2006 Status Report on the Accomplishments of the Mussel Coordination Team (MCT)

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Abstract

In 2003, the interagency Mussel Coordination Team (MCT) prepared a report *Saving the Higgins Eye Pearlymussel (Lampsilis higginsii) from Extinction: 2002 Status Report on the Accomplishments of the Mussel Coordination Team* (Mussel Coordination Team 2003). The following report summarizes MCT activities from 2003 through 2006 including winged mapleleaf and other species. Select information from the 2003 report is also included to provide continuity.

Since 2000, a variety of measures have been implemented for the federally endangered Higgins eye (*Lampsilis higginsii*) and winged mapleleaf (*Quadrula fragosa*) by the U.S. Army Corps of Engineers (Corps) with assistance from the MCT. These activities were required by a Biological Opinion for continued operation and maintenance of the federal 9-Foot Channel Project on the upper Mississippi River (UMR). Major accomplishments include: 1) identifying host fish for winged mapleleaf; 2) hatchery and cage propagation of Higgins eye and winged mapleleaf; 3) stocking adult, subadult, and juvenile Higgins eye into the UMR and tributaries to establish five new and viable populations; 4) collecting sexually mature Higgins eye at age 4 that were stocked as subadults; 5) collecting subadult Higgins eye at population establishment sites where infested fish were released; 6) developing marking techniques for stocked subadults; 7) testing a floating cage system; 8) developing a Geographic Information System mussel database and Internet web site; 9) determining thermal requirements for transformation of Higgins eye and winged mapleleaf glochidia; and 10) implementing measures for other native mussels.

Higgins Eye Activities

Higgins eye (*Lampsilis higginsii*) historic range includes the upper Mississippi River (UMR) and major tributaries (Figure 1). In 1976, Higgins eye was listed as a federally endangered species, and the subsequent recovery plan might have been successful if zebra mussels (*Dreissena polymorpha*), an exotic species, had not invaded. During the 1980's, they entered the Illinois/Mississippi River System by a man-made connection with Lake Michigan at Chicago, Illinois (Figure 2). Today, they have infested nearly the entire navigable portion of the UMR (Figure 3).

Zebra mussels attach to nearly all underwater objects. They encrust hard objects and can form a thick carpet on the bottom of the river. Unfortunately, native freshwater mussels serve as an excellent substratum for attachment (Figure 3). At high densities, zebra mussels harm native mussels and entire beds by competing for food, preventing opening/closing of shells, degrading habitat conditions, and preventing successful reproduction and recruitment.

Between 1998 and 2000, the U.S. Army Corps of Engineers (Corps) and U.S. Fish and Wildlife Service (Service) were involved in formal consultation under Section 7 of the Endangered Species Act for impacts to Higgins eye from operation and maintenance of

the existing 9-Foot Channel Project for another 50 years. Zebra mussels were a primary issue because they are transported by towboats and other large craft to upstream areas on the UMR using the federal navigation system (Figure 3). In the April 2000 Biological Opinion, the Service determined that operation and maintenance of the navigation project for an additional 50 years would jeopardize the continued existence of Higgins eye because it provides for a steady upstream transport of zebra mussels on the UMR (U.S. Fish and Wildlife Service 2000). To avoid jeopardy, the Corps was required to establish new and viable populations of Higgins eye in areas of the UMR and tributaries protected from heavy colonization from zebra mussels. They were also required to implement a zebra mussel control program, if technically feasible. In 2000, the Corps established the MCT to assist in implementing the Biological Opinion requirements.

Establishing Five New and Viable Populations

The MCT includes representatives from the Wisconsin, Minnesota, Illinois, and Iowa Departments of Natural Resources , U.S. Geological Survey, U.S. Coast Guard, National Park Service, Science Museum of Minnesota, Corps, and Service; assistance is also provided by Iowa State University, Macalester College, and the University of Minnesota (Figure 4). An important accomplishment of the MCT is the Higgins Eye Pearlymussel Relocation Plan (U.S. Army Corps of Engineers 2002). The interim goal of the plan to be accomplished within the next 10 years is to establish five new and viable populations of Higgins eye in areas of the UMR and tributaries that are protected from heavy colonization by zebra mussels. The plan recommended spending $2,542,000 through 2010 on a variety of activities including: genetics studies; mussel propagation at the Genoa National Fish Hatchery (NFH) in Wisconsin; propagation of mussels in cages in the UMR and tributaries; stocking juvenile, subadult and adult mussels; stocking fish infested with Higgins eye glochidia; cleaning and stockpiling adult mussels; and monitoring these activities.

To establish five new and viable populations of Higgins eye, the MCT selected ten population establishment sites on the UMR and tributaries (Figure 5). Sites were selected based on a variety of factors including historic range of Higgins eye, abundance and future risk of zebra mussels, existing mussel community (Table 1), presence of suitable host fish, water quality, and other suitable habitat conditions.

Site 1, Upper Pool 2, UMR, St. Paul, Minnesota, Figure 6, adults and subadults
Site 2, Lower Pool 2, UMR, Hastings, Minnesota, Figure 7, subadults
Site 3, Pool 3, UMR, Hastings, Minnesota, and Diamond Bluff, Wisconsin, Figure 8, adults and subadults
Site 4, Pool 4, UMR, Red Wing, Minnesota, Figure 9, subadults
Site 5, Rock River, Rock Island, Illinois, Figure 10, subadults
Site 6, Pool 16, UMR, Fairport, Iowa, Figure 11, subadults
Site 7, Lower Wisconsin River, Orion and Prairie du Sac, Wisconsin, Figure 12, juveniles and subadults
Site 8, Iowa River, Iowa City, Iowa, Figure 13, juveniles
Site 9, Cedar River, Palisades-Kepler State Park, Iowa, Figure 14, juveniles

Site 10, Wapsipinicon River, Central City and Anamosa, Iowa, Figure 15, juveniles

The Twin Cities reach (Coon Rapids Dam to mid- Lake Pepin in Pool 4) has been described as the last remaining mussel refuge from zebra mussels on the UMR (Mussel Coordination Team 2003). We currently have 28 live species of mussels in the Twin Cities reach, and their increasing numbers and densities are primarily due to water quality improvements (Kelner and Davis 2002). While zebra mussels occur in this reach, they have remained at low densities and do not appear to be harming native mussels. Consequently, all of our UMR population establishment sites are located in the Twin Cities reach (Figure 16; an exception is Site 6 in Pool 16 which will be initiated in 2008).

Four methods are used to establish viable populations of Higgins eye at the ten population establishment sites:

1. stocking adults from the wild,
2. stocking juvenile (less than one year old) Higgins eye produced in the hatchery, from open-bottom cages, or from release of infested host fish,
3. stocking subadults (one year and older) collected from closed-bottom cages, and
4. combination of these methods.

The RAMAS population model was used to estimate the number of juveniles, subadults and adults required to produce viable populations (Corps of Engineers 2002). Kelner and Heath (2003) developed protocols for stocking and monitoring Higgins eye. Table 2 shows the number of mussels stocked/to be stocked at each population establishment site from 2000 through 2010.

Stocking Juveniles

Like many freshwater mussels, Higgins eye require a host fish to complete their life cycle (Figure 17). Eggs are fertilized and stored in the gills of the female where they transform into parasitic larvae (glochidia). When gravid, adult females display a unique mantle lure that resembles a small fish (Figure 17). The lure attracts predatory fish like largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieui*), and walleye (*Stizostedion vitreum vitreum*). When a fish strikes the lure, it ruptures the gill chambers of the mussel (Figure 18). If gill chambers are ruptured, glochidia are expelled into the mouth of the fish and attach to its gills. If the fish is a suitable host, glochidia are encysted, transform into juvenile mussels, detach from the gills, and fall to the substrate, where they burrow and grow to adulthood.

In nature, the female mussel lures a host fish to glochidia. Because this portion of the life cycle is a potential weak link in the perpetuation of the species, in hatchery situations we bring glochidia to fish. In April of each year, gravid female Higgins eye are collected by divers and transported to Genoa NFH, where they are measured and marked. Glochidia are flushed from the gills of donor females with a syringe and water into a glass container (Figure 19). Glochidia are tested for viability with table salt; viable glochidia quickly

snap their valves closed when in contact with salt water. Two to 10 milliliters of viable glochidia are added to a bucket containing host fish, water, and an airstone to keep the glochidia in suspension (Figure 20). Contents are stirred for 2 to 5 minutes, and a fish is examined under a microscope to estimate the number of attached glochidia. If an adequate number of glochidia are attached to the gills (150/300 per fish), all of the fish in the bucket are placed in a holding tank. If not enough glochidia are attached, the sample fish is returned to the bucket, the contents stirred, and the process continued until attachment occurs.

