a depth effect characterized by slightly higher detection at intermediate depths (0.25-0.75 m) than in shallow (<0.25 m) or deeper (0.75-1.5 m) water (Fig. 1). The lowest detection was found in deeper water (0.75-1.5 m). Differences in

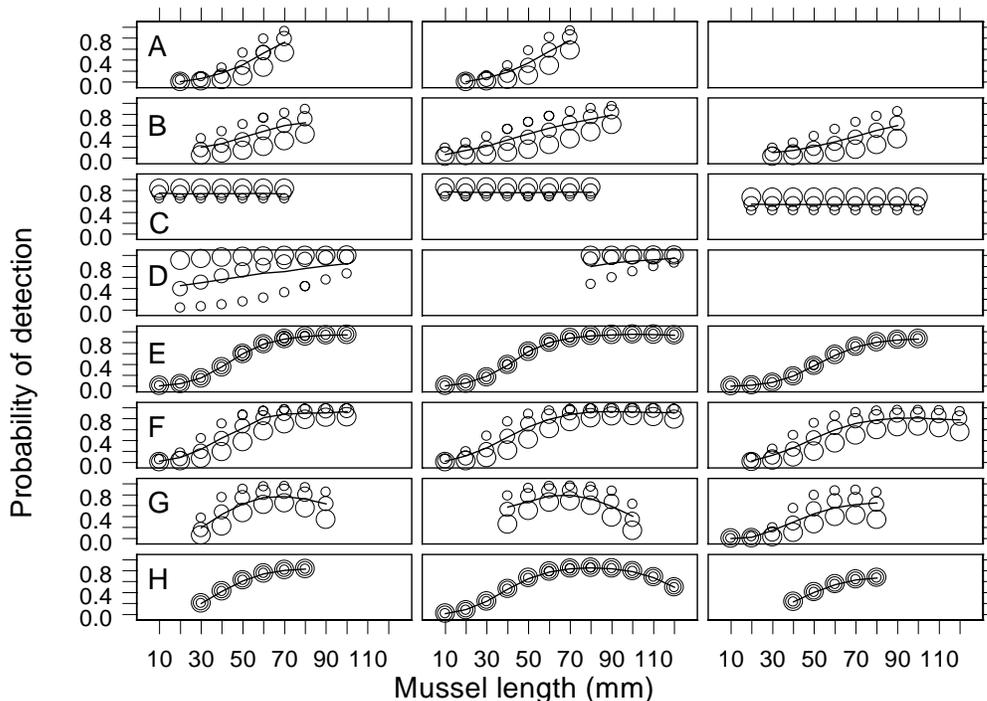


Figure 1. Probability of detection as a function of mussel length (measured along its longest axis) and habitat. Habitats are defined by substrate type (boulder: row A, large cobble: row B, small cobble: row C, gravel: row D, sand: row E, silt: row F, clay: row G, and organic debris: row H), depth (<0.25 m: left column, 0.25 to 0.75 m: middle column, and 0.75 to 1.5 m: right column), and percent rooted vegetation (0: small circle, 0 to 33: medium circle, and >33: large circle). Probability of detection is the predicted probability that an *E. complanata* is detected during a search of the substrate surface. The probability of detection is based on a logistic regression model of data from 8 Atlantic Slope streams where 521 0.25 m² were excavated to a depth of approximately 10 cm after a visual or tactile search of the surface.

Table 3. Relative abundance based on surface and total counts for 2 sites where abundance rankings differed between surface and excavated samples. Total counts are mussels on the surface plus mussels detected by excavating to a depth of approximately 10 cm.

Site	Species	Total Count		Surface Count		% Detected at the Surface
		%	Rank	%	Rank	
Ashuelot River ^a	<i>Alasmidonta heterodon</i>	36.7	1	20.0	3	22
	<i>Alasmidonta undulata</i>	34.7	2	35.0	2	41
	<i>Strophitus undulatus</i>	26.5	3	40.0	1	62
	<i>Pyganodon cataracta</i>	2.1	4	5.0	4	100
French Creek	<i>Villosa fabalis</i>	41.5	1	23.3	2	25
	<i>Actinonaias ligamentina</i>	34.1	2	57.8	1	71
	<i>Elliptio dilatata</i>	7.9	3	1.4	7	8
	<i>Alasmidonta marginata</i>	6.7	4	5.5	3	36
	<i>Lasmigona costata</i>	3.0	5	4.1	4	38
	<i>Amblema plicata</i>	1.8	6	4.1	4	100
	<i>Epioblasma torulosa rangiana</i>	1.2	7	2.7	6	100
	<i>Fusconaia subrotunda</i>	0.6	8	1.4	7	100
	<i>Lampsilis cardium</i>	0.6	8	1.4	7	100
	<i>Quadrula cylindrica</i>	0.6	8	1.4	7	100
	<i>Lampsilis fasciola</i>	0.6	8	0	-	0
	<i>Pleurobema sintoxia</i>	0.6	8	0	-	0
	<i>Ptychobranthus fasciolaris</i>	0.6	8	0	-	0

^a Relative abundance of species other than *Elliptio complanata* are shown for the Ashuelot River. *E. complanata* was the most abundant species at the Ashuelot River; however, its numbers were not recorded.

detection among substrate types were greatest for younger (and smaller) mussels (Fig. 1).

