Winged Mapleleaf
(Quadrula fragosa)

5-Year Review:
Summary and Evaluation

U.S. Fish and Wildlife Service
Twin Cities Field Office
Bloomington, Minnesota

May 2015

5-YEAR REVIEW

Species reviewed: winged mapleleaf (*Quadrula fragosa*)

Contents

5-YEAR REVIEW
Winged mapleleaf/*Quadrula fragosa*

1.0 GENERAL INFORMATION

1.1 Reviewers

Lead Regional Office: Jessica Hogrefe, Midwest Region, (612) 713-5346

Lead Field Office: Phil Delphey, Twin Cities Field Office, (612) 725-3548 ext. 2206

Cooperating Field Office(s):

Chris Davidson
Conway, Arkansas Field Office

Andy Roberts
Columbia, Missouri Field Office

David Martinez
Tulsa, Oklahoma Field Office

Cooperating Regional Office(s):

Southeast Region
Atlanta, GA

Southwest Region
Albuquerque, NM

1.2 Methodology used to complete the review:

Public notice of this 5-year review and a 60-day comment period was given in the Federal Register on March 18, 2009 (74 FR 11600-11602). This review was conducted by reviewing all substantial information regarding *Q. fragosa* that has been published, reported, or otherwise made available since the approval of the species' recovery plan in 1997. Phil Delphey, Twin Cities (Minnesota) Ecological Services Field Office, drafted the review, which was subsequently reviewed by biologists in cooperating U.S. Fish and Wildlife Service field offices, regional offices, and by members of the winged mapleleaf recovery team.

1.3 Background:

1.3.1 FR Notice citation announcing initiation of this review:

The Service notified the public of the initiation of the 5-year review in the *Federal Register* on March 18, 2009 (74 FR 11600-11602).

1.3.2 Listing history

Original Listing

FR notice: 56 FR 28345-28349
Date listed: June 20, 1991
Entity listed: species
Classification: endangered

1.3.3 Associated rulemakings:

Nonessential Experimental Population Status

FR notice: 66 FR 32250-32264
Date listed: June 14, 2001
Area listed: Free-Flowing Reach of the Tennessee River below the Wilson Dam, Colbert and Lauderdale Counties, Alabama

1.3.4 Review History: N/A

1.3.5 Species' Recovery Priority Number at start of 5-year review: 2C – indicates that the species faces a high degree of threat, has a high recovery potential, and that there is an actual or imminent conflict between the species and development activities.

1.3.6 Recovery Plan or Outline

Name of plan: Winged Mapleleaf Mussel Recovery Plan (*Quadrula fragosa*)
Date issued: June 1997

2.0 REVIEW ANALYSIS

2.1 Application of the 1996 Distinct Population Segment (DPS) policy

2.1.1 Is the species under review a vertebrate?
_____Yes, go to section 2.1.2.
__X__No, go to section 2.2.

2.2 Recovery Criteria

2.2.1 Does the species have a final, approved recovery plan containing objective, measurable criteria? Yes.

2.2.2 Adequacy of recovery criteria.

2.2.2.1 Do the recovery criteria reflect the best available and most up-to date information on the biology of the species and its habitat? No.

2.2.3 List the recovery criteria as they appear in the recovery plan, and discuss how each criterion has or has not been met, citing information.

The recovery plan's criteria for reclassification of *Q. fragosa* from endangered to threatened are (U.S. Fish and Wildlife Service 1997, p. 19):

(a) The existence of three discrete populations in at least two tributaries of the Mississippi River drainage basin.

(b) Each population must be viable as defined in Task 5A of the recovery plan's narrative outline;

(c) Each population must demonstrate persistence as defined in the narrative outline under Task 5B;

(d) Each population must have long-term habitat protection as defined in the narrative outline under Task 5C.

The **delisting criteria** for *Q. fragosa* are (U.S. Fish and Wildlife Service 1997, p. 17):

(a) The existence of five discrete populations in at least three tributaries of the Mississippi River drainage basin, unless Task 2D4 determines otherwise;

(b) Each population must be viable as defined in Task 5A of the recovery plan's narrative outline;

(c) Each population must demonstrate persistence as defined in the narrative outline under Task 5B;

(d) Each population must have long-term habitat protection as defined in the narrative outline under Task 5C.

