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Development and Assessment of a Sampling Design for Mussel Assemblages in Large Streams

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ABSTRACT.—Freshwater mussel beds of the lower 68 km of the Cache River, Arkansas, were delineated, sampled using dive techniques and a stratified random sampling methodology and analyzed for density and species richness. A total of 38 mussel beds were delineated, 14 major beds (*M*beds) and 24 minor beds (*m*beds), and defined by areal extent and mussel density. Analysis of our sampling precision indicated 80% or better confidence levels for a majority of our sites and suggested that a sample size of 15 1-m^2 quadrats is sufficient to obtain 80% or better confidence. Our large river diver-assisted sampling methodology has been shown to be a useful and appropriate methodology for obtaining large geographic scale baseline distribution (bed and species), species richness, density and population and community numerical standing crop estimates information where tradeoffs are required in order to complete a project within time and budget constraints.

INTRODUCTION

Over 300 species of freshwater pearly mussels reside in the continental United States (Turgeon *et al.*, 1998). However, within the last 50 y, this rich fauna has been decimated by impoundments, sedimentation, channelization, dredging, water pollution and invasive species (National Native Mussel Conservation Committee, 1998). Approximately 67% of the freshwater mussel species in the United States are vulnerable to extinction or are already extinct, and more than 1 in 10 species may have become extinct in this century (Williams *et al.*, 1993; Bogan, 1997; Master *et al.*, 1998).

Freshwater mussels are renewable resources that provide both ecological and economic benefits. They are ecologically important as a food source for aquatic and terrestrial animals, improve water quality by filtering contaminants, sediments and nutrients from aquatic systems and serve as an early warning system for water quality problems (National Native Mussel Conservation Committee, 1998; Vaughn and Hakenkamp, 2001). Currently, endangered species are regarded as indicator organisms in an ecosystem and efforts are being made to preserve not only the species and its habitat, but as much of the entire ecosystem as possible in order to prevent further degradation (Watters, 1992, 1993; Anderson, 1993; Doppelt, 1993; Richter, 1993). Economic benefits have been derived historically from the harvest of pearls and the production of buttons from shells. Recent economic benefits are found in the sale of mussels to the Far East where they are formed into beads for the thriving cultured pearl industry. However, the demand for beads has declined since 1995 due to the loss of the Akoya pearl oysters in Japan and new techniques for cultured pearl production in China, reducing the $50 million dollar industry of the mid 1990s to a much lesser valued industry (Neves, 1999).

In order to conserve native freshwater mussels in the U.S., the National Native Mussel Conservation Committee (1998) identified 10 specific problems. Among these problems was the lack of knowledge regarding current distribution and health of mussel populations

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(Problem 4). Strategies 4.1–4.3 regarding alleviation of this problem are germane to this study. These strategies include: (4.1) determining location, density, species composition and status of existing mnssel commnnities, (4.2) gathering historic mussel distribution data and making it readily available and (4.3) gathering information on the occurrence and abundance of mussel stocks that have valne for the commercial mussel industry. Surveys that address this problem have proliferated in recent years (Williams and Schuster, 1989; Ahlstedt and McDonough, 1993; Miller *et al.*, 1993; Siemsen, 1993; Ahlstedt and Tuberville, 1997).

Historically, status surveys for freshwater mussels usually consisted of wading in a stream or walking along the stream bank picking up live mussels or relic shells (*e.g.*, Gordon, 1982; Hoggarth, 1992; Watters, 1994), which would hamper collecting from deeper and/or more turbid waters. Furthermore, until recently (Smith *et al.*, 2001; Strayer and Smith, 2003), there were few standardized protocols for sampling freshwater mussels for status surveys and research purposes with little information for sampling large blackwater streams (Isom and Gooch, 1986; Huebner et al., 1990).