Some of the patterns that emerged from modeling detection could be spurious although patterns were largely consistent with our perception of how detection changed with habitat and mussel length. For example, the model indicated that in gravel and small cobble the probability of detection increased with rooted vegetation. At first this result seemed counterintuitive, however we offer the following heuristic argument that the result may be accurate. We suggest that rooted vegetation increases the proportion of mussels at the surface similarly for all substrates, however “visibility” of mussels at the surface varies among substrates. Increased root mass will occupy space that otherwise would be available for mussels. Thus, in a sense, mussels are forced to the surface by the increase in rooted vegetation regardless of substrate. However, the effect on detection of mussels at the surface might differ among substrates. There are three cases to consider: fine sediment (*e.g.*, silt and clay), intermediate sediment (*e.g.*, gravel and small cobble), and coarse sediment (*e.g.*, large cobble and boulder). In fine sediment, presence of vegetation hinders visibility of mussels at the surface because turbidity will increase when vegetation is parted to search for mussels and because vegetation interferes

with tactile searching. Increased turbidity might be less of a problem in the other two substrates. In coarse sediment, mussels are hidden amongst larger material, and the presence of vegetation compounds that problem. In intermediate sediment, there is less turbidity than in fine sediments and less surface roughness than in coarse sediments. Thus, we hypothesize that in intermediate sediment as rooted vegetation increases more mussels are at the surface, yet visibility is not greatly reduced, and the net effect is higher detection.

Another interesting result was that detection was greatest at intermediate depths. We offer a possible explanation. At shallow depths the observer’s field of view is restricted because his/her face is close to the substrate. Consequently, coverage is compromised. At intermediate depths the observer can “pull back” and enjoy a wider and possibly more effective field of view of the substrate in the quadrat. As depth increases the effect of turbidity increases, thus decreasing visibility.

At 12 (86%) of the 14 sites relative abundance as measured by ranks from surface counts matched that from total counts. However, at 2 (14%) of the sites (Ashuelot River [P=0.08] and French Creek [P= 0.06]) the ranking of the most abundant species changed order

Table 4. A comparison of densities among sites for two Federally endangered species (*Alasmidonta heterodon* and *Epioblasma torulosa rangiana*) as calculated from surface and total counts.

Species	Site	Total Density (no. m ⁻²) SE	Surface Density (no. m ⁻²) SE	% Detected at the Surface
<i>A. heterodon</i>	Ashuelot River	1.11 ± 0.424	0.25 ± 0.148	22
	Connecticut River	2.45 ± 0.592	1.31 ± 0.456	55
	Neversink River	0.71 ± 0.216	0.45 ± 0.188	64
<i>E. t. rangiana</i>	Allegheny River	0.31 ± 0.098	0.20 ± 0.081	67
	French Creek	0.29 ± 0.286	0.29 ± 0.286	100

when based on surface counts rather than total counts (Table 3). For example, at the Ashuelot River site *Alasmidonta heterodon* (Lea, 1830), a federally listed species, was ranked 1st and *Strophitus undulatus* (Say, 1817) ranked 3rd based on total counts (not including *E. complanata*). However, when based on surface counts *A. heterodon* ranked 3rd and *S. undulatus* ranked 1st because 22% of *A. heterodon* and 62% of *S. undulatus* were detected at the surface (Table 3).

Because percent detected at the substrate surface varied among sites, comparisons of species status among sites depended on whether density was calculated from total or surface counts (Table 4). Based on surface counts, the Ashuelot River site appeared to have the lowest density (0.25 m⁻²) of *A. heterodon* (Table 4). However based on total counts, *A. heterodon* at the Ashuelot River site was 1.11 m⁻², an intermediate density compared to the other two sites where we found the species. Similarly, surface density of *Epioblasma torulosa rangiana* (Lea 1838) was 45% greater at the French Creek site than at the Allegheny River site, but total density was comparable between the 2 sites (Table 4).

We observed mortality in 2 of the 12 species held for 7 weeks (Table 5): *Villosa fabalis* (Lea 1831) and *Alasmidonta undulata* (Say 1817). In neither case was mortality related to excavation (for *V. fabalis*: $\chi^2=0.402$, df=1, exact P=0.643; and for *A. undulata*: $\chi^2=2.27$, df=1, exact P=0.259). Overall, mortality was 11.7% (8 of 68) for *A. undulata* over 67 d and 6.7% (4 of 60) for *V. fabalis* over 50 d.

The optimal proportion to excavate

Excavation was 3 to 12x more time consuming than surface counts. Typically, excavation took 6x longer than surface counts; at 75% of the sites excavation took > 4x longer. Strength of the relationship between total counts and surface counts varied among sites and depended on detectability of mussels at the site (Table 6).

The optimal proportion to excavate (*i.e.*, the proportion that minimized variance for fixed cost as determined from Eqn. 1) ranged from about 10% to 100% and was related to the percent detected at the surface (Fig. 2; on log scale $r = -0.94$, $t = -9.24$, df=12, $P < 0.0001$), but was not related to density (on log scale $r = 0.07$, $t = 0.24$, df=12, $P = 0.81$). Although we used Eqn. 1 and Eqn. 2 to calculate the optimal proportion to excavate using observations from our 14 sites, we used Eqn. 3 and Fig. 3 to illustrate graphically the numerical procedure. For example, to use Fig. 3 to find the proportion that minimizes the variance (*i.e.*, to find the optimal proportion of quadrates to excavate), follow 1 of the variance curves to its lowest point then drop down to the x -axis. This is in effect what was done by the use of Eqn. 1, although Eqn. 1 provides an exact numerical result. The minima depended on the strength of the relationship between surface and total counts (*i.e.*, R^2_{adjusted}), which in turn is determined by the percent detected at the substrate surface (*i.e.*, the higher the percent the stronger the relationship). By examining the variance curves, we noticed that the variance curve flattened around the minimum as R^2_{adjusted} decreased (Fig. 3). Thus, as R^2_{adjusted} decreased a wider range of the proportion to excavate came close to minimizing the variance.

