Sampling Adequacy in Population Studies of Freshwater Mussels

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Abstract. Sample sizes in 16 recent mussel surveys of the Upper Mississippi River were compared. Diving (either scuba or surface-supplied) was the primary sampling technique. The quadrat size was usually 0.25 m^2, and some type of handpicking or whole-bottom sample removal was used. The sample size, when statistically determined, seemed to be based on total density of all mussels in the bed and assumed random distribution. The importance of aggregation and the density of noncommercial species in the determination of sample size was evaluated. Based upon these factors, it seems that there is a need to increase sample size in future studies. The need for whole-bottom samples to obtain juvenile size classes is stressed.

Introduction

Before 1980, sampling of mussel populations on the Upper Mississippi River, with the exception of Ellis' 1931 semiquantitative survey (van der Schalie and van der Schalie 1950), consisted primarily of qualitative collections by handpicking, shore line collections of dead shells, and brailing (Fuller 1978, Perry 1978). Although these techniques were adequate to describe species assemblages and reflect relative abundances, they could not reflect productivity, density, recruitment, or quantitative changes in the population as a result of commercial or natural modifications of the riverine environment. With an increased awareness of the need to address these problems, brought on by the demands of the National Environmental Policy Act and the Endangered Species Act, sampling studies of mussel populations in the river began to use linear transects or defined area quadrats to supplement or replace brailing and expressed results as density per square meter.

Since 1980, papers dealing with the sampling of mussels in large rivers have reflected growing concern with the need for adequate quantitative sampling of mussel populations (Nelson 1982, Kovalak et al. 1986, Isom and Gooch 1986, Cawley 1989, Miller and Payne 1988). To investigate the impact of these concerns, 16 surveys conducted on the Upper Mississippi River since 1985 were reviewed. The studies ranged from biologic assessments of potential impacts of construction projects along the river (Berlocher and Wetzel 1987, 1990; EA Science and Technology 1986; Heath 1989; Helms 1989, 1990; Sparks and Blodgett 1985; Stanley Consultants 1987a, b, 1988), evaluation of commercial harvest effects (Heath et al. 1988), base line studies of mussel refuges (Cawley 1989, 1990; Koch 1990), and the long-range monitoring program (Miller et al. 1990, Miller and Payne 1991). Although some studies conducted since 1985 are not included, an attempt was made to include studies from most of the research organizations presently at work on the freshwater mussel populations of the Mississippi River.

Results

The sampling techniques were divided into three categories: sampling criteria, quadrat placement, and sample removal (Table 1). Sampling criteria could be separated into three types: qualitative, defined area, and quantitative. All of the studies reported relative values, but one-third of them sampled in such a way (brailing or handpicking by divers over an undefined area) that they could only express their results in a qualitative form. Half of the studies used some type of a frame to define a sampling area, but if there was no estimate of variance between the samples it was not considered a quantitative sample since it was impossible to evaluate possible error. Forty-four percent of the studies used some measure of error in their estimate of population density and were considered quantitative for this paper.

All the studies used some level of randomness in quadrat placement. Thirty-one percent set out quadrats "at random" to get the samples distributed over a given area. One-quarter of the studies distributed the samples in much the same way but took subsamples at the selected locations so they could calculate variability. The remainder used a struc-

tured sampling plan to evaluate the spatial distribution and density of mussel populations.

Some topics were covered in almost all of the study reports, but some information was found in only a few reports (Table 2). In general, all the reports discussed the samples in such a way that species variability within the study area could be examined. In two studies (12%), absolute numbers of mussels in sample frames were reported by the divers, but then the samples were pooled on the bottom so it was impossible to determine any patterns in species composition.

With the exception of two studies concentrating on the harvest of particular species, all the reports included the biotic parameters of richness, relative abundance, and density. Size demography was discussed in 38% of the reports, and only 25% calculated any of the most commonly used indices of diversity.

