EVALUATION OF RELOCATION OF UNIONID MUSSELS TO *IN SITU* REFUGIA

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ABSTRACT

The aim of this study was to evaluate the recovery and survival of four species of unionid mussles [pimpleback, *Quadrula pustulosa pustulosa* (I. Lea, 1831); spike, *Elliptio dilatata* (Rafinesque, 1820); Higgins eye, *Lampsilis higginsii* (I. Lea, 1857); and pocketbook, *Lampsilis cardium* (Rafinesque, 1820)] that were experimentally relocated to *in situ* refugia in the St Croix River of Minnesota and Wisconsin, USA. In 1996, 150 mussels of each of the first three species (450 total) were relocated to three 5 × 5 m study grids (Site A), one near Lakeland, Minnesota, which served as a source-site control, and two in the experimental refuge 48 km upstream, near Franconia, Minnesota. In a second relocation in 1997, *L. cardium* was substituted for *L. higginsii* and 150 mussels of this and each of the other two species (450 total), were relocated to two study grids (Site B). The source site control was near Sunrise, Minnesota and the experimental refuge was 14 km downstream near Almelund, Minnesota. Mussel recovery, survival and substratum characteristics were evaluated annually at Site A for 2 years and for 3 years at Site B. Mean annual recovery of all three species ranged from 90 to 100% at Site A, and from 34 to 70% at site B. The mean annual survival of recaptured mussels ranged from 85 to 100% at Site A, and from 88 to 100% at Site B. The textural characteristics of the substratum differed significantly between the control and the two refuge locations at the beginning of the study, but did not differ from this initial status among subsequent years at Site A. At Site B, there was a significant shift in textural characteristics from large to smaller fractions over the four years. The relatively high survival of mussels during this study demonstrates the importance of proper handling and transport protocols when relocating mussels and the selection of suitable relocation habitat with stable substratum. When established correctly, *in situ* refugia may be a viable tool for preserving unionid mussels.

INTRODUCTION

Freshwater mussels (Unionidae) are one of the most rapidly declining faunal groups in North America. About 67% of the nearly 300 freshwater mussel species found in North America are considered vulnerable to extinction or already extinct (Bogan, 1993; Williams, Warren, Cummings, Harris & Neves, 1993). The decline of mussel populations in North America began by the mid-1800s and has been attributed to over-harvest, construction of dams and impoundments, sedimentation, navigation, pollution and habitat degradation (Fuller, 1974; Bogan, 1993; Brim Box & Mossa, 1999; Vaughn & Taylor, 1999). An additional recent threat to the unionid fauna has come from the introduction of the zebra mussel *Dreissena polymorpha* (Pallas), which colonizes unionids and impedes their movement, reduces their ability to feed and eliminate wastes, and competes for food and space (Mackie, 1991; Schloesser, Nalepa & Mackie, 1996; Strayer, 1999a).

Because of the declines in diversity and abundance of unionid mussels, and the rapid and severe impacts of zebra mussels on native unionids (e.g. Gillis & Mackie, 1994; Nalepa, Hartson, Gostenik, Fanslow & Lang, 1996), a National Strategy for the Conservation of Native Freshwater Mussels was developed to provide a framework for preventing further mussel extinctions and population declines (National Native Mussel Conservation Committee, 1998). This document identified a number of conservation needs, and outlined various goals, strategies and tasks to address these needs. Listed among these items was the recommendation to investigate relocation of native mussels to various types of refugia for their protection and conservation.

The relocation of unionids has been used as a conservation and management tool by state and federal agencies for the past several decades to remove mussels from bridge or other construction zones, to restore or supplement populations that had been adversely affected by pollution or other factors, and to protect populations from colonization by zebra mussels (Sheehan, Neves & Kitchel, 1989; Layzer & Gordon, 1993; Ogawa & Schloesser, 1993; Dunn & Sietman, 1997). Cope & Waller (1995), who evaluated the success of 37 mussel relocations conducted between 1967 and 1994, found that the average rates of survival and recovery were relatively low (51 and 43%, respectively) and were largely influenced by environmental factors such as water and air temperature extremes, improper collection, handling and transport methods, and substratum instability at the relocation site. The objectives of this study were to conduct two experimental mussel relocations, each with a similar design, to evaluate the effectiveness of establishing *in situ* refugia, and to evaluate the influence of refugia characteristics, substratum composition and mussel density on survival and recovery.