This study was part of a larger project identifying, delineating, mapping, sampling and estimating population and community numerical standing crops of mussel heds along 1380 total km in 10 rivers and 185 total km in three impoundments and one natural (oxbow) lake in Arkansas from 1991–1997. The results of these studies are not discussed here, but are summarized in other sources (Harris *et al.*, 1993b; Rust, 1993; Christian, 1995; Davidson, 1997; Posey, 1997). The objectives of this paper were to describe the methodology used to survey large river mussel populations and assemblages and to determine the effectiveness of this sampling methodology. This methodology may be useful in other large blackwater streams where density, species richness, size frequency, population and community numerical standing crops and impact assessments for freshwater mussel assemhlages are needed.

FIELD-SITE IDENTIFICATION

The Cache River headwaters originate on the western slope of Crowley's Ridge in Butler County, Missouri, and the river flows southwesterly in the Western Lowlands portion of the Mississippi River Alluvial Plain (Saucier, 1974; Royall, 1988) until joining the White River near Clarendon, Monroe County, Arkansas (Fig. 1). The watershed is approximately 230 km long with a maximum width of approximately 29 km and a drainage area of >5240 km^2 (Smith, 1996). Discharge at Patterson, Woodruff County, Arkansas, approximately 49 river km upstream of Cotton Plant, Woodruff County, Arkansas, ranges from a 7-d average low flow of 3.0 m^3/s to a seasonal high flow of 225 m^3/s (Smith, 1996), with a mean annual discharge is 35.7 m^3/s (Kleiss *et al.*, 1989). The main channel and tributaries in the upper third of the watershed were dredged and channelized during the 1920s and 1930s to drain the land for agricnltural use (U.S. Army Corps of Engineers, 1974). The entire river channel upstream of Grubbs, Jackson County, Arkansas, was straightened and, in one segment, a double channel was constructed. The chaunelized segment of river drains 2072 km^2 of the 5180 km^2 basin. Downstream of Grubbs, and throughout most of the study area (the lower 68 km), the Cache River flows in a meandering natural channel.

Most mussel heds in the lower 68 km of the Cache River begau at the head of a lateral scour pool (sensu Bisson *et al.*, 1981) (bendway) with point bars associated across from the high clay bank (Christian, 1995). Upstream of the high clay bank and lateral scour pool, a small but distinct area of slower current and high siltation, *i.e.*, the secondary channel pool (sensu Bisson *et al.*, 1981), usually marked tbe beginning of substantial mussel densities. Moving downstream into the lateral scour pool, the current and water depth increased. Concurrently, the thalweg (deepest portion of channel) narrowed and substrate was swept clear of encroaching sands. The thalweg substrate, which provided the most suitable mnssel habitat,