We summarized results on how much to excavate to yield robust estimates of population density given percent detection at the substrate surface (Table 7). We reduced the results to 4 possible cases. If >60% were detected at the substrate surface then variance was approximately minimized by excavation of 25% (or 1 out of 4) of the quadrats. Similarly, 50 to 60% detection at the surface resulted in 33% excavation, 40 to 50% detection at the surface resulted in 50% excavation, and <40% detection at the surface resulted in 100% excavation.

Coefficient of variation was a function of density and sample size; the higher the density and sample size, the lower the CV (Fig. 4). For sample size = 200, CVs were 0.25, 0.33, 0.45, and 0.69 for densities of 1.0,

Table 5. Evaluation of the survivorship of mussels removed by excavation and held in cages to determine if excavation increased mortality. Mortality was observed for 2 species: *Villosa fabalis* at French Creek and *Alasmidonta undulata* at Piscataquog River. In neither case was mortality attributable to excavation.

Site	Days Held	Species	Excavated			Surface		
			Count	Mean Length (SD)	% Dead	Count	Mean Length (SD)	% Dead
Cacapon River	119	<i>Elliptio complanata</i>	42	46.3 (9.7)	0	31	59.6 (13.1)	0
French Creek	50	<i>Actinonaias ligamentina</i>	8	42.3 (23.1)	0	5	69.2 (16.2)	0
		<i>Alasmidonta marginata</i>	4	69.1 (12.9)	0	12	59.4 (10.9)	0
		<i>Amblema plicata</i>				1	82.2	0
		<i>Elliptio dilatata</i>	8	71.2 (23.7)	0	8	65.4 (20.1)	0
		<i>Lasmigona costata</i>	2	87.8 (39.9)	0	6	99.0 (16.7)	0
		<i>Lampsilis fasciola</i>	1	35.1	0			
		<i>Pleurobema sintoxia</i>	1	60.5	0			
		<i>Ptychobranthus fasciolaris</i>	1	52.1	0	1	102.2	0
		<i>Strophitus undulatus</i>				1	61.8	0
		<i>V. fabalis</i>	36	23.5 (5.2)	8.3	24	26.2 (5.5)	4.2
Piscataquog River	67	<i>A. undulata</i>	34	38.9 (8.5)	5.9	34	44.1 (7.6)	17.6
		<i>Elliptio complanata</i>	35	56.2 (17.7)	0	51	61.0 (15.1)	0

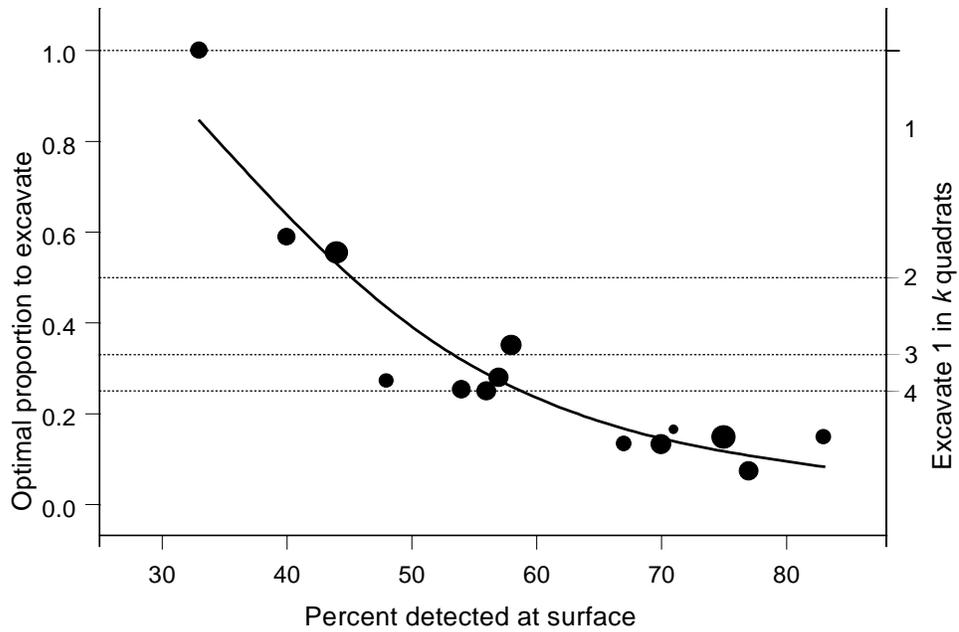


Figure 2. Optimal proportion excavated as a function of percent detected at the substrate surface based on mussel surveys at 14 sites. The proportion excavated is optimal in the sense that it yields a population estimate with minimum variance for a fixed cost. The size of the symbols is proportional to mussel density at each site. On the 2nd y-axis is the interval between excavated quadrats when those quadrats are selected systematically.

