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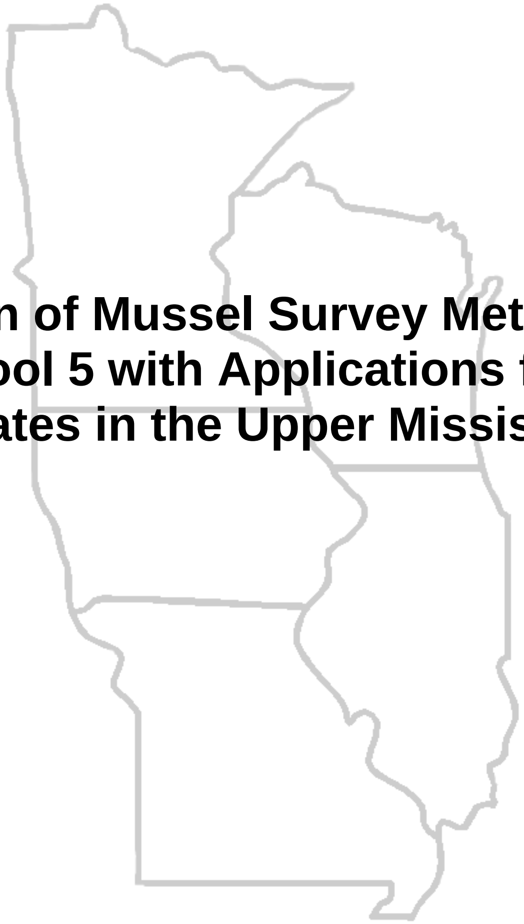

Documentation of Mussel Survey Methodology Deployed in Pool 5 with Applications for Future Poolwide Estimates in the Upper Mississippi River

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1. Background

Freshwater mussels (Unionoidea) are among the most imperiled of all taxa groups. For example, over the past 50 years, about 20 species have been lost from the Upper Mississippi River (UMR) basin (Wiener et al. 1995). Mussels are threatened by habitat changes due to dams, dikes, and levees; by increases in sediment loads; by contaminants; by exploitation; and by invasive species, such as zebra mussels and Asian carps that compete with mussels for food (Strayer et al. 2004). In addition, mussels are now potentially threatened by ecosystem restoration activities such as construction of barrier islands and water level drawdowns. Thus, recent years have seen a considerable amount of research into mussel biology and ecology.

Although not sufficiently documented, freshwater mussels may play important roles in riverine ecosystems. Mussels can dominate benthic biomass and production in certain environments (Hanson et al. 1988, Strayer et al. 1994). Adults can be important suspension-feeders, influencing water chemistry and clarity, and the amount and kind of suspended particles in the water (Welker and Walz 1998, Vaughn and Hakenkamp 2001). Recent data also suggest that their waste products can enhance local populations of algae and macroinvertebrates (Howard and Cuffey 2006, Vaughn and Spooner 2006). Collectively, these data suggest that mussels may play important functional roles in riverine food webs.

Much of the information on mussel populations has been developed on streams or small rivers, whereas large rivers (such as the UMR) have received much less attention. Large rivers that contain substantial floodplains are fundamentally different from smaller systems in their lateral complexity; thus, physical and biological processes may be quite different (Johnson et al. 1995). The lack of published studies on large rivers may be due in part to the difficulty and expense of sampling mussels in this environment. Small streams and rivers can often be sampled inexpensively by wading and hand collection, but adequate sampling of large rivers frequently requires more expensive diver-assisted sampling (e.g., Holland-Bartels 1990, Christian and Harris 2005) due to areas of deep and turbid water.

River managers use water-level drawdowns to rehabilitate habitats, particularly side channels and backwaters for vegetation and other desirable species on the UMR. Concerns for the immediate effects of a 2005 water-level drawdown on mussels in Navigation Pool 5 (Wisconsin Department of Natural Resources 2006) prompted scientists from the U.S. Geological Survey Upper Midwest Environmental Sciences Center, the U.S. Army Corps of Engineers, and the state of Minnesota to conduct a systematic population estimate of mussels during a second drawdown of the pool in 2006. The objectives of the Pool 5 mussel survey were to (1) estimate the number of live mussels in Pool 5 and (2) estimate the number of live mussels within the area expected to be dewatered in 2006 (to estimate the proportion of the total population that might be affected by the drawdown). To our knowledge, a systematic approach over such large spatial scales had not previously been conducted with native mussels. This report is an assessment of the design used in Pool 5, and discussion of suggested methods to be used in future surveys.

