Appendix L-15

Mussel Survey Protocols

These protocols are currently being prepared and will be included in this MSHCP when available from the Service. These protocols will be based, in part, on the specifications provided in Smith 2006, *Survey design for detecting rare freshwater mussels* (attached).

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Survey design for detecting rare freshwater mussels

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Abstract. A common objective when surveying freshwater mussels is to detect the presence of rare populations. In certain situations, such as when endangered or threatened species are potentially in the area of a proposed impact, the survey should be designed to ensure a high probability of detecting species presence. Linking survey design to probability of detecting species presence has been done for quantitative surveys, but commonly applied designs that are based on timed searches have not made that connection. I propose a semiquantitative survey design that links search area and search efficiency to probability of detecting species presence. The survey can be designed to protect against failing to detect populations above a threshold abundance (or density). I illustrate the design for surveys to detect clubshell (*Pleurobema clava*) and northern riffleshell (*Epioblasma torulosa rangiana*) in the Allegheny River. Monte Carlo simulation indicated that the proposed survey design performs well under a range of spatial distributions and low densities (<0.05 m^2) where search area is sufficient to ensure that the probability of detecting species presence is predicted to be ≥ 0.85.

Key words: unionid, probability of species detection, detectability, qualitative sampling, rare populations, species presence, timed search, occupancy.

A common objective of surveys of freshwater mussels is to detect the presence of rare populations, e.g., when assessing site-specific impacts on endangered or threatened species (Wilcox et al. 1993, Smith et al. 2001a) or when delineating the range of a rare species (Strayer et al. 1996). An important application of this objective is determining the presence of an endangered or threatened species in an area of a proposed impact. In that case, confirmation of species presence would halt or influence the activity that would cause the impact, whereas failure to detect a species when it was in fact present (analogous to a Type II error) could permit an adverse impact to occur. Thus, a survey designed to achieve this objective should ensure a high probability of detecting species presence.

Intuition tells us that the probability of detecting species presence is related to species abundance and spatial distribution, sampling effort, search efficiency within the area sampled (i.e., detectability), and the distribution of sampling effort within a study site. McArdle (1990) and Green and Young (1993) related detection of rare species to the number of sampling units taken in a quantitative, quadrat-based survey assuming perfect search efficiency. Near-perfect search efficiency would be achieved in a freshwater mussel survey by sediment excavation (Hornbach and Deneka 1996, Smith et al. 2001b). Green and Young (1993) provided guidelines for designing a quantitative survey that would ensure a high probability of detecting rare species. However, their guidelines have not been widely adopted for freshwater mussel surveys, in part because quantitative sampling is perceived as time-consuming and expensive (Obermeyer 1998), and timed-search surveys result in more species detections per unit time than quadrat-based surveys (Strayer et al. 1997, Vaughn et al. 1997, Obermeyer 1998).

Timed searches are qualitatively more efficient than quadrat-based surveys, but an explicit method to relate search time to the probability of detecting species presence does not appear to exist. Strayer et al. (1997) calculated probability of detection for timed searches for *Elliptio complanata*, but cited high variance of catch per unit effort statistics as a limitation on the generality of a timed-search-based detection curve. Metcalfe-Smith et al. (2000) found that >50% of species present are missed when typical search times are used and that increased search time resulted in more species detections. However, the essential question of how much search time is enough to ensure a high

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probability of detecting a rare species remains unanswered.

I propose an alternative survey design that is intermediate between a timed search and Green and Young's (1993) quadrat-based sampling. The design relates probability of detecting species presence to search area and search efficiency. The semiquantitative approach does not require sediment excavation, but does require a priori information on search efficiency. Search effort is constrained to defined areas (i.e., sampling units), so the survey design can be linked to probability of detecting species presence. I describe an example survey designed to detect clubshell (*Pleurobema clava*) and northern riffleshell (*Epioblasma torulosa rangiana*) in the Allegheny River. Last, I evaluate the design using a Monte Carlo simulation that includes spatially clustered populations because the survey design relies on assumptions about spatial distribution of rare populations.

Survey Design

I developed the survey design by specifying the survey objective and applying a model to link the objective to elements of the design. In particular, I considered factors that affect search efficiency (e.g., detectability) because it is an important element in mussel survey design. I also considered relevant statistical principles that could guide how best to distribute the area to be searched within a site.

Survey objective

Clear, specific, and quantifiable objectives are central to successful survey design (Strayer and Smith 2003, McDonald 2004). The primary objective of our survey was to detect the presence of a rare population, but a survey objective should be defined further and stated quantitatively to allow for evaluating whether a proposed design will meet the objective. For example, the objective might be stated quantitatively: "To detect the presence of any of the endangered or candidate species in a site with probability $\geq$0.85 given that species abundance is $\geq$100 individuals." This statement has 2 important elements: 1) the minimum threshold for the probability of detecting presence of a species, and 2) a species abundance or density that is deemed biologically meaningful. I used an abundance of 100 individuals only as an example. The determination of a biologically meaningful threshold should involve multiple considerations including legal mandates, life history, population viability, and comparisons of densities throughout a local watershed, region, or range.

Modeling the sampling process

A model of the sampling process is needed to relate the proposed objective to the survey design. The model represents the expected survey results (counts of mussels) as a function of the controlling factors—mussel abundance, search area, and search efficiency. Search efficiency, which is also termed detectability, is the probability of detecting an individual mussel given that it is within the search area.

The expected number of individuals counted in a survey of a site can be represented as

$$E(C) = \alpha\beta T \quad [1]$$

where C is the count of individuals, $E(C)$ is the expected count based on a repeatable sampling process, α is the fraction of the site that is searched, β is the probability of detecting an individual given that it is in the search area, and T is the total number of individuals in the site (Williams et al. 2002:244). The expected number of individuals in the search area is $\alpha T = a\mu$ where a is the search area and μ is species density. Note that the fraction of the site that is searched is $\alpha = a/A$ where A denotes the area of the site. The search area is the sum of the areas of each unit in the sample, i.e., $a = \sum_{i=1}^{n} a_i$ where n is the sample size and a_i is the area of the i^{th} sampling unit (typically a_i is the same or nearly the same for all sampling units).

Search efficiency, which refers to the probability of detecting an individual given that it is in the search area, is a function of search rate (time per unit area) and search area (Fig. 1). In eq. 1, search efficiency is denoted by β. In theory, if one spends enough time and effort searching an area, all individuals that are present within the search area will be detected, in which case $\beta = 1$. However, in actual sampling situations, search time and effort are restricted so that not all individuals in the sample area are detected and $\beta < 1$.

Mussel sampling techniques have been classified as quantitative, semiquantitative, or qualitative (Strayer and Smith 2003). This classification can be related to the parameters in eq. 1 (Table 1). Quantitative and semiquantitative sampling are distinguished from qualitative sampling by α. α is known when sampling is quantitative or semiquantitative, but α is not known when sampling is qualitative. Quantitative and semiquantitative sampling are distinguished by β. Quantitative sampling is the case where $\beta = 1$ or $\beta < 1$ and is estimated. In either case, β can be accounted for in eq. 1. Semiquantitative sampling is the case where β is unknown. Unbiased estimation of abundance or density is possible only when α and β are known or

TABLE 1. Contrast of sampling techniques (classified as qualitative, semiquantitative, or quantitative) based on fraction of site searched (α), search efficiency or detectability (β), and which parameter(s) are known or estimated. C = count of mussels in a sample at a site, T = total number of individuals in the population at a site, $\hat{T}$ = estimated total number of individuals at a site, $\hat{\beta}$ = estimated probability of detecting an individual given that it is within the search area.

Sampling technique	α	β	Survey result
Qualitative	Unknown	Unknown	Incomplete count
Semiquantitative	Known	Unknown	Incomplete count within searched area
Quantitative	Known	Known or estimated	Abundance estimate: $\hat{T} = C/(\alpha\hat{\beta})$

estimated, i.e., $\hat{T} = C/(\alpha\hat{\beta})$ where $\hat{T}$ is the estimated total number of individuals in the study site (i.e., the abundance estimate) and $\hat{\beta}$ is an unbiased estimate of the probability of detecting an individual given that it is in the search area.

Detecting the presence of a rare species within a site is equivalent to detecting at least one individual of that species, and it follows from eq. 1 that this event is a function of α, β, and T. That is:

$$\text{Prob(detecting at least one individual)} = \text{Prob}(C > 0) = f(\alpha\beta T). \quad [2]$$

Green and Young (1993) considered sampling rare populations of freshwater mussels in quadrats and derived a formula for the probability of detecting the presence of a low-density population (i.e., $\mu < 0.10/m^2$) using a Poisson probability distribution:

$$\text{Prob(detecting at least one individual)} = 1 - e^{-mn} \quad [3]$$

where m is the number of individuals within a sampling unit and n is the number of random sampling units searched. The Poisson assumption implies that mussels at very low density have a spatially random distribution. This assumption does not imply an absence of underlying ecological relationships, such as habitat associations and dispersal mechanisms, which affect distribution (Downing and Downing 1991). Rather, it indicates that when mussels are geographically rare at a site (i.e., $\mu < 0.1/m^2$), their low density masks underlying ecological relationships and their spatial distribution is random from a statistical perspective. Green and Young (1993) presented empirical data to support this contention. In addition, Smith et al. (2003) found that low-density mussels on the Cacapon River, West Virginia, had random distributions as evidenced by variance-to-mean ratios. A variance-to-mean ratio of 1 indicates a Poisson distribution (Elliott 1977). Downing and Downing (1991) presented a formula for variance as a function of the mean number of individuals collected that was developed empirically from surveys in lentic and lotic habitats. The Downing and Downing (1991) formula indicates that the variance-to-mean ratio approaches 1 (spatial randomness) as the mean approaches 0.10, the threshold for rarity used by Green and Young (1993). I used data from Smith et al. (2001b) and found variance-to-mean ratios for 60 species/site combinations (31 species at 14 sites) that indicated mussel distributions were statistically spatially random for $\mu \leq 0.10/m^2$. The same relationship between density and spatial distribution has been found in other populations (McArdle 1990, Welsh et al. 1996). Therefore, I propose eq. 3 as a useful approximation for guiding survey design, and I evaluate its use in a simulation that includes spatially clustered populations and sampling units other than quadrats (see below).