In approximately 2 to 4 weeks, glochidia transform into juvenile mussels and excyst from the gills of the host fish (Figure 21). Based on hatchery data in 2005, transformation rates for attached glochidia were approximately 86% on largemouth bass and 29% on smallmouth bass. In 2001 and 2002, we used walleye as host fish; transformation rates were approximately 40% for attached glochidia. However, walleye are more difficult to raise and hold in captivity; therefore, largemouth and smallmouth bass are preferred.

In 2006, the threshold temperature required for transformation of glochidia on largemouth bass was estimated to be 10.9 °C using a model developed for winged mapleleaf (*Quadrula fragosa*; Steingraeber et al. 2007). The cumulative temperature units of development needed for Higgins eye glochidia to complete metamorphosis on largemouth bass (i.e., from the date of encystment until the start of peak excystment) was subsequently estimated to be 169 °C days. The developmental threshold temperature for Higgins eye is about 1.6 °C greater than that of the winged mapleleaf (9.3 °C). This indicates that transformation of Higgins eye glochidia, which typically occurs in the spring, is less productive in cold water temperatures than winged mapleleaf. However, Higgins eye transformation requires a fraction (43%) of the cumulative thermal energy needed for winged mapleleaf transformation on channel catfish (*Ictalurus punctatus*) (395 °C days) and occurs more rapidly. Thermal criteria for Higgins eye can be used to attain targeted rates of metamorphosis in the hatchery by making knowledgeable water temperature adjustments, quantitatively estimate cumulative mussel development at any time, and guide the selection of when to place infested fish into rivers to maximize juvenile mussel production.

Our target is to use 100 donor females at each population establishment site to establish viable populations of Higgins eye with adequate genetic composition; Table 3 shows the number of donor females contributing to each cohort. We collected donor females from five sites: lower St. Croix River at Hudson, Wisconsin; lower Wisconsin River at Prairie du Sac, Wisconsin; UMR Pool 10 at Prairie du Chien, Wisconsin; UMR Pool 11 at Cassville, Wisconsin; and UMR Pool 14 at Cordova, Illinois. Higgins eye has a high level of genetic variation but does not have genetically distinct populations in the UMR and lower St. Croix River (Bowen 2004). To err on the side of caution, fish released into open cages in the upper reaches of the UMR are infested with glochidia from the lower St. Croix River; likewise, fish released into open cages or the wild in lower reaches receive glochidia from Pools 10, 11, or 14, depending on which is closer to the population establishment site. Tissue samples are taken from each donor female; in the future, we hope to use genetic analysis (e.g., microsatellites) to determine if Higgins eye

collected from monitoring activities came from our donor females (i.e. did they come from this project or nature?).

Figure 22 shows the annual production of host fish at Genoa NFH. Since 2000, we used 343 donor females to infest 39,387 host fish with Higgins eye glochidia (Table 4). Based on transformation rates in the hatchery, potential production of Higgins eye from these inoculations was 5,918,276 juveniles, although we do not expect that they all survived. Infested fish are used in cage propagation activities, released into the wild, or kept for transforming juveniles in the hatchery. We use three methods to stock juvenile Higgins eye at population establishment sites:

1. Stocking hatchery-propagated juveniles. Approximately 8,000 juvenile (< 30 days old) Higgins eye propagated at Genoa NFH were stocked into the lower Wisconsin and Black Rivers (Table 5; Figure 23). Juveniles were placed in a container and contents poured on the river bottom in proximity to existing mussel beds. In 2001, we monitored exact sites where we placed hatchery juveniles on the river bottom in 2000; none were found (live or dead). We found similar results for thousands of hatchery juveniles we placed on substrate in closed propagation cages to try and grow subadults; only three live juveniles were found in one cage in the lower St. Croix River. For unknown reasons, mortality of hatchery juveniles in the river environment is extremely high; therefore, this method was discontinued.

2. Placing infested fish in open-bottom cages. Since 2001, we placed 2,802 infested fish into 78 open cages at Site 7 on the lower Wisconsin River; potential production was 385,950 juvenile Higgins eye (Table 6). In 2001, we confined infested fish in cages in existing mussel beds. Theoretically, once juveniles excyst from the fish, they will burrow and grow in the substrate below. Open cages are constructed of aluminum frame and ½-inch wire mesh screen (12 by 24 by 36 inches; Figure 24). Wire screen is secured to the frame with pop rivets. These cages are slanted in front to provide stability in currents and have a 3/8-inch plywood board attached to the front to give host fish a refuge from these currents. Each cage contains approximately 4 feet^3 of water and is designed to hold approximately 50 fish. Workers open the top of the cage to insert host fish, secure the top closed with plastic ties, and place the cage in the river. Glochidia transformed from the gills of host fish pass through the cage bottom directly to the substrate below. All cages have a separate bottom frame with four pointed legs that is driven into the river substrate to provide a stable platform for the cage. After transformation, the cage and fish are removed from the river, leaving the bottom frame and legs to mark the release site.

3. Stocking infested host fish (free release). Compared to the above stocking techniques for juveniles, free release of infested fish mimics the life cycle of Higgins eye. Infested fish are transported from Genoa NFH to the population establishment site, or wild fish are captured and infested on-site with Higgins eye glochidia (Gritters 2001; Figure 25). Infested fish are released into the river where glochidia transform and fall to the substrate. This technique is simple and avoids labor and construction costs associated with open cages or producing juveniles at the hatchery. Since 2000, we

released 21,429 infested host fish at four population establishment sites (Table 6). Potential production was 2,188,214 juveniles, although we do not expect actual juvenile production numbers to be this high.

Monitoring. Trying to collect juvenile Higgins eye in the wild is truly a "needle in a haystack" adventure. We do not know how far river currents carry transformed juveniles from open cages, or where host fish travel during transformation. Juvenile Higgins eye have a byssal thread which holds them to substrate or debris. We usually find their byssal threads tangled together, attached to the wire mesh inside cages, or attached to collection screens. To take advantage of their byssal threads, in 2003 we constructed a juvenile collector by modifying a crowfoot bar and dragging it across the river bottom with a boat. Metal hooks on the bar were replaced with strips of Velcro hooks hoping they would snare juvenile mussels by their byssal threads (Figure 26). Collection attempts were made near population establishment Sites 1 and 3 on the UMR and cage propagation sites at Prescott and Hudson on the lower St. Croix River. Although no Higgins eye were collected, we did collect several juveniles of other species on the Velcro hooks. We abandoned this technique and decided to wait until 2005 to monitor the four juvenile sites so mussels would be large enough to find by diving/wading:

1. Site 7, Lower Wisconsin River, Wisconsin. Since 2000, we stocked a maximum of 721,321 juvenile Higgins eye (direct stocking, open cages or free release of fish; Table 2). In 2005, we monitored areas downstream of open bottom cages and free fish releases and found 4 subadult (1 year or older) Higgins eye (Figure 27).

2. Site 8, Iowa River, Iowa. Since 2002, we stocked a maximum of 442,374 juvenile Higgins eye from host fish releases (Table 2). In 2005 and 2006 we monitored these areas and found no subadult Higgins eye. However, we did find 3,426 live mussels of 22 species (Table 7).

3. Site 9, Cedar River, Iowa. Since 2001, we stocked a maximum of 338,112 juvenile Higgins eye from host fish releases (Table 2). In 2004, we conducted a radio tracking study of stocked host fish to identify areas of the river where they resided during peak transformation of attached glochidia (Cook and Bierl 2005). Over the 9-day tracking period, host fish moved between zero and 28 kilometers. In 2005, we monitored these and other areas and found no subadults. Only 43 live mussels of 9 species were found (Table 7). It also appeared that the native mussel community had declined significantly compared to previous mussel surveys so we decided to defer future stocking activities on the Cedar River.

