Propagation and Restoration of Higgins' Eye Pearlymussels in the Upper Mississippi River Basin

Partnership Efforts and Achievements in 2000-2001

June 2002

Executive Summary

Artificial propagation of the Higgins' eye pearlymussel was initiated in spring 2000 at the Genoa National Fish Hatchery where more than 1,300 cultured host fish were inoculated with viable Higgins' eye glochidia collected from females brooding in the lower St. Croix River. This effort resulted in the production of about 92,000 juvenile mussels. Later that summer, containers with more than 4,800 of these juveniles were placed in the lower Wisconsin River at several sites. Subsequent efforts to relocate these individuals were unsuccessful. Juveniles that were not relocated to the river were kept at the hatchery for continued growth in several different rearing systems. However, repeated pulses of turbid water in the rearing chambers deposited layers of sediment that apparently smothered all of the remaining juveniles here by late summer.

Higgins' eye propagation efforts resumed at the Genoa hatchery in spring 2001 when nearly 3,800 cultured host fish were inoculated with glochidia from gravid females brooding in the lower St. Croix River. By early summer, more than 90% of these glochidia-infected fish were stocked in cages or free-released at six sites in the upper Mississippi River basin with suitable mussel habitat. These reintroduction efforts were estimated to have placed more than 175,000 juvenile Higgins' eye mussels in the river environment in as natural a process as possible. Hundreds of young mussels were found inside several of the cages later that summer and most were identified as Higgins' eye juveniles that ranged in length from 5 to 32 mm. Moreover, a single cage inspected in spring 2002 contained 37 juvenile Higgins' eye mussels that successfully overwintered and appeared healthy almost one-year after the start of this reintroduction effort. About 16,000 juveniles were also retained at the hatchery in summer 2001 for continued growth, but only a small fraction of these mussels (12%) were released before the remainder were consumed by predators in the hatchery water supply. Propagation efforts in 2001 concluded in the fall when nearly 1,800 host fish were inoculated in the field with glochidia

gathered from gravid female Higgins' eye mussels brooding in upper Mississippi River Pool 14 and immediately released at a site on the lower Iowa River.

Propagation and Restoration of Higgins' Eye Pearlymussels in the Upper Mississippi River Basin: Partnership Efforts and Achievements in 2000-2001

Artificial propagation of the Higgins' eye pearlymussel (*Lampsilis higginsi*) is considered an essential component of the survival strategy for this federally endangered species. Such efforts were initiated in spring 2000 at the Genoa National Fish Hatchery (NFH) in Genoa, Wisconsin. More than 1,300 cultured host fish yearlings (592 largemouth bass and 752 walleye) were inoculated here with viable Higgins' eye glochidia collected from five gravid females brooding in the lower St. Croix River Essential Habitat Area (EHA) near Hudson, Wisconsin. Transformed juveniles were first detected about three weeks after inoculation and continued to detach from host fish over a period of about four weeks. An estimated total of 91,528 glochidia transformed into juveniles as a result of this initial propagation effort. Approximately 80% of the glochidia that encysted on the gills of largemouth bass and 23% of the glochidia that encysted on the gills of walleye successfully transformed, yielding estimated totals of 87,403 and 4,125 free-living juveniles produced by these host fish species (respectively).

Juvenile mussels were held at the hatchery in several different flow-through culture treatments among substrates of uniformly-sized crushed rock. After several weeks of growth and development at the hatchery, an estimated 3,750 juveniles (mean diameter 250 µm) were transported early in the summer to a site on the lower Wisconsin River (river mile 4.4) near Prairie du Chien, Wisconsin (Figure 1) and placed in wooden-framed, screen-covered trays that were anchored to the substrate (Figure 2). Several days later, the trays were moved upstream to the site of an existing mussel bed (river mile 6.5) where the contents of each was gently removed and transplanted. Survival and growth rates for these

individuals are currently unknown. Later in the summer, several other screened trays containing an estimated total of 1,100 juveniles (mean diameter 510 µm) were placed in the lower Wisconsin River at the Orion EHA near Muscoda, Wisconsin (river mile 44.4; Figure 1) and anchored to the substrate. Only one of these trays was found during a dive survey conducted 15 months later (fall 2001) and it was filled with an accumulation of river sediment, suggesting this reintroduction effort did not succeed.