Recovery Criterion (a) – Number of Discrete Populations

In the recovery plan, the Service stated that "*Quadrula fragosa* is probably extirpated from its entire historic range except for one remnant population in the St. Croix River between Minnesota and Wisconsin" (U.S. Fish and Wildlife Service 1997, p. 4). Since then, live *Q. fragosa* have been found in the Ouachita River and Saline River, Arkansas; in the Bourbeuse River, Missouri; and, in the Little River, Oklahoma and Arkansas.

Due to the discovery of four additional populations, each of which inhabits rivers within the Mississippi River basin[1], the first recovery criterion has been met. In the recovery plan, this criterion (a) refers to Task 2D4, which states: "Estimate the number of discrete populations needed to maintain the species and the optimal geographic distribution for those populations" (U.S. Fish and Wildlife Service 1997, p. 27). Task 2D4 has not been completed; therefore, the number and distribution of populations that would be necessary to meet this recovery criterion remains as stated in the recovery plan – that is, three populations would be needed to consider the species for reclassification and five for delisting.

Recovery Criterion (b) – Population Viability Analyses

Criterion (b) refers to Task 5A, which states that "A population may be counted toward reclassification or delisting only after the following tasks are performed to demonstrate its viability:

> Task 5Al, Recruitment: Conduct surveys until data demonstrate recruitment to the population in 8 of the 11 age classes aged 2 to 12 years.
>
> Task 5A2, Population size: Conduct surveys until data demonstrate the population likely exceeds the MVP[2] determination made in Task 2D1.
>
> Task 5A3, Age structure: Conduct surveys until data demonstrate the population has an age structure consistent with the MVP determination made in Task 2D1.
>
> Task 5A4, Genetic structure: Conduct surveys until data demonstrate the population has a genetic structure consistent with the MVP determination made in Task 2D1."

Three of the sub-tasks described above refer to Task 2D1, which is to "Conduct a Population Viability Analysis (PVA) to determine the Minimum Viable Population (MVP) for a discrete population of *Q. fragosa*" (U.S. Fish and Wildlife Service 1997, p. 27). We have not completed a PVA; thus, Task 5A has not been completed and recovery criterion (b) has not been met. In the review

[1] St. Croix River is a direct tributary to the Mississippi River; Bourbeuse River flows into the Meramec River, which flows into the Mississippi River. The other three populations – Little River, Ouachita River, and Saline River – are all tributaries of the Red River, whose waters discharge into Atchafalaya River and Mississippi River.

[2] Minimum Viable Population

below, we present the best available information for each population to address its size and viability, age structure, genetic structure, and evidence for recruitment.

Recovery Criterion (c) – Population Persistence

This criterion refers to "the narrative outline under Task 5B":

> Task 5B1, Longevity: The population must have been extant for 24 years following colonization or establishment.
>
> Task 5B2, Population surveys: Three consecutive surveys taken at approximately 5-year intervals must demonstrate population levels to exceed the MVP determination made in Task 2D1.

This criterion has not been met because Task 2D1 has not been completed to determine the minimum size of a viable *Q. fragosa* population. Nevertheless, we summarize in the body of the review below, the best available information regarding each population's longevity and population sizes.

Recovery Criterion (d) – Long-Term Habitat Protection

The narrative outline under the recovery plan's Task 5C defines long-term habitat protection:

> A watershed management plan must be drafted and approved by the Service which demonstrates all potential threats to the population have been identified and either eliminated, mitigated, or otherwise provided for. The factors to be included in this plan should be similar to those outlined in this document for protection of the St. Croix Population in Task 1 and must include:
>
> a) Physical habitat.
>
> b) Chemical habitat.
>
> c) Biological habitat.
>
> d) Protection from commercial harvest.
>
> e) Protection from toxic spills.

According to Task 1 in the recovery plan, these plans must also address adequacy of stream flow, potential threats from "exotic mussels" (e.g., zebra mussels, *Dreissena polymorpha*), prevention of habitat degradation, designation of appropriate areas as critical habitat, prevention of human disturbance and destruction, and depredation by wildlife.

This is the only threat-based recovery criterion in the recovery plan. It would address threats in the following categories that the Service considers when evaluating the status of species under the Endangered Species Act:

- the present or threatened destruction, modification, or curtailment of a species' habitat or range;
- overutilization for commercial, recreational, scientific, or educational purposes;

- disease or predation;
- the inadequacy of existing regulatory mechanisms;
- other natural or manmade factors affecting the species' survival

This criterion has not been met, although some of the aspects mentioned above have been addressed for *Q. fragosa* populations through a variety of mechanisms, which are summarized later in this document.