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MATERIAL AND METHODS

Study area

The St Croix River, which flows through northwestern Wisconsin and east-central Minnesota, USA, is managed by the US National Park Service (upper 365 km), and the Minnesota and Wisconsin Departments of Natural Resources (lower 43 km). The St Croix Riverway includes the St Croix, Namekagon and other tributary rivers, and encompasses a total catchment of 22,196 km^2 (Fig. 1). The St Croix River forms part of the north-central border between Wisconsin and Minnesota and terminates at the confluence of the Mississippi River, near Prescott, Wisconsin (Fig. 1).

The St Croix River supports 43 species of unionid mussels (Heath & Rasmussen, 1990; Graf, 1997), including two species listed as endangered by the US Fish and Wildlife Service [the Higgins eye, *Lampsilis higginsii* (I. Lea, 1857) and the winged mapleleaf, *Quadrula fragosa* (Conrad, 1835), Code of Federal Regulations, 1993]. Additionally, 23 other species in the river are listed by the States of Wisconsin and Minnesota as endangered, threatened or of special concern. Because the upper St Croix River still supports a relatively large and diverse mussel fauna that has not yet been colonized by reproducing populations of the exotic zebra mussel (Karns, 2002), it represents one of the few remaining natural refuges for unionids in the Upper Mississippi River Basin.

Experimental design

Two study sites were established in the St Croix River to quantify mussel recovery and survival among individuals relocated into mussel beds of differing densities. Site A was located in the lower St Croix River downstream of the dam at St Croix Falls, Wisconsin and Site B was located upstream of the dam (Fig. 1). Pre-relocation surveys done near each site indicated differences in the diversity and density of mussels. Locations sampled for Site A near Franconia, Minnesota (n = 240 ¼ m^2 quadrats) revealed 27 mussel species with a mean density of 11.25 m^{-2} and locations sampled near Lakeland, Minnesota (n = 100 ¼ m^2 quadrats) in the lower river had 19 species and an average mussel density of 18.8 m^{-2}. Surveys at Site B, conducted just downstream of the refuge site near Almelund, Minnesota (n = 200 ¼ m^2 quadrats), found 21 mussel species with an average density of 32.4 m^{-2}.

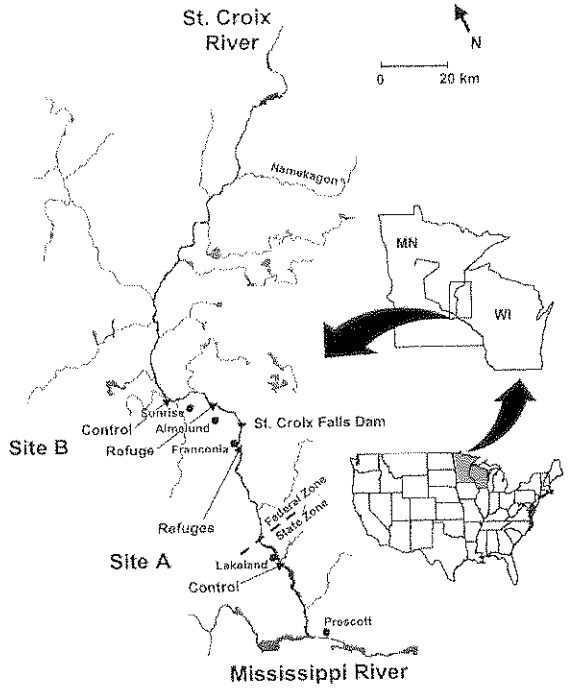

Figure 1. Study area for the experimental relocation of unionid mussels to *in situ* refugia on the St Croix River, Minnesota and Wisconsin from 1996 to 2000, showing the location of the control sites near Lakeland and Sunrise, Minnesota and the refuge sites near Franconia and Almelund, Minnesota.

Study Site A was established between July 29 and August 2, 1996, and Site B between July 3–15, 1997. A total of 150 of each of four species of unionid mussels, two representing the subfamily Ambleminae [pimpleback, *Quadrula pustulosa pustulosa* (I. Lea, 1831); and spike, *Elliptio dilatata* (Rafinesque, 1820)] and one representing the subfamily Lampsilinae (at Site A, Higgins eye, *Lampsilis higginsii* and at Site B, plain pocketbook, *Lampsilis cardium* Rafinesque, 1820), were relocated to *in situ* refugia. The mussels for study Site A were collected by divers from the lower St Croix River, near Lakeland, Minnesota (control site, St Croix River kilometre, SCRKM 27.4, Fig. 1) and relocated into two areas (experimental refugia), near Franconia, Minnesota (SCRKM 78.0). Mussels for Site B were collected by divers from the St Croix River near Sunrise, Minnesota (control site, SCRKM 114.2) and a refuge site was established near Almelund, Minnesota (SCRKM 99.7). Site boundaries were delineated and recorded from permanent landmarks with standard surveying techniques and by a Global Positioning System. A grid (5 × 5 m) composed of 1-m^2 cells was placed at each of these sites. A random (PROC Plan procedure in PC-SAS, SAS Institute Inc., 1996) nested block design (adapted from Waller, Rach, Cope & Luoma, 1993) was used to monitor mussel survival and recovery, to assess the potential effects of physical habitat on unionids and to assess the impact of introducing relocated mussels into an established mussel bed.