2. Methods Utilized for Sampling in Pool 5 in 2006

STRATIFICATION

The sampling frame (i.e., the population of potential sampling locations) for the Pool 5 survey in 2006 was determined using geographical information system (GIS) coverage of aquatic area types (Owens and Ruhser 1996). The sampling design included stratification of the sampling effort across two zones of interest: the shallow water zone where effects of a drawdown were most probable, and a deep water zone covering the rest of the pool. The shallow water zone was delineated as the area determined by aerial photography to be dewatered in 2005 and all depths less than 0.5 m as determined from an existing bathymetric coverage (Rogala 1999). The area with depths less than 0.5 m were added because our dewatered coverage was based on aerial photography taken early in the 2005 drawdown and did not include some areas that were later dewatered. This zone undoubtedly included areas that were unaffected by the drawdown, but was the best available estimate of the appropriate frame for estimating mussel abundance in the dewatered area.

SAMPLE SIZE SELECTION

Resource managers and scientists set the level of precision for the estimates to be within 20% relative error in the 95% confidence interval estimates. The required sample size (n) was estimated using

$$n = (1.96 * CV / (RP))^2 \qquad (1)$$

where CV = coefficient of variation = standard deviation/mean; and
RP = relative precision (relative error).

Sample size requirements were made using CV estimates based on two previous studies. Data from studies in Pools 9 (Sietman 2006) and 10 (Holland-Bartels 1990) suggested a range of CV between 0.5 and 2.0, with a lower CV found in shallow water surveys. Estimates of required sample size did not account for the cluster sample design (described below), thus the estimates of relative precision were conservative (i.e., actual precision would be expected to be better).

The target sample size and the expected relative precision are summarized in Table 1. The sample allocation was performed by first selecting the sample size needed in the shallow stratum, and then putting the remainder of the sites into the deep stratum. The total of 402 sites selected exceeded the target sample size of 375, but we expected that some locations would be inaccessible for sampling, so the final sample size would be less.

Table 1. Target sample allocation and expected performance of proposed mussel survey design for Pool 5 of the Upper Mississippi River. The best and worst case scenarios present a range for the expected performance based on the selected low and high coefficient of variation (CV) values.

					Best Case Scenario		Worst Case Scenario	
Stratum	**Area (ha)**	**Area**	**Sample Allocation**	**Allocation**	**Assumed CV**	**Expected RP**	**Assumed CV**	**Expected RP**
Shallow	545	12%	112	28%	0.5	9%	2	38%
Deep	3,820	88%	290	72%	1.0	12%	2	23%
Total	**4,364**		**402**					

SYSTEMATIC SAMPLING

A systematic sampling design using regularly spaced square grids was implemented for the Pool 5 survey, using the aquatic areas coverage from the Long Term Resource Monitoring Program (Owens and

Ruhser 1996). The area of each stratum (shallow and deep) was estimated, and the square root of the area was calculated to estimate the size of the sampling grid in each stratum based on the sample size allocation. The resultant grids had sampling densities of about one sample per 5 ha in the shallow zone and one sample per 13 ha in the deeper zone.

CLUSTER DESIGN

We used a simple cluster design deploying only two samples (subplots) per site. The samples within clusters were not randomly spaced, but were spaced about 10 m apart. Data from the subplots will allow us to infer if the cost savings and dissimilarity within clusters make the selected cluster design effective. The two subplots should not be considered as replicate samples because they are not continuous and there is some variability expected among the subplots.

RANDOM SAMPLING OF SITES IN SPACE OVER TIME

Particular care was given to ensure bias was not introduced from sampling over space through time. The primary reason was that if a drawdown influenced mussel densities over the sampling period, we did not want samples to be acquired in some spatial pattern (e.g., sample all sites in the upper pool first). There are other possible temporal patterns (e.g., naturally occurring trends in discharge over the sampling period) that could influence mussel distribution or detectability as well. Therefore, we assigned sampling locations to blocks that represented a day of sampling (about 15 to 20 sites per day). The sequence for sampling of these blocks was random, thus minimizing the potential for bias from sampling point locations. Additionally, the design provided for an unbiased sample if all sampling locations could not be visited in the allotted study period; missing data would be at random.