Winged Mapleleaf Recovery Criteria and Estimation of the Minimum Viable Population Size

The recovery criteria rely on the identification of the minimum viable population size – a threshold population size below which extinction risk is "deemed unacceptably high" (Flather et al. 2011, p. 307). Formal application of the methods to develop MVPs "requires extensive, high-quality data, usually drawn from intensive, long-term studies" (Flather et al. 2011, p. 307). The cost of acquiring the data necessary to develop reasonably precise MVPs for each of the five *Q. fragosa* populations may be too high to be feasible (Hornbach et al. 2010, p. 256). In addition, it could result in unacceptable levels of disturbance to benthic (bottom dwelling) organisms. Moreover, even with high-quality data "extinction probabilities will often be estimated with considerable uncertainty" when developing MVPs, "unless populations are rapidly growing or declining" (Flather et al. 2011, p. 307). The Service will need to develop methods to assess the status of *Q. fragosa* populations that are feasible to implement, result in acceptable levels of disturbance to the species and other benthic organisms, and that provide conservative estimates of population viability.

2.3 Updated Information and Current Species Status

In this section, we discuss information that has become available since the species' listing in 1991.

2.3.1 Biology and Habitat

2.3.1.1 New information on the species' biology and life history:

Breeding Behavior and Characteristics

Our understanding of the breeding behavior of *Q. fragosa* has improved substantially since the species was listed in 1991and even since the recovery plan was approved in 1997. At that time, *Q. fragosa* was assumed to behave in a manner typical of members of the subfamily, Ambleminae – i.e., to brood its young and to infest its host in the summer (U.S. Fish and Wildlife Service 1997, p. 5). To confirm the brooding period, Heath et al. (2000, p. 2) collected and inspected *Q. fragosa* about once every two weeks in 1999 from early April until late October in the St. Croix River. They inspected any *Q. fragosa* that they found by "gently prying apart the two valves" and visually inspecting for inflated gills (Heath et al. 2000, p. 3). They found no evidence of brooding until 31

August and none after 6 October, confirming *Q. fragosa* as a fall short-term (tachytictic) brooder.

The appearance and behavior of brooding *Q. fragosa* changes markedly when they are ready to infest their host. For a few days during its approximately six-week brooding period the posterior mantle around the excurrent aperture of brooding *Q. fragosa* becomes “greatly expanded” with swelling and development of “black-ridged crenulations overlaying the gray mantle” (Heath et al. 2000, p. 5; Figure 1, Hove et al. 2000, p. 3; Fig. 1, Barnhart et al. 2008, p. 376-378; Hove et al. 2012). *Q. fragosa* brood glochidia in this “mantle magazine”, gape widely, and are “reluctant to close the shell when touched” (Barnhart 2009, p. 6). When in this condition, movement of water into and out of the incurrent and excurrent siphon, respectively, appears to cease due to blockage of the excurrent aperture, as an adaptation to avoid flushing glochidia from the magazine, or both (Barnhart 2009, p. 6-7). The prominent gape and emersion of the mussel from the substrate may also be mechanism for maintaining the ventilation of the ctenidia (gills) while siphoning is ceased (Barnhart 2009, p. 7).

The prominent display developed by brooding *Q. fragosa* allows host fish to trigger rapid release of glochidia, although spontaneous release of glochidia and conglutinates may also occur on occasion as an alternative strategy (Barnhart 2009, p. 7; Sietman et al. 2012, p. 44). As is typical with species that use catfish as hosts, *Q. fragosa* emerges from the substrate when brooding glochidia; during the brooding period a greater proportion of *Q. fragosa* are exposed at the surface than outside the brooding period (Hove et al. 2000, p. 3; Sietman et al. 2012, p. 43). There are no obvious differences in appearance of brooding *Q. fragosa* between night and day (Hove et al. 2000, p. 3).

Figure 1. ***Q. fragosa*** **showing typical brooding display (expanded mantle, top) and emerged at substrate surface without expanded mantle (bottom). The posterior mantle is only expanded as shown for a few days during an approximate six-week brooding period whose precise timing varies from year-to-year. (USFWS photos)**

Variations in external environmental factors among years affect the timing of *Q. fragosa*’s late summer/early fall brooding period and may affect the proportion of the population that becomes gravid (Hove et al. 2012, p. 49). In 1998, for example, Heath et al (2000, p. 2-4) found indications of gravidity in *Q. fragosa* as early as 10 September whereas in 1999 they did not find any gravid specimens until 21 September and none thereafter. In 2001, *Q. fragosa* “apparently failed to reproduce” in the St. Croix River and in 2002 only about three percent of the *Q. fragosa* he inspected were gravid, compared to 10-20 percent in other years (Hove 2003, p. 1-6). In 1997 only 3.7% of the *Q. fragosa* examined in the St. Croix River were gravid compared to 21.3% in 1998 and 1.8% in 1999 (Heath et al. 2000, p. 7). The authors suggested that warmer water temperatures in 1998 may have contributed to the higher proportion of brooding by increasing plankton (food) productivity.