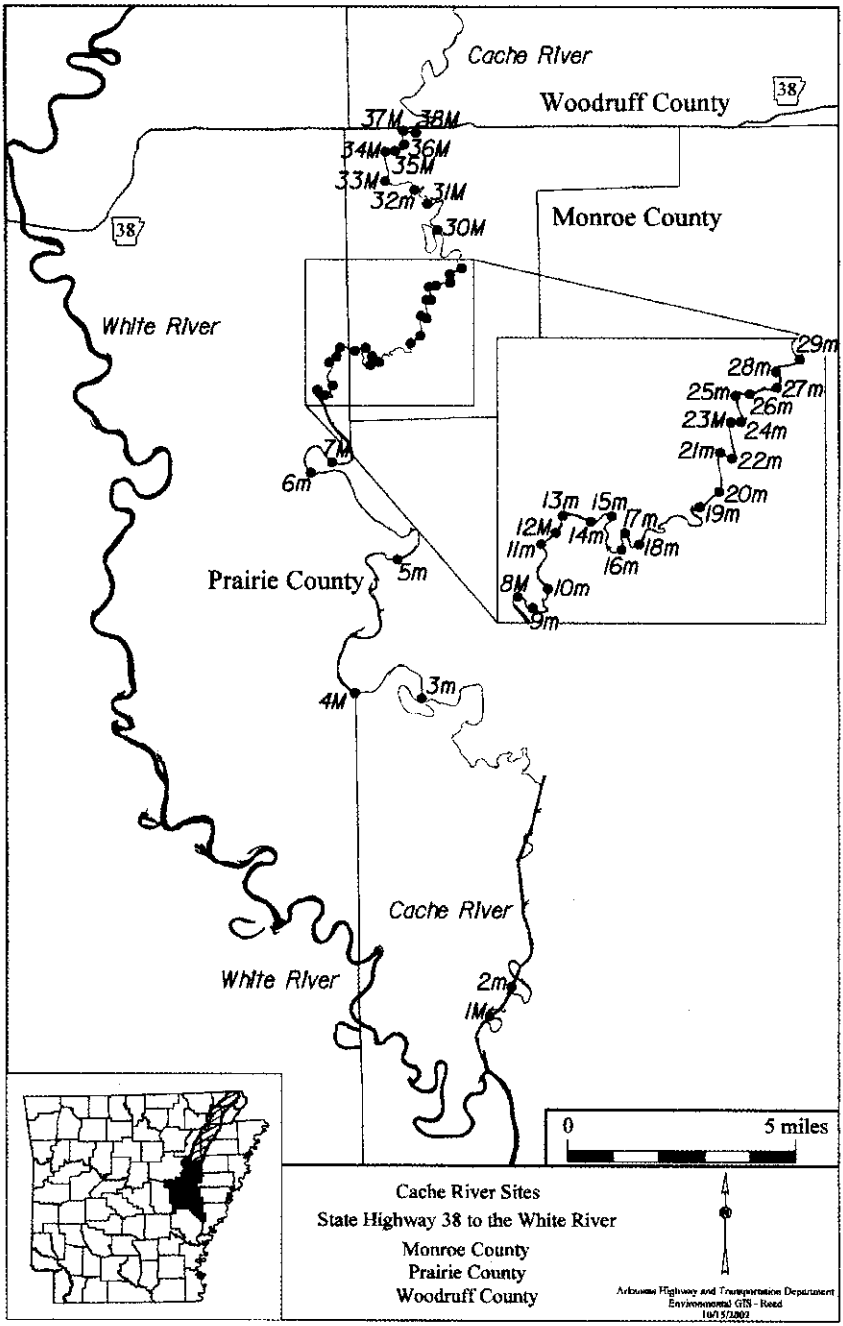

FIG. 1.—Cache River watershed (crossbar and shaded area of lower left figure) and study area (shaded area of lower left figure) and major (*M*) and minor (*m*) beds of the lower 68 km of the Cache River, Arkansas, from the State Highway 38 bridge to the confluence with the White River in Monroe, Prairie and Woodruff counties, Arkansas

consisted of soft-to-hard clay and usually extended to the soft clay of the near ascending bank. Thalweg width increased and water depth decreased downstream of the lateral scour pool into the mid channel pool where substrate usually consisted of sand (Christian, 1995).

Center channel substrate in the mid channel pool consisted of an ascending mid-channel bank of sand substrate grading into a soft or hard clay substrate. On the opposite margin, an ascending near-shore bank of soft clay met the expanded clay substrate. After the widening of the thalweg, bed width decreased as the encroaching sands covered the clay substrate, leaving little suitable mussel habitat. Smaller beds consisted of only an encroaching sand-soft clay interface at the ascending near-shore clay bank, and this was generally located in the thalweg, where depth increases slightly in comparison to surrounding substrates. This narrow band, defined here as the transition zone, was usually one meter or less in width but often extended several hundred meters downstream. Within this band, mussel densities commonly reached $>10/m^2$ (Christian, 1995). Visibility during the entire survey time was effectively <0.01 m and water depths generally ranged from 2 to 10 m, with an average of ~5 m.