A variety of statistical measures were used (Table 3). The most common approach was to report the mean density per quadrat, but since the quadrat samples were often intentionally taken in those parts of a mussel bed with the greatest abundance, the values are biased and can only be used as an estimate of maximum density in a particular part of the bed. Mean density per square meter was reported in 50% of the studies, using variance to estimate confidence limits. However, in all but two of the reports normal distribution was assumed in the calculations, so the estimate can only be used to reflect the density in the areas within the quadrats. In two of the studies, data were transformed to take into consideration the aggregation found in the mussel bed, and these studies reported the data for total area. Range, and more commonly variance, was used to evaluate variability within the populations.

Only 25% of the reports attempted to evaluate the sampling adequacy of the study (Table 4). These reports determined that sufficient samples were taken to estimate the total population of all species. These estimates assumed a normal (Poisson) distribution; however, the aggregation present in most mussel populations results in a biased estimate using parametric statistics. One group of studies (12%) evaluated aggregation, transformed the data to the negative binomial distribution, and reestimated sampling adequacy.

Table 1. Sampling techniques.

Method	% of studies surveyed
Sampling criteria *	
Qualitative	37
Defined area	56
Quantitative	44
Quadrat placement	
Random	31
Random, subsamples	25
Structured random	44
Sample removal	
Underwater handpicking	62
Complete bottom sample, surface sort	38

* Some sampled using more than one technique.

Table 2. Report format.

Topics included	% of studies surveyed
Sample treatment	
Pooled samples	12
Separate samples	88
Biotic parameters	
Species richness and abundance	88
Species diversity indices	25
Species density/area	88
Size demography	38

Table 3. Statistical treatment.

Measures included *	% of studies surveyed
Mean: number / quadrat	56
Mean: number / m^2 – quadrat area	50
Mean: number / m^2 – total area	12
Range: number / quadrat	31
Variance – standard deviation	50

* Some reported more than one statistical measure.

Table 4. Sampling evaluation.

Topics included	% of studies surveyed
Population normal or aggregated	12
Sampling adequacy – normal distribution	25
Sampling adequacy – neg. binomial distribution	12

Discussion

Sampling of a population can be used to characterize an assemblage of mussels in a particular locality, compare it with other geographic regions, examine trends over time, or evaluate changes in populations in response to environmental modification.

Qualitative studies can be used to assess broad geographic changes, changes in species richness over time, or major changes in the mussel fauna resulting from large-scale environmental modifications. In general, diving acquired greater numbers of mussels than brailing and supplied more data for the time expended. Multiple small plot samples laid out, with subsamples, along transects were more efficient than random searches by divers.

Quantitative studies of mussels can reflect the biomass or productivity of an area along a stretch of

river (a bed). They can be used to describe populations of common species in this same area of the river, including density and diversity indices as well as species richness and abundance and size demographics. If spatial and temporal comparisons are planned, it is important that density is reported on an absolute basis. In general, the more quantitative the sampling methods, the more sensitive the study will be in reflecting changes in populations in response to environmental modification. There are two restrictions to this general statement. First, changes in rare species can seldom be monitored by quantitative sampling due to the low probability of their occurrence in sufficient numbers in the samples (Kovalak et al. 1986). Second, the sensitivity of the method will depend upon the adequacy of the sample. A sample with an error of 30% can not be used to determine a 10% change in a critical population.

Quantitative sampling not only requires that a defined area quadrat be used but also assumes that an adequate number of samples are taken (Eckblad 1991). Sample size can be estimated with the following formula, assuming 95% confidence limits (Krebs 1989):

$$n \approx \left(\frac{200CV}{r}\right)^2$$

where n = sample size, r = desired relative error (as a percentage), and CV = coefficient of variation = $1/\sqrt{\text{mean}}$. We can use this same formula and solve for the relative error using the sample size.