Q. fragosa take several years to reach maturity after which growth may slow. The smallest *Q. fragosa* that Heath et al. (2000, p. 8) observed brooding was 65 mm in total length and the youngest was about eight years old, based on counts of external annuli. Due to the small relative sample size and the difficulty in determining gravidity in small individuals the authors concluded that the earliest reproduction may occur before age 8 – as early as age 4 to 6 (Heath et al. 2000, p. 8). Heath et al. (2000, p. 8) also suggested that the observed decline in relative grow rates may be associated with the onset of reproduction.

Some work with *Q. fragosa* has yielded information that is particularly useful to help guide propagation efforts. Barnhart (2009, p. 6) found that "females that were placed directly into reconstituted hard fresh water or a 50/50 mix of reconstituted water and native water released viable glochidia within a few hours and generally voided the entire contents of the demibranchs" whereas females "held in native water were less likely to void the demibranchs and several females held glochidia for up to two weeks." Brooding females develop a swollen mantle magazine that is used to hold glochidia, "fragmentary" and whole conglutinates, gape widely, and are "reluctant to close the shell when touched" (Barnhart 2009, p. 6; Hove et al. 2012, p. 50). In contrast, non-brooding females – including females that had emptied the contents of their magazine – "showed no more than slight extension of the mantle, did not gape widely, and closed immediately when touched" (Barnhart 2009, p. 6).

Host Fish and Juvenile Development

Channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) are the only known suitable reproductive hosts for *Q. fragosa*. Trials to narrow the list of potential hosts initially included 67 fish species in 20 families and allowed researchers to narrow the focus of host studies to the two catfish species (Hove et al. 2012, p. 50). Hove (2002) and Hove et al. (2000) infested 39 fish species and the salamander, mudpuppy (*Necturus maculosus*), with *Q. fragosa* glochidia and obtained juvenile *Q. fragosa* only from channel catfish. Later trials yielded 743 and 2995 transformed juveniles on channel catfish and blue catfish, respectively (Hove 2004, p. 8). Steingraeber et al. (2005, p. 4-5) carried out additional studies, using glochidia that had been collected from brooding females in the St. Croix River, and recovered more than 24,000 juveniles from the two catfish species. Barnhart (2009, p. 8-9) conducted additional trials with five fish species, using glochidia from Saline River in Arkansas, and also found successful metamorphosis only from blue catfish and channel catfish.

Channel catfish occurs in all five rivers inhabited by *Q. fragosa*, whereas blue catfish are present only in the Little, Ouachita, and Saline rivers (Hove et al. 2012, p. 53). Channel catfish "is currently common and widely-distributed in the

Mississippi River basin", suggesting that host abundance may not be a threat to *Q. fragosa* despite its "very specific host requirements" (Hove et al. 2012, p. 53). In some trials blue catfish have produced greater numbers of metamorphosed *Q. fragosa* than channel catfish, but fish size and other factors would have to be controlled to adequately compare the relative suitability of the two host species (Steingraeber et al. 2005, p. 5; Barnhart 2009, p. 9).

The development of *Q. fragosa* that are attached to their host fish may be lengthy and is temperature dependent. Using the results from the infestation trials, Steingraeber et al. (2005, p. 8) determined that glochidia that are attached to host fish cease development at water temperatures of about 9.24°C. The authors also developed a quantitative model to predict the number of days after infestation when drop-off of juvenile *Q. fragosa* would reach peak levels. In two tests of the model, it predicted that the peak of drop-off would occur four days before and one day after the actual observed date, respectively (Steingraeber et al. 2007, p. 308) – total encapsulation periods during the two tests were 262 and 260 days, respectively. Peak detachment from hosts occurred after an accumulation of at least 395 °C x days (cumulative temperature units) and after water temperatures rose to 17 – 20°C (Steingraeber et al. 2007, p. 308).