Juveniles produced in 2000 that were not relocated to river sites (about 84,000 individuals) remained at the hatchery for continued growth and development by means of several different captive rearing methods. By the end of summer however, repeated pulses of highly turbid water had deposited substantial layers of sediment over the crushed rock substrate in all of the rearing chambers and apparently smothered the remaining mussels. Subsequent efforts to locate viable juveniles in these treatments were unsuccessful with only a few empty shells encountered.

Higgins' eye pearlymussel propagation efforts resumed at the Genoa NFH in spring 2001. A total of 19 gravid female Higgins' eye pearlymussels were collected from the lower St. Croix River EHA in early May and brought to the hatchery. Later that month, viable glochidia were removed from these mussels to inoculate nearly 3,800 yearling cultured fish (2,782 smallmouth bass, 940 walleye, and 92 largemouth bass) over a two-day period using the same procedures as in 2000. More than 90% of these glochidia-infected fish were randomly selected and held in raceways for later stocking at sites within the Mississippi River basin. The remaining glochidia-infected fish were distributed among 24 ten-gallon aquaria that were supplied with a continuous flow of fresh aerated water in order to monitor the glochidial transformation process, as well as to provide a supply of juvenile Higgins' eye mussels for hatchery propagation and release trials.

Glochidia began to excyst from host fish in the aquaria 31 days after inoculation. The excystment process peaked 8 days later and concluded within 2 weeks. Estimated rates of glochidial transformation in 2001 ranged from 73% for the smaller-sized smallmouth bass (i.e., fish less than 80

mm total length) to 41% for walleye (Table 1). These results were similar to those achieved in 2000 and may reflect observed differences in the depth of the gill epithelial growth response by the various host fish species to the parasitic glochidia. Cumulative mortality of host fish in the aquaria totaled 21% when excystment had concluded and was primarily associated with interruptions in the water supply and intraspecific aggression among smallmouth bass. The total number of transformed mussels collected from host fish in the aquaria and available for subsequent hatchery propagation and release trials was estimated at 11,300.

In an effort to improve upon the methods used in 2000 to reintroduce hatchery propagated young-of-year juvenile Higgins' eye mussels to suitable river habitats for continued growth, two new release strategies were used 2001. In both cases, glochidia-infected fish were stocked into appropriate receiving streams where conditions appeared to be suitable for long-term mussel survival. Here, the fish were either released to freely disperse in the stream or confined in submerged cages that were positioned directly over substrates thought to provide high quality mussel habitat. Juvenile mussels that excysted from both the free-released and confined fish could thus follow a more natural process as they settled to the substrate for continued growth. The free-release method was initially utilized early in the 20th century as a management tool to help increase Unionid populations. The caged-release procedure offers the added benefit that upon completion of mussel excystment, the host fish could be released and the cage removed, leaving behind only a bottom support structure to act as a marker for later relocating the site to assess mussel survival and development here.

Nearly 1,200 host fish infected with Higgins' eye glochidia were free-released in the Cedar River at Palisades-Kepler State Park in east-central Iowa during spring 2001 while 500 glochidia-infected fish were likewise released in the lower Wisconsin River at a site below the dam at Prairie du Sac, Wisconsin (Table 2; Figure 3). These fish produced an estimated cumulative total of more than 82,000 Higgins' eye juveniles with the majority of these mussels (84%) introduced in the Cedar River. Higgins' eye pearlymussels historically occurred at low but detectable levels near the free-release sites

in both of these rivers but are now much less abundant or completely absent. Therefore, any increase in the population of this species near the free-release sites in the immediate future could most likely be attributed to the current propagation and reintroduction program.