It has been widely recognized that mussel aggregations or beds are not evenly distributed in rivers. Mussels may be locally abundant, but broad areas of the river may have few or no mussels. Even within a bed, mussels can exhibit extreme aggregation. Many studies in the past have only quantitatively sampled these "high density" areas and then reported bed density from these selected samples. This obviously results in a bias toward high density. If the studies are to be used to evaluate changes in mussel populations, they must be converted to number per total area of the bed, not to areas of the quadrats sampled.

Even studies that have used a structured sampling plan and reported mean density from the entire area may be biased if the population is aggregated. An index of aggregation (variance/mean) was calculated for all studies that reported adequate data. Only 7% of the beds were not aggregated.

As a result of the aggregation, normal parametric statistics should not be used in interpretation or comparison of these populations. Krebs (1989) suggested that many aggregated populations could be fitted to a negative binomial distribution. If this is true, the data can be transformed and comparisons may be possible. Sample size can be estimated for a negative binomial distribution with the following formula, assuming 95% confidence limits (Krebs 1989; a Macintosh computer program to carry out these calculations and fit data to the negative binomial distribution is available from the author):

$$n \approx \frac{(200)^2}{r^2}\left(\frac{1}{\bar{X}} + \frac{1}{K}\right)$$

where n = sample size for negative binomial variable, $\bar{X}$ = estimated mean of counts, K = estimated negative binomial exponent, and r = desired level of error (percent).

This transformation was carried out in two of the studies. As a result of these transformations, mean density of the beds decreased, and required sampling intensity increased. This procedure may result in as much as a 10-fold increase in sample intensity, but using these transformations makes it possible to report total density of the bed rather than just the quadrat areas.

The low number of juvenile mussels found in past studies has been attributed to sampling bias from brailing or hand picking in low visibility conditions. Six of the studies reviewed in this paper use "total quadrat samples." Sampling in five of them used transfer of the complete sample into a container, a bucket or fine mesh bag, on the bottom for sorting on the surface with 5–6 mm sieves (Cawley 1989, 1990; Heath 1989; Miller et.al. 1990; Miller and Payne 1991); the remaining study used a hydraulic siphon to remove the entire sample to the surface, where it was passed through 2-mm sieves (Koch 1990). With the exception of one bed, they all found a similar low density of juveniles under 10 mm, ≈ 0.05% of total mussels sampled. These consistent numbers with sieve sizes from 6 mm to as small as 2 mm seem to indicate that a 5-mm sieve is adequate to sample juvenile mussels. The smallest mussel reported in any of the studies was 6 mm, in a sample sieved through a 5-mm bag. These low juvenile densities do not fit any normal population model and may reflect a serious decrease in all mussel species. These results are from a five-year span so they do not seem to reflect a "low year." This trend needs to be closely monitored since in long-lived species the abundance of adults may mask recent reproductive failure, especially when low densities were assumed to be due to sampling artifact.

Summary

The knowledge gap in our understanding of the population dynamics of naiad mussels, even after almost 20 years of surveys on the Upper Mississippi River, indicates that future studies should be structured to address questions of density, diversity, and size demographics rather than just taxonomy and relative abundance. The use of common surrogate species as indicators of impacts that are affecting rare species (which are difficult to sample) has been recognized (Miller et al. 1990).

Based on this situation the sampling design of all studies that seek to evaluate changes in mussel density over time should include:

1. Total bottom samples, sieved through a 5–6 mm mesh.
2. A structured sampling plan that includes subsampling to allow calculation of variance within a bed.
3. Preliminary sampling to use in estimating a sample size with sampling error at a level appropriate to the experimental design.
4. A calculation of an index of aggregation following the sampling.
5. If aggregation is present, fit the data to a negative binomial distribution. Calculate K to use in transforming the data.
6. Use the original or transformed values to calculate sampling error. If the error is insufficient to fit the experimental design, expand the number of samples.
7. Report species richness and abundance, density, diversity indices, and size demography.

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