4. Site 10, Wapsipinicon River, Iowa. Since 2002, we stocked a maximum of 1,332,842 juvenile Higgins eye from host fish releases (Table 2). In 2005, we monitored these areas and found one subadult Higgins eye (species identification confirmed by genetic testing in 2006 by Kevin Roe, Iowa State University). We returned in 2006 and found 9 additional subadults (Figure 28). While we cannot be certain that these juveniles came from our released host fish, their ages are consistent with stocking events and may indicate successful transformation in several different years. In 2005

and 2006, we found 1,243 live mussels of 16 species (Table 7).

Stocking Subadults from Closed Propagation Cages

Cages Placed on the River Bottom. In the early 1900's, fatmucket (*Lampsilis siliquoidea*) were successfully propagated in large wooden corrals in Pool 4 (Lake Pepin) on the UMR near Frontenac, Minnesota (Figure 29). Corrals were surrounded with wire mesh to exclude predators and confine mussels. Our current site at Frontenac is at or close to this original propagation site (Figure 30). To mimic these past propagation efforts, two large propagation cages were constructed of wood, metal and screen in 2000 (Figure 31). Unfortunately, these cages were heavy and trapped sediment and zebra mussels; however, one produced three juvenile Higgins eye. We now use closed propagation cages that are similar in construction to open cages (18 by 24 by 36 inches; Figure 24) but have a (3/8-inch) plywood bottom to confine juvenile mussels. Cages are attached to the bottom frame with bungee cords or plastic ties. Before host fish are added, a 3- to 6-inch layer of sediment is placed on the bottom of the cage as substrate for transformed juveniles.

Our original cages had a wire mesh bottom similar to open cages. However, when attached to the frame it created a ¼ -inch space between the plywood and wire mesh that trapped transformed juveniles. Because juveniles grow rapidly, some larger individuals became trapped, could not open their shells, and died. Alternatively, mussels grew between the mesh and were deformed (Figure 32). We now eliminate the wire mesh bottom; however, cages must be strongly secured to the metal frame and plywood to ensure mussels are confined. In 2003, we also constructed two large 4 by 8-foot wooden sleds to propagate and hold juveniles and subadults (Figure 33). While heavy, our sleds are still in use and each can hold a larger number of subadults than a standard cage.

Cage propagation techniques are described in Davis 2001 and 2002. In May, infested fish and closed-bottom cages are transported by boat to the population establishment site. Depending on their size, approximately 30 to 50 infested fish are placed in each cage. Tags are attached to each cage for identification. We initially used metal tags that subsequently rusted or corroded at their attachment point or fouled and made underwater identification difficult. We then tried plastic livestock ear tags; while they are made of a rubber/plastic compound and seem durable, some deteriorated at their attachment point and fell off. We also used a variety of colored livestock tags to track donor strain and age cohort; all tended to foul or fade making identification difficult. Our newest tag is also a livestock tag which we now attach with plastic zip ties. We also write information on each tag with a permanent marker and etch it with a Dremel tool (Figure 34).

At the population establishment site, divers transport and secure cages to the river bottom. Cage locations are marked with Global Positioning System (GPS) coordinates, lines/buoys, and shoreline references. After approximately 3 to 4 weeks, glochidia transform, excyst from the gills of the host fish, and drop to the substrate on the bottom of the cage. After transformation, divers return, open the top of the cage, and carefully release host fish to the river. In late September, we inventory the contents of closed

cages (Figure 35). Cages are brought to the surface by divers, waders, or a winch and placed in the boat or on shore. In 2005, we tested an underwater communication system that provides voice communication between divers, and between divers and shore/boat attendants (Figure 36). This system is useful in waters like the UMR that have limited visibility. Water is used to wash contents; benthic sampling trays are used to screen sediment and collect mussels (Figure 37). Larger mussels and sediment can be washed using the cage as a screen. Juveniles are counted, measured, and returned to cages at a density no greater than 500 per cage until they are large enough to stock as subadults (usually Age 2 or 3).

Propagation of Higgins eye in closed cages has been successful beyond our expectations. Since 2000, we placed 491 closed cages with 15,753 infested fish at propagation sites in the upper Mississippi, lower St. Croix, and lower Wisconsin Rivers; maximum total production was 3,255,217 juveniles (Table 8, Figure 38). Our best propagation site is Pool 4, UMR, at Frontenac, Minnesota (Figure 39). In August and September 2006, we collected approximately 23,700 juvenile Higgins eye (2005 Cohort) from 111 closed propagation cages at Frontenac; one cage contained 1,400 juveniles, the current record. It is interesting to note that subadults have three color phases; yellow, green, and red (Figure 40). Subadults ready for stocking at population establishment sites are marked and measured (Figure 41). Each age cohort gets a unique set of dots using black super glue. In addition, a subset of 100 or so are individually marked with plastic oyster tags attached with clear super glue. To speed the drying process of the glue, an accelerant is sprayed or applied with a syringe. Mussels ready for stocking were placed in nylon mesh bags inside aerated coolers and transported to population establishment sites.

Approximately 35,823 subadults are currently being held in closed propagation cages at Frontenac for future stocking (Table 9). Security of Higgins eye has always been a concern given the large number of cages and mussels concentrated at the Frontenac Site. Since 2000, a few cages were vandalized or damaged by outboard propellers. To restrict boating, fishing and swimming in our propagation area, we marked the corners and mid point with floating signs. In 2007 we will replace our old signs with a new format (Figure 42).

We tried fourteen cage propagation sites on the UMR and tributaries (Figure 38). Production of Higgins eye juveniles varied between propagation sites and cages and ranged from zero to 1,400 per cage. At locations with no or few juveniles, cages usually filled with fine or coarse sediment limiting propagation success and sometimes finding cages. Low dissolved oxygen at sites with fine sediments and aquatic vegetation may have also limited production. Our best cage production sites were in UMR Pool 4 at Frontenac, Minnesota, and floating cages in UMR Pool 12 (Ice Harbor) at Dubuque, Iowa. The advantages of Frontenac include easy vehicle and boat access, a publicly owned shoreline that offers little conflict with recreational use, probability of less fine particle deposition due its location farther downstream in Lake Pepin, and its protection from wind-driven waves. While Frontenac continues to be our primary propagation site, abundance of zebra mussels has gradually increased and its continued use is questionable if this trend continues.

Production was usually higher for the St. Croix than Cassville or Cordova strains. From 2004 through 2006, average number of juveniles per cage using largemouth bass for hosts was 347 for the St. Croix Strain, 106 for the Cassville Strain, and 59 for the Cordova Strain. This trend is also consistent with transformation rates observed at Genoa NFH; average transformation rates of Higgins eye glochidia on largemouth bass at the hatchery in 2003 and 2004 were 25% for the St. Croix strain, 15% for the Cassville strain, and 16% for the Cordova strain.

Besides Higgins eye, we collected juveniles of 14 other species in our closed propagation cages (Table 10). We were surprised to find the number and variety of other species considering that all host fish were raised in isolated hatchery ponds at Genoa National Fish Hatchery and not subject to infestation by other riverine mussels. Perhaps cages act as fish attractors and glochidia excysting from fish immediately outside the cage flow into the cage with river currents and settle to the bottom. Alternatively, there may be natural drift of transformed juveniles or glochidia that infest our host fish inside cages.

Survival of Higgins eye in cages was variable and ranged from 60 to 100% (Table 11). For example, in September, 2003 we collected 4,818 juveniles at Frontenac and placed them back into cages to grow; in July 2004 we monitored these cages and collected 4,380 14-month–old subadults. Survival was 91%, although only 28 empty shells were found of the 438 missing animals. It is common to find very few empty shells in propagation cages, especially those containing juveniles. In 2004, we collected 7,439 juveniles from 29 cages and found only 14 dead shells. Juvenile shells are very thin and lightweight; dead shell may dissolve or be swept out of cages by river currents.

Most juveniles less than four months old are small enough to pass through the wire mesh sides of closed cages. We assume that many transformed juveniles escaped from cages to the river on their own or passed through the wire mesh by river currents or during cage inventories. Consequently, many young Higgins eye should theoretically be living in the river in the vicinity of our propagation cages. In 2004, divers conducted a 64-minute search within the Frontenac cage propagation site to locate juveniles and subadults. No living or dead Higgins eye were found, suggesting that mortality is extremely high for juveniles in the wild compared to cages, at least at this site. The RAMAS model assumed mortality of 99.9 % for Higgins eye juveniles from Age 0 to Age 1. We believe that survival of juveniles and subadults is enhanced in cages probably due to protection from large bottom-feeding predators like common carp (*Cyprinus carpio*), river redhorse (*Moxostoma carinatum*) and freshwater drum (*Aplodinotus grunniens*).