A total of 1,645 glochidia-infected fish were also distributed among 25 cages (about 65 fish per cage) deployed at four sites including two EHAs in the lower St. Croix River, one EHA in the lower Wisconsin River, and a location in upper Mississippi River Pool 3 just downstream of the St. Croix River confluence (Table 3; Figure 3). These fish were estimated to have produced and contributed more than 94,000 juvenile Higgins' eye mussels to the river environment, with nearly 80% of these juveniles released over substrates in EHAs. Cages deployed at the Orion EHA in the lower Wisconsin River were removed following excystment and no juvenile mussels were detected in the underlying substrates here. Meanwhile, cages deployed in the lower St. Croix River and the upper Mississippi River were also fitted with solid bottoms (plywood) and remained submerged after excystment was complete to aid later efforts to locate and monitor the growth of any apparent hatchery-reared Higgins' eye mussels found in the substrates here.

Six of the cages with bottoms were retrieved late in the summer by divers from the Minnesota Department of Natural Resources (DNR) who sieved the sediments that had accumulated within them in search of mussels. Several hundred young mussels were found inside of these cages and most were identified as Higgins' eye juveniles that ranged in length from 5 to 32 mm (Figure 4). For example, thirteen weeks after glochidia-infected fish were placed in cages at the Hudson EHA, 67 Higgins' eye juveniles (8 to 22 mm length range) were found here in a cage that had been stocked with walleye while another 40 Higgins' eye juveniles (10 to 22 mm length range) were found here in a second cage that had been stocked with smallmouth bass. Small juvenile Higgins' eye mussels (5-32 mm length range) were also found at this time in cages that were similarly inspected at both the Prescott EHA in the lower St. Croix River and in upper Mississippi River Pool 3 downstream of the St. Croix River confluence. Thus, Higgins' eye glochidia appeared to transform successfully from both species of cage-released

host fish at these sites and grow rapidly at near equivalent rates as independent juveniles. Higgins' eye juveniles recovered from the retrieved cages were subsequently returned to the water and redistributed among the remaining undisturbed cages at these sites for continued growth. In addition to these Higgins' eye mussels, a variety of other juvenile native mussel species were found inside the cages retrieved for inspection. Meanwhile small numbers of zebra mussels were found on exterior surfaces of the cages and on the legs of the structures supporting them. These findings suggest that in locations where the abundance of zebra mussels is relatively low, the cages may likewise act as a refuge for juveniles of other native mussel species that drift into them from the surrounding mussel bed and settle among the silty sediments that accumulate within them (as much as 15 cm over the summer). Thus, it seems just as likely that some of the
recently introduced Higgins' eye juveniles could also be emigrating from within the cages to the substrates surrounding them. The most recent inspection of a cage deployed at the Hudson EHA in the lower St. Croix River (May 2002) revealed a total of 37 juvenile Higgins' eye mussels successfully overwintered here (i.e., no empty shells were observed: Figure 5). These findings optimistically suggest that many of the Higgins' eye juveniles propagated in 2001 were apparently in good condition one-year after efforts to reintroduce these individuals to portions of the species' historic range had commenced.

Juvenile Higgins' eye mussels that were recovered from host fish held in aquaria were subsequently distributed among two different culture treatments to evaluate methods for continued early-life rearing at the hatchery. In the first treatment, nearly 8,100 juveniles were distributed among 24 baskets, each constructed of a 25-cm length of large diameter (20-cm) plastic pipe with a nylon-screened bottom (150-µm mesh) that was filled with 12 mm of gravel (1-2 mm diameter) and supplied with a continuous flow of pond water. This design provided a vertical flow of oxygenated water throughout the substrate and delivered a variety of potential food items (e.g., algae, bacteria, detritus) for the growing mussels. In the second treatment, nearly 3,200 juveniles were distributed in a series of miniature raceways constructed of drain gutters that were sloped slightly and lined with gravel (1-2 mm

diameter). The horizontal flow of water in this recirculating system was supplemented with daily additions of cultured algae (primarily *Scenedesmus sp.* and *Chlamydomonus sp.*) to provide a more lotic-like environment for continued mussel development. A third culture treatment was also evaluated using juveniles that excysted from about 100 host fish that were placed in two fiberglass rearing tanks (1 m x 6 m) immediately after inoculation. The bottoms of both tanks were lined with 5 cm of a sand-gravel mixture and a screen partition was inserted to divide the tanks in half with the fish retained near the water inflow (194 L pond water/min). Fish were removed from these tanks at the conclusion of mussel excystment. About 4,500 glochidia were estimated to have successfully transformed into juvenile mussels in this treatment and were assumed to be distributed throughout the bottoms of the tanks.