At the beginning of the study, ten ¼-m^2 quadrats were collected at fixed points (within 1 m) from peripheral cells (P1-P10, Fig. 2) of each grid at both the control (Lakeland and Sunrise, Minnesota) and refuge (Franconia and Almelund, Minnesota) sites, and analysed for mussel density, species richness, live to dead ratio and sediment characteristics. The experimental design of the grids (Fig. 2) consisted of five randomly selected 1-m^2 cells that served as undisturbed (non-handled) resident controls (RC). Because future conservation and recovery efforts will probably require relocating mussels to existing mussel beds, we also assessed the effects of increased mussel density on overall survival and recovery. Therefore, mussels from an additional five randomly selected cells within each grid were removed and mussel density, species richness and live to dead ratio were determined, and then the mussel density was doubled with mussels from the peripheral cells (double-density controls, DDC). Each of the three species representing the subfamilies Ambleminae and Lampsilinae had five replicates (grid cells) with 10 mussels per replicate for a total mussel density of 10 m^{-2}, plus the natural mussel density in each cell (Fig. 2).

Mussel processing and relocation

At the time of processing, mussels were measured (total length to nearest mm), weighed (total weight to nearest g) and uniquely marked (given a sequential number) by etching the periostracum with a rotary grinding tool. In addition, the shells of all mussels from Site A were thoroughly scrubbed with a wire brush, visually inspected and rinsed in distilled water prior to relocation to ensure that zebra mussels would not be inadvertently transported to the un-infested waters of the upper St Croix River. This study was conducted before unionid mussel quaran-

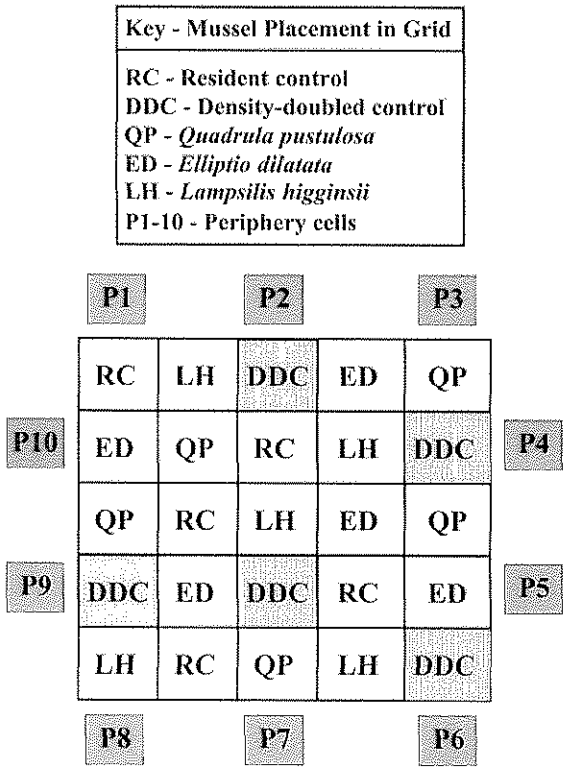

Figure 2. Example of the experimental design used for the mussel treatments and sediment sampling locations at each of the 5 × 5 m monitoring grids at both sites A and B during the experimental relocation of unionid mussels to *in situ* refugia on the St Croix River, Minnesota and Wisconsin.

tine procedures (Newton, Monroe, Kenyon, Gutreuter, Welke & Thiel, 2001) were necessary to prevent the inadvertent introduction of zebra mussels during relocation activities in the St Croix River. The emersion (exposure to air) of mussels was kept to a minimum (<3 min) during processing; average water and air temperatures during collection and processing were 23 and 25°C, respectively.

After processing, 10 mussels from each treatment were placed into labelled mesh dive bags corresponding to their randomly selected placement cell in each of the control and refuge grids. The bags were dampened with distilled water and placed into ice-chests. Mussels were maintained to within ±2°C of the water temperature during transport overland in a single trip (duration of 1 h) to the refuge sites near Franconia and Sunrise, Minnesota. The mussels used in the control grids at Lakeland and Almelund, Minnesota, were given similar transportation exposure conditions and duration as the mussels relocated to the experimental refugia. Once at the refuge and control sites, mussels were removed from the ice-chests and hand-placed in their predetermined grid and treatment cell by a diver.