MATERIALS AND METHODS

A questionnaire was sent to each state licensed mussel taker in Arkansas during late 1990 to gain preliminary information on commercial mussel bed locations. Additional information on Cache River commercial mussel beds was compiled from interviews and field reconnaissance with experienced commercial shellers between 1991 and 1994. Areas mapped as commercial beds and unmapped freshwater mussel habitats were searched by dive techniques using a Hookah System, an oil-less surface air-compressor with a breathing line attached to a regulator. Initial searches of probable freshwater mussel bed habitats were conducted in an upstream-to-downstream fashion to determine the limits of a potential bed. Divers counted mussels within arms reach and estimated the number of live mussels within a 1 m^2 area while searching and substrates were explored by feel while moving across the area in question. If the number of mussels was determined by the diver to be $\geq 10/m^2$, the diver estimated the width of the bed by traversing from the inshore to mid-river limits in 1 m increments. Additional searches (or transects), which always consisted ≥ 3 search areas per potential mussel habitat reach, were conducted downstream within the reach until the substrate was determined to be uninhabited by mussels. Total bed length was measured using a distance finder. Site locations were recorded on a reproduced 7.5 min topographic quadrat map and recorded in degrees, minutes and seconds based on a Global Position System. Water depth, determined by a depth finder, and river morphology (*e.g.*, bendway or straightaway) were recorded. Additional information, such as substrate type, density of mussels, dimensions of the investigated bed and species composition in each area searched, was compiled based on a briefing given by the diver subsequent to each dive. This information was used as a prerequisite for determining whether a more intensive sampling effort should be initiated.

Following the initial searches, an area was categorized and sampled by one of three methods, as follows: (1) relative abundance, species composition and general habitat information were recorded for areas with mussel densities averaging $<10/m^2$, but with no limit as to total bed area, (2) minor beds (*m*beds) were areas >500 m^2 with sporadic densities $>10/m^2$ or areas <500 m^2 with mean densities $>10/m^2$ and were inventoried with five 1-m^2 quadrates taken in areas of highest density along the length of the bed, and (3) major beds (*M*beds) were mussel beds with mean densities $>10/m^2$ and occupying an area >500 m^2 and were the most intensively sampled areas with a sample size of 10–25 1-m^2 quadrats.

If appropriate, *M*beds were first divided into strata based on substrate composition (*e.g.*, gravel, sand, silt, clay), physical river morphology (*e.g.*, bendway or straightaway) and/or river depth. The number of samples taken from a bed was determined by total bed area: a minimum

of 10 1-m^2 samples from beds of 500–999 m^2 area; a bed with area between 1000–2500 m^2 was sampled by one percent of the area (*i.e.*, 10–25 samples); and a bed with area >2500 m^2 was sampled by a maximum of 25 1-m^2 samples. The number of samples taken from each stratum was based on the proportion of stratum size to total bed area; however, a minimum of three samples was taken from each stratum for statistical validity. Quadrat sample sites were determined from a random numbers table generated in SYSTAT (Wilkerson, 1990).

Divers hand collected mussels within a 1-m^2 2.5 cm weighted PVC pipe quadrat. Mussels were placed in a mesh dive bag and transported to the surface for identification. Nomenclature followed Turgeon *et al.* (1998). Length or depth measurements, collected in accordance with the legal harvest dimensions set by Arkansas Game and Fish Commission, and total wet mass were recorded. Mussels were then returned to their collection location.

The relative error of our sampling protocol was estimated using two methods. One described by Southwood (1978) is represented by:

$$n = (s \div (Ex))^2$$

where n = sample size, s = standard deviation, E = standard error as a decimal and x = mean richness or density. The second, discussed by Downing and Downing, (1992) is represented by:

$$\check{n} = m^{-0.5}D^{-2}$$

where m is the average density and D is SE/m where SE is the standard error of the samples to estimate the relative error of sampling sizes in determining mean species richness and density. These were calculated to determine the number of quadrat samples needed to estimate mean species richness and mean density with 80% and 90% confidence limits.