DATA ANALYSIS

We analyzed the data using survey sampling statistical software (Surveymeans procedure, SAS 2003). These methods accommodated our stratified cluster sampling design and the unequal probabilities across strata.

We used the intra-cluster correlation coefficient (ICC) as a measure of the variation between clusters relative to the total variance. The ICC estimates within-site correlation and was calculated separately for “shallow” and “deep” strata using analysis of variance (Surveymeans procedure; SAS 2003):

$$\mathrm{ICC} = s_b^2 / (s_b^2 + s_w^2) \qquad (2)$$

where s_b^2 = variance between clusters, and
s_w^2 = variance within clusters.

Effective sample size (ESS), a measure of the sample size adjusting for the information from within clusters, was calculated using

$$\mathrm{ESS} = mk/(1 + \mathrm{ICC}(m - 1)) \qquad (3)$$

where m = number of samples in a cluster,
k = the number of clusters, and
ICC = the intra-cluster correlation coefficient (Equation 2).

3. Evaluation of Methods

OVERALL PRECISION OF ESTIMATES

The estimated coefficient of variation (CV) for the shallow zone of Pool 5 was larger than the estimates from previous surveys. This resulted in a less than acceptable relative precision of 55% when compared to the predefined goal of 20% (Table 2).

Table 2. Actual Sample Allocation and Performance of the Survey Design for Pool 5 of the UMR

Zone	Sample Size	Mussel Density (Per M^2)	CV	Relative Precision	Sample Size Needed To Acquire 20% Relative Precision	Intra-Cluster Correlation Coefficient	Effective Sample
Shallow	93	0.32	2.73	55%	715	0.33	140
Deep	266	5.03	1.52	18%	212	0.63	326

The 2005 drawdown may have resulted in the low abundance of mussels observed in the shallow water zone in the 2006 survey. This would cause our estimates of relative precision to be high, because very low densities may result in larger CVs.

There are several reasons why this design might not be suitable for future attempts to estimate mussel abundance in shallow areas likely to be affected by drawdowns. First, it is nearly impossible, even with existing bathymetry, to estimate the area that might be dewatered. This frame error will lead to poor inferences on the potential effects of drawdowns on mussels. Second, even with a good frame, the effect on mussel mortality will depend on their ability to move to deeper water or withstand submersion into the sediments during the drawdown. With this unknown, the estimate would represent only the maximum of the true range in mortality. There may be effects on mussels other than mortality (e.g., reproductive disruption) where this concern is not valid. Third, the Pool 5 data suggest that about 715 sites would be needed in the shallow water zone to obtain our desired precision. This estimate is perhaps lower if the mussel mortality from the 2005 drawdown influenced the mean and its standard deviation obtained in 2006, but it would have to be significantly lower to make this design practical.

The relative precision in the estimate for the deep zone was found to be acceptable at 18%. The goal of 20% could have been attained at a somewhat reduced sample size of 212 (Table 2).

SYSTEMATIC SAMPLING

A systematic sampling layout with a random starting point was selected because, with modest assumptions about positive spatial autocorrelation, systematic designs are more precise (i.e., lower variance) than simple random sampling designs. This is largely because systematic sampling eliminates the potential for poor interspersion, such as odd clusters of locations that can be obtained with simple random sampling (Cochran 1977). If surveys are to be repeated over a period of years, it is possible to select a new systematic grid each year (resulting in independent samples) or revisit the same sample locations (resulting in a repeated measures design).

Variance from samples obtained with a systematic design can be estimated as though obtained from a random sample if the sampled units can reasonably be conceived as being in random order (Thompson 2002). Given the rather large minimum distance between sample points on the grid (225- and 375-m grid spacing for shallow and deep, respectively) and the general patchy distribution of mussels at small spatial scales, we assumed the sample units were close to being in random order. This assumption is conservative; variance will be overestimated if this assumption is false (Thompson 2002).