Sediment collection and analysis

To assess the potential differences in physical habitat among sites, sediment samples were collected from the peripheral cells (P1-P10) at the control and refuge sites (Fig. 2). At each site, a diver obtained ten ¼-m^2 quadrat sediment samples by hand excavating the substratum to a depth of 15 cm and placed each sample into a 20-l plastic bucket. The whole-bucket sediment samples were then weighed on-site and a subsample was taken, placed in a Ziploc© bag, stored on ice and transported to the laboratory within 48 h for further analysis of the fine sediment fractions. The remaining sample from the bucket was passed through a set of three sequential sieves (mesh size openings of 12, 6 and 3 mm) at Site A and five sequential sieves (mesh size openings of 67, 57, 12.5, 6.5 and 0.5 mm) at Site B to obtain a field estimate of sediment particle fractions. At the laboratory, sediment samples were analysed for wet weight, dry weight and particle size (Guy, 1969; Plumb, 1981). Dried samples were analysed for particle size by passing the sediment through a series of nested sieves (11.4, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.062 mm) on a sieve shaker. Particle sizes were classified as cobble and gravel (>2.0 mm), sand (0.062–2.0 mm), and silt and clay (<0.062 mm). Subsamples collected from Site A were also analysed for volatile matter (a surrogate for organic carbon; APHA, AWWA & WPCF, 1995). The volatile matter content of sediment was estimated by loss on ignition at 550°C.

Monitoring

A quantitative assessment of mussel survival, recovery and sediment characteristics was made annually for 2 years following the relocation at Site A and for 3 years following the relocation at Site B. At each annual evaluation, mussels within all cells of each of the grids (except the RC cells in 1997) were collected by a diver, placed into numbered dive bags, identified, enumerated, measured, weighed and replaced into their respective cells. Mussels in the DDC cells were sampled each year to assess natural mortality and population structure. Mussels in the RC cells at Site A were evaluated only in 1998 and mussels in RC cells at Site B were evaluated annually in 1998–2000. Sediment and mussel population samples (n = 10) were taken annually from the peripheral cells (P1-P10) of the grids (Fig. 2) at the control and refuge sites to assess potential changes in physical habitat characteristics or resident mussel density after the relocation. At Site A, mussels were collected from peripheral cells (P1-P10) for the duration of the study, those from Site B were collected from cells oriented like peripheral cells (P1-P10), but set an additional metre out from P1-P10 in 1998, 2 m in 1999 and 3 m in 2000. The latter approach, at Site B, was used to aid recovery of both marked and unmarked mussels outside of the grid due to the high natural density at this site.

Statistical analyses

Statistical analyses were performed with PC-SAS (SAS Institute Inc., 1996). Variation among treatments in mean mortality and recovery of mussels and particle size, and volatile matter content of sediments was evaluated with one-way analysis of variance (ANOVA). All variables were examined for normality and homogeneity of variance (PROC Univariate and Bartlett's test in SAS), and transformed, if necessary, to meet assumptions of statistical tests. The data for textural characteristics and volatile matter content of sediment were arcsine transformed prior to analysis. A Tukey's *hsd* test was used to identify significant differences among treatment means. A Type I error (α) of 0.05 was used to judge statistical significance.

RESULTS

The relocation of mussels to *in situ* refugia in the St Croix River was successful, based on the short-term recovery and survival of mussels after at least 2 years of monitoring. At Site A, recovery of all three species at the sites was greater than 90% (Fig. 3), with the lowest recovery occurring with *Q. p. pustulosa* at the control site in 1997 and with *E. dilatata* at the refuge I site in 1997. At Site