We also assessed our ability to sample all species within a bed by comparing our observed species richness to first and second order jackknife estimates using PC-ORD software (Mccune and Mefford, 1999) where:

$$\text{Jack1} = S + r1(n - 1)/n$$

where S = the observed number of species, r1 = the number of species occurring in one sample unit, and n = the number of sample units and

$$\text{Jack2} = S + r1(2n - 3)/n - r2(n - 2)^2/(n(n - 1))$$

where r2 = the number of species occurring in exactly two sample units.

RESULTS

Survey of the lower 68 km of the Cache River required 94 person days to complete. Approximately 138 river reaches were explored by multiple dives resulting in delineation and sampling of 14 *M*beds and 24 *m*beds (Fig. 1). Twenty-six species were identified during this survey from among 5686 specimens examined. A total of 170 1-m^2 quadrats from 14 *M*beds yielded 3621 mussels. *M*beds ranged in size from 500–3420 m^2 and were sampled by 10–25 1-m^2 quadrats representing 0.3–2.0% of the bed area (Table 1) and possessed a variety of substrates including silt, sand, soft and hard clay, gravel and gastropod shells.

A total of 24 species was identified with richness ranging from 7–18 at 1*M* and 38*M*, respectively (Table 1). Sampling effort located 7–18 species within *M*beds, whereas the first and second order jackknife estimates of bed richness were slightly higher at 7–25 and 7–29, respectively (Table 1). Mean species richness per m^2 ranged from 3.1–7.5 with calculated confidence levels of 79–94% (Table 1). The number of m^2 quadrats needed for 80% confidence in mean species richness ranged from 1–11, whereas 5–43 samples were required to obtain a 90% confidence level (Table 1).

TABLE 1.—Cache River *M*bed areas, samples taken, species richness, first and second order Jacknife estimates and estimated sampling error associated with species richness

*M*bed	Area (m^2)	Samples (n)	Proportion sampled (%)	Total richness	1st order Jacknife estimate of richness	2nd order Jacknife estimate of richness	Mean spp./m^2	Confidence level (Southwood 1979)	n required for 80% conf. level (Southwood 1979)	n required for 90% conf. level (Southwood 1979)
01	900	10	1.1	7	7	7	3.5	86	5	20
04	8000	25	0.3	13	14	14	5.5	93	4	14
07	3420	25	0.7	17	25	29	7.3	94	3	10
08	600	10	1.7	13	16	15	4.2	83	7	29
12	1026	10	1.0	14	19	18	5.4	85	6	24
23	800	10	1.3	13	18	20	5.0	88	3	13
30	533	10	1.9	11	16	19	4.2	83	8	30
31	600	10	1.7	12	15	16	6.0	90	2	9
33	600	10	1.7	13	19	23	3.1	79	11	43
34	550	10	1.8	11	13	12	4.9	93	1	5
35	750	10	1.3	13	12	9	6.2	87	4	17
36	675	10	1.5	16	21	26	5.3	89	3	13
37	500	10	2.0	15	18	18	6.1	83	7	30
38	800	10	1.3	18	19	17	7.5	89	3	11

Mean densities ranged from 6.2 to 44.1 mussels/m^2 with an overall mean of 20.4 mussels/m^2 (SD $\pm$ 10.4) (Table 2). Based on the Southwood (1978) calculation, the confidence level of density estimates ranged from 75–89% with a range of 5–16 and 18–63 samples needed to obtain 80% and 90% confidence, respectively (Table 2). Meanwhile, the number of samples needed to obtain 80% and 90% confidence based on the Downing and Downing (1992) calculation was 4–10 and 15–40, respectively, and were generally lower than the Southwood (1978) calculations. This indicated that our sampling effort obtained 80% confidence levels at all 14 *M*beds (Table 2).