Equation 3 can be revised to account for search efficiency by including the parameter β, thereby making a connection to the sampling-process model in eq. 1. The expected number of individuals detected is $\beta nm = \beta\alpha T$. Thus:

$$\text{Prob(detecting at least one individual)} = 1 - e^{-\beta\alpha T} = 1 - e^{-\beta a T/A} = 1 - e^{-\beta a \mu}. \quad [4]$$

Equation 4 can be used to examine the effect of search efficiency (β), search area (a), and density (μ) on the probability of detecting at least one individual or, analogously, the probability of detecting species presence. Figure 2 shows the probability of detecting species presence for $\mu = 0.01$, 0.05, and $0.10/m^2$, $\beta = 0.2$, 0.4, 0.6, and 0.8, and $a = 100$ to 1000 m^2. Equation 4 also could be used to examine the effect of abundance (T) for a given study site area (A) instead of μ. Table 2 shows probability of detecting species presence for $T = 100$ to 500 and $A = 16{,}000$ and 32,000 m^2.

Factors that affect search efficiency

Search efficiency is a function of search area and search time (Fig. 1). The exact form of that relationship is not known and will vary over time and area. For a given search area, the more time spent searching, the higher the search efficiency. It is likely that search efficiency will increase quickly as search time is

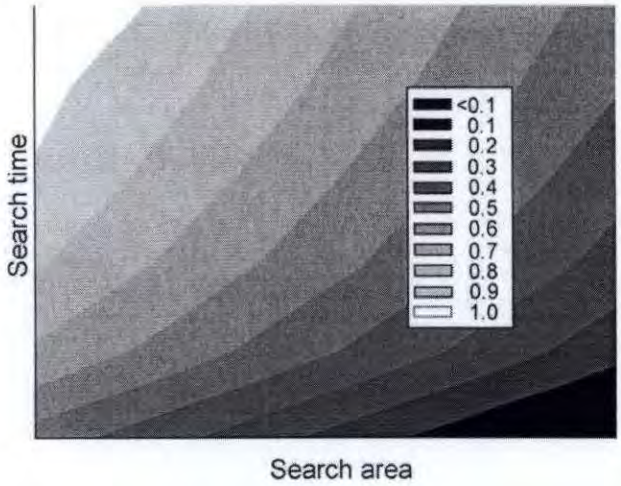

FIG. 1. Search efficiency (β; legend) as a function of search time and search area (*a*). The axes are not labeled because the exact form of the relationship is determined by a variety of factors involving mussel biology, physical environment, and observer capabilities.

increased from low to moderate levels and the rate of increase in search efficiency will slow as it approaches complete detection, exhibiting a point-of-diminishing-returns-type phenomenon. These relationships between search time and search efficiency also have been shown empirically (Metcalfe-Smith et al. 2000).

The exact form of the relationship between search efficiency and search time will depend on a number of factors (Strayer et al. 1997), some of which are inherent to the biology and natural history of the mussel species. For example, some species are more cryptic than others by virtue of their size, coloration, or reproductive behavior (Miller and Payne 1993, Obermeyer 1998, Haag and Warren 2000). Mussels exhibit seasonal patterns in vertical migration associated with day length and water temperatures (Amyot and Downing 1991, Watters et al. 2001, Perles et al. 2003). Other biological factors include gender and demographics. For example, female northern riffleshell (*Epioblasma torulosa rangiana*) are more visible than males (Smith et al. 2001a), and small mussels are difficult to detect (Miller and Payne 1988, Hornbach and Deneka 1996, Richardson and Yokley 1996, Smith et al. 2001b). Other factors, such as turbidity, hydrologic variability, substrate, and vegetative cover, are associated with the physical environment (Di Maio and Corkum 1997, Smith et al. 2001b). Last, some factors, such as observer experience, visual acuity, and fatigue, are associated with the observer (Strayer et al. 1997).

Only those mussels that are epibenthic or not buried can be found in a search restricted to the substrate surface (Amyot and Downing 1991). If an area is searched thoroughly so that all mussels on the substrate surface have been found, then search efficiency will be capped at the proportion of mussels that are on the surface. Beyond that level of effort, excavation would be required to increase search efficiency to the point that all or nearly all mussels within the searched area are found (Smith et al. 2001b).

Impact of search efficiency on survey design

Because search efficiency directly affects the probability of detecting species presence, it should be considered when designing a survey. Two approaches could be used to incorporate search efficiency in survey design. First, one could be conservative and assume that search efficiency (β) was low. Then the relationship from eq. 4 (Table 2, Fig. 2A–D) could be used as a guide to find the search area (*a*) that would ensure that the probability of detecting species presence is sufficiently high (Fig. 2A–D). For example, if β were assumed to be ≤ 0.2, then *a* would have to be >1000 m^2 to have a probability of detecting at least one individual = 0.85 for $\mu = 0.01/\text{m}^2$ (Fig. 2A). This *a* would be equivalent to ten 1-m-wide × 100-m-long transects (distribution of search effort throughout the site is discussed below). The assumed β could be based on life-history traits, such as likelihood that an individual would be endobenthic (Amyot and Downing 1991). This approach would be precautionary.

Second, β could be estimated at another time and place where the rare species was numerous or by a pilot survey based on a related, but more common, species. For example, β could be estimated by searching the surface of quadrats before excavating sediment (cf. Haukioja and Hakala 1974, Smith et al. 2001b). In this case, the estimate of β and eq. 4 could be used to predict the *a* that would result in the desired probability of detecting species presence. For example, if β for a search rate of 2 min/m^2 were estimated as 0.4, then $a = 500$ m^2 would ensure a probability of detecting species presence = 0.85 for $\mu = 0.01/\text{m}^2$ (Fig. 2B), and 1000 min (16.67 h) of search time would be required. The shortcoming of using an estimate from another time and place is that β would be estimated under one set of conditions and applied under a similar, but not identical, set of conditions. If an overestimate of β were used in survey design, then the probability of detecting species presence also would be overestimated, and the design would not be precautionary. The number of quadrats needed to estimate β would depend on μ at the site and the

TABLE 2. Probability of detecting species presence given the study site area (A), search efficiency (β), abundance (T), and search area (a). Bold font indicates probability of species detection ≥ 0.85.

			a (m^2)								
A (m^2)	β	T	100	200	300	400	500	600	700	800	900
16,000	0.2	100	0.12	0.22	0.31	0.39	0.46	0.53	0.58	0.63	0.68
		200	0.22	0.39	0.53	0.63	0.71	0.78	0.83	**0.86**	**0.89**
		300	0.31	0.53	0.68	0.78	**0.85**	**0.89**	**0.93**	**0.95**	**0.97**
		400	0.39	0.63	0.78	**0.86**	**0.92**	**0.95**	**0.97**	**0.98**	**0.99**
		500	0.46	0.71	**0.85**	**0.92**	**0.96**	**0.98**	**0.99**	**0.99**	**1.00**
	0.4	100	0.22	0.39	0.53	0.63	0.71	0.78	0.83	**0.86**	**0.89**
		200	0.39	0.63	0.78	**0.86**	**0.92**	**0.95**	**0.97**	**0.98**	**0.99**
		300	0.53	0.78	**0.89**	**0.95**	**0.98**	**0.99**	**0.99**	**1.00**	**1.00**
		400	0.63	**0.86**	**0.95**	**0.98**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
		500	0.71	**0.92**	**0.98**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	0.6	100	0.31	0.53	0.68	0.78	**0.85**	**0.89**	**0.93**	**0.95**	**0.97**
		200	0.53	0.78	**0.89**	**0.95**	**0.98**	**0.99**	**0.99**	**1.00**	**1.00**
		300	0.68	**0.89**	**0.97**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		400	0.78	**0.95**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		500	**0.85**	**0.98**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
32,000	0.2	100	0.06	0.12	0.17	0.22	0.27	0.31	0.35	0.39	0.43
		200	0.12	0.22	0.31	0.39	0.46	0.53	0.58	0.63	0.68
		300	0.17	0.31	0.43	0.53	0.61	0.68	0.73	0.78	0.82
		400	0.22	0.39	0.53	0.63	0.71	0.78	0.83	**0.86**	**0.89**
		500	0.27	0.46	0.61	0.71	0.79	**0.85**	**0.89**	**0.92**	**0.94**
	0.4	100	0.12	0.22	0.31	0.39	0.46	0.53	0.58	0.63	0.68
		200	0.22	0.39	0.53	0.63	0.71	0.78	0.83	**0.86**	**0.89**
		300	0.31	0.53	0.68	0.78	**0.85**	**0.89**	**0.93**	**0.95**	**0.97**
		400	0.39	0.63	0.78	**0.86**	**0.92**	**0.95**	**0.97**	**0.98**	**0.99**
		500	0.46	0.71	**0.85**	**0.92**	**0.96**	**0.98**	**0.99**	**0.99**	**1.00**
	0.6	100	0.17	0.31	0.43	0.53	0.61	0.68	0.73	0.78	0.82
		200	0.31	0.53	0.68	0.78	**0.85**	**0.89**	**0.93**	**0.95**	**0.97**
		300	0.43	0.68	0.82	**0.89**	**0.94**	**0.97**	**0.98**	**0.99**	**0.99**
		400	0.53	0.78	**0.89**	**0.95**	**0.98**	**0.99**	**0.99**	**1.00**	**1.00**
		500	0.61	**0.85**	**0.94**	**0.98**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**

proportion of individuals on the substrate surface (Smith et al. 2001b, Strayer and Smith 2003). Therefore, the environmental conditions in the pilot survey should be as close as possible to the conditions likely to be encountered at the site where species presence will be determined. Information on species-specific densities and search efficiencies are available in the literature in some cases (e.g., Smith et al. 2001a), and unpublished agency surveys are likely to provide relevant data.