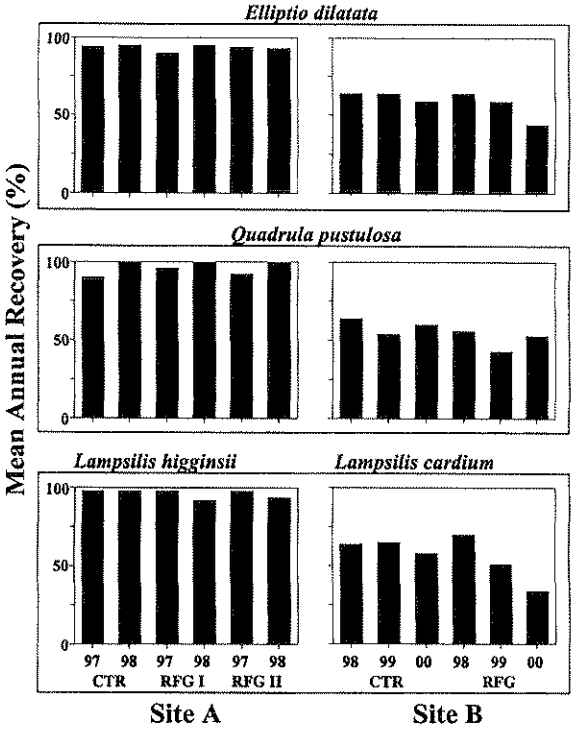

Figure 3. Mean annual recovery (%) of *Elliptio dilatata, Quadrula p. pustulosa,* and *Lampsilis higginsii* at the control (CTR), refuge I (RFG I) and refuge II (RFG II) at Site A, and *Elliptio dilatata, Quadrula p. pustulosa* and *Lampsilis cardium* at the control (CTR) and refuge (RFG) at Site B during the experimental relocation of unionid mussels to *in situ* refugia on the St Croix River, Minnesota and Wisconsin.

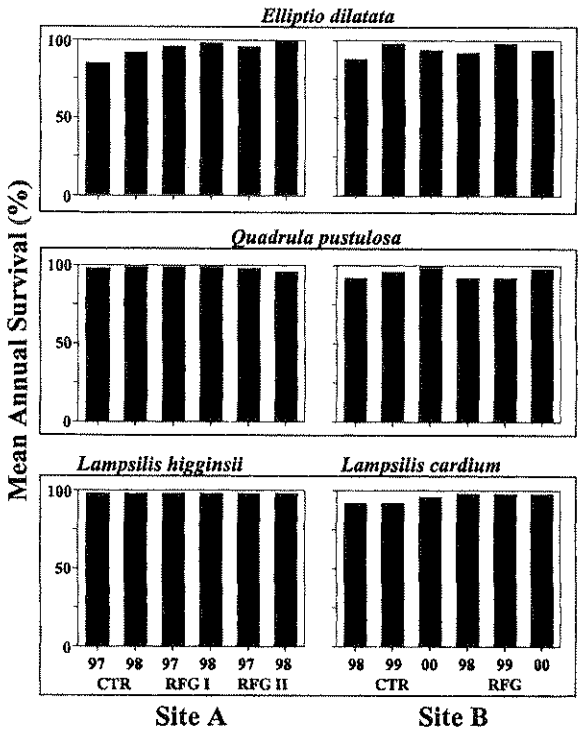

Figure 4. Mean annual survival (%) of *Elliptio dilatata, Quadrula p. pustulosa* and *Lampsilis higginsii* at the control (CTR), refuge I (RFG I) and refuge II (RFG II) at Site A, and *Elliptio dilatata, Quadrula p. pustulosa* and *Lampsilis cardium* at the control (CTR) and refuge (RFG) at Site B, during the experimental relocation of unionid mussels to *in situ* refugia on the St Croix River, Minnesota and Wisconsin.

B, recovery was between 56 and 70% in 1998 for all three species, and ranged between 34 and 65% in 1999 and 2000. Annual mussel survival was calculated based on recaptured mussels, making no assumptions on the condition of non-recovered mussels and, therefore, the annual survival rate was greater than 85% at Site A and greater than 88% at Site B (Fig. 4). At Site A, the lowest annual survival (85%) occurred with *E. dilatata* at the control site in 1997 and was most likely due to deposition of sand over several of the randomly selected cells in the grid where *E. dilatata* was placed.

The mean natural density of mussels at Site B was roughly triple the density at Site A, although density varied between locations and over time (Tables 1 and 2). The mean natural density of mussels at Site B appeared to decline between 1997 and 2000, although the only statistically significant decrease occurred between 1997 and 1998 at the control site (Table 2). Doubling mussel density at the control and refuge sites, as measured by the DDC, had no effect on mussel recovery or survival. The mean density of mussels from the DDC cells at each of the Site A and B locations remained similar during 1997 and 2000 (Tables 1 and 2). In addition, the overall density of mussels in two of the three grids at Site A, and in both grids at Site B, increased throughout the duration of the study due to the immigration of unmarked mussels (Table 3).

During the course of the study, the species richness of the natural mussel populations peripheral to the sites did not change significantly. At Site A, the number of live species ranged from 18 to 20 at the control site, 18 to 22 at refuge site I and 15 to 17 at refuge site II between 1996 through 1998. No significant change in the average number of species per quadrat was observed over this time period. The dominant species of mussel found at the Site A control grid was *E. dilatata*, whereas, *Truncilla truncata* dominated at the two refuge sites. At Site B, there were 10–15 species at the control site and 13–16 species at the refuge between 1997 and 2000. The average number of species per quadrat did not change significantly over this time period. The two dominant species at the Site B control and refuge grids were *Actinonaias ligamentina* and *E. dilatata*.