DISCUSSION

Our large river, diver assisted sampling methodology included delineating the mussel bed area, defining strata if applicable and then randomly sampling the strata using 10–25 1 m^2 samples to reduce the variability in heterogeneous nature of mussel distribution. Based on the overall project objectives to survey the mussel beds of 10 rivers and reservoirs in Arkansas, our ability to adequately sample mussel beds was achieved on multiple levels. This methodology also produced valuable information on mussel bed distribution, population and community numerical standing crop estimates, species composition, species size frequency distribution, species percent legally harvestable information and species and assemblage densities (Harris *et al.*, 1993a; Rust, 1993; Christian, 1995; Davidson, 1997; Posey, 1997).

For this paper an average of 78% of the species in a bed was accounted for and for a majority of the beds mean species/m^2 was observed at the 80% confidence level. In fact, a mean of 11 samples per bed gave the 80% confidence level, whereas a mean of 43 samples per bed would have yielded the 90% confidence level for the mean species/m^2 at all *M*beds.

Meanwhile, with almost all mean densities over 10/m^2, the goal of 80% confidence was met using the Downing and Downing (1992) method. However, sampling effort of 40 quadrats/bed would have been required to obtain the 90% confidence interval. Alternatively, using the Southwood (1978) equation, a sampling effort of 14 quadrats/bed would have been required to obtain 80% confidence at all beds and 55 quadrats/bed would

TABLE 2.—Cache River *M*bed mean density and estimated density sampling error

*M*bed	Mean density/m^2	Calculated confidence level (Southwood 1979)	n required for 80% conf. level (Southwood 1979)	n required for 90% conf. level (Southwood 1979)	n required for 80% conf. level (Downing & Downing 1992)	n required for 90% conf. level (Downing and Downing 1992)
01	11.2	77	14	55	8	30
04	13.6	89	8	31	7	27
07	37.6	88	9	37	4	16
08	15.8	84	6	25	6	25
12	18.2	85	6	23	6	23
23	21.4	78	12	47	5	22
30	11.3	83	7	29	7	30
31	22.0	83	7	28	5	21
33	6.2	76	14	56	10	40
34	25.4	80	10	38	5	20
35	26.5	85	6	24	5	19
36	14.8	87	5	18	7	26
37	44.1	75	16	63	4	15
38	18.3	82	8	33	6	23

have been needed to obtain 90% confidence levels. Therefore, based on our sampling of the Cache River, it seems that in large rivers where mussel densities average $>10/m^2$, a 15 sample minimum would ensure 80% confidence in bed richness, mean species/m^2 and density estimates. Others have also found that in beds with average mussel densities of over $10/m^2$ or higher, 15 samples provides precision >80%, many times over 95% (Strayer *et al.*, 1997; Vaughn *et al.*, 1997). Vaughn *et al.* (1997) also found that a sample size of 15 provided high confidence levels for beds with an average >5 mean species/m^2. Overall, the 10-sample minimum gave no less than 75% confidence in estimates.

In an effort to provide guidance for gathering status and monitoring information for freshwater mussels, Strayer and Smith (2003) proposed appropriate standardized sampling designs and methods based on the needs of a study. In their publication, Strayer and Smith (2003) provide design-based (*e.g.*, simple random sampling, stratified sampling, double sample) and model-based (regression designs, mark-recapture models, distance sampling) designs and gave examples of how to implement these designs. Methods described in this study are a large blackwater river mussel sampling modification of the stratified sampling design proposed by Strayer and Smith (2003) and have been shown to be an effective way to obtain baseline information of freshwater mussels.

Our large river diver-assisted sampling methodology has been shown to be a useful and appropriate methodology for obtaining baseline distribution (bed and species), species richness, density and population and community numerical standing crop estimates in large streams. This level of effort seems appropriate for large geographic scale, baseline surveys where tradeoffs are required in order to complete a project within time and budget constraints.

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