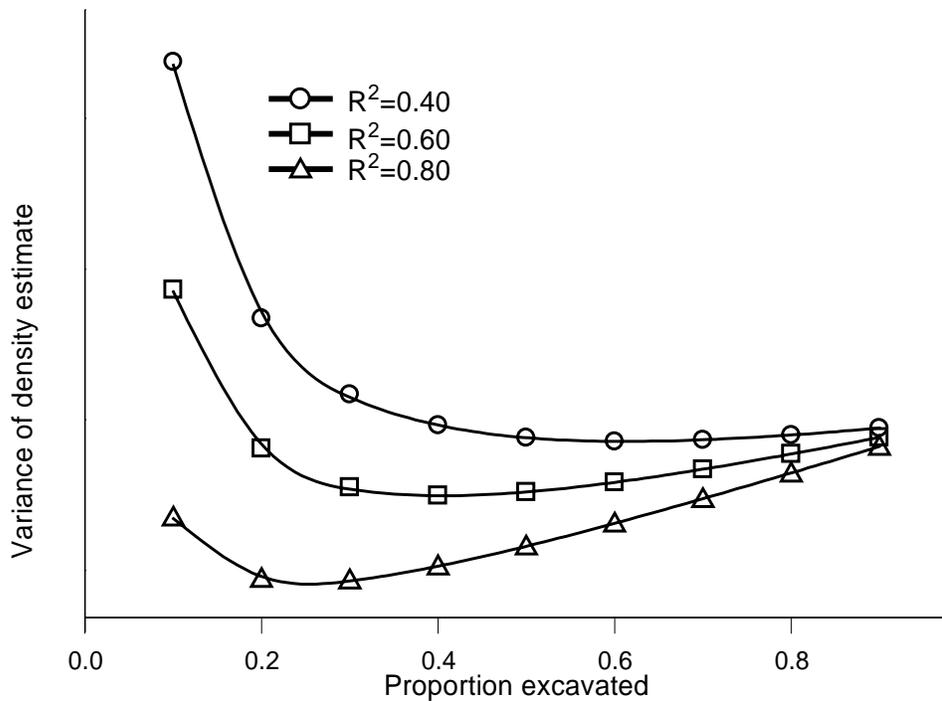


Figure 3. Variance of a density estimate as a function of the proportion excavated. The proportion excavated refers to the proportion of an initial sample of quadrats that is excavated in a double sampling design. On the initial sample, only a surface count is conducted. Variance is based on a regression estimator. The shape of the variance curve is related to the strength of the relationship between surface counts and total counts as measured by R^2 .

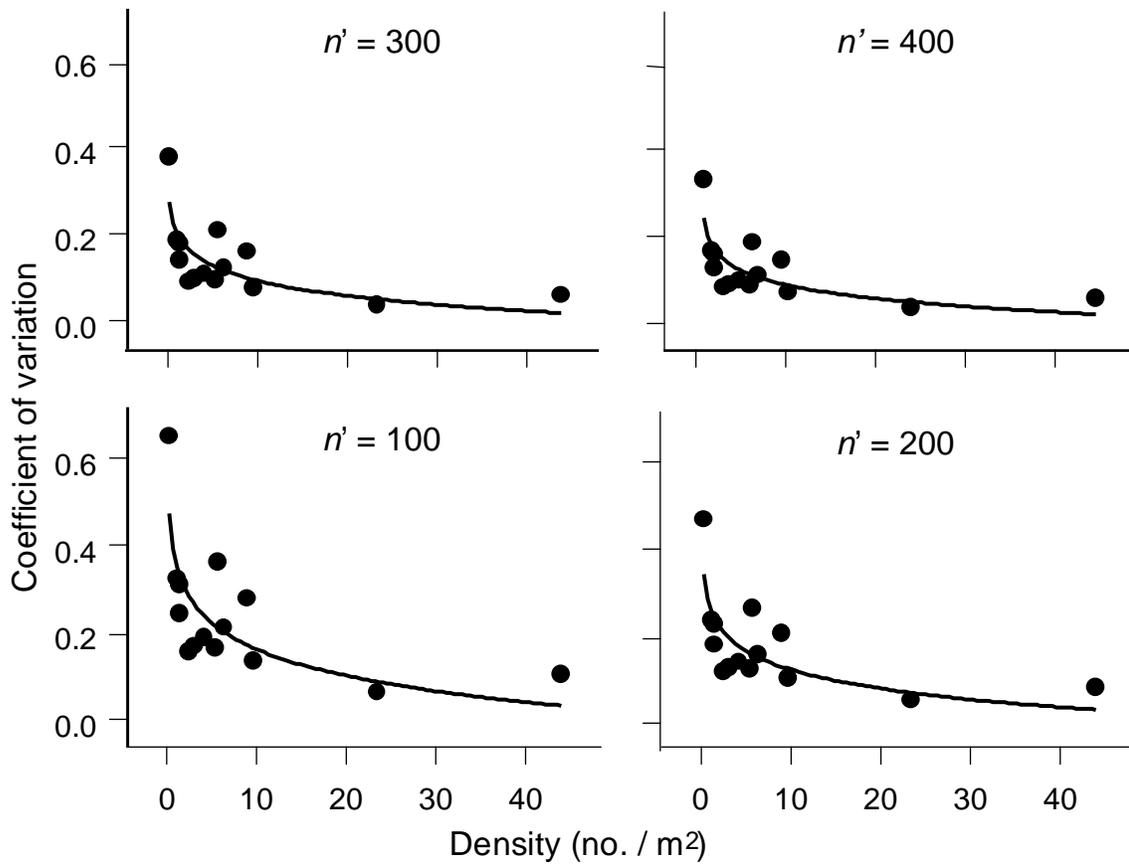


Figure 4. Coefficient of variation calculated for a range of sample sizes and based on a double sampling design where only a proportion of sampled quadrats are excavated. Calculations were based on data from the 14 sites.