The particle size distribution (cobble-gravel, sand and silt-clay fractions) of the sediment remained stable at Site A and changed significantly at Site B over the course of the study. At Site A, the mean percentage of cobble-gravel and sand fractions differed significantly (ANOVA, $P < 0.01$) between the control and the two refuge locations at the beginning of the study, but did not differ from this initial status among subsequent years (Table 4). The mean percentage of the silt-clay fraction did not differ among locations (ANOVA, $P > 0.05$). The mean volatile matter content of sediments at Site A did not differ among locations (ANOVA, $P = 0.33$). The overall percentage of volatile matter content averaged 1.4% (range 0.14–23) at all three locations. At Site B, substratum fractions were analysed in two different ways. In 1997, textural characteristics of Site B were analysed with the same substratum size fractions as those used at Site A. The proportions of cobble-gravel, sand, and silt-clay between the refuge and control site (overall mean 78, 22 and 0.06%, respectively) did not differ statistically from one another

Table 1. Mean density (range in parentheses) of mussels from the natural population, non-handled, resident control cells, and density-doubled control cells at the Site A control and refuge locations on the St Croix River from 1996 to 1998.

	Mean density (no. m^{-2})				
	Natural population[a] for year	Resident control[b] for year	Density-doubled control for year		
Grid and location	1996	1998	1996	1997	1998
Control	29	15	48	49	44
Lakeland, MN	(4–64)	(7–24)	(41–62)	(31–62)	(36–54)
Refuge I	15	15	27	28	28
Franconia, MN	(8–24)	(9–24)	(19–36)	(23–35)	(16–34)
Refuge II	15	6	23	23	19
Franconia, MN	(0–20)	(0–20)	(18–26)	(16–28)	(14–25)

[a]Estimated from ten ¼-m^2 quadrats taken from the periphery cells of each grid.
[b]Estimated from five 1-m^2 non-handled, resident control cells.

Table 2. Mean density (range in parentheses) of mussels from the natural population and density-doubled control cells at the Site B control and refuge locations on the St Croix River from 1997 to 2000.

	Mean density (no. m^{-2})							
	Natural population[a] for year				Density-doubled control for year			
Grid and location	1997	1998	1999	2000	1997	1998	1999	2000
Control	78	52	50	44	117	126	113	107
Sunrise, MN	(32–136)	(24–88)	(20–84)	(24–72)	(84–134)	(101–153)	(84–128)	(64–161)
Refuge	77	72	51	49	103	99	84	78
Almelund, MN	(20–168)	(20–152)	(24–116)	(20–124)	(86–137)	(73–141)	(48–108)	(51–112)

[a]Estimated from ten ¼-m^2 quadrats taken from the periphery cells of each grid.

Table 3. Mussel immigration (as indicated by unmarked mussels) at Sites A and B (both control and refuge grids) on the St Croix River from 1997 to 2000.

	Mussel immigration (cumulative no./grid)[a]			
Grid and location	1997	1998	1999	2000
Site A				
Control—Lakeland, MN	253	295	—	—
Refuge I—Franconia, MN	299	323	—	—
Refuge II—Franconia, MN	208	201	—	—
Site B				
Control—Sunrise, MN	—	805	1201	1372
Refuge—Almelund, MN	—	605	1094	1266

[a]Estimated from ten ¼-m^2 quadrats taken from the periphery cells of each grid.

(ANOVA, $P > 0.05$), nor were they statistically different from the two refuge sites (ANOVA, $P > 0.05$) at Site A. Size fractions for additional substratum samples collected between 1997 and 2000 at Site B, were categorized as follows: (1) boulder and cobble; (2) gravel; and (3) fine gravel, sand and silt. The mean percentage of boulder and cobble at the Site B control changed significantly between 1997 and 1999, and significant differences were observed at the refuge site between 1997 and 2000, and 1999 and 2000 (Table 5). The mean percentage of the gravel fraction did not differ within sites over the study (ANOVA, $P > 0.05$). There was a significant difference in the mean percentage of the fine gravel and sand fraction at the Site B control between 1997 and 1999, and 1997 and 2000, and at the refuge between 1997 and 2000, and 1999 and 2000 (Table 5).