Statistical principles guiding the distribution of search effort within the site

Two statistical principles, in particular, are useful for guiding distribution of search effort. First, spatially balanced sampling has been recognized as efficient for sampling natural resources (Christman 2000, Stevens and Olsen 2004). A spatially balanced sample is one that is distributed throughout a site or population. Various systematic or grid sampling methods qualify as spatially balanced. Second, it is generally more efficient (reduces sampling error) to distribute effort among many small units than a few large units. This principle is particularly relevant when the population is spatially clustered (Elliott 1977). The mitigating factor is the effort required to move among units. Many small units require more between-unit travel than few large units. Thus, the challenge is to find a sampling-unit size that represents a compromise between cost and sampling error. These principles can be combined with stratification to allocate effort efficiently and to ensure that sampling is done in all habitats. For example, a site can be stratified by macrohabitat (e.g., riffle, run, pool) and search area can be allocated proportionately or according to anticipated habitat value (i.e., more effort in better habitat). On the other hand, the survey could be conducted in phases as suggested by Kovalak et al. (1986) and implemented recently by Villella and Smith (2005). During the 1st phase, an informal search or surveillance can be conducted to delineate mussel beds or

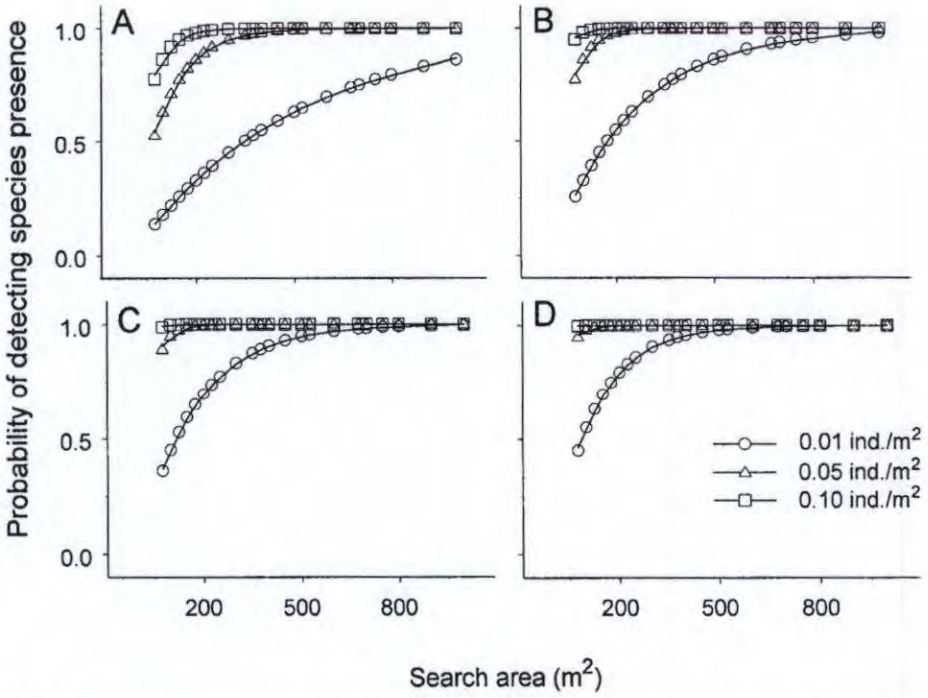

FIG. 2. Probability of detecting species presence as a function of search area (a) and density (0.01, 0.05, 0.10 individual/m^2) of mussels when search efficiency (β) was 0.2 (A), 0.4 (B), 0.6 (C), and 0.8 (D).

habitat. During the 2nd phase, the semiquantitative approach can be applied after the search area (a) has been determined to ensure a sufficiently high probability of detecting species presence. The predetermined a should be allocated so that most, but not all, of the area occurs within the bed or habitat identified during the 1st phase.

A cautionary note is warranted regarding the distribution of sampling effort according to an explicit or implied habitat model. If the habitat model is a good approximation, then it can be helpful in distributing search area. Depth and hydrological variability are useful predictors of mussel density (Haukioja and Hakala 1974, Strayer and Ralley 1993, Di Maio and Corkum 1995). However, if the model is a poor approximation, as Strayer and Ralley (1993) found for microhabitat variables, then model-based distribution can be inefficient at best and misleading at worst. A poor habitat model could lead to omission of the actual habitat from the area searched.

Detection of Clubshell (*Pleurobema clava*) and Northern Riffleshell (*Epioblasma torulosa rangiana*) in the Allegheny River

I used data from the clubshell and northern riffleshell in the Allegheny River to illustrate the design of a survey to detect their presence. In previous surveys on the Allegheny River, Smith et al. (2001a) reported that a thorough search of the substrate surface required 2 min/m^2 of search time. At the West Hickory bridge site, ~30% and 50% of clubshell and northern riffleshell were found at the substrate surface, respectively.

Suppose the goal was to protect a site against adverse impact if either species was present at $\mu \geq 0.01/m^2$ with a probability of detecting species presence ≥ 0.85. (Tolerance for risk is a subjective decision that often would be set during the regulatory process.) To protect either species, the β corresponding to the least detectable species, the clubshell, would be used. In this case, we assume that the substrate surface within a will be searched thoroughly so that β is the proportion of mussels on the substrate surface. Given this information, we can design a survey using eq. 4:

$$0.85 = 1 - e^{-0.30a0.01}$$

and solve for a:

$$a = \frac{\ln(1 - 0.85)}{-0.003}$$

$$= 632 m^2.$$

Based on the principle of spatially balanced sampling, at least 632 m^2 of search area should be

distributed throughout the site. A reasonable design would be to search within transects oriented perpendicular to shoreline or the thalweg. Following the rule that more small units are better, use of 0.5-m-wide transects would allow greater spatial dispersion of sampling effort; however, logistics and tradition might favor 1-m-wide transects, especially at sites where SCUBA is required. Transect length would depend on site dimensions. For example, if the site was 100 m across the river, then seven 1-m-wide transects would be required. Good spatial balance and coverage would be achieved by selecting a random start and placing transects at equal intervals. An improvement on that plan would include 2 random starts. To increase probability of detecting species presence to 0.95, ten 1 × 100 m transects would be required.

After a has been determined based on $\hat{\beta}$ and a μ that is to be protected, the time required to conduct the survey can be calculated. Based on 2 min/m^2 to search the surface substrate thoroughly, searching seven 1 × 100-m transects would require ~23 h, which could be divided among multiple observers. The survey could be accomplished in ~1 d with a crew of 4. This time and effort does not seem to be an unreasonable survey cost when the objective is to detect a rare or endangered species before an adverse impact occurs. Budgets for construction projects, for example, can amount to hundreds of thousands to millions of dollars. The cost to conduct a rigorous mussel survey is trivial by comparison.

Monte Carlo Simulation

To evaluate the proposed survey design, a computer program was used to generate locations for individual mussels within a site of 16,000 m^2 (100 m × 160 m), apply search efficiencies so that different proportions of the mussels were detectable, and count detectable mussels within systematically placed 1-m transects. Abundance at the site was a Poisson random variable with means of 100, 300, and 500 mussels representing population densities of 0.006, 0.02, and 0.03 (individuals/m^2). Individual mussels were in clusters with mean sizes of 1, 3, or 5 individuals (a cluster size of 1 represented complete spatial randomness). The location of the cluster center was random within the site, and individuals were distributed from the cluster center at a uniform random angle and exponential random distance, with mean distance of 1 m. Search efficiencies of 0.2, 0.4, or 0.6 were applied to determine whether each individual in the population was detectable. Detectable individuals were counted within 1-m transects oriented across the short axis of the site (100 m). Areas searched were 400, 600, 800, and 1000 m^2. The probability of detecting species presence was calculated as the proportion of 1000 replications where at least one individual was counted. Computations were done in SAS (version 9.1 SAS Institute, Cary, North Carolina).

TABLE 3. Abundance (T), search efficiency (β), and cluster size for the populations used to simulate the proposed survey design. The study site was 16,000 m^2 (160 m × 100 m). Variance-to-mean ratios were calculated for individuals within 1 m × 100 m transects.

			Variance-to-mean ratio	
T	β	Cluster size	Entire population	Detectable portion of the population
100	0.2	1	1.19	0.91
		3	2.03	1.11
		5	2.33	1.04
	0.4	1	1.21	1.17
		3	1.93	1.40
		5	2.17	1.29
	0.6	1	1.05	1.04
		3	1.91	1.58
		5	2.84	1.77
300	0.2	1	0.87	0.85
		3	2.44	1.08
		5	2.53	1.20
	0.4	1	0.83	1.03
		3	1.85	1.39
		5	2.11	1.36
	0.6	1	0.94	1.08
		3	1.77	1.31
		5	2.62	1.92
500	0.2	1	1.13	1.15
		3	1.84	1.19
		5	2.91	1.44
	0.4	1	1.03	1.04
		3	1.61	1.11
		5	2.19	1.39
	0.6	1	1.03	0.80
		3	2.01	1.45
		5	2.81	2.04

The populations showed differing degrees of spatial clustering (Table 3, Fig. 3). Variance-to-mean ratios increased with cluster size were lower when calculated using detectable individuals only. Thus, the detectable portion of the population appears less spatially clustered than the actual population.

Simulated probabilities of detecting species presence generally tracked the probabilities predicted from eq. 4 (Table 4). Variability in the simulated probabilities was caused by variability in abundance, search efficiency, cluster size, and sample selection. This result is relevant because abundance, search efficiency, and spatial distribution would not be known exactly when using eq. 4 for survey design. The simulations indicated that eq. 4 is a useful guide under a range of conditions. Most important, the survey design

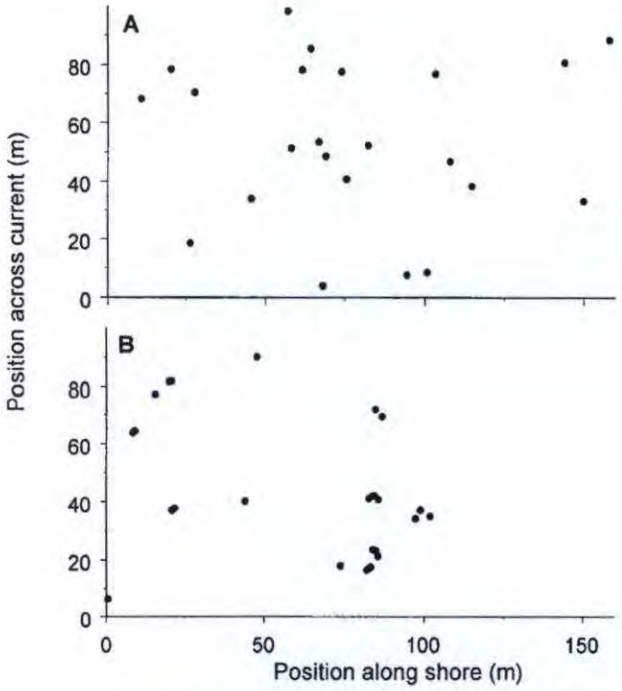

FIG. 3. Example spatial distributions of detectable mussels used to evaluate the survey design when simulated abundance was 100, search efficiency was 0.2, and cluster sizes were 1 (A) and 5 (B). Detectable mussels were a random subset of the abundance determined by the search efficiency. There were 23 detectable mussels in A and 29 in B.

performed well when a was predicted to result in a high probability of detecting species presence. Simulated probabilities of detecting species presence were ≥ 0.85 in 92% (77 of 84) of cases where eq. 4 predicted the probabilities would be ≥ 0.85 (Table 4).