0.50, 0.25, and 0.10, respectively. For sample size = 400, CVs were 0.18, 0.23, 0.32, and 0.49 for densities of 1.0, 0.50, 0.25, and 0.10, respectively. When density was transformed to an inverse square root scale, CV was a linear function of density ($t = 6.06$, $df=12$, $P < 0.0001$); and this relationship was used to generalize the power analysis.

The minimum detectable change in density over 5 or 10 y, like variance, depended on density and sample size (Fig. 5). Also, the more years of monitoring the smaller the density change that would be detected. Sample size had a qualitatively more dramatic effect on detecting increases in density. When the focus was on detecting drops in density below a certain level, say below 0.10 m^{-2} for example, detection of change depended on initial density and sample size. To detect a drop below 0.10 m^{-2} for 5 y of monitoring, initial density needed to be $\geq 0.6 \text{ m}^{-2}$ with sample size ≥ 200 . In contrast, to detect a drop below 0.10 m^{-2} for 10 y of monitoring, initial density could be as low as 0.2 m^{-2} with sample size ≥ 400 ; that would be equivalent to detecting a drop of 100 individuals per 1000 m^2 .

Discussion

Detection at the substrate surface was related to observer, habitat, mussel length, and mussel species. Thus, changes in any of these variables will confound comparison of populations across time, sites, habitat, or taxa when based exclusively on surface counts. For example, in the absence of rooted vegetation the probability of detecting a 40 mm *E. complanata* was 74% higher in small cobble (probability = 0.68) than in sand (probability = 0.39), and comparing surface densities across these microhabitats would lead to erroneous conclusions about habitat use. At a larger scale, we found that surface density was an unreliable indicator of population status because detection varied among sites. For example, detectability of *A. heterodon* at the Neversink site (0.64) was 191% of that at the Ashuelot site (0.22) so that densities (based on surface counts) appeared higher at the Neversink site when, in fact, the opposite was true. Because detection was species-specific, comparison of relative abundance based on surface counts within the same site can be misleading. Even ranked

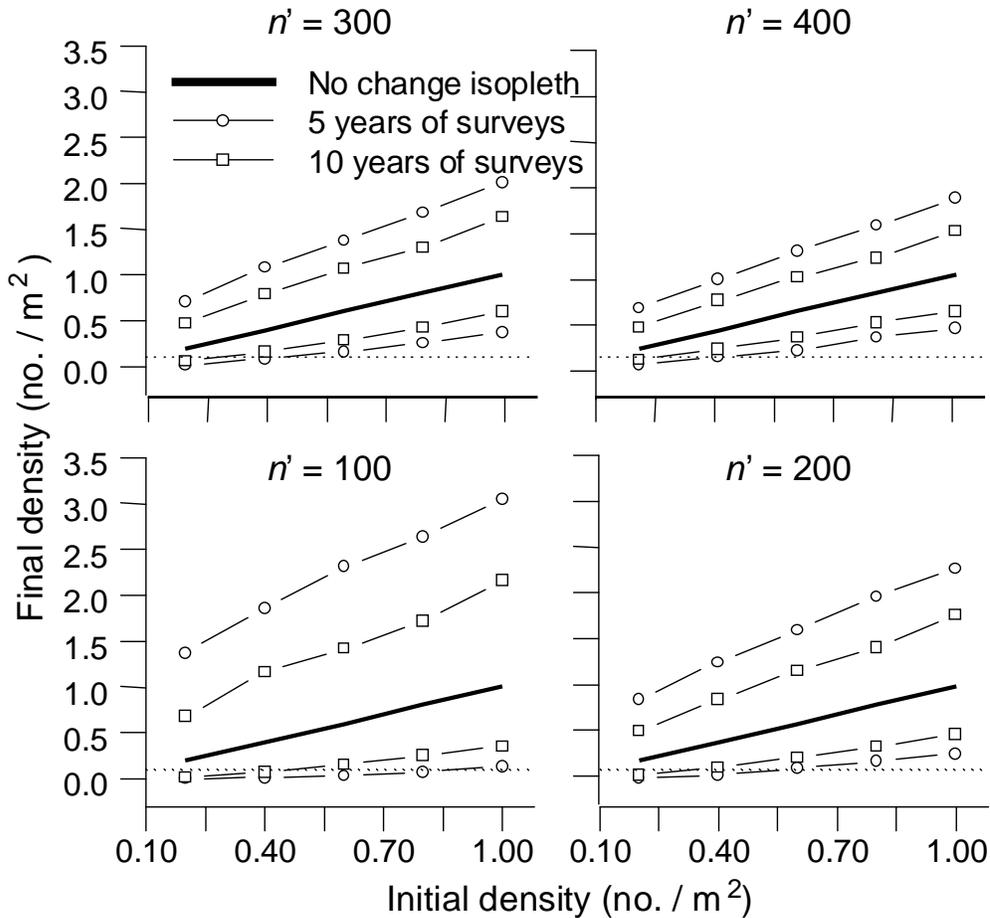


Figure 5. Minimum detectable changes in density for monitoring over 5 or 10 y and for various levels of sample size. The change in density is the smallest that would be detected with 80% probability given a 10% Type I error rate, a one-tailed test, and assuming a trend that is proportional to density. The underlying sampling design is double sampling with an optimal proportion of the sample being excavated.

abundance based on surface counts was an inaccurate measure of relative abundance at 2 (14%) of the 14 sites.