DISCUSSION

Our study shows that *in situ* refugia may be a viable tool for protecting and conserving populations of unionid mussels. The relocation of mussels to *in situ* refugia in the St Croix River was successful, based on the recovery and survival of mussels after 2 and 3 years of monitoring. The overall mean recovery of all three species at the sites after 2 years was 95% at Site A and 57% at Site B after 3 years (Fig. 3). The lower recovery of mussels at Site B was probably due to the relatively high natural density (32.4 m^{-2}) of mussels at the site and, thereby, the greater rate of movement of mussels into and out of the grid areas. The overall mean survival of recaptured mussels was 97% at Site A and 95% at Site B (Fig. 4). Several other studies have also shown that *in situ* mussel relocations can be successful (Waller, Rach, Cope & Miller, 1995; Havlik, 1997; Dunn & Sietman, 1997; Dunn, Sietman & Kelner, 2000). For example, Dunn & Sietman (1997), who used methods for handling and monitoring mussels similar to those in this study at four geographically diverse relocation sites, found that survival and recovery of mussels after 1 year of monitoring were favourable (average survival 99%, average recovery 72%). In addition, our study shows that doubling or tripling the density of mussels at an existing mussel bed does not adversely affect survival of mussels in the natural or relocated populations, which is a potential concern for establishing future mussel refuges. Havlik (1997) similarly found that increased mussel densities (tripled) at a relocation site in the Wolf River, Wisconsin did not negatively affect survival or recovery.

Biological and physical habitat characteristics at the relocation site are other important factors influencing mussel survival and recovery (Dunn & Sietman, 1997; Dunn *et al.*, 2000). We selected the two sites for this study because they were in areas of

Table 4. Mean particle size characteristics (SD in parentheses) of sediment taken from around the periphery ($n = 10$ locations) of each mussel relocation grid at the Site A control and refuge locations on the St Croix River from 1996 to 1998.

	Mean particle fraction (%)								
	Cobble and gravel for year			Sand for year			Silt and clay for year		
Grid and Location	1996	1997	1998	1996	1997	1998	1996	1997	1998
Control	57[a]	54[a]	52[a]	43[a]	46[a]	48[a]	0.13	0.08	0.12
Lakeland, MN	(13)	(15)	(11)	(13)	(15)	(11)	(0.06)	(0.04)	(0.03)
Refuge I	71[b]	78[b]	78[b]	29[b]	22[b]	22[b]	0.12	0.14	0.23
Franconia, MN	(10)	(4)	(7)	(10)	(4)	(7)	(0.11)	(0.05)	(0.24)
Refuge II	76[b]	76[b]	78[b]	25[b]	23[b]	21[b]	0.07	0.12	0.14
Franconia, MN	(5)	(5)	(16)	(5)	(4)	(5)	(0.02)	(0.07)	(0.10)

For a given particle fraction and year, any two means not accompanied by a common letter were judged to be significantly different ($\alpha = 0.05$) based on a Tukey's *hsd* test.

Table 5. Mean particle size characteristics (SD in parentheses) of sediment taken from around the periphery ($n = 10$ locations) of each mussel relocation grid at the Site B control and refuge locations on the St Croix River from 1997 to 2000.

	Mean particle fraction (%)											
	Boulder and cobble for year				Gravel for year				Fine gravel and sand for year			
Grid and location	1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000
Control	54[a]	41[a,b]	32[b]	34[a,b]	19	19	21	22	27[a]	40[a,b]	47[b]	44[b]
Sunrise, MN	(21)	(22)	(17)	(12)	(6)	(6)	(6)	(7)	(17)	(18)	(14)	(6)
Refuge	58[a]	49[a,b]	57[a]	35[b]	17	15	16	14	25[a]	36[a,b]	27[a]	51[b]
Almelund, MN	(18)	(10)	(15)	(14)	(7)	(6)	(6)	(6)	(15)	(8)	(10)	(19)

For a given particle fraction and year, any two means not accompanied by a common letter were judged to be significantly different ($\alpha = 0.05$) based on a Tukey's *hsd* test.