Juvenile mussels remained in hatchery propagation treatments until mid-summer when stocking efforts were scheduled to start. About 1,900 juveniles in 6 rearing baskets were initially transferred at this time to the Wisconsin DNR for placement at a mussel bed in the lower Black River (river mile 60.6) near Black River Falls, Wisconsin (Figure 1). This site will be inspected in the summer of 2002 to search for one-year old juveniles that may have resulted from this introduction. Within one week of this release however, and before any other stocking occurred, hatchery staff noted an abundance of predacious micro-crustaceans in all of the 18 rearing baskets that remained at the hatchery. Subsequent efforts to retrieve as many of the estimated 6,200 juvenile mussels that may have remained in this treatment yielded a recovery rate of only 1%. The near total lack of dead shell material encountered in these baskets, coupled with the great abundance of voracious Cladocera (e.g., *Leptodora* sp.) found here, indicated that predation was the primary factor responsible for low survival of juvenile mussels in this treatment. Following the discovery of predators in the rearing basket treatment, an abundance of these and other predacious invertebrate taxa (e.g., flatworms) was also noted in both of the other hatchery treatments. Less than 10% of juvenile mussels in the miniature raceways and only 31 juveniles in the large fiberglass tanks were later recovered from the hatchery treatments and transferred to the Minnesota DNR for placement in river cages. The small size (600 to

700-µm range) of most juveniles that survived predation at the hatchery indicated that early-life growth was much slower here than in the river and may reflect nutritional deficiencies (i.e., quality and/or quantity) in the diet supplied to mussels at the hatchery.

Annual Higgins' eye propagation efforts for 2001 concluded in October with the inoculation and free-release of glochidia-infected host fish in the lower Iowa River. Twelve gravid females collected from the Cordova EHA in upper Mississippi River Pool 14 supplied an estimated 750,000 glochidia that were used to inoculate nearly 1,800 host fish during a one-day event held in the field at a site on the Iowa River near Iowa City, Iowa (Figure 3). The majority of the host fish used here (1,000) were hatchery-reared largemouth bass fingerlings while most of the remainder represented a variety of feral host fish (walleye, freshwater drum, largemouth bass, and white bass) that were caught locally. The estimated mean rate of glochidial attachment for all host fish during this event (13%) was slightly lower than that attained in earlier inoculations conducted at the hatchery and may have reflected operational field constraints that altered the typical number of fish used per inoculation sequence. Despite such differences however, more than 100,000 glochidia were estimated to have attached to these host fish, making this the single largest field propagation-release effort for Higgins' eye mussels to date.

Acknowledgments

Funding for this work was provided by the National Fish and Wildlife Foundation (Project Grant 97-282-001), the U.S. Army Corps of Engineers (St. Paul District), and the U.S. Fish and Wildlife Service (Region 3 Ecological Services Program and the National Aquatic Nuisance Species Program). River diving services for the collection of gravid female Higgins' eye mussels and the placement/retrieval of cages, fish, and juvenile mussels were provided by scuba-certified staff from the Wisconsin and Minnesota Departments of Natural Resources. These and many other staff members from upper Mississippi River project offices of the Wisconsin, Minnesota, Iowa, and Illinois

Departments of Natural Resources, the U.S. Geological Survey, and the U.S. Fish and Wildlife Service have willingly assisted in executing this continuing multi-partner endangered species recovery effort from its inception to the present. This report was prepared by the U.S. Fish and Wildlife Service Fishery Resource Office in Onalaska, Wisconsin.

Table 1. Estimated production statistics for host fish species inoculated with Higgins' eye glochidia at the Genoa National Fish Hatchery in spring 2001.

	Host fish species				
	Walleye	Smallmouth bass			Largemouth bass
		< 102 mm total length	≥102 mm total length	Mass inoculation[†]	
Fish inoculated	908	2,263	90	380	79
Glochidia encysted/fish	151	76	NA	30	NA
Glochidia transformed/fish	62	56	48	15	53
Glochidia transformation rate (%)	41	74	NA	50	NA
Juveniles produced	56,296	125,822	4,293	5,568	4,155

[†]More than 10 fish of different sizes were inoculated in each batch during this procedure.