One strategy to cope with variable detection in “qualitative” or “semi-quantitative” sampling (Miller and Payne 1988) is to standardize survey methods and hold constant the conditions under which surveys are conducted. This strategy will not be successful because it is not possible to hold constant habitat variables such as depth, vegetation, and substrate. Biomass of submerged aquatic vegetation varies temporally and spatially, substrate is altered by fluvial processes, and flow may not drop to a base level in years with higher than average precipitation. Thus, critical microhabitat conditions vary temporally and spatially in spite of the intentions of the surveying biologist.

Our results are consistent with earlier work regarding vertical position of *E. complanata*. Amyot and Downing (1991) and Balfour and Smock (1995) found variation across season and mussel length. Because we found that probability of detection was lowest and differences in detection across all microhabitats were greatest for small mussels, our results underscore the recommendations of Miller and Payne (1988), Richardson and Yokley (1996), and Vaughn *et al.* (1997) that recruitment will be more difficult to observe without including excavation in the survey protocol.

We conclude that when the objective is to rigorously compare population density across sites, time, habitat, or taxa, it is not a question of whether to excavate, but rather how much to excavate. However, the benefit of excavation in terms of increased accuracy must be weighed against the added cost due to increased effort required.

Table 6. Results from linear regression of total count as a function of surface count. The $\hat{\beta}_0$, which is defined in the text, can be interpreted as conventional R^2 . Percent detected at the surface is a ratio of the surface count over total count. Density (no. m^{-2}) is estimated from total counts.

Site	Regression Parameters ($\hat{\beta}_0, \hat{\beta}_1$)	R^2_{adjusted}	% Detected at the Surface	Density (no. m^{-2}) \pm SE
Ashuelot River	(0.53, 1.07)	0.31	41	3.02 \pm 0.519
Cacapon River	(0.42, 1.27)	0.78	58	6.36 \pm 1.369
Connecticut River	(0.38, 1.16)	0.69	55	4.16 \pm 0.808
Little River	(2.37, 1.04)	0.93	75	44.00 \pm 6.050
Neversink River	(1.01, 1.00)	0.65	58	9.68 \pm 1.280
Norwich Creek	(0.51, 1.07)	0.92	71	8.92 \pm 3.159
Piscataquog River	(0.02, 0.99)	0.81	71	0.28 \pm 0.130
St. George River	(0.12, 1.16)	0.53	48	1.08 \pm 0.236
West River	(0.02, 1.39)	0.87	68	1.44 \pm 0.396
Farmington River, W. Branch	(0.04, 1.07)	0.87	83	1.44 \pm 0.375
Allegheny River	(0.53, 1.07)	0.74	57	5.39 \pm 0.611
French Creek	(3.27, 0.99)	0.47	44	23.44 \pm 2.352
Little Tennessee River	(0.42, 0.98)	0.14	33	2.52 \pm 0.792
Poultney River	(0.19, 1.11)	0.94	78	5.72 \pm 1.538

We make recommendations on how much to excavate that are based on the double sampling design and a minimizing of variance for fixed survey cost. We recommend that under the double sampling design the proportion of quadrats excavated should be determined by the percent of mussels likely to be detected at the substrate surface. We summarized results into a simple set of rules for determining how much to excavate (Table 7). To use this set of rules, information on the percent likely to be detected at the substrate surface for the species of interest is needed. These preliminary

data may exist from similar surveys or may be obtained through a pilot survey.

Table 7. Recommended rules for determining the optimal proportion of quadrats to excavate. Percent detection at the surface refers to the percent of the species that is likely to be detected at the substrate surface, which could come from a pilot survey or similar surveys. A convenient and valid method to select a subset of quadrats for excavation is by excavating every k^{th} quadrat (or excavate “1-out-of- k ” quadrats). In other words, which quadrat to excavate can be determined systematically with the first chosen at random among the first k quadrats.

% detection at the surface	Optimal proportion to excavate	k
> 60%	0.25	4
50-60%	0.33	3
40-50%	0.50	2
<40%	1.00	1

In our calculation of the optimal proportion to excavate, we formulated survey costs simply in units of time. However, the true cost of excavation includes disturbance to mussels and their habitat. If quantified, then disturbance can be incorporated in the analysis to find the optimal amount of excavation. If disturbance were much greater in excavation than in surface counts, then the optimal proportion to excavate would be lower than what we report here. However, we found no evidence that excavation increased mortality. We did observe mortality in 2 of the 12 species held after sampling; 11.7% of *A. undulata* over 67 d and 6.7% of *V. fabalis* over 50 d. If not due to excavation per se, then the mortality was likely related to the action of removal from and re-embedding to substrate, which occurs during most survey techniques. Nevertheless, we conclude that significant effects of sampling on population-level survival are unlikely because a small percentage of a site is sampled in a typical survey. Thus, most of the sampled mussels will not be affected adversely. For example, if 10% of a site is sampled (*i.e.*, 400 0.25 m^2 quadrats in a 1000 m^2 site) and sampling causes 20% mortality of sampled mussels (this level of mortality was higher than we observed, cf. Table 5), then sampling would cause only 2% mortality for the population. This would be a worse than expected scenario because more often than not we observed no mortality among

sampled mussels and typically <10% of a site is sampled (Table 5).