stable substrata that had unionid communities similar in species diversity and abundance to the control area. During our study, the particle size distribution (cobble-gravel, sand and silt-clay fractions) of the sediment remained stable at Site A. However, the lowest survival (85%) among the three species tested occurred with *E. dilatata* at the Site A control in 1997, where there was deposition of sand over several of the randomly selected grid cells containing *E. dilatata*. The lower St Croix River, in the area of the Site A control, experienced extremely high flows and flooding during the spring of 1997, which probably contributed to the deposition of sand over these cells. At Site B, there were significant differences in textural substratum characteristics among years. However, variation was relatively large and a consistent trend was not observed at either the control or refuge sites. Several relocation efforts in the past have shown decreased survival of relocated mussels due to substratum instability at the relocation site (Sheehan *et al.*, 1989; Layzer & Gordon, 1993). Although we selected relocation sites with relatively stable substrata and dense mussel populations, the deposition of sand over several cells in the control grid at Site A shows that microhabitat within a given area may vary, and that certain patches of otherwise stable sediment, may be unsuitable for long-term retention of unionids. The stability of substrata and other characteristics of sediment, and their relation to the presence of dense and diverse mussel beds at specific sites have been difficult to quantify (Holland-Bartels, 1990; Strayer & Ralley, 1993; Hornbach, March, Deneka, Troelstrup & Perry, 1996), but are probably due to complex interactions among many physical and hydrological variables (Vannote & Minshall, 1982; Strayer, 1993; Di Maio & Corkum, 1995; Vaughn, 1997; Johnson & Brown, 2000). As such, the overall high recovery and survival of mussels in our study may have been due to the selection of relocation sites with many of these combined, yet unmeasured, suitable habitat characteristics, rather than to the lack of difference in sediment particle size measured over time (Tables 4 and 5). For example, our relocation sites may have served as refuge areas (portions of the river bed with low hydraulic stress) during periods of high flow (Strayer, 1999b) and, thereby, contributed to the relatively high recovery and survival of mussels at these sites.

Establishing *in situ* refugia seems to have a greater potential for the successful long-term preservation of unionids than artificial refugia, based on the relatively high rates of recovery and survival observed in our study. For example, Newton *et al.* (2001), who relocated five species of unionids from the Upper Mississippi River to a fish hatchery pond, found that mussel survival averaged only 35% 3 years after relocation. However, their study also included a riverine control group, in which the mussels were treated similarly to the hatchery pond held mussels, but placed back into the Mississippi River. They found that 75% of the mussels survived in the river after 3.3 years, illustrating the utility of *in situ* refugia over artificial refugia. Newton *et al.* (2001) attributed the differences in survival between the hatchery pond and riverine mussels to inadequate nutritional resources in the pond. Gatenby, Parker, Smith, Duncan & Neves (1999) observed similar decreases in survival of six large river species relocated to pond refugia over time. They found that, despite an abundance of a suitable algal food supply and adequate water quality conditions in the ponds, the survival of unionids decreased to 44% after 2 years and to 5% after 3 years.

Obviously, mussels should only be relocated from existing areas as a last resort (Cosgrove & Hastie, 2001). However, if

mussel relocations are required to protect or conserve localized populations, the results of our study and those of others (Gatenby *et al.*, 1999; Newton *et al.*, 2001) show that relocating mussels to *in situ* refugia offers advantages over certain types of artificial refugia (e.g. aquaculture pond facilities). These advantages are largely due to the similarity of water quality, substratum characteristics, food and necessary fish hosts in the system. Moreover, *in situ* refugia would permit the retention of genetic diversity of the mussel and host fish populations in the system. However, these important ecological and evolutionary concerns (Villella, King & Starliper, 1998; Storfer, 1999) need to be carefully considered before adopting widespread use of mussel relocation to *in situ* refugia, especially if the mussels are to be relocated between river basins or between sub-basins of the same river system. Because the mussels in our study were from the same river system and relocated only a short distance (about 48 km) from the source site, these ecological and evolutionary concerns did not apply.

In addition to relocating mussels to *in situ* refugia for conservation purposes, certain areas within many aquatic systems may provide natural refuges for protecting unionids from zebra mussels and other stressors (Nichols & Amberg, 1999; Nichols, Black & Allen, 2000). Findings such as these may have direct implications for protecting the mussel fauna of the upper St Croix River because zebra mussels are expanding their range from the upper Mississippi River (Cope, Bartsch & Hayden, 1997) into the lower St Croix River (Karns, 2002). Identifying and studying these types of natural refugia habitats may provide an additional tool for the conservation and management of imperilled unionid populations in certain systems.

Our relocation of mussels to *in situ* refugia in the St Croix River was successful, as measured by recovery and survival of mussels over 2 years at Site A and over 3 years at Site B. However, the long-term viability of the populations will be demonstrated by reproduction and growth of the relocated mussels. During our follow-up monitoring in years 2 and 3, we qualitatively evaluated the *Lampsilis* species from the sites for gravidity and found evidence of fertilization among relocated individuals. There is additional recent evidence that mussels can successfully reproduce following relocation. Heinricher & Layzer (1999) found that 49% of *Megalonaias nervosa* relocated from the Cumberland River to Kentucky Lake reproduced after a minimum of 2 years following relocation. The documentation of reproduction and other important physiological events such as growth will certainly require monitoring of relocated populations for longer than 2–3 years, and these long-term measures of success will be necessary to fully evaluate the efficacy of relocating mussels to *in situ* refugia for protecting or conserving mussel populations.

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