Discussion

Clear, specific, and quantitative objectives are prerequisites to a successful survey design (McDonald 2004). For example, the objective for a pre-dredging survey could be to detect the presence of any endangered or candidate species with probability ≥ 0.85 given that species density is $\geq 0.01/m^2$. An important question to ask when designing a survey is whether the proposed design will meet the stated objective (Strayer and Smith 2003). The survey design described here provides a method for answering that question by linking survey elements, i.e., search area and search efficiency, to the probability of detecting species presence.

The proposed survey design, which is intermediate between timed search and quadrat methods, requires that the search area be constrained within sampling units, but excavation is not required because search efficiency is assumed to be less than perfect. Distribution of the search area within the site is flexible within guidelines. Based on well-established principles of sampling natural resources, it is best to distribute sampling effort throughout a study site in relatively small sampling units. The size of the sampling units is mitigated by logistic considerations with transects recommended in some cases because of ease of field application. A Monte Carlo simulation confirmed that use of systematically placed transects is a good approach for the objective of species detection. However, use of transects would not be a good approach when the objective is to estimate abundance or density because some amount of excavation would be required and, therefore, quadrats would be required (Smith et al. 2001b, Strayer and Smith 2003). Information on habitat or mussel beds can be used to stratify the site and to allocate the search area within strata either proportionately or with more of the search effort allocated to better habitats. More complex sample-selection procedures, such as unequal probability sampling, could be applied. However, ease of application should be an overarching concern, and simple selection procedures, such as systematic sampling, would be preferable.

Some population abundances or densities are unlikely to be detected without substantial sampling effort by increasing search efficiency or search area (Table 2). This constraint is unavoidable in any protocol. The proposed survey design incorporates sampling techniques (i.e., transect-based, semiquantitative sampling) that are part of many existing protocols. However, the user of the proposed design can be fully aware of population sizes that are likely to be detected by explicitly stating the probability of detecting species presence for given population size and sampling effort. As one reviewer noted, a main advantage of the proposed design is that the user has an answer to the question: "How much sampling effort is enough?"

A reasonable concern with the proposed design is the cost to survey a site. The recommended sampling effort is likely to exceed the costs associated with currently applied protocols. Few protocols for rare species detection have been published; however, Young et al. (2001) recommended at least 2 person-hours of search time in optimal habitat before concluding that a species was absent if no individuals were detected. At a search rate of 2 min/m^2, a 2-h search would be equivalent to <100 m^2 of search area, which appears to be an insufficient effort for detecting rare species. A search area of 100 m^2 resulted in a probability of detecting species presence as low as 0.12

TABLE 4. Probabilities of detecting species presence observed from a computer simulation and predicted by eq. 4. Abundance (T), search efficiency (β), and cluster size are mean values used in the simulation, but were random variables in the simulation. Cluster locations were random within a 16,000-m^2 study site. Searches were conducted within 1 m × 100 m transects. The search area (a) was the sum of the transect areas. Bold font indicates combinations with predicted probabilities ≥0.85.

			a (m^2)							
			400		600		800		1000	
T	β	Cluster size	Simulated	Predicted	Simulated	Predicted	Simulated	Predicted	Simulated	Predicted
100	0.2	1	0.45	0.39	0.78	0.53	0.55	0.63	0.51	0.71
		3	0.36	0.39	0.73	0.53	0.61	0.63	0.89	0.71
		5	0.43	0.39	0.57	0.53	0.70	0.63	0.75	0.71
	0.4	1	0.76	0.63	0.95	0.78	**0.82**	**0.86**	**0.91**	**0.92**
		3	0.63	0.63	0.89	0.78	**0.79**	**0.86**	**1.00**	**0.92**
		5	0.49	0.63	0.65	0.78	**0.82**	**0.86**	**0.92**	**0.92**
	0.6	1	0.89	0.78	**0.97**	**0.89**	**1.00**	**0.95**	**0.95**	**0.98**
		3	0.67	0.78	**0.87**	**0.89**	**0.96**	**0.95**	**0.94**	**0.98**
		5	0.72	0.78	**0.86**	**0.89**	**0.95**	**0.95**	**0.73**	**0.98**
300	0.2	1	0.75	0.78	**0.97**	**0.89**	**0.95**	**0.95**	**1.00**	**0.98**
		3	0.73	0.78	**0.78**	**0.89**	**1.00**	**0.95**	**1.00**	**0.98**
		5	0.79	0.78	**0.69**	**0.89**	**0.97**	**0.95**	**0.96**	**0.98**
	0.4	1	**0.92**	**0.95**	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
		3	**0.83**	**0.95**	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
		5	**0.94**	**0.95**	**1.00**	**0.99**	**0.93**	**1.00**	**1.00**	**1.00**
	0.6	1	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		3	**0.88**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		5	**0.95**	**0.99**	**0.97**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
500	0.2	1	**0.92**	**0.92**	**1.00**	**0.98**	**1.00**	**0.99**	**1.00**	**1.00**
		3	**0.90**	**0.92**	**0.92**	**0.98**	**1.00**	**0.99**	**1.00**	**1.00**
		5	**0.95**	**0.92**	**0.96**	**0.98**	**1.00**	**0.99**	**1.00**	**1.00**
	0.4	1	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		3	**0.96**	**0.99**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		5	**1.00**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	0.6	1	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		3	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		5	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**

and <0.85 for all but one combination of abundance and search efficiency in Table 2. If this result is any indication, using the proposed survey design would lead to increased sampling effort and higher survey costs than currently practiced. A legitimate and reasonable question is whether the added cost is worthwhile and affordable. Ultimately, that question will have to be answered on a case-by-case basis by the organizations that are funding the survey. One counterbalancing consideration is the cost of failing to detect the presence of a rare population within the area of a pending adverse impact. Cost would be reduced if searching stopped as soon as one individual of the rare species was detected; however, that practice would limit the utility of the survey. There certainly are circumstances when designing a survey to achieve a high probability of detecting species presence will be worthwhile. Surveys of federally endangered species in areas of proposed adverse impacts would probably be one of those circumstances.

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Guidelines Used in Four Geographically Diverse Unionid Relocations

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Abstract. The endangered status of many unionids has prompted the use of relocations as a mitigation measure. However, current data suggest that relocations have been only minimally successful possibly due to factors such as improper site selection and handling techniques. Guidelines for relocation site selection and unionid handling were developed from reviewing literature and contacting knowledgeable researchers. These guidelines were used during unionid relocations on the Elk River, West Virginia; Meramec River, Missouri; St. Croix River, Wisconsin-Minnesota; and the Wolf River, Wisconsin. Stream characteristics, collection and relocation conditions, and species varied among relocations. Preliminary results suggest that these relocations were successful. Observed mortality 1 month and 1 year following relocations was negligible (0 to 1%), and recovery ranged from 50% to 96%. We suggest the following guidelines for future relocations: (1) use field personnel that are familiar with unionids, (2) select a relocation area with stable substrate and a similar unionid community that is near the collection area, (3) keep animals moist or in water and minimize out-of-water time, (4) avoid extreme temperatures, and (5) avoid crowding animals.

Introduction

Modifications of our rivers, such as impoundment, channelization, dredging, instream construction, and the resulting siltation and hydrological changes, are often cited as the primary reasons for the decline of unionid species (Stansbery 1970, 1971; Stein 1972; Yokley 1976; Suloway et al. 1981; Miller et al. 1984; Williams et al. 1992; Parmalee and Hughes 1993; Hartfield 1993). A high percentage of North American unionids are presumed extinct, threatened, endangered, or in need of conservation (see Neves 1993). Currently, Section 7(a)(2) of the Endangered Species Act as well as equivalent legislation within some states requires that impacts to these species be minimized, and if impacts are unavoidable, that they be mitigated. Relocating unionids from instream construction, impoundment, and channelization areas has often been used to mitigate impacts to unionids (Oblad 1980; Harris 1984, 1986, 1989; Harris et al. 1992; Jenkinson 1985, 1989; Dunn 1993). However, monitoring studies suggest that in most cases unionid recovery and/or survival may be less than ideal (Sheehan et al. 1989; Burke 1991; Aquatic Resources Center 1993; Dunn 1993; Koch 1993; Layzer and Gordon 1993).

Dunn (1994) and Cope and Waller (1995) reviewed literature and Dunn (1994) contacted knowledgeable researchers on previous relocation studies and found that habitat stability in the relocation area (Sheehan et al. 1989; Dunn 1993; Layzer and Gordon 1993) and handling methods (Ahlstedt, pers. comm.; Harris, pers. comm.; Neves, pers. comm.) were consistently noted as possible reasons for relocation success or failure. Unionid survival and/or recovery following relocation also varied among species within most studies (Oblad 1980; Sheehan et al. 1989; Dunn 1993).

Habitat stability in the relocation area seems to be a key factor. Low recovery was attributed to changes in substrate or habitat in some relocations (Sheehan et al. 1989; Hubbs et al. 1991; Dunn 1993; Layzer and Gordon 1993), and Dunn (1993) recovered fewer relocated unionids from areas with less stable substrate in the Ohio River. Handling methods, such as overcrowding, prolonged periods out of water, exposure to extreme temperature, and improper placement in the substrate, have frequently been speculated as possible causes of low recovery and survival. However, researchers disagree on unionid sensitivity to handling and few studies have tested these effects (Waller et al. 1995).

Recommendations outlined by Dunn (1994) were incorporated into four unionid relocations that varied in geographic location, riverine characteristics, and unionid species: St. Croix River (Minnesota and Wisconsin), Wolf River (Wisconsin), Meramec River (Missouri), and Elk River (West Virginia) (Figure 1). Each relocation was monitored to determine protected species survival. The Wolf, St. Croix, and Elk River monitoring also included other relocated species and the St. Croix and Wolf River

relocations included tests to substantiate handling and placement techniques. Other monitoring objectives included determining if mortality was immediate or long term (St. Croix, Wolf, and Meramec rivers), determining nonrelocated unionid mortality (all relocations), determining adequacy of buffer zones (St. Croix River), and determining if removal areas were recolonized (Meramec and Elk rivers). Monitoring for most of these relocations is not complete; therefore, results in this paper will be limited to recovery and observed mortality of relocated unionids. This paper compares preliminary monitoring results and offers guidelines for future relocations.