NA - Data not available (this group was not sampled due to the relatively small number of fish inoculated).

Table 2. Estimated production of Higgins' eye juveniles from host fish species inoculated with Higgins' eye glochidia at the Genoa hatchery and stocked at free-release sites in spring 2001.

Release site	Number of host fish released		Estimated juvenile production
	Walleye	Smallmouth bass	
Cedar River Palisades-Kepler State Park	405	793	69,200
Wisconsin River Prairie du Sac	0	500	13,485

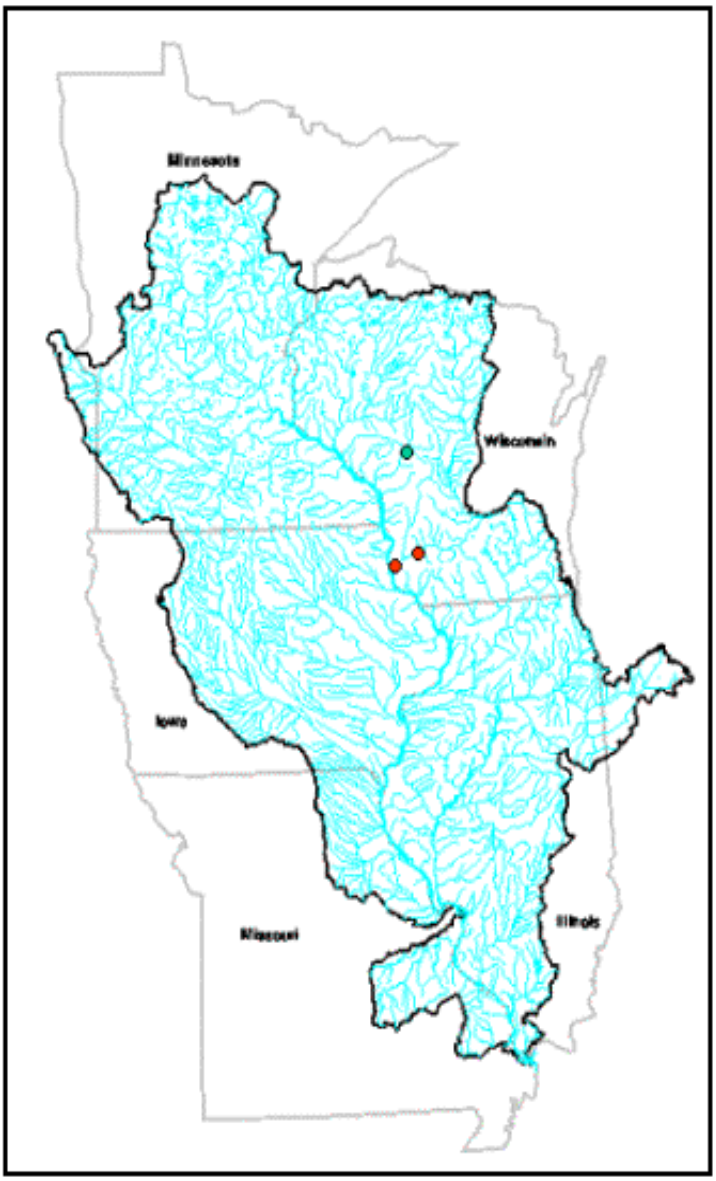
Table 3. Number of host fish stocked in cages and estimated number of juvenile Higgins' eye mussels produced at caged-release sites in the upper Mississippi River basin in 2001.

Release site	Cages deployed	Host fish species		Estimated juvenile production
		Smallmouth bass	Walleye	
Wisconsin River, Orion EHA	9	445	150	34,042
St. Croix River, Prescott EHA	7	300	150	25,980
St. Croix River, Hudson EHA	4	150	100	14,540
Upper Mississippi River, mile 810.8	5	300	50	19,780

Figure 2. Staff from the Wisconsin Department of Natural Resources and the U.S. Fish and Wildlife Service prepare to submerge a tray containing juvenile Higgins' eye mussels at a Wisconsin River reintroduction site in summer 2000 *(U.S. Fish and Wildlife Service photo)*.