In the UMR, growth of Higgins eye in cages appears to be excellent; at Age 1, 2, and 3 they are approximately 10 to 20 mm, 20 to 40 mm, and 40 to 50 mm in length, respectively (Mussel Coordination Team 2003). In comparison, we have been unable to produce similar results at Genoa NFH. Juveniles at 3 months of age in the hatchery are less than 1 mm, versus 8 to 22 mm in cages in the river (Gordon 2002). The lack of growth in laboratory/hatchery reared situations compared to field environments is consistent with food limitation (Teresa Newton, personal communication). In September,

2004 we measured individuals from the 2002 and 2003 cohorts that were propagated in closed cages in the UMR at Frontenac; mussels from the 2002 cohort were approximately 10 mm longer than the 2003 cohort (Figure 43). Age at maturity was also consistent with previous observations for Higgins eye. In September 2006, females from the 2003 cohort were gravid; they were 39 months old (Age 3) and had completed four growing seasons (Figure 44).

We used to open all propagation cages in the fall to count our 3-month old juveniles. We now sample only a few cages to get an estimate of production because juveniles are small and can easily wash through the sides of the cage; additionally, we now have hundreds of new cages each year. However, due to zebra mussels, each cage must be opened at least every other year to clean Higgins eye of zebra mussels. We assume the numerous zebra mussels attached to small juveniles negatively affect their growth and survival (Figure 45).

Floating Cages. In 2005, we tested floating cages which may reduce water quality and sedimentation problems by placing propagation cages higher in the water column where fewer sediments will settle out (Figure 46). Standard closed cages were placed on a floating rack system constructed at Genoa NFH. In 2005, over 5,000 juvenile Higgins eye were collected from nine closed floating cages in the UMR, Pool 12, Iowa (Dubuque Ice Harbor). In 2006, eight floating cages at this site produced an estimated 870 juveniles. Newton et al. (2001) found that survival was higher for mussels placed in suspended versus buried trays. Floating cages may also be safer for recreational boating, angling, and swimming in the area since they are visible from the surface, although they may also attract vandals.

Monitoring Subadults Stocked in the Wild.

Since 2003, we stocked 9,754 subadults at six population establishment sites on the UMR and tributaries (Table 2). Most were stocked using an underwater grid system (Figure 47; Kelner and Heath 2003). The grid was constructed of ¾-inch diameter PVC pipe that enclosed four 1-meter square cells. The grid is placed on the river bottom perpendicular to the current and positioned with steel rods in the upper and lower left corner of each cell. Ten to twenty subadult Higgins eye are placed into each cell. When cells are filled, the grid is flipped over in the downstream direction and additional pins placed in the downstream corners of each cell. The process is continued until all mussels are stocked. A closed propagation cage containing subadults is placed next to the grid so mortality and growth may be compared at the time of monitoring. Shore markers and GPS readings are taken so it can be located for future monitoring. In a year or two when the site is monitored, the metal stakes will be located, the grid repositioned on the bottom, and mussels collected from the individual cells (assuming they have not moved from their locations).

Survival of our subadults in the river was variable between population establishment sites and apparently influenced by predation (Table 12). In 2004, we searched 1 of 19 cells at Site 3 and found 90% of the mussels placed in 2003. Conversely, on this same date we

monitored Site 4 and found very few subadults in those cells. No empty shells were found and it was impossible to determine their fate. However, all subadults inside the cage at Site 4 were alive and appeared to be in good condition ruling out water quality or other environmental factors. Mussels could have crawled out of the grids at Site 4. We also found that smaller individuals were stocked at Site 4 suggesting they could have been carried off by currents or were more susceptible to predation (Figure 48).

In 2004, one of our large wooden sleds that held 958 subadults was apparently vandalized and the protective top screen removed sometime during the summer. We found 742 live subadults and many broken shells. At first we thought that shells were crushed by vandals walking/jumping on the subadults inside the sled. However, we could not crush similar unbroken shells by standing/jumping on them in the sled. We concluded that opening the wire top let muskrat, otter, carp, drum or other predators feed on the subadult Higgins eye. In 2004, we stocked more subadults at Site 4. To reduce the risk of predation, we constructed large 5x12-foot blankets constructed of an aluminum frame and mesh wire or plastic (Figure 47). These blankets were placed over grids to protect stocked subadults from large predators. For comparison, we also placed subadults in uncovered grids. In 2005, we monitored an uncovered grid at Site 4 and found only 5 of 262 subadults (1.9%). We also found many crushed shell fragments, some bearing our identification tags (Figure 49). At blanketed grids, it appeared that predators seeking our subadults had excavated bottom sediment around the perimeter of the blanket. Excavated areas were characterized by a chain of deep pockets and broken shell, suggesting the feeding behavior of common carp or other bottom feeders. By reaching beyond this excavated area into the middle of the blanket, the diver was able to feel many live subadults. In 2005, we observed similar browse zones around blankets at Site 2. Many live mussels were felt under the middle of the blanket where they were probably safe from predators. While not a long-term solution, blankets may be useful in minimizing mortality of small mussels for a short time. However, they trap sediment at some locations and can bury mussels, inhibit their movement, and interfere with their ability to attract host fish once they reach sexual maturity.

Placing subadults in the grid system made it easier to monitor for survival, growth, and movement. However, the grid system concentrates individuals much more closely than in nature and probably makes it easier for predators to find them (once found, the predators are essentially at a mussel buffet). To minimize predation, we now stock subadults randomly instead of using our standard grids; monitoring is more difficult, but this method may improve survival. We also stock larger individuals, which may help reduce predation; Table 13 shows the size distribution of subadults stocked at each population establishment site which ranged from 33 to 73 mm.

In September, 2006, we conducted a mussel survey of the tailwaters below Lock and Dam 3 to evaluate its potential as a replacement for 4th Cut at Site 3 where predation of stocked subadult Higgins eye seems to be a severe problem. A total of 12 live species were collected from four locations in the tailwater. An evaluation site was selected and 290 juvenile plain pocketbook (*Lampsilis. cardium*) placed on the bottom substrate; juveniles came from propagation cages placed at the Frontenac Site in 2005. Each

juvenile was marked with a black dot of super glue to differentiate it from other mussels in the area. In 2007, we will attempt to recapture a subset of these mussels and any shell remnants to determine the extent of predation or other mortality at the site before stocking subadult Higgins eye.

For Higgins eye, the RAMAS population model assumed annual mortality was 10% for Age 1 to Age 2, and only 5% for Age 2 and older (U.S. Army Corps of Engineers 2002). Unfortunately, we found significant mortality of subadults in the wild at some population establishment sites. Apparently, that mortality is caused by large common carp, freshwater drum, or other fish having the capability to crush thick mussel shells. As the exotic common carp has been in the UMR since the 1800's, our observations raise important ecological questions concerning their effects on native mussels since they were introduced. It is interesting to note that pharyngeal teeth of common carp are similar in appearance to exotic black carp (*Mylopharyngodon piceus*) which are known to eat mollusks (Figure 49). Also, broken shells are always found in the water near release sites suggesting the predator feeds underwater. We cannot rule out predation by muskrat (*Ondatra zibethicus*) or river otter (*Lutra cacadensis*) which also eat mussels (Parmalee 1967; Parmalee and Bogan 1998).

Estimating survival of juveniles and subadults in the wild is important; if mortality rates for juveniles and subadults are higher than the RAMAS model estimates, more juveniles and subadults will need to be stocked to reach the short-term goal of 5 new and viable populations of Higgins eye. In August 2006, we started experiments to measure predation of juvenile mussels using small plain pocketbooks (*Lampsilis cardium*) as a surrogate. One study was conducted in lower Pool 2 near the subadult reintroduction site. Forty sites were randomly generated within a 40-acre area (Figure 50). At each site, five pocketbook were tethered to a 40-cm metal rod by 15 cm of monofilament nylon line cemented to the shells to prevent escape. At half the sites, tethered mussels were covered with a wire mesh basket to exclude predators; mussels at the remaining sites were left uncovered. In October 2006, we monitored two uncovered sites. At one site, all five mussels were found alive; two were still tethered to the metal rod and three were loose. At the second site, four were found alive and one was missing; of the four live individuals, three were still tethered and one was loose. In 2007, we will monitor the remaining treatment sites. In 2006 we also attached fishing line and hooks (to fish for predators) to small plain pocketbook and placed them in Pool 8; no predators were caught (Figure 51). In the future, we hope to place large carp and freshwater drum in an enclosure with subadult plain pocketbook and observe any predation.