The next step, after finding the optimal proportion to excavate, is to calculate sample size. Under the double sampling design, sample size is the total number of quadrats on which to conduct a surface count (*i.e.*, n'); of these, a proportion is excavated. Sample size is primarily a function of density. Sample size needed to achieve precise density estimates for densities $\geq 1.0 \text{ m}^{-2}$ is in the order of 100-200 0.25 m^2 quadrats. If, for example, 40-50% of the mussels are likely to be detected on the surface, then 50% of the quadrats should be excavated. The time required to conduct the survey would be 6.7-13.3 h (not including time to set up and break down), assuming an average of one minute for a surface count and six minutes for an excavation. The actual time to complete the survey will depend on the crew size. In our experience, such a survey would take 1-2 d with a crew of five or six. However, to achieve precise density estimates for densities $< 1 \text{ m}^{-2}$ will require 2-4 times the effort required to effectively sample densities $\geq 1.0 \text{ m}^{-2}$. Double sampling can be combined with more complex designs useful for sampling rare populations, such as stratification based on density and adaptive cluster sampling (Thompson 1992, Strayer *et al.* 1996).

We disagree with Payne *et al.* (1997) who suggest it is not worthwhile to estimate density of low-density populations (*i.e.*, those $< 0.5 \text{ m}^{-2}$). First, Payne *et al.* (1997) do not consider possible gains in efficiency due to improved survey design. We calculate that use of the double sampling design would result in a CV = 0.23 for a population of 0.5 m^{-2} and a sample size of 400. This is less than half the sample size that Payne *et al.* (1997) predict would be needed for a similar CV and population density; the difference is due to improved survey design. Second, in survey planning there is an overemphasis on CV to determine adequacy of sample size. CV measures variance relative to the magnitude of the density estimate; as density decreases it takes a smaller absolute variance to achieve a low CV. Whereas relative variance (as measured by CV) is very useful for survey planning, absolute variance and power analysis should also play a role. For example, at a site where density is 0.2 m^{-2} , sample size of 400 would result in an estimate with CV of 0.36, a SE of 0.07, a “margin of error” (2 SE) of 0.14 or 140 individuals per 1000 m^2 site, and high power (>80%) to detect drops to 0.02 m^{-2} over 5 y or 0.07 m^{-2} over 10 y. Although the CV of 0.36 does not meet the criteria of CV=0.20 suggested by Payne *et al.* (1997:154), we argue that this estimate is informative

and when applied to a monitoring program meaningful changes in population density would likely be detected.

To document presence of rare species it is clear that some amount of directed qualitative sampling is required (Strayer *et al.* 1997, Vaughn *et al.* 1997). However, we offer the following reasons for quantitative sampling even for rare populations (*i.e.*, densities $\leq 0.1 \text{ m}^{-2}$). First, in the absence of quantitative sampling (used here to be analogous to probability sampling) there is no measure of uncertainty and therefore no way to gauge the reliability of the survey results. Second, even though target species may occur at low density, often other species at a site occur at higher density and can be used to monitor changes in the mussel community. Third, if quantitative sampling is used to monitor a population, then population recovery can be documented. Fourth, quantitative sampling allows a probability statement to be made regarding species presence and maximum density. For example, to model the probability of detecting rare species Green and Young (1993) used the Poisson distribution. That same approach can be adapted to make statements regarding species presence and maximum density when only a proportion of quadrats are excavated. For our double sampling design the equivalent formulae to Eqn. 3 in Green and Young (1993) is

$$n' = \frac{-4 \ln(\beta)}{m([1-f_2]\lambda + f_2)}$$

where m is density (no. m^{-2}), n' is number of quadrats in the sample, f_2 is proportion of n' that is excavated, λ is the proportion of mussels detected at the substrate surface, and β is the probability of not detecting any mussels in the n' quadrats. The multiple ‘4’ is needed because mussel density is expressed in numbers m^{-2} , whereas quadrat area is 0.25 m^2 . Take, for example, the following scenario: percent detected at the surface was 60% ($\lambda=0.60$) based on excavation of 33% of the quadrats ($f_2=0.33$) and suppose 200 quadrats are sampled ($n'=200$). In this scenario, if the target species of mussel was not found then with 95% confidence ($\beta=0.05$) we can state that species density at the site was $\leq 0.08 \text{ m}^{-2}$. The time to sample the 200 quadrats, 67 of which are excavated, would require approximately 10 h of search time (using 1 min for surface counts and 6 min to excavate). Search time can be divided among several observers so that field time would be less than 10 h.

We agree with Thompson *et al.* (1998) who state, “sampling rare populations will likely be a very costly

endeavor regardless of how it is performed". We offer suggestions on sampling techniques that provide some gains in efficiency. However, we ultimately conclude that to successfully monitor populations of mussels, particularly those that occur at densities $<0.4 \text{ m}^{-2}$ requires a substantial investment to collect the necessary data. If monitoring population density is an objective given high priority then managers must be prepared to allocate adequate time and money to the task.

Acknowledgements

Funding was provided by USGS Biological Resources Division through the Species at Risk Program and U.S. Fish and Wildlife Service, Silvio O. Conte National Fish and Wildlife Refuge. We are indebted to the following individuals for their participation in data collection: John Alderman, J. Allen, Bob Anderson, J. Baldizar, Henry Barbour, Bill Bartles, Glen Black, Marc Blouin, D. Braden, H. Brooks, D. Brown, Janet Butler, Janet Clayton, Tom Cox, Mark Ferguson, Chris Fichtel, Steve Fiske, T. Flynn, John Fridell, Marea Gabriel, M. Gibson, Beth Goettel, T. Gola, Marnee Gormley, Tom Gloria, Jamie Haskins, Carrie Horton, Pat Huckery, Judith Johnson, John Kanter, Bill Lellis, Madeleine Lyttle, H. Malcom, Paul Marangelo, Mark Maurer, David McLain, Mark McCollough, Debbie Mignogno, Robert Monroe, Patty Morrison, Tom Muir, Kathleen O'Brien, Amy Pasternak, Mike Penko, Lois Richards, Kathy Schneider, David Strayer, Rebecca Suomala, Nissa Thomsen, Keith Voges, J. Weitzel, Dave Weller, Barry Wicklow, M. Wood, and Priscilla Young. We offer a special thanks to Paul Marangelo, Nissa Thomsen, and Carrie Horton for their coordination of field work. We thank Penelope Pooler, Paul Johnson, and 2 anonymous reviewers for helpful comments on an earlier draft of the manuscript.