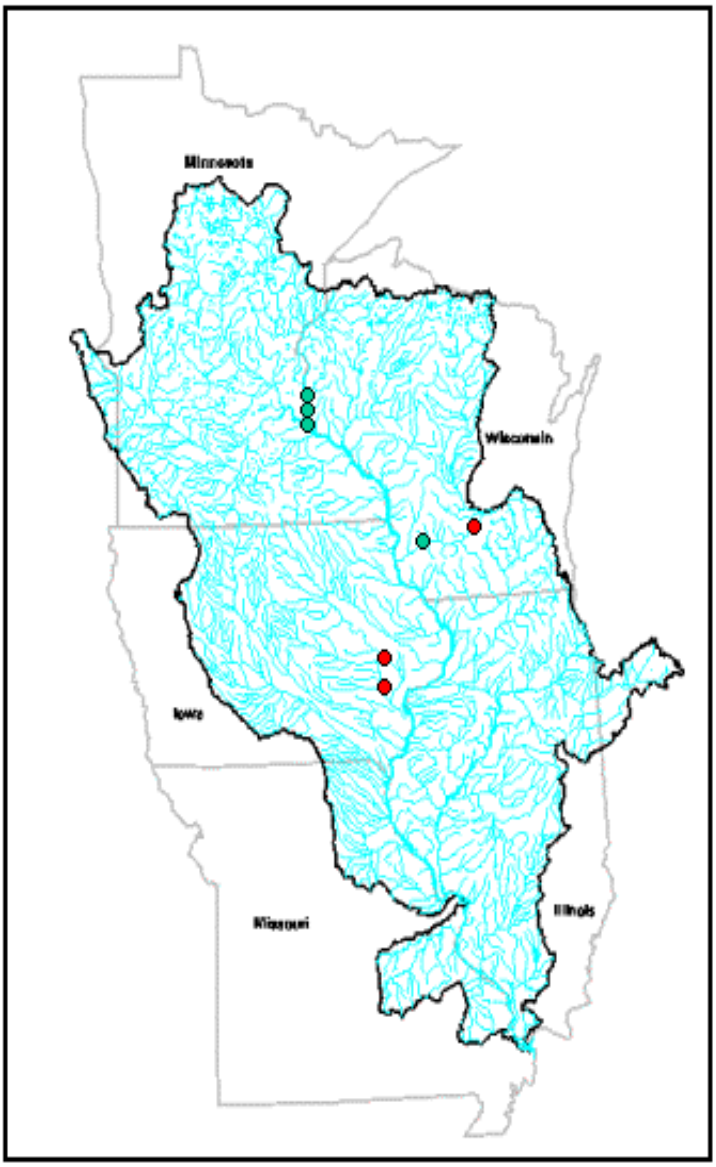
Figure 4. Juvenile Higgins' eye mussels recovered from cages deployed in Essential Habitat Areas of the lower St. Croix River and stocked with glochidia-inoculated host fish in spring 2001. These photographs were taken 13 to 15 weeks after host fish were placed in the cages *(courtesy of Minnesota Department of Natural Resources)*.

Figure 5. Juvenile Higgins' eye mussels recovered in May 2002 from a cage deployed at the Hudson



Essential Habitat Area in the lower St. Croix River that was stocked with glochidia-inoculated host fish in spring 2001 *(Minnesota Department of Natural Resources photos)*. A total of 37 juvenile Higgins' eye mussels successfully overwintered here (no empty shells were observed) and were apparently in good condition one-year after efforts to artificially propagate and reintroduce these individuals to the species' historic range had commenced.

Figure 1. Locations in the upper Mississippi River basin where containers with hatchery propagated young-of-year Higgins' eye mussels were placed during summer 2000 (🔴) and summer 2001 (🟢).



Base map courtesy of the U.S. Geological Survey.

Figure 3. Locations in the upper Mississippi River basin where glochidia-inoculated host fish

were stocked for free-release (●) and caged-release (●) during 2001. *Base map courtesy of the U.S. Geological Survey*.