Growth of subadult Higgins eye stocked at population establishment sites was surprisingly uniform within cohorts (Table 14). Average growth 1 year from stocking was 7 and 9 mm for 2001 cohorts at two sites, 7 and 8 mm for 2002 cohorts at two sites, and 11, 15 and 15 mm for 2003 cohorts at three sites. At Site 4, average two-year growth was 29, 20 and 28 mm for 2001, 2002 and 2003 cohorts, respectively. From placement in 2001, one individual at Site 4 grew 52 mm over three years. An example of individual shell growth is shown in Figure 52.

Higgins eye subadults stocked in the UMR reached sexual maturity in approximately 4 years, similar to observations for those in cages (Figure 53). In 2005 and 2006, females were gravid at population establishment sites; it is our hope that they are infesting fish and founding new populations in UMR Pools 2, 3 and 4. Monitoring over the next several years will determine if successful reproduction and recruitment are occurring.

Stocking Adults

Zebra mussels have not infested the UMR upstream of Lock and Dam 3 to levels approaching those in downstream areas below Lake Pepin in Pool 4. Adult zebra mussels are transported upstream of Lock and Dam 3 by towboats and barges and are present at upper St. Anthony Falls, the most upstream navigation lock and dam on the UMR (Yager 1993). We also know they successfully reproduce above Lock and Dam 3; veligers have been collected below Lock and Dam 2 in previous studies. However, zebra mussels do not proliferate in this area possibly due to the Minnesota River, which enters the UMR approximately 2 miles downstream of Lock and Dam 1. It provides a significant amount of fine sediment from Minnesota's farming country. Perhaps the fine sediments interfere with survival, growth, and/or attachment of veligers in Pools 2, 3, and upper 4. Alternatively, perhaps flow velocities and growth rates do not allow for settlement of veligers until they reach lower Pool 4. Regardless of the reason, the reach of the UMR from the Coon Rapids Dam in the Twin Cities Metropolitan Area to upper Pool 4 in Lake Pepin has a diverse mussel community that does not appear to be adversely affected by zebra mussels (Kelner and Davis 2000, 2002).

Where zebra mussels pose a significant threat to existing Higgins eye populations, adults are cleaned of zebra mussels and relocated to areas of the UMR within its historic range having no or few zebra mussels (we move them out of harm's way). We relocated adults to two population establishment sites: Site 1 in UMR Pool 2 above the confluence of the Minnesota River in the Twin Cities; and the upstream portion of Site 3 in UMR Pool 3 near Hastings, Minnesota (Figure 16). Both sites have good populations of native mussels and few zebra mussels (Kelner and Davis 2000, 2002). Since 2000, we relocated 472 adult Higgins eye and approximately 2,100 state-listed species from Pool 11 at Cassville, Wisconsin (Anderson 2002), and Pool 14 at Cordova, Illinois, to Sites 1 and 2 in the Twin Cities reach (Table 2). Native mussels at Cassville and Cordova were highly infested with zebra mussels. The Cordova relocation project and methods are described in Anderson et al. 2002; this relocation was accomplished as part of a mussel workshop conducted by the Illinois Chapter of the American Fisheries Society. Mussels were cleaned of zebra mussels by scrubbing, placed in burlap sacks that were suspended in water in a hatchery truck, and transported to the population establishment sites (Figure 54). At the population establishment sites, they were transported by boat and hand-placed in the river bottom by divers usually along a lead line to facilitate future monitoring. The location of each population establishment site was noted by GPS coordinates, rope and buoys, and shoreline references.

Female-to-male ratios for adult Higgins eye stocked at Sites 1 and 3 were 159:212 and 41:60, respectively, indicating a good mix of males and females at each site. Monitoring

since 2000 showed generally good growth of stocked adults (Table 15). Mortality appeared to be low at both locations; only 2 dead were found at Site 1 and 6 dead at Site 3. In addition, gravid females were observed in each sampling year since 2000, and we hope they are establishing populations at the new sites (Figure 54).

1. Site 1, UMR, Upper Pool 2, Twin Cities, Minnesota. In 2000 and 2001, 371 adult Higgins eye were relocated from UMR Pools 11 (Cassville, Wisconsin) and 14 (Cordova, Illinois). In September 2004, we monitored Site 1 and found 14 adults that were stocked in 2000 and 2001; all ten of the females were gravid. In 2004, shell growth appeared normal suggesting that abnormal growth observed in prior years may have been due to repeated handling, use of several females as glochidial donors in 2002, or overly aggressive scrubbing to remove attached zebra mussels prior to stocking. Average growth of adults at Site 1 between stocking and September, 2004 was 6 mm (range 1 to 14 mm; Table 15).

2. Site 3, UMR, Pool 3, Hastings, Minnesota. In September, 2000, 101 adult Higgins eye and 1,380 state-listed mussels were placed at this site from Pool 11, UMR, at Cassville, Wisconsin. In 2006, we monitored Site 3 and collected 24 live mussels placed in 2000; 18 were males and 6 females. All six females were gravid (4 of 5 females were gravid in 2003). In addition, 46 live state-listed mussels (5 of the 6 species relocated) were collected from those relocated in 2000. Average growth of adults at Site 3 between stocking and September, 2006 was 6 mm (range -1 to 19 mm; Table 15).

Long-term Monitoring of New and Viable Populations

Our interim goal (2001 – 2010) is to establish five new and viable populations of Higgins eye in areas of the UMR and tributaries that have no or few zebra mussels. For a population to be viable, it must have a minimum of 500 individual Higgins eye and a minimum of 2 year classes between 4 and 10 years of age as evidence of successful reproduction and recruitment (U.S. Army Corps of Engineers 2002). Once a population is viable, it must be monitored at least 20 years under supervision of a qualified malacologist and continue to meet the viability requirements over this time.

Monitoring survival and growth of stocked Higgins eye at population establishment sites provides important information and will be continued. However, collecting progeny from stocked juveniles, subadults, and adults will be critical in future years. The first ten years of the Higgins eye relocation program focuses on stocking enough individuals to establish populations at the ten population establishment sites. The next phase of the program will focus on long-term monitoring to assess population establishment and viability over time (Table 16). We will begin looking for juvenile Higgins eye at the adult population establishment sites in 2007 as their progeny should be large enough to find by diving and wading. In 2008, we will include population establishment sites where subadults were stocked. A challenge will be to locate new populations of Higgins eye and assess their viability over time. We assume that new populations are just as likely to establish at other locations as they are at the same locations where adults,

subadults, and juveniles were stocked. The location of host fish at the time of juvenile excystment ultimately determines where transformed juveniles drop to the river substrate, mature to adulthood, and complete their life cycles.

Maintaining Existing Populations

In waters infested with zebra mussels, one way to increase survival of native mussels is to periodically clean them of zebra mussels and return them to their habitat (Hallac and Marsden 2001). Since 2001 we cleaned 1,363 adult Higgins eye at five locations on the UMR and tributaries (Table 17). Our primary sites are in Pools 11 (Cassville) and 14 (Cordova) which we call Adult Clean and Stockpile Sites (Figure 55). Mussels are collected by diving or wading, cleaned of zebra mussels by scrubbing with a stiff brush or gloves, measured, sexed and individually marked (Anderson et al. 2002; Figure 56). Instead of randomly releasing mussels, they are hand-placed on the bottom by divers in a 10 – 20 m^2 area (stockpile) so they can be easily recaptured. Stockpile sites are marked by GPS coordinates, rope and buoys, or shoreline references. A year later, mussels are inspected and cleaned, if necessary. Another benefit of the stockpile sites is that gravid females can be easily collected for host fish infestation.