CLUSTER DESIGN

Cluster sampling designs can sometimes be an effective way to sample populations that are difficult to survey. The effectiveness of the cluster design is related to the cost savings from obtaining samples closer together, and the amount of similarity among the closely selected subplots. For mussel surveys, most of the costs are associated with traveling from site to site, setting up to dive (in deep sites), and taking individual dives (for deep sites). Mussels are expected to be distributed in such a way that some dissimilarity among samples within clusters (i.e., correlation among close sites is not high) might be expected. The effectiveness of cluster designs can be tested using the effective sample size and the cost associated with sampling primary and secondary units.

In the cluster design used in the 2006 Pool 5 survey, the sites are the primary units and the two quadrats (subplots) are the secondary units. Intra-cluster correlation coefficient values near one suggest that within cluster correlation was high relative to among cluster correlation (i.e., subplots are relatively similar; sites are dissimilar), which in turn suggests little benefit from the cluster design. Values near zero would suggest that correlation within clusters was low relative to among cluster correlation (i.e., subplots and sites are equally dissimilar), and each sample at the secondary unit (each subplot) can be thought of as a simple random site. Seldom are ICC values near zero, as we expect more correlation among measures that are closer to each other in space.

The ICC values from the Pool 5 survey were 0.33 and 0.63 for the shallow and deep water zones, respectively (Table 2). Another meaningful measure is the ESS, which adjusts for the extra information gleaned from the two subsamples per site. The ESS can be compared to the cluster (site) sample size to determine design efficiency. For the shallow water zone (n=93), the ESS was 140; for the deep water zone (n=266), the ESS was 326 (Table 2). Interpreting these values for the purposes of determining the effectiveness of the cluster design is somewhat subjective, as the true cost of sampling another site versus the subplot is unknown. However, the cost ratio of the subplot to site would have to be less than 0.50 (i.e., cost ratio = [140-93]/93) for the shallow water zone to benefit from the clustered sampling design, and less than 0.23 (i.e., cost ratio = [326-266]/266) for the design to be beneficial in the deep water zone.

Field personnel estimated a second quad to a new site ratio of 0.24 for the shallow water sites, and 0.38 for the deep water sites. Using these subjective estimates of sampling cost, it appears that the cluster design was beneficial in the shallow zone (0.24 < 0.50), but not beneficial in the deep zone (0.38 > 0.23). Better estimates of relative cost may be determined in the future by analyzing sampling time data from various surveys. Given the apparent advantage of the cluster design in shallow areas and closeness of the actual cost ratio estimate to the beneficial ratio estimate (0.38 compared to 0.23) in the deep areas in Pool 5, we suggest collecting additional data with the cluster design before switching to another design.

MISSING DATA

Only 359 sites out of the 402 selected were sampled. The missing data can provide the potential for a biased sample of the population. The sources for the missing data included sites found to be terrestrial, sites in isolated aquatic areas that would require extreme efforts to reach, sites in locations where diving was not feasible for safety reasons, and samples not acquired due to the ending of the sampling period.

We have not attempted to evaluate the potential for bias as a result of missing data, but will suggest ideas for consideration in subsequent poolwide surveys. Selecting a study area (frame) in a prudent way can minimize the number of edge sites that are terrestrial. The risk with this approach is that the shallow portion of a pool may be under-sampled. Using a liberal frame (i.e., one where sites are more likely to be aquatic) assures that samples are obtained in the shallow areas. Although the sites that are terrestrial could be handled as frame errors, it is not necessary to do so when estimating total abundance (i.e., terrestrial sites will not influence the total abundance as the extra area they represent will always be associated with zero abundance). However, these frame errors could affect the variance estimate because these sites add only zeros to the sample.

4. Recommendations for Future Mussel Poolwide Surveys

FRAME SELECTION

As discussed above, the best frame is one that encompasses all aquatic areas. The Long Term Resource Monitoring Program has GIS coverages of land/water that are based on vegetation cover types and absence of vegetation in water and on land. Coverages of aquatic area types provide additional information for developing the sampling frame. For general poolwide surveys, we recommend including all isolated areas that may be connected during high discharges. However, isolated areas will probably have low abundance and different species composition, so, depending on the objective of the survey, these areas could be considered outside the area of inference. Those areas isolated by high levees, or those managed as moist soil units, should always be excluded from the sampling frame. We recommend excluding tributaries from the sampling frame, as the relevance of mussels in tributaries to the study reach would extend further upstream than their extent within the floodplain.