Literature Cited

- Amyot, J.P. and J.A. Downing. 1991.** Endo- and epibenthic distribution of the unionid mollusc *Elliptio complanata*. *Journal of the North American Benthological Society* 10:280-285.
- Balfour, D.L. and L.A. Smock. 1995.** Distribution, age structure, and movements of the freshwater mussel *Elliptio complanata* (Mollusca: Unionidae) in a headwater stream. *Journal of Freshwater Ecology* 10:255-268.
- Burnham, K.P. and D.R. Anderson. 1998.** Model selection and inference: a practical information – theoretic approach. Springer-Verlag, New York.
- Gerrodette, T. 1987.** A power analysis for detecting trends. *Ecology* 68:1364-1372.
- Green, R.H. and R.C. Young. 1993.** Sampling to detect rare species. *Ecological Applications* 3:351-356.
- Gordon, N.D., T.A. McMahon, and B.L. Finlayson. 1992.** Stream hydrology: an introduction for ecologists. Wiley, New York.
- Hedayat, A.S. and B.K. Sinha. 1991.** Design and inference in finite population sampling. Wiley, New York.
- Hosmer, D.W. and S. Lemeshow. 1989.** Applied logistic regression. Wiley, New York.
- Luo, M., L. Stokes, and T. Sager. 1998.** Estimation of the CDF of a finite population in the presence of a calibration sample. *Environmental and Ecological Statistics* 5:277-289.
- Mehta, C.R. and J.F. Hilton. 1993.** Exact power of conditional and unconditional tests: going beyond the 2 x 2 contingency table. *The American Statistician* 47:91-98.
- Miller, A.C. and B.S. Payne. 1988.** The need for quantitative sampling to characterize size demography and density of freshwater mussel communities. *American Malacological Bulletin* 6:49-54.
- Payne, B.S., A.C. Miller, and R. Whiting. 1997.** Designing a riverine mussel survey. Pages 150-156. *In* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors) Conservation and management of freshwater mussels II: initiatives for the future. Proceedings of a UMRCC Symposium, October 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.
- Richardson, T.D. and P. Yokley, Jr. 1996.** A note on sampling technique and evidence of recruitment in freshwater mussels (Unionidae). *Archiv für Hydrobiologie* 137:135-140.
- Ryan, T.P. 1997.** Modern regression methods. Wiley, New York.
- Strayer, D.L. and J. Ralley. 1993.** Microhabitat use by an assemblage of stream-dwelling unionaceans (Bivalvia), including two rare species of *Alasmidonta*. *Journal of the North American Benthological Society* 12:247-258.
- Strayer, D.L., S.J. Sprague, and S. Claypool. 1996.** A range-wide assessment of populations of *Alasmidonta heterodon*, an endangered freshwater mussel (Bivalvia:Unionidae). *Journal of the North American Benthological Society* 15:308-317.
- Strayer, D.L., S. Claypool, and S.J. Sprague. 1997.** Assessing unionid populations with quadrats and time searches. Pages 163-169. *In* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors) Conservation and management of freshwater mussels II: initiatives for the future. Proceedings of

a UMRCC Symposium, October 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Thompson, S.K. 1992. Sampling. Wiley, New York.

Thompson, W.L., G.C. White, and C. Gowan. 1998. Monitoring vertebrate populations. Academic Press, San Diego, California.

Vaughn, C.C., C.M. Taylor, and K.J. Eberhard. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. Pages 157-162. *In* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors) Conservation and management of freshwater mussels II: initiatives for the future. Proceedings of a UMRCC Symposium, October 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Williams, D.A. 1982. Extra-binomial variation in logistic linear models. Applied Statistics 31:144 -148.

Appendix

Formulae for estimating density using the regression estimator under the double sampling design are (Thompson 1992, Hedayat and Sinha 1991)

$$\hat{\mu}_{lr} = a^{-1} \left[\bar{y}_2 - \hat{\beta}_1 (\bar{x}_2 - \bar{x}_1) \right], \quad (\text{A.1})$$

with variance

$$\text{var}(\hat{\mu}_{lr}) = a^{-2} \left\{ \left(\frac{N-n'}{N} \right) \frac{s^2}{n'} + \left[\frac{n'-n}{n'n(n-2)} \right] \sum_{i=1}^n (y_i - \hat{\beta}_0 - \hat{\beta}_1 x_i)^2 \right\}, \quad (\text{A.2})$$

where a is the quadrat area, \bar{y}_2 is the mean total count from second-phase sample, \bar{x}_1 and \bar{x}_2 are the mean surface counts from the first and second-phase samples, $\hat{\beta}_0$ and $\hat{\beta}_1$ are estimates of the regression parameters (*i.e.*, intercept and slope), s^2 is the variance of total counts in the second-phase sample, N is the total number of quadrats at a site (*i.e.*, total site area/quadrat area), n' is the size of the first-phase sample, and n is the size of the second-phase sample.