Long-term Monitoring of Higgins Eye and Zebra Mussels

Since 2000, Higgins eye and zebra mussels were monitored at several locations on the UMR and tributaries (Table 18). Of historic importance are Essential Habitat Areas (EHA) considered to be the best remaining habitat for Higgins eye and important for its recovery (U.S. Fish and Wildlife Service 2004): Interstate Park, lower St. Croix River; Hudson, lower St. Croix River; Prescott, lower St. Croix River; Orion, lower Wisconsin River; Whiskey Rock, Pool 9, UMR; Harpers Slough, Pool 10, UMR; Prairie du Chien, Pool 10, UMR; McMillian Island, Pool 10, UMR; Cassville, Pool 11, UMR; Cordova, Pool 14, UMR; and Sylvan Slough, Pool 15, UMR.

In 1991, the first zebra mussel was collected on the UMR in Pool 8 near La Crosse, Wisconsin (Mussel Coordination Team 2003). In 1992, the Corps began monitoring zebra mussels at the navigation locks and dams from upper St. Anthony Falls to Pool 10. In 1993, densities in the lockworks ranged from zero at lower St. Anthony Falls at Minneapolis, Minnesota, to 6.5 per m^2 at Lock and Dam 7 at La Crescent, Wisconsin; in general, densities increased in a downstream direction (Yager 1993). In 1998, the Wisconsin and Iowa Departments of Natural Resources began collecting zebra mussel veligers below locks and dams on the UMR. In 2001, the Corps expanded veliger studies to include the UMR and select tributaries from Lock and Dam 2 at Hastings, Minnesota, to Lock and Dam 19 at Keokuk, Iowa (Stoeckel 2002). Unfortunately, zebra mussel veligers have been collected at nearly all sites (Farr and Antwine 2005).

Since 1991, zebra mussels have expanded their range in the UMR and tributaries within the historic range of Higgins eye, which results in fewer zebra mussel-free locations for possible relocation of Higgins eye and other species. Since 1995, densities of native and zebra mussels increased at some locations and decreased at others (Figures 57 and 58).

In 2001, we observed a die-off of zebra mussels in several navigation pools in the upper reaches of the UMR the including Pools 11 (Cassville) and 14 (Cordova). Unfortunately, this trend did not persist, and zebra mussels returned in 2002; their abundance now seems to be highly variable from year to year and from season to season. Currently, zebra mussels may be extremely abundant on native mussels in spring and summer but absent in fall collections. This mortality of zebra mussels may be due either to high water temperatures in the late summer or fish predation. Since 2000, we noticed a dramatic decline in the number of zebra mussels attached to the outside of cages at our Frontenac propagation site while at the same time zebra mussels may be abundant inside cages (Figure 45), suggesting predation as the cause of mortality, rather than unfavorably high water temperatures. Bottom feeders like freshwater drum or common carp may feed on exposed zebra mussels attached to cages or other substrates. Magoulick and Lewis (2002) also found that native fish fed on zebra mussels.

In 2006, the Corps summarized data collected on abundance and distribution of zebra mussel veligers (Tables 19 and 20; Figure 59). Since 2000, the abundance of zebra mussels continued to increase on the St. Croix River and this location is now a source population for the UMR (Figure 60). Zebra mussels were recently found in large reservoirs within the UMR basin upstream of the Twin Cities Metro Area (Ossawinnamakee Lake, Mille Lacs Lake and Rice Lake, a backwater of the UMR near Brainerd, Minnesota). However, no zebra mussel veligers have been found upstream of upper St. Anthony Falls, indicating there is not yet a source population upstream of the Twin Cities Metro Area.

Lake Pepin, Pool 4, UMR, Minnesota – Wisconsin. Lake Pepin is a natural feature of the UMR formed by the delta of the Chippewa River near Reads Landing, Minnesota. Lake Pepin is considered the source population of zebra mussels for downstream pools on the UMR. While zebra mussels occur upstream of Lake Pepin, in the lower St. Croix River and UMR, they are probably a self-sustaining population in the lake, no longer requiring an upstream source of veligers for maintenance.

The Minnesota Department of Natural Resources has monitored zebra mussels in Lake Pepin since 1996. The lake is divided into four sampling quadrants, MN 1 through 4, from upstream to downstream. The pattern of zebra mussel distribution in Lake Pepin has remained similar over time. Highest densities were found at MN-4 near the outlet and lowest densities at MN-1, at the upper end of the lake. In 2006, densities of zebra mussels declined substantially at MN-3 and MN-4 (Figure 61). However, some individuals were covered with small zebra mussels presumably from the 2006 cohort.

MN-4 (LaCupolis) is also the site of diverse and abundant mussel bed that has been monitored periodically since 1991. This bed contains 29 species of native mussels including an abundance of spike (*Elliptio dilatata*). Zebra mussel infestations from 1998 through 2000 significantly reduced the abundance of native mussels at LaCupolis. In 2006, zebra mussels were predominantly young of the year with no evidence of 2005 recruits having survived at this site. We remain hopeful this trend will continue to enable the LaCupolis bed to recover.

Essential Habitat Areas

1. East Channel Reference Site, Prairie du Chien EHA, Pool 10, UMR, Wisconsin. The East Channel Reference Site is part of the Prairie du Chien EHA. Abundance of Higgins eye and other native mussels were routinely monitored for many years by the Corps. Zebra mussels significantly impacted Higgins eye and other native mussels at this location after 1996. Density of zebra mussels was 10,853 per m^2 in 1996 and peaked in 1999 at 56,507 per m^2. In comparison, density of native mussels dropped from 59.2 per m^2 in 1996 to 1.7 per m^2 in 1999 (U.S. Fish and Wildlife Service 2000). Likewise, catch-per-unit-effort of Higgins eye dropped from approximately 1.0 per minute in 1995 to nearly zero in 2000 (Figure 62).

   Since 2002, abundance of native mussels has increased, likely due to a dramatic decrease in zebra mussels (Figure 63). Abundance of juvenile mussels (< 30 mm long) also increased during this time, as did mussels less than 4 years old, indicating recently successful recruitment (Figure 64). Indications are that Higgins eye may tolerate zebra mussel infestation better than other species. The percent composition of Higgins eye in the overall mussel community in the East Channel has increased from pre-zebra mussel conditions (Figure 64). While this is good news, Higgins eye and the native mussel community in the East Channel have not recovered to pre-zebra mussel conditions.

   Dead zebra mussels also adversely affect native mussels in the East Channel; in 2005 some locations still had a thick layer (two feet or more) of zebra mussel shells and associated fine sediment covering the former rich and diverse mussel community (Figure 65). We remain concerned that if native mussels do recover in the East Channel, they will simply provide substrate for zebra mussel attachment, cause high mortality of native mussels, and the cycle will continue.

2. Harpers Slough EHA, Pool 10, UMR, Iowa**.** Native mussels at the Harpers Slough EHA were also significantly impacted by zebra mussels after 1996. In 2000, the density of zebra mussels was over 10,000 per m^2, and native mussels fell to less than 5 per m^2 (Figure 66). However, abundance and recruitment of native mussels are improving as zebra mussels decline; in 2005 zebra mussel density fell to less than 500 per m^2. While this is good news, in 2005 zebra mussels still formed a carpet of dead shells and fine sediment on the river bottom (Figure 67).

3. Cassville EHA, Pool 11, UMR, Wisconsin. While not an original EHA, Cassville has been a source of adults for relocation and propagation since 2000 and qualifies for EHA designation according to Recovery Plan criteria. Zebra mussels have declined from 2,465 per m^2 in 2002 to 0.1 per m^2 in 2006. In 2006, 78% of live mussels collected had no attached zebra mussels and only 8 live zebra mussels were collected in 40 samples (1/4 m^2) (Dunn and Winterringer 2006). A total of 5,461 live mussels were collected, representing 28 species; 405 represented 12 state or federally listed species, including Higgins eye. Higgins eye ranged from 3 to 15 years of age and

represented 1.1% of the community. They were evenly distributed through the bed, and 91% of females were gravid. Because of these improvements, Cassville became an EHA for Higgins eye in 2006.

4. Cordova EHA, Pool 14, UMR, Illinois. The Cassville and Cordova EHA's are now considered to be the best remaining Higgins eye populations in the UMR. One of the most interesting discoveries since 2000 is the abundance of adult Higgins eye now found in shallow water at Cordova, Cassville, and Prairie du Chien EHAs. Historic surveys found Higgins eye to be associated with deeper water (U.S. Fish and Wildlife Service 2004). Since 2000, we cleaned 1,834 adult Higgins eye from these areas and nearly all were collected in shallow water less than three feet deep. Theories for this habitat shift include: 1) Higgins eye always lived in shallow marginal habitat, while surveys were concentrated in deeper preferred habitat; 2) Higgins eye always lived in shallow marginal habitat and that is all that remains today due to zebra mussel colonization of deeper waters; 3) habitat conditions have changed in recent years, rendering shallow habitats preferable; or 4) Higgins eye have always used shallow and deep habitats without preference.