Study Area

In May 1994, 4,514 unionids were relocated from construction areas of the I-55 bridge over the Meramec River near St. Louis, Missouri (Figure 1). In this area, the Meramec River (river mile 6.9) is typically a flowing gravel run, about 120 m wide and 1.5 m deep; however, the area is often pooled by the Mississippi River, resulting in little to no flow and silt deposition. Substrate is mostly loose gravel and sand, with some silt particularly near the riverbanks. Areas with boulders over gravel and silt are found under and downstream of the bridge. A total of 33 species has been collected in this area (Table 1) including *Lampsilis abrupta* (federally endangered); *Elliptio crassidens, Fusconaia ebena,* and *Leptodea leptodon* (Missouri endangered); *Arcidens confragosus* and *Plethobasus cyphyus* (Missouri rare); and *Obovaria olivaria* (Missouri watch list).

In July and August 1994, 202 unionids were relocated from a pipeline construction area in the Elk River near Clendenin, West Virginia (Figure 1). Elk River is a small (55 m wide and less than 1.2 m deep in the study area), clear, high-gradient tributary of the Kanawha River. Habitat in this area of the river consists of riffles, runs, and deeper pools with cobble, gravel, and sand substrate. A total of 20 species has been collected in the study area (Table 1) including *Epioblasma torulosa rangiana, Pleurobema clava,* and *L. abrupta* (federally endangered).

In August 1994 and August 1995, 8,996 and 14,027 unionids, respectively, were relocated from I-94 bridge construction and demolition areas in the St. Croix River near Hudson, Wisconsin (Figure 1).

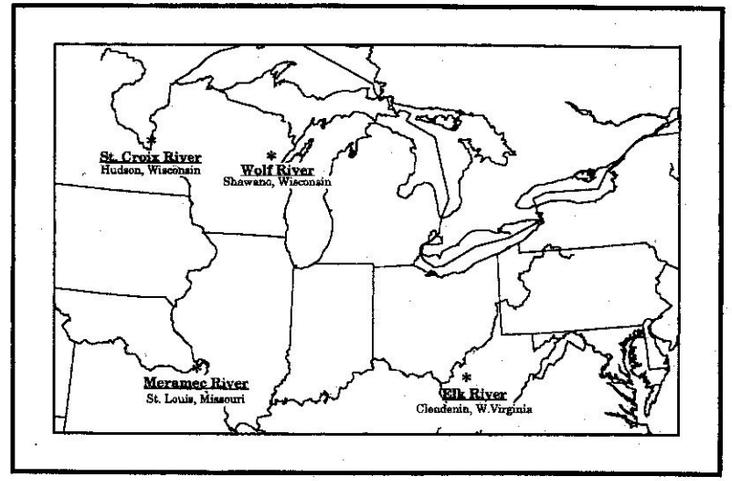

Figure 1. Distribution of unionid relocation studies.

Table 1. Unionid species recorded from each of the relocation areas.

Species[1]	Meramec River[2]	Elk River[3]	St. Croix River[4]	Wolf River[5]
Actinonaias ligamentina	X	X	X	X
Alasmidonta marginata			X	X
Amblema plicata plicata	X	X	X	X
Arcidens confragosus	X			
Cumberlandia monodonta			X	
Cyclonaias tuberculata			X	
Ellipsaria lineolata	X		X	
Elliptio crassidens	X		X	
Elliptio dilatata	X	X	X	X
Epioblasma torulosa rangiana		X		
Epioblasma triquetra		X		
Fusconaia ebena	X		X	
Fusconaia flava	X	X	X	X
Fusconaia subrotunda		X		
Lampsilis abrupta	X	X		
Lampsilis cardium	X	X	X	X
Lampsilis fasciola		X		
Lampsilis higginsii			X	
Lampsilis ovata		X		
Lampsilis siliquoidea	X	X	X	X
Lampsilis teres	X			
Lasmigona complanata complanata	X		X	
Lasmigona costata		X	X	X
Leptodea fragilis	X		X	X
Leptodea leptodon	X			
Ligumia recta	X	X	X	X
Megalonaias nervosa	X		X	
Obliquaria reflexa	X		X	X
Obovaria olivaria	X		X	X
Obovaria subrotunda		X		
Plethobasus cyphyus	X			
Pleurobema clava		X		
Pleurobema coccineum	X		X	X
Potamilus alatus	X	X	X	X
Potamilus ohiensis	X		X	
Ptychobranchus fasciolaris		X		
Pyganodon grandis	X		X	X
Quadrula metanevra	X		X	
Quadrula pustulosa pustulosa	X	X	X	X
Quadrula quadrula	X		X	X
Simpsonaias ambigua			X	
Strophitus undulatus undulatus	X	X	X	X
Toxolasma parvus	X		X	
Tritogonia verrucosa	X		X	X
Truncilla donaciformis	X		X	X
Truncilla truncata	X		X	
Utterbackia imbecillis	X			
No. of species	33	20	34	22

[1]Nomenclature follows Turgeon et al. (1988) and Hoeh (1990).
[2]ESI (1995a)
[3]ESI (1993), USFWS (unpubl. data)
[4]Heath and Rasmussen (1990), ESI (1995b)
[5]Miller (1993), ESI (unpubl. data)

The St. Croix River at this point (approximately river mile 16.2) is a wide, riverine lake, which is pooled by Mississippi River Lock and Dam 3. The navigation channel follows the Minnesota bank and current is mostly restricted to the channel. At the I-94 bridge, the river is approximately 950 m wide and 4 to 6 m deep. The water is fairly clear and substrate is primarily sand with cobble and gravel in areas with flow. The reach between river mile 17.6 and 16.2 is listed as Essential Habitat for *Lampsilis higginsii* (USFWS 1983). A total of 34 species has been collected in the area (Table 1) including *L. higginsii* (federally endangered); *Cumberlandia monodonta, Cyclonaias tuberculata, Ellipsaria lineolata*, and *Elliptio crassidens* (Wisconsin endangered); and *Quadrula metanevra, Simpsonaias ambigua*, and *Tritogonia verrucosa* (Wisconsin threatened).

In July 1995, 24,557 unionids were relocated from the U.S. Highway 20 bridge construction area in the Wolf River near Shawano, Wisconsin. The Wolf River is a small high-gradient Lake Michigan drainage stream with riffles and pools, and is 70 m wide and up to 1.5 m deep in the relocation area. Substrate is mostly cobble and gravel with areas of hard pan clay and loose sand. A total of 22 species was collected in the project area including *Epioblasma triquetra* (Wisconsin endangered), *T. verrucosa* (Wisconsin threatened), and *Alasmidonta marginata* and *Pleurobema coccineum* (Wisconsin special concern species).

Materials and Methods

Before beginning each relocation, construction and potential impact areas were delineated. An area near the construction zone, with stable substrate and an existing unionid community, was selected as the relocation area, and 0.25 m^2-quadrat samples were collected by divers to determine substrate composition and existing unionid community characteristics.

Handling methods during collection and relocation varied somewhat among study areas, but several guidelines were followed. Unionids were collected and handled by experienced people, were not relocated during extreme hot or cold weather, were kept in water most of the time, and were handled in small batches.

In general, animals were kept moist or in water throughout each relocation. However, handling during transport varied among relocations. During the St. Croix and Wolf River relocations, unionids were quickly transferred from the river to a large (1.1 m x 0.8 m x 0.8 m) flow-through holding tank in the transport boat. Water siphoned from at least 0.5 m beneath the river's surface was continually pumped into the tank and allowed to flow out through a surface drain. A rack in the bottom of the tank prevented animals from lying in accumulated debris. Water temperature and dissolved oxygen were continually monitored in the tank, which was drained and cleaned at the end of each day.

Unionids were also quickly transferred out of the tank and into the river. Individuals were out of water for brief intervals during sorting, counting, marking, and measuring.

Unionids in the Meramec River relocation were transported between the collection and relocation area in moist burlap. Animals were briefly removed from the burlap for processing. Unionids collected in the Elk River were walked upstream to the relocation area in collecting bags, but were out of water during most of the 180 m-walk.

Monitoring grids adapted from Waller et al. (1993) were established in relocation areas. A limited number of unionids were marked with a sequential number (using a Dremel tool), measured (length in mm), weighed (g), aged (external annuli count), and placed in the grids. Unionids not placed in grids were identified, counted, and distributed in a designated general area.

Relocated unionids were monitored 1 month following relocation to assess immediate mortality and 1 year after relocation to assess long-term mortality. One month following the St. Croix, Meramec, and Wolf River relocations, a diver searched grid and general relocation areas, collecting any marked and unmarked dead shells. One year following all relocations (Meramec, St. Croix, and Elk River completed to date), one half of the grid cells were sampled by excavating the cells and collecting all unionids.

Observed mortality refers to mortality estimated from recovered shells and live unionids, since the fate of nonrecovered marked shells is unknown. Recovery refers to the percentage of marked unionids recovered during monitoring.

Results and Discussion

Preliminary data for relocations conducted in different rivers with different species and different handling techniques indicate that relocation can be successful if a few simple guidelines are followed. Recovery during the first year was high for most species in all studies even though handling methods varied among sites.

Observed mortality was minimal (< 1%) 1 month following the St. Croix, Wolf, and Meramec River relocations. Almost 600 unionids were marked and placed in monitoring grids in each of

the St. Croix River relocations, and only one marked shell was recovered 1 month following each relocation (Table 2). No marked shells were found in Meramec or Wolf River grids, although shells could have been carried away by flow or predators, or buried and not readily observable. All unionids placed in the St. Croix and Wolf River general relocation areas were not examined. However, many live marked individuals were found while only three dead marked shells were observed in the 1994 St. Croix River general relocation area, and only six dead marked shells were observed in the 1995 St. Croix River and in the Wolf River general relocation areas (Table 2).

Recovery was high and observed mortality was minimal 1 year following the St. Croix, Meramec, and Elk River relocations, although recovery and observed mortality did vary among species within each relocation (Table 3). Recovery ranged from 50% in the Meramec River, a dynamic area with fairly unconsolidated gravel substrate and fairly high flow, to 96% in the Elk River, a dynamic small river with high flow but very consolidated substrate. Recovery of live marked unionids was 71% in the St. Croix River, although some mortality (1%) was observed.