SITE SELECTION

For the Pool 5 sampling, a size for the regularly spaced square grid was estimated based on the square root of the surface area of the sampling frame. These methods produced a sample size that was not as close to the desired sample size as might be needed. A different method using a GIS can achieve the target sample size much better. We have written a GIS program to create grids with spacing that more accurately provides a selected sample size within the sampling frame. This is accomplished through an iterative process to adjust the grid as needed to produce the desired sample size.

SAMPLE SIZE

The sample size needed to estimate poolwide abundance in previously unsampled pools at given precision levels is still subject to some uncertainty. The 2006 Pool 5 survey provides an estimate for the deep water zone, and, given that the deep water zone was 88% of the area, that might reflect an approximate sample size needed. However, the mean and standard deviation of the mean may differ greatly across pools in the UMRS, and therefore a larger (or smaller) sample size may be needed in some pools. Additional poolwide surveys will provide a measure of where Pool 5 falls on the distribution of poolwide abundance among all pools. We recommend continued use of a sample size greater than the number that the Pool 5 data suggest until we know more about the differences in abundance and variances among pools.

If the goal of the poolwide surveys is to provide information of some metric other than total mussel abundance, then sample size may need to be adjusted. For example, abundance of common species may be desired, but these estimates would probably require a larger sample size to obtain similar relative precision in the estimates. It is obvious that good abundance estimates of uncommon and rare species will not be obtained with the methodology used in Pool 5. Similarly, if abundance estimates for mussels in specific age classes is desired, the sample size must be increased to obtain the precision equivalent to that obtained for poolwide total mussel abundance in Pool 5.

OTHER DESIGN CONSIDERATIONS

Other sampling designs that show promise in estimating mussel abundances in large rivers are discussed below. Please note that we are currently working with a collaborator at the USGS Leetown Science Center in West Virginia to evaluate efficiency of multiple sampling designs for obtaining relative density and population estimates of mussels over large (poolwide) and smaller (e.g., footprint of an island project) spatial scales.

A systematic sampling with multiple random starting points could be considered in the future. This design is recommended by Strayer and Smith (2003) to avoid random selection of a single set of many possible random sets of sampling locations. Given our present recommendation to use a clustered design (e.g., sample multiple subplots at each site), we suggest using multiple starts provides some challenges in data analyses. Those challenges are related to clusters (subplots at sites) within clusters (multiple random sets of sites) in a design that would use multiple starts. For now, we suggest that the assumptions about spatial distribution are valid, and the variance estimate obtained by treating the systematic sample as though it is random is acceptable. Applying a random start design in the future would provide some evidence as to whether the assumption is truly valid.

The cost of obtaining samples by diving and the heterogeneous spatial distribution of mussels make an adaptive sampling design attractive. Adaptive sampling provides for more samples in some areas based on some *a priori* criteria being met during the sampling. Additional samples are obtained where we anticipate a higher likelihood of the organism to be present. For mussels, which often have clustered distributions (e.g., beds), the advantage of a cluster design is obvious. However, the benefit from an adaptive sampling design would be less for poolwide surveys of total mussel abundance than the benefit for abundance estimates of individual species or details at smaller spatial scales.

Two-phase sampling might be another sampling method to consider for obtaining estimates of similar precision with less cost (Strayer and Smith 2003). These designs integrate quantitative methods (i.e., excavation of quadrats) with semi-quantitative (e.g., surface detection of mussels) or fully qualitative methods to determine detection probabilities. As with adaptive sampling methods, these should be considered for surveys where more resolution is needed with regard to individual species or detail at small spatial scales. The two-phase sampling and adaptive sampling designs can be integrated into a single design for specific surveys.

We recommend using a cluster design in future surveys until stronger evidence suggests the design should be dropped. The efficiency of the design would be greater as mussel density decreases, time of travel from site to site increases, or dive preparation time increases. There are also other cluster designs that might be considered, particularly adaptive sampling designs.

In conclusion, we recommend using a systematic cluster design in future surveys until additional data suggest that a different design may be more robust and cost effective. The present design can be expected to be altered as new information regarding these methods is obtained from repeated use. In addition, slightly different objectives for surveys may require considering other designs.

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