Other Exotic Species. Future concerns include the exotic quagga mussel (*Dreissena bugensis*), which is similar in appearance to zebra mussels and a threat to native mussels (Figure 68). For unknown reasons, no quagga mussels have been found on the UMR and tributaries. Approximately 12 years ago, zebra mussels outnumbered quaggas by 100 to one in Lake Erie. Today, this trend is reversed, and quaggas now outnumber zebra mussels by 10 to one (Mississippi Interstate Cooperative Resource Association 2002). Quagga mussels survive in deeper water and spawn in colder water than zebra mussels, which may allow them to become established in more northern reaches of the UMR and tributaries. Another exotic species with a similar life history as zebra mussels is the golden mussel (*Limnoperna fortunei*). It is native from rivers and streams in China ans soustheast Asia. It has not yet been found in the United States but has become established in South America probably due to ballast water exchange (Darrigran and Pastorino 1995). Like zebra mussels, the golden mussel attaches to native mussels and other hard objects using byssal threads. Because the golden mussel has a wider tolerance of ecological parameters than the zebra mussel, it is expected to invade a broader range of habitats in North America. Another concern for native mussels are exotic black carp (*Mylopharyngodon piceus*) which feed on mollusks; they have recently been found on the UMR in Illinois and are likely to spread upstream.

Will Native Mussels Survive Zebra Mussels?

Before zebra mussels became established, the Prairie du Chien EHA was considered the best remaining habitat for Higgins eye and other native mussels on the UMR. All Higgins eye populations on the UMR were adversely affected by zebra mussels, but none were affected to such an extent as Prairie du Chien. Habitats that were ideal for native mussels were also ideal for zebra mussels. Marginal areas for Higgins eye often had higher flows and unstable substrates, and these habitat conditions were also less favorable to zebra mussels, which probably limited attachment and carpeting of the bottom. As

long as zebra mussels persist in the UMR, habitat once considered marginal for Higgins eye may become critical to the future persistence of native mussels, including Higgins eye. These marginal areas are less hospitable to zebra mussels but still provide suitable habitat for native mussels. While zebra mussels may not extirpate native mussels from the UMR and tributaries, they are likely to keep their populations at low densities. Strayer and Malcom (2007) suggest that native mussels in North America may persist at population densities about an order of magnitude lower than pre-invasion by zebra mussels.

Zebra Mussel Management Feasibility Study

The second requirement of the Biological Opinion was development of a reconnaissance study by the Corps to determine the necessary measures, projected costs, and likelihood of success in controlling zebra mussels in the UMR (U.S. Fish and Wildlife Service 2000). In 2003, the Corps of Engineers released the *Mississippi River Between the Missouri River and Minneapolis, 9-Foot Channel Project, Measures for Managing Zebra Mussels – Reconnaissance Report* (U.S. Army Corps of Engineers 2003). The report identified potential control measures and recommended $1,990,000 for completion of a feasibility study. In 2005, the Corps completed the report *Distribution and Density of Zebra Mussel Veligers at Sites in the Upper Mississippi River System and its Major Tributaries, 2004* (Farr and Antwine 2005). In 2006, funds were authorized to expand zebra mussel sampling in the lower St. Croix River and popular recreational waters in the basin. Existing data was compiled on habitat requirements of zebra and quagga mussels to develop the basic framework for the risk assessment. The study area was also expanded to include the lower St. Croix River basin and UMR from the mouth of Lake Pepin in Pool 4 to upper St. Anthony Falls in Minneapolis, Minnesota.

Winged Mapleleaf Activities

The winged mapleleaf (*Quadrula fragosa*) was listed as a federally endangered species in 1991, and a recovery plan completed in 1997. Unlike Higgins eye, the historic range of winged mapleleaf is large and included 14 states (Figure 69). Today, the species occurs at only a few locations: immediately below Taylors Falls on the lower St. Croix River in Minnesota – Wisconsin, the Bourbeuse River in Misssouri, and the Ouachita and Saline Rivers in Arkansas (Figure 70).

Like Higgins eye, the Service determined that operation and maintenance of the 9-Foot Channel Project for an additional 50 years is likely to adversely affect winged mapleleaf (U.S. Fish and Wildlife Service 2000). While zebra mussels have not currently infested winged mapleleaf locations, there is a likelihood of infestation occurring over the next 50 years if zebra mussels persist on the UMRS. In order to minimize take of winged mapleleaf, the Service issued Reasonable and Prudent Measures, which required the Corps to establish populations of winged mapleleaf in areas with no or few zebra mussels and implement a zebra mussel control program, similar to the Reasonable and Prudent Alternatives for Higgins eye (U.S. Fish and Wildlife Service 2000). The Corps is using the MCT to assist in implementing these Reasonable and Prudent Measures for winged

mapleleaf.

Establishing New and Viable Populations

The Corps' activities for winged mapleleaf began in 2003 and focused on implementing the *Plan for Controlled Propagation, Augmentation, and Reintroduction of Winged Mapleleaf (Quadrula fragosa)* (U.S. Fish and Wildlife Service 2004). Because the historic range of winged mapleleaf extends beyond the 9-Foot Channel Project, the Corps agreed to lead reintroduction efforts within the UMR and tributaries and the Service agreed to lead reintroduction activities within the remaining area (Figure 69). A draft Winged Mapleleaf Relocation Plan will be completed in 2007 identifying population establishment sites and methods.

Until recently, the last known reproducing population of winged mapleleaf was in the lower St. Croix River in Minnesota - Wisconsin. Today, the Saline River in Arkansas contains the largest known population of winged mapleleaf; in 2005, the population at a single site was estimated to be approximately 10,000 individuals (Harris 2006). In November, 2005, 7 gravid females were collected in only 15 minutes to confirm fish hosts for this southern population; the blue catfish (*Ictalurus furcatus*) was the only successful host from these trials, although follow up trials in 2006 yielded the channel catfish (*Ictalurus punctatus*) as a marginal host, as well (Chris Barnhart, MSU, pers. comm). Survey efforts will continue in Missouri and Arkansas to collect individuals for genetics research at Iowa State University to evaluate populations and develop techniques for identifying juveniles produced from host fish inoculations.

Producing Juveniles

Hatchery Efforts. In 2004, host fish for St. Croix River winged mapleleaf were found to be channel and blue catfish (Steingraeber et al. 2005). Over 10,000 juvenile winged mapleleaf were propagated in aquaria at the Upper Midwest Environmental Sciences Center in Wisconsin from host fish experiments and released into the lower St. Croix River (Figure 71). None were subsequently found at the release sites. We also released hatchery juveniles into substrate of closed propagation cages with no apparent survival. Like Higgins eye, mortality of hatchery juveniles in the wild is very high.

In 2004 and 2005, glochidia from 8 female winged mapleleaf were used to infest 900 channel catfish at Genoa NFH. The maximum number of juveniles produced from these infestations is approximately 169,324. All infested host fish were used in experimental cage propagation activities; none were released to the river while infested.

Cage Propagation of Juveniles and Subadults. We found that propagation and population establishment activites are more challenging for winged mapleleaf than Higgins eye; mussels of the subfamily Lampsilinae (Higgins eye) tend to be faster growing and more prolific than their counterparts in the Ambleminae subfamily (winged mapleleaf). Not only was the identification of host fish for winged mapleleaf completed only recently, but winged mapleleaf are tachytictic, breeding in the late summer and spawning only for a

few days or weeks; their glochidia must remain attached to the gills of the host fish throughout the winter until transformation and excystment in the spring. We cannot feed or protect captive fish in cages during ice conditions so we hold them overwinter at Genoa NFH. Doing so requires a source of food (fathead minnows), indoor raceways, and water chillers to keep fish at a similar water temperature as the lower St. Croix River, to prevent premature excystment. Holding fish this long in the hatchery is expensive and puts the fish at a higher risk for diseases. Every female may not be gravid each year, making it more labor-intensive than Higgins eye to collect gravid females in the fall for infestation. Once females are collected, they tend to spontaneously abort glochidia if mishandled, making collection and transportation difficult.