In the Meramec River no marked shells were recovered and recovery of *L. abrupta* and *F. ebena* was over 80% 1 year after the relocation (Table 3). The only species with a low recovery rate (29%) was *A. confragosus*, which is typically an active species (Dunn, pers. obs.). In the St. Croix River, recovery was lowest for *Truncilla truncata* (50%) and highest for *Q. metanevra* (83%). Mortality was observed for *L. higginsii* (one individual) and *T. verrucosa* (two individuals). In the Elk River recovery was 100% for all species except *Quadrula p. pustulosa* (60%) and no marked shells were found.

Factors contributing to the success of these relocations appear to be careful handling and selection of the relocation area. Guidelines we think should be followed for a successful relocation include:

1. Use field personnel familiar with unionids.

Handling errors, such as roughly removing animals from the substrate, leaving animals out of water or in stagnant water, and not replacing animals in a natural position in the substrate, are minimized by using personnel familiar with unionid biology.

2. Select a relocation area with stable substrate and a similar unionid community that is near the collection area.

We agree with Sheehan et al. (1989) and Cope and Waller (1995) that site selection is one of the key factors in successful unionid relocations. Our goal was to select areas with stable substrate and an existing unionid community at least as species rich and dense as the construction area and that was as close as possible to the construction area. Placing unionids in stable substrate should enhance relocated unionid recovery (Sheehan et al. 1989). In many cases unionids occur in an unstable substrate, such as unconsolidated sand or gravel. However, these areas probably have a high degree of substrate and therefore unionid movement (Golightly 1982; Vannote and Minshall 1982; Huehner 1987). Although this may be natural (Matteson 1955) and may not result in unionid mortality, the probability of recovering relocated unionids is greater if the animals remain in a designated area.

Selecting a relocation area with an existing unionid community near the collection area should ensure that habitat conditions are suitable for unionids and similar to those under which the animals are currently living (such as water quality and fish species) as well as minimize transport time between construction and relocation areas. The variables determining unionid distribution are complex and attempts to quantify microhabitat and

Table 2. Monitoring results 1 month after relocation.

Site	Year	No. marked unionids placed in grids	No. marked shells recovered in grids	No. marked unionids placed in general area	No. marked shells recovered in general area
St. Croix River	1994	598	1	8,398	3
Meramec River	1994	61	0	4,453	NS
Elk River	1994	100	NS	102	NS
Wolf River	1995	831	0	23,726	6
St. Croix River	1995	591	1	13,436	6

NS=Not sampled

determine variables useful in predicting unionid distribution have met with little success (Strayer 1981; Holland-Bartels 1980; Strayer and Ralley 1993). A seemingly suitable area currently devoid of unionids may support unionids; however, unknown variables may be preventing natural unionid colonization. Selecting a relocation area with an existing unionid community reduces the chance of choosing unsuitable unionid habitat.

3. Keep animals moist or in water and minimize out-of-water time.

Unionids require humid conditions for gas exchange, and keeping animals moist will enhance survival (Waller et al. 1995).

4. Avoid extreme temperatures.

Unionid mortality during relocation has been attributed to extremely cold water and air temperature (Heath, pers. comm.; Miller, pers. comm.) and an extreme difference between water and air temperature (Koch, pers. comm.). Although temperature effects on survival have not been tested (Cope and Waller 1995), stress is typically evident in unionids held out of water on hot or cold days. Unionids should be relocated under moderate air and water temperatures, and animals should not be exposed to extreme cold or heat.

5. Avoid crowding animals.

Overcrowding may have negative effects on unionids due to waste accumulation and oxygen

Table 3. Monitoring results 1 year after relocation.

Species	No. placed in sampled cells[1]	No. collected live	No. shells collected	Percent recovered live	Percent observed mortality
Meramec River					
Arcidens confragosus	14	4	0	29	0
Fusconaia ebena	1	1	0	100	0
Lampsilis abrupta	7	7[2]	0	86	0
Obovaria olivaria	0	1[2]	0		0
Total	22	13	0	50	0
St. Croix River					
Amblema plicata plicata	129	98	0	76	0
Cyclonaias tuberculata	0				
Ellipsaria lineolata	10	7	0	70	0
Elliptio crassidens	4	3	0	75	0
Fusconaia flava	48	37	0	77	0
Lampsilis higginsii	20	13	1[2]	65	7
Obliquaria reflexa	43	28	0	65	0
Quadrula metanevra	24	20[2]	0	83	0
Tritogonia verrucosa	8	4	2	50	33
Truncilla truncata	30	15	0	50	0
Total	316	225	3	71	1
Elk River					
Actinonaias ligamentina	29	29	0	100	0
Elliptio crassidens	1	1	0	100	0
Fusconaia flava	1	1	0	100	0
Fusconaia subrotunda	10	10	0	100	0
Lampsilis ovata	5	5	0	100	0
Quadrula pustulosa pustulosa	5	3	0	60	0
Total	51	49	0	96	0

[1]Only half of the cells in each grid were sampled.
[2]Animal moved from adjacent cell into sampled cell.

depletion; however, research on these effects is currently lacking. The number of unionids placed in collecting bags was limited to approximately 100 and the number transported in the flow-through holding tank was limited to approximately 500.

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PIT tags increase effectiveness of freshwater mussel recaptures

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Abstract. Translocations are used increasingly to conserve populations of rare freshwater mussels. Recovery of translocated mussels is essential to accurate assessment of translocation success. We designed an experiment to evaluate the use of passive integrated transponder (PIT) tags to mark and track individual freshwater mussels. We used eastern lampmussels (*Lampsilis radiata radiata*) as a surrogate for 2 rare mussel species. We assessed internal and external PIT-tag retention in the laboratory and field. Internal tag retention was high (75–100%), and tag rejection occurred primarily during the first 3 wk after tagging. A thin layer of nacre coated internal tags 3 to 4 mo after insertion, suggesting that long-term retention is likely. We released mussels with external PIT tags at 3 field study sites and recaptured them with a PIT pack (mobile interrogation unit) 8 to 10 mo and 21 to 23 mo after release. Numbers of recaptured mussels differed among study sites; however, we found more tagged mussels with the PIT-pack searches with visual confirmation (72–80%) than with visual searches alone (30–47%) at all sites. PIT tags offer improved recapture of translocated mussels and increased accuracy of posttranslocation monitoring.

Key words: PIT tags, freshwater mussels, survival, recapture, *Lampsilis radiata radiata*, translocation.

A goal in the national strategy for the conservation of native freshwater mussels is to "develop, evaluate, and use the techniques necessary to hold and translocate large numbers of adult mussels" (National Native Mussel Conservation Committee 1997). Successful recovery of translocated mussels is essential for accurate assessment of translocation success. Previous studies of freshwater mussel translocation used visual searches to recover mussels with varied success (Layzer and Gordon 1993, Havlik 1995, Bolden and Brown 2002, Cope et al. 2003). Survival estimates of translocated mussels often are based on the number of mussels recaptured or found dead, and mussels that are not recaptured are assumed to have emigrated from the study site (Dunn and Sietman 1997, Hamilton et al. 1997, Dunn et al. 2000). A review of 33 mussel translocation studies found a mean estimated survival rate of 51% (but mortality was not reported in 27% of the studies); the average recapture rate was 43% (range: 1–97%) (Cope and Waller 1995).

Passive integrated transponder (PIT) tags may be an effective tool for tracking translocated mussels to increase accuracy of survival estimates. PIT tags are electronic glass-encased microchips that are activated by an inductive coil. They can be attached to an organism internally or externally. The tag is passive until activated by a fixed or portable reader with an antenna. When activated, the tag transmits a unique code to the reader, identifying the individual organism (Gibbons and Andrews 2004). Tag longevity is indefinite because an internal power source is not needed. In aquatic systems, PIT tags have been used extensively to study fish passage past stationary antennae or readers (Zydlewski et al. 2001). Portable PIT-tag systems are used in shallow waters to assess spatial distributions of local fish populations, fine-scale

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movements, and microhabitat preferences (Roussel et al. 2000, Hill et al. 2006). This mobile application is ideally suited to freshwater mussel translocation studies because mussel movements often occur over short distances.

Traditional mussel recapture methods depend on visual encounters and excavation to locate burrowed mussels. PIT tags may enhance mussel recapture at sites where visibility is poor (e.g., turbid water) or when mussels are burrowed in sediments. Reliability of any tagging method depends on tag retention. The tagging method selected for freshwater mussels depends on shell thickness and the type of habitat into which the tagged mussels will be placed. Internal tagging may be best for thick-shelled species, whereas external PIT-tag placement may be more appropriate for thin-shelled species. In a fast-flowing environment with a rocky substrate, an external PIT tag might be dislodged, whereas an internal PIT tag would be protected from abrasion.

We designed an experiment to evaluate the use of PIT tags to mark and track individual freshwater mussels as part of a larger study to determine the feasibility of translocations of 2 state-listed threatened mussel species (tidewater mucket [*Leptodea ochracea*] and yellow lampmussel [*Lampsilis cariosa*]) in response to an impending dam removal. The objectives of our study were to evaluate internal and external PIT-tagging methods, retention, and posttagging survival in freshwater mussels and to determine the effectiveness of PIT-tag technology for mussel recaptures. We used the relatively common eastern lampmussel (*Lampsilis radiata radiata*) as a surrogate for the listed species to develop the method. We tested internal tagging methods for future use with thick-shelled species (e.g., yellow lampmussel) and external attachment for use with thin-shelled species (e.g., tidewater mucket).

Methods

Internal PIT tagging: mantle separation

We used 2 methods to place internal PIT tags. For method 1 (mantle separation), we placed the mussels in sandy substrate, waited until they were actively siphoning and slightly gaped, and then inserted a micropipette tip between the valves to separate them by ~5 mm. We teased the mantle tissue away from the shell and inserted the PIT tag (Digital Angel, South St. Paul, Minnesota) between the mantle and shell along the midventral margin. We also marked all mussels externally with numbered bee tags (The Bee Works, Orillia, Ontario) cemented (GC Fuji I Glass Ionomer Luting Cement; Henry Schein, Melville, New York) to the posterior end of the left valve. We sealed the bee tags with Delton Light Curing Pit and Fissure Sealant (Henry Schein). Control mussels received only the numbered bee tags. We were able to tag ~20 mussels/h with this method. Most of our time was spent waiting for mussels to gape so we could insert the micropipette tip.

In October 2004, we collected eastern lampmussels (55–101 mm length, $n = 164$) from the impoundment that will be dewatered following the Fort Halifax dam removal in the Sebasticook River near Winslow, Maine. In November 2004 (24–35 d after capture), we partitioned the mussels into a control ($n = 40$) and 3 tag-type treatment groups: 23-mm tags ($n = 40$), 12-mm tags ($n = 44$), and 12-mm tags with an antimigration cap (a plastic sleeve encasing one end of the 12-mm tag to encourage tissue adherence; Biomark, Boise, Idaho; $n = 40$). Each group consisted of mussels of all sizes (control: length 55–99 mm, 23-mm tags: length 58–101 mm, 12-mm tags: length 58–99 mm, 12-mm tags with cap: length 58–96 mm).

We maintained mussels in the Aquaculture Research Center (ARC), University of Maine, Orono, Maine, in three $2.44 \times 0.61 \times 0.30$-m fiberglass tanks filled with sand (13 cm deep) and recirculating water. We divided the mussels in each group among 3 replicates (13–15 mussels/replicate) and distributed 1 replicate from each group in each tank.

We fed the mussels an algal diet (*Phaeodactylum tricornutum*, *Chaetocerus-B.*, and *Nannochloropsis oculata*; Algae Spat Formula [Innovative Aquaculture Solutions, Inc., Vancouver, British Columbia]) 3 times/wk. During each feeding, we stopped water recirculation and applied 40 to 50×10^9 algal cells/tank (R. Mair, Virginia Polytechnic Institute and State University, personal communication). To simulate changes in seasonal water temperature, we gradually reduced water temperature from 18°C (October) to 10°C (December) and maintained 10°C until the following April, then gradually increased the temperature to 18°C by June. We monitored the mussels for mortality 3 times/wk and examined them for tag retention in November 2004 and in February, April, and June 2005.

Internal PIT tagging: mantle incision

We developed a 2nd internal PIT-tagging method (mantle incision) with techniques from the cultured pearl industry (H. Dan, Virginia Polytechnic Institute and State University, personal communication). We implanted PIT tags by inserting a micropipette tip between the mussel valves to separate them by ~5 mm, making an incision with a scalpel in the midventral mantle tissue, inserting the tag between the mantle and the shell through the incision, and then removing the

micropipette tip. We also marked all mussels externally with bee tags on the posterior end of the left valve. Inserting the tags took little time (20 mussels/h). Most of our time was spent waiting for mussels to gape so we could insert the micropipette tip.

In June 2005, we collected 112 eastern lampmussels (43–101 mm length) from the Sebasticook River impoundment and randomly assigned the mussels into 3 groups consisting of a control ($n = 27$) and 2 tag-type treatment groups (23-mm tags: $n = 43$, 12-mm tags with cap: $n = 42$) with 3 replicates/group (9–15 mussels/replicate), being careful to include mussels of all sizes in each group. We did not test the 12-mm tags without caps because of poor retention in the mantle-separation experiment.

We maintained tagged mussels in the ARC for 21 d to ensure tag retention and then placed 1 replicate from each group in sand in each of 3 enclosures (1 × 2-m polyvinyl chloride [PVC] pipe and rebar frames covered in hardware cloth) in Unity Pond, Maine. Unity Pond is a 1039-ha lake connected to the Sebasticook River upstream of the Winslow mussel collection site. Unity Pond contains a natural population of eastern lampmussels and thus is suitable habitat for the species. Before placing the mussels in the enclosures, we reinserted rejected tags ($n = 9$). We examined the mussels to assess tag retention and survival 60 d (August 2005) and 371 d (June 2006) after tagging.

External PIT tagging

We tested the reliability of external PIT-tag attachment and determined the probability of recapturing translocated PIT-tagged mussels that were not confined to enclosures (as in the previous experiment). We placed external PIT tags on 238 eastern lampmussels (41–88 mm length) collected during September and October 2004 from various sites in Unity Pond ($n = 90$), Sandy Stream (a 1st-order, spring-fed stream that drains into Unity Pond; $n = 88$), and the Sebasticook River impoundment near Winslow ($n = 60$). We chose these water bodies because they had naturally occurring populations of eastern lampmussels and the 2 listed species, and because, based on neutral markers, Sebasticook River and Sandy Stream populations of these mussels were genetically similar (Kelly 2004).

We tagged mussels by cementing a PIT tag to the posterior end of the right valve and a numbered bee tag to the posterior end of the left valve. After the first 30 tags (at Unity Pond), we completely encapsulated the PIT tag in dental cement to increase tag retention. We placed tagged mussels in water before the cement was fully cured (~5 min after application) to avoid overdrying and cracking of the cement. We tagged ~30 mussels/h with this method. Most of our time was spent waiting for the bee-tag sealant to dry. We used 23-mm tags at all sites. We also used some 12-mm tags at Sandy Stream and Unity Pond because of a limited supply of cement.

TABLE 1. Numbers of mussels tagged with passive integrated transponder tags in each translocation treatment during September and October 2004.

Site	Tagged and replaced (site control)	Moved within water body	Translocated from Sebasticook River
Sandy Stream	30	26	32
Unity Pond	30	30	29
Sebasticook River	30	30	–

We compared survival of translocated mussels among within-water body, between-water body, and within-site (control) translocation treatments. We measured, tagged, and moved mussels to 1 × 2-m plots or replaced them where they had been found (Table 1). We marked the corners of the plots with stakes with flagging, and recorded Global Positioning System (GPS) locations for each plot and for each of the tagged mussels that were returned to their original location.

We recaptured externally PIT-tagged mussels with a mobile PIT detection unit (PIT pack). The PIT pack used Destron Fearing FS1001A DC-powered, full duplex transceivers and custom-designed portable antennas. When a PIT tag was within range of an antenna (~0.5 m), the tag emitted a 134.2-kHz (ISO standard frequency) radio frequency, which was transmitted back to the receiver for decoding. The antennas, enclosed in an airtight PVC wand and attached to the transceiver, consisted of several wraps of 12- to 18-gauge wire, with inductance values ranging from 325 to 375 μH and a set of capacitors (Hill et al. 2006). The capacitors were attached to an antenna lead cable from the transceiver, fixing the capacitance between 33 and 44 nF. The fixed capacitance was used within the transceiver in conjunction with the adjustable capacitance to tune the resonance frequency of the system to 134.2 kHz (Hill et al. 2006). We tuned the adjustable capacitor while antennas were submerged. We conducted all field experiments with the PIT pack tuned to phase 0 to 2%, signal 1 to 20%, and current 2.5 to 5.0 amps.

We searched the release sites for externally PIT-tagged mussels ~30 d after tagging (October 2004) and visually confirmed recaptures with snorkeling. If the PIT-tag reader registered a tag but no mussel was observed, we assumed the mussel had burrowed into the substrate. To minimize substrate disturbance, we did not excavate burrowed mussels preparing to

overwinter. These data were not used in the calculations of recapture success because the signals may have been from detached tags.

During June and July 2005 (271–355 d after tagging) and July and August 2006 (670–750 d after tagging), we searched again for PIT-tagged mussels at the release sites, beginning at the last location recorded with GPS during October 2004. In 2005, we conducted initial searches without the PIT pack to provide recapture percentages with visual searches only. We visually searched each site for 2 d. Approximately 1 wk later, we searched the sites using PIT-pack searches with visual confirmation and excavation to confirm recaptures (3–4 d/site). In 2006, we repeated the PIT-pack searches with visual confirmation (3 d/site). Water clarity was too poor to conduct visual searches in 2006. If the PIT pack detected a tagged mussel, but we did not see the mussel, we excavated the area within 0.5 m of the signal to 15 to 45 cm deep to determine if the signal was coming from a burrowed mussel or an unattached tag. If we found no tagged mussel after excavation, we assumed the tag had become detached. We searched (with snorkeling and the PIT pack) the sites at Unity Pond and the Sebasticook River 4 times each to at least 3 m beyond the perimeter of the original study area to detect mussels that may have moved. We also searched the shorelines for valves from dead mussels. Extensive ice scouring and spring flooding substantially reconfigured the substrate at the Sandy Stream site, so in addition to searching the study area plus 3 m beyond the perimeter, we also swept the antenna bank to bank downstream of the site for 200 m over a total of 3 d. We calculated recapture rates by dividing the number of mussels recaptured at each site by the number tagged.

Data analysis

We used adjusted χ^2 for small sample sizes (Gotelli and Ellison 2004) for all analyses.

We compared long-term tag retention among tag types and mussel mortality among treatments and controls for both mantle separation and mantle incision methods. We compared the percentages of recaptures using visual searches alone with the number of recaptures using PIT-pack searches with visual confirmation.

Results

Mussel retention of internal PIT tags in the laboratory (mantle separation)

Five percent of the PIT tags were rejected within 2 wk of internal placement via mantle separation. By 100 d after tagging, rejection had increased to 10% for 12-mm tags with caps, 12.5% for 23-mm tags, and 30% for 12-mm tags without caps. High mortality with this method was more troubling than the rejection rates. By 100 d after tagging, mortality rates were 3% for the control group (no tags), 10% for the group with 12-mm tags with caps, 25% for the group with 23-mm tags, and 27% for the group with 12-mm tags without caps. This mortality may have been caused by inexperience with the tagging procedures and mussel aquaculture husbandry (mortality in control mussels was 3% 100 d after tagging and 73% 244 d after tagging), so we discontinued using the 12-mm tags without caps, switched to the mantle-incision method, and retained the tagged mussels in field enclosures.

Long-term tag retention did not differ among tag types (adjusted $\chi^2 = 5.61$, $p = 0.691$, df = 8), and mortality did not differ among the tag-type and control groups (adjusted $\chi^2 = 7.97$, $p = 0.716$, df = 11) 100 d after tagging. We examined the condition of the PIT tags in all mussels that died over winter. By 90 d after tagging, all 12-mm PIT tags with caps were coated with nacre and attached to a valve. By 120 d after tagging, 23-mm and 12-mm PIT tags without caps that had not been rejected were similarly attached.

Mussel retention of internal PIT tags in field enclosures (mantle incision)

All mussels in the control and tag-type groups (mantle incision) were still alive 60 d after tagging (40 d after transport from the ARC to the Unity Pond enclosures) (Table 2). One 23-mm tag was rejected after the mussels were placed in the enclosures; this rejected tag was not one of the tags that had been rejected and reinserted within the 2-wk posttagging observation period. By June 2006 (371 d after tagging), 2 mussels in the enclosures had died (1 control, 1 with a 23-mm tag), and one 12-mm tag with cap was rejected. Long-term tag retention did not differ among tag types (adjusted $\chi^2 = 4.26$, $p = 0.833$, df = 8), and mortality did not differ among control and tag-type groups (adjusted $\chi^2 = 3.72$, $p = 0.882$, df = 11) 371 d after tagging.