To increase our chances of finding gravid females, we have 5 aggregation sites in the lower St. Croix River. In the summer, we collect adult winged mapleleaf by diving and wading and place them at the aggregation sites. Each aggregation site has a maximum of 20 individuals such that a single catastrophic event does not eliminate our entire pool of aggregated animals. Mussels are measured, marked with etched numbers and superglued shellfish tags, and placed by hand into the substrate at aggregation sites (Figure 72). Sites are located by GPS, lead lines, and shoreline markers. In September, divers return and search for displaying gravid females. Those displaying and gravid are placed in a plastic bag to prevent losses of glochidia due to spontaneous abortion, and taken to the lab to test glochidia for viability. Viable freshwater mussel glochidia snap shut when salt is introduced to the water. Donor females with viable glochidia are transported to Genoa NFH and used to infest host fish. They are later returned to the aggregation sites.

In 2004, 100 channel catfish were infested with winged mapleleaf glochidia from 2 females and held at Genoa NFH overwinter. In April 2005, catfish were released into six closed propagation cages at two sites in the lower St. Croix River (Figure 73). Cages were a new cage-on -cage design that allowed easy removal of host fish from the river (Figure 74). On October 4, 2005, 11 juvenile winged mapleleaf were collected from one of six closed propagation cages (Figure 75). Juveniles were only a few millimeters long and placed in a cage next to two other cages that were not monitored, leaving three total cages in the river for another year.. Flows in the lower St. Croix River on this date went from approximately 2,000 cfs during the collection to over 20,000 cfs within 24 hours due to a major rainstorm. We were concerned that this flood event would transport mussels and perhaps cages downstream. In September 2006, the three remaining cages were monitored and 24 subadults collected (Figure 75). Subadults were approximately 10 to 15 mm long after two growing seasons. The good news is that the upper Hudson site is resilient and can withstand major flood events. The bad news is that subadults inside cages were covered with zebra mussels and we are unsure of its continued use as a propagation site (Figure 75)

In September 2005, 300 channel catfish were infested with winged mapleleaf glochidia and held at Genoa NFH over the winter period. In April 2006, catfish were placed in 5 closed propagation cages at the upper Hudson site in the lower St. Croix River and in 4 cages at Frontenac (UMR Pool 4). These cages will be monitored in the fall of 2007. Because the Frontenac site has been successful for cage propagation of Higgins eye, we

are hopeful the same will be true for winged mapleaf. In September 2006, four gravid female winged mapleleaf were collected in the lower St. Croix River and used to infest 620 channel catfish. Catfish are being held overwinter at Genoa NFH for cage propagation in 2007.

Due to the high mortality of catfish in propagation cages in the spring (approximately 40% mortality over one month), we would like to place these fish in propagation cages as close to juvenile excystment as possible. A significant accomplishment was the development of a model estimating the threshold temperature of 10.9 °C required for transformation of winged mapleleaf glochidia on largemouth bass (Steingraeber et al. 2007). Using this model, we can predict when peak transformation of glochidia is likely to occur and therefore the best time for placement of host fish in cages to minimize their time spent in the river.

Propagation cages can weigh several hundred pounds if sediment accumulates inside. In some areas of the lower St. Croix River, it is not possible to work from shore or in shallow water where non-divers can provide assistance in hauling cages. In 2005, Roger Marks (USFWS) installed a portable hoist system in a large pontoon boat (Figure 76). The system includes a 12-volt winch on a steel A-frame, operated by a conventional 12-volt deep cycle battery. Divers attach the winch cable to the cage on the bottom of the river and attendants on the boat winch it up through a hole cut in the deck of the pontoon boat. A nice addition is a 12-volt bilge pump and hose, which provides water for washing cage contents in collection screens on deck or on shore.

Other Species and Activities

The following is a list of other mussel activities of the MCT since 2000:

- In 2000, approximately 2,100 state-listed species were relocated from Pool 11 at Cassville, Wisconsin, and Pool 14 at Cordova, Illinois, to adult population establishment sites in Pool 2 and 3, UMR.
- In 2003, we created an internet web site on *Freshwater Mussels of the Upper Mississippi River System* (www.midwest.fws.gov/mussel; Figure 77).
- In 2003, we collected live elephant ear (*Elliptio crassidens*) and ebonyshell (*Fusconaia ebena*) in the lower St. Croix River near Prescott, Wisconsin (Figure 78). The host fish for both species is skipjack herring (*Alosa chrysochloris*), which can no longer migrate upstream to the St. Croix River due to construction of navigation locks and dams on the UMR. Consequently, they are extremely rare in areas upstream of Lock and Dam 19 at Keokuk, Iowa, which was the first dam constructed on the UMR and has a high head.
- In 2004, The Mussel Coordination Team and other partners revised the popular booklet *Freshwater Mussels of the Upper Mississippi River*, first produced by the Wisconsin Department of Natural Resources in 1985. The booklet has been a popular outreach tool for mussel identification and conservation. A free copy can be obtained from Gary Wege, U.S. Fish & Wildlife Service, 4101 East 80th

Street, Bloomington, Minnesota, 55425-1665, 612-725-3548 extension 207, gary_wege@fws.gov.

- In 2004, the Upper Mississippi River Conservation Committee's ad hoc mussel committee completed the *Conservation Plan for Freshwater Mussels of the Upper Mississippi River System*. This plan follows the format and recommendations of the National Strategy, includes portions of the 1988 strategic plan that have not been implemented, and uses the implementation of the 2000 Biological Opinion as a real-world model.
- Because of the effects of zebra mussels, the *Recovery Plan for the Higgins eye Pearlymussel* was revised in 2004. Also in 2004, sheepnose (*Plethobasus cyphyus*) and spectaclecase (*Cumberlandia monodonta*) became candidate species under the Endangered Species Act.
- In 2004, the U. S. Fish and Wildlife Service completed the *Final Biological Opinion for the Upper Mississippi River-Illinois Waterway System Navigation Feasibility Study*. Measures were included to minimize impacts to Higgins eye from future navigation and ecological restoration projects.
- We have successfully propagated other mussels in closed cages including black sandshell (*Ligumia recta*) (Figure 79). Over 3,000 juvenile black sandshell were collected in 2005 from three closed floating cages in the UMR, Pool 12, Iowa (Dubuque Ice Harbor). Over 200 mucket (*Actinonaias ligamentina*) and 500 fatmucket juveniles were collected in 2005 from closed propagation cages in the UMR, Pool 4, Minnesota (Frontenac).
- In 2005, the Minnesota Department of Natural Resources drafted a report *Freshwater Mussels of Minnesota: A Plan for Controlled Propagation, Reintroduction and Augmentation within the Mississippi River from St. Anthony Falls to Lake Pepin*.
- Work continues in 2006 to identify host fish and propagation techniques for a variety of UMR mussels. Some species like black sandshell, mucket, fatmucket, and plain pocketbook have been successfully propagated. Identifying a host fish for spectaclecase, a federal candidate species, still eludes researchers.
- For the first time, pool-wide mussel surveys were conducted to estimate the population size and depth distribution of native mussels on the UMR, starting with Pool 5 in 2006 (Davis 2007).
- In 2006, work continued on developing standard mussel sampling protocols on the UMR for large- and small-scale projects.
- During 2006, divers collected additional video and photographs of mussel displays in the St. Croix River.
- The proposal *Conservation of Native Freshwater Mussels as a Program Element of the Navigation and Ecosystem Sustainability Program (NESP)* was redrafted in 2006.
- In 2006, the manuscript *Saving the Higgins Eye Pearlymussel (*Lampsilis higginsii*) from Extinction: 2002 Status Report on the Accomplishments of the Mussel Coordination Team* was accepted for publication in the Journal of the Iowa Academy of Science.
- Data management is becoming a serious issue as mussel activities continue. To facilitate storage, retrieval and evaluation of data, a new Geographic Information

System (GIS) mussel database for the UMRS is nearing completion by the Corps (Figure 80). Initially, the database will contain 208 mussel surveys and projects from 1984 to the present. Ultimately, it will include historic mussel surveys on the UMR and data from MCT activities.

- A list of MCT publications from 2000 through 2006 is found in Appendix 1.

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