Retention of external PIT tags and recapture of mussels in the field

Overall, ~93% of the recaptured tagged mussels retained the PIT tag (Table 3). Recapture rates with PIT-pack searches with visual confirmation exceeded recaptures from visual searches alone at all study sites during June and July 2005 (adjusted $\chi^2 = 10.198$, $p = 0.0014$, df = 1; Fig. 1). During June and July 2005 and July and August 2006, we used a combination of visual searches alone and PIT-pack searches with visual

TABLE 2. Percent mortality and % tag retention (60 d and 371 d after tagging using the mantle-incision method) of eastern lampmussels with internal passive integrated transponder tags in field enclosures in Unity Pond, Maine.

Treatment	60 d after tagging		371 d after tagging	
	% mortality	% tag retention	% mortality	% tag retention[a]
23-mm tag ($n = 43$)	0	98	2.5	97.5
12-mm tag with cap ($n = 41$)	0	100	0	97.4
Control (no tag) ($n = 27$)	0	–	4.3	–

[a] Includes mussels that died with retained tags

confirmations to recapture 77% of externally tagged mussels at Unity Pond and 80% of externally tagged mussels in the Sebasticook River (combined results from 2005 and 2006 recaptures). In Sandy Stream, where ice scouring and spring flooding reconfigured the substrate, we recovered only 25% of the tagged mussels. Ninety-five percent of the mussels we did recapture were found using PIT-pack searches with visual confirmation, and only 1 mussel was found using visual searches alone. In Sandy Stream, we found 71% of recaptured mussels >100 m from their October 2004 locations, whereas we found recovered mussels in Unity Pond and the Sebasticook River <2 m from their September–October 2004 locations. Seventeen percent (Unity Pond), 17% (Sebasticook River), and 3.5% (Sandy Stream) of the recaptured mussels found with the PIT pack were completely burrowed into the substrate (Fig. 1). We found most burrowed mussels within 6 cm of the sediment surface. However, the PIT pack detected 1 tagged (23-mm tag) living mussel burrowed 45 cm into the substrate and 3 tagged dead mussels 20 to 30 cm below the substrate surface in Sandy Stream. We also found 1 dead mussel with a PIT tag during shore sweeps at the Sebasticook River site.

Discussion

Tagging methods

Low mortality (<2%), high tag retention (~97%), and evidence that tags had fused to the shell 3 to 4 mo after tagging suggest that internal PIT tagging using the mantle-incision method may be a viable method of tagging thick-shelled freshwater mussel species that can be pried open for tag insertion without damaging the shell. Long-term survival of captive freshwater mussels is low (Patterson et al. 1997, 1999, Nichols and Garling 2002), and high mortality of captive mussels in our study (73–93% 255 d after tagging) might be attributed to inadequate nutrition, winter water temperatures in the ARC that exceeded temperatures at the mussel collection sites, and physiological stresses experienced by captive mussels that were gravid when captured. The low mortality of mussels tagged with the mantle-incision method and placed in the enclosures at Unity Pond supports this assertion. We strongly recommend field trials rather than aquaculture experiments for testing methods intended for use in the field to remove uncertainty of the effects of captivity on mussel survival.

External PIT-tag retention also was high (~93%)

TABLE 3. Percent recapture, % mortality, and % tag retention of externally passive integrated transponder–tagged eastern lampmussels in translocation experiments within and among sites (~21 mo after tagging) in Maine.

Site[a]	Treatment	Number tagged	% recapture	% mortality[b]	% tag retention[c]
Unity Pond	Translocated from Sebasticook River impoundment	29	93.1	0	100
	Translocated within Unity Pond	32	74.2	0	78.3
	Site control (not moved)	30	63.3	0	89.5
Sebasticook River	Translocated within Sebasticook River impoundment	30	93.3	0	96.4
	Site control (not moved)	30	66.7	6.7	100
Total		151	78.0	1.3	93.2

[a] Sandy Stream data omitted because of winter ice scouring and spring flooding
[b] Percent mortality calculated only for recaptured mussels
[c] Retention calculated as % recaptured mussels retaining tags

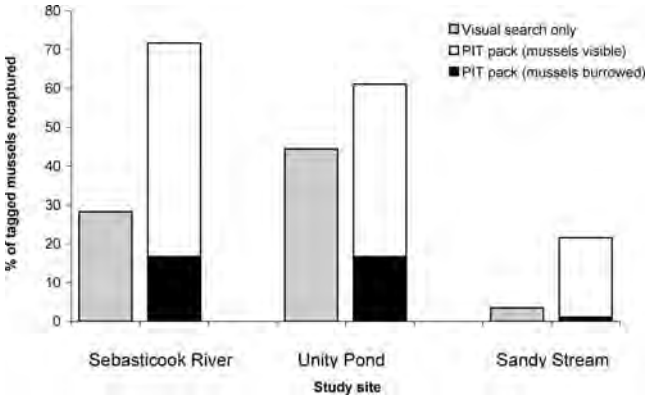

FIG. 1. Percentages of mussels externally tagged with passive integrated transponder (PIT) tags recaptured using different methods during June and July 2005.

when the PIT tag was completely encapsulated in cement and the mussel was placed in water within 5 min of cementing. However, retention was more variable with external tagging than with internal tagging methods, and ranged from ~78 to 100% at the Unity Pond site 9 mo after tagging. We attribute low retention to incomplete coverage with cement. Retention of tags completely encapsulated with cement ranged from 89.5 to 100%. We observed evidence of some cement loss from recaptured mussels; occasional reapplication of cement will ensure long-term retention of external PIT tags. Internal tag placement via mantle incision is a viable alternative to external attachment in environments where tag loss from abrasion is likely.

Previous studies assessed external freshwater mussels tagging methods with visual searches to relocate mussels marked with numbered tags (Lemarié et al. 2000) or coded wire tags inserted into mussels held in suspended pocket-nets (Layzer and Heinricher 2004). Both of these tagging methods resulted in higher tag retention than in our study, but mussels tagged using these methods can be detected only with visual searches. PIT tags provide an alternative tool for finding mussels, and this method is especially useful for long-term monitoring or where visual searches are impractical or time consuming.

Mussel recapture efficiency

The proportion of mussels visible at the substrate surface may vary by locality, time of year, species, and gender. Smith et al. (2001) detected only 31% of clubshells (*Pleurobema clava*) at the substrate surface, whereas 52% of northern riffleshells (*Epioblasma torulosa rangiana*; 80% females, 45% males) were visible. Wick (2006) observed that >90% of eastern lampmussels had burrowed to 10 to 15 cm at Sandy Stream by August, but only 26% had burrowed in the Sebasticook River impoundment at that time.

Because the water was turbid, we found burrowed mussels and mussels that would have been overlooked had the sites been searched only visually. For example, water clarity in Unity Pond was routinely poor, and only 47% of tagged mussels were recaptured visually, whereas 72% of tagged mussels were recaptured with the PIT pack and visual confirmation. In the Sebasticook River, where the visibility was compromised by silt covering the mussels, the recaptures with the PIT pack and visual confirmation (80%) were >2× those of the visual searches alone (29%). Initially, PIT tags also provided a visual cue of tagged mussels in clear water, but after several months in the water, the cement was stained or covered with algae and indistinguishable from the shell. When first applied, the white cement might provide a visual cue to predators, but only 1 shell was found in a shoreline midden in our study. Tinting the cement a dark color might eliminate this possible problem.

Low recaptures in Sandy Stream probably were caused by extensive downstream displacement of mussels in late winter and early spring when ice scour and high water flows during snowmelt reconfigured the stream bottom. The low recapture rates of PIT-tagged mussels at this site were attributed to tag loss from severe abrasion, burial in sediment beyond the detection limit, or transport beyond the regions searched.

Limitations of PIT tags in field applications

Debris on the substrate and signal interference caused by nearby iron objects (Hill et al. 2006) can affect reliability of the PIT pack. The antenna configuration we used also is limited to sites with water depth <2 m. Maximum effective depth and antenna range are not necessarily uniform among sites; these limitations should be identified at each field site so that mussel absence can be distinguished from nondetection caused by equipment limitations. Reducing the antenna size for use while snorkeling, waterproofing the PIT pack for diver use, and lengthening the antenna handle are modifications that will broaden field use of this tool. At present, PIT-tag use is limited to larger mussels (length >20 mm). However, smaller tags with greater detection ranges are in development, and eventually it should be possible to tag smaller mussels, at least externally. Although internal tags were retained, the ~3-wk captive period to ensure tag retention could limit the usefulness of internal tags. Internally tagged mussels should be held in field

enclosures during the initial posttagging period when tag rejection may occur. Retaining a subset of internally tagged mussels may be a viable alternative for estimating tag retention proportions when large numbers of mussels are translocated.

The initial cost of the PIT tags and reader may exceed start-up costs for other mussel-tagging methods. The PIT pack (transceivers, batteries, antenna) we used cost ~$10,000 to construct and was designed for research on a variety of organisms such as fish, mussels, and amphibians. Smaller units can be developed for ~$2500. The PIT tags we used cost $3.50 each, but the tags work indefinitely. On the other hand, the percentage of tagged mussels recaptured using PIT tags far exceeded the percentage recaptured during visual searches. Visual searches can be time consuming and labor intensive. For long-term monitoring of individuals and populations, the added initial costs may be recouped over time, and it may be possible to share the costs with other investigators using PIT tags.

In conclusion, PIT tags permit repeated, nondestructive sampling of individuals with little disturbance, last indefinitely, and appear to have negligible effects on short-term survival of freshwater mussels. PIT tags were retained using both internal and external attachment methods. Thus, the choice of tagging method will depend on shell thickness, habitat characteristics, and ease of implementation in the field.

The need for freshwater mussel translocations to protect and conserve threatened and endangered mussel species will increase as aquatic habitat alteration continues. Superior recapture rates with PIT tags suggest that this tool is valuable for use in mussel translocations and monitoring and may improve accuracy of survival estimates for assessing translocation success. Because PIT tags have indefinite longevity, they can be used in monitoring both translocated mussels and populations at sites of concern, especially populations of endangered or threatened species. Moreover, because PIT tags provide reliable individual identification, they may be a useful tool for monitoring the growth and survival of individual mussels.

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