Although Hove et al. (2012) indicated that "*Q. fragosa* infesting a host early in the brooding period could metamorphose during a warm fall," juveniles in the St. Croix River are likely to remain on host fish throughout winter and detach in the spring of the following year. *Q. fragosa* encapsulated on their fish hosts grow considerably before metamorphosis is complete – increasing about 2 to 4 times in length (Barnhart 2009, p. 10; Hove et al. 2012, p. 51) – but temperatures in the St. Croix River are typically below the minimum for glochidia development (9.24°C) for five to six consecutive months. Nevertheless, encapsulated glochidia may continue to develop for about five weeks in the spring and are likely to be significantly larger than those that detach in the fall (Steingraeber et al. 2007, p. 308; Barnhart 2009, p. 10).

Water temperatures should be carefully controlled to ensure normal development and survival of glochidia because water temperatures that do not closely mimic those in the natural environment of *Q. fragosa* may affect normal shell development. Shells of juvenile *Q. fragosa* that transformed from fish held at water temperatures higher than in the St. Croix River typically appeared "lopsided" and were smooth, lacking the surface features that typify *Q. fragosa* (Steingraeber et al. 2005, p. 10). *Q. fragosa* that transformed from fish held in water that was closer or identical to water temperatures in the St. Croix River did not exhibit anomalous valve development. None of the juveniles survived longer than four weeks due to factors unrelated to shell morphology, so it is unknown whether the anomalous shell development would have resolved itself under water temperature regime that approximated that of the St. Croix River (Steingraeber et al. 2007, p. 307).

2.3.1.2 Abundance, population trends (e.g. increasing, decreasing, stable), demographic features (e.g., age structure, sex ratio, family size, birth rate, age at mortality, mortality rate, etc.), or demographic trends:

The discovery of four additional populations has expanded the known range of *Q. fragosa* since the species was listed in 1991 and has greatly changed the context for recovery planning (Fig. 2, U.S. Fish and Wildlife Service 1991). The discovery of live *Q. fragosa* in Arkansas' Ouachita River in 1996 preceded approval of the recovery plan, but there was some initial uncertainty regarding the identity of the specimens found there and the Service based its plan on the assumption that there was still only a single extant population (Posey et al. 1996, p. 97; U.S. Fish and Wildlife Service 1997). After the discovery in the Ouachita River, biologists have also confirmed extant populations in Arkansas in the Saline River (Davidson and Clem 2002;2004), in Missouri in the Bourbeuse River (A. Roberts, U.S. Fish and Wildlife Service, pers. comm. 17 September 2008; S. McMurray, Missouri Department of Conservation, pers. comm. 19 September 2008), and in Oklahoma in the Little River (Allen and Vaughn 2008, p. 3).

Figure 2. Locations of extant populations of winged mapleleaf (*Quadrula fragosa*).

St. Croix River – Minnesota and Wisconsin

Except for the recent discovery of about 400 *Q. fragosa* that were inadvertently released from a propagation cage near Hudson, Wisconsin, the species' status and distribution in the St. Croix River appears to have changed little since listing. Nearly all of the *Q. fragosa* population occurs in a 9-km (6-mile) reach that comprises only about 9% of the species' original distribution in the river (Fig. 3, Wisconsin Department Natural Resources 2002, p. 2; Hornbach and Hove 2008,

p. 13). From 1988 through 2014, Wisconsin Department of Natural Resources and others have periodically sampled the area of highest *Q. fragosa* density in the St. Croix River – the 'Interstate' area (Table 1, Doolittle and Heath 1997, p. 5; Heath et al. 2001; Wisconsin Department of Natural Resources et al. 2004, p. 1; Mussel Coordination Team et al. 2010). Except for 2004 when none of the 164 quadrats contained *Q. fragosa*, estimated density has remained in the range of 0.02-0.07/m^2 (Table 1). In 1988 – before listing – Wisconsin DNR had found a density of 0.02/m^2 (U.S. Fish and Wildlife Service 1991, p. 28346).

Detecting trends in the abundance of *Q. fragosa* in the St. Croix River with a high degree of precision may not be feasible using standard approaches. Hornbach and Hove (2008, p. 6) concluded that when using the standard method of simple random sampling, over 16,000 samples would have to be taken "to have an 80% chance of detecting a 30% change in the population with 80% confidence." This level of effort would require funding beyond what is currently available and would result in a level of disturbance that may have unacceptable effects on mussels, including *Q. fragosa* (e.g., Heath et al. 2000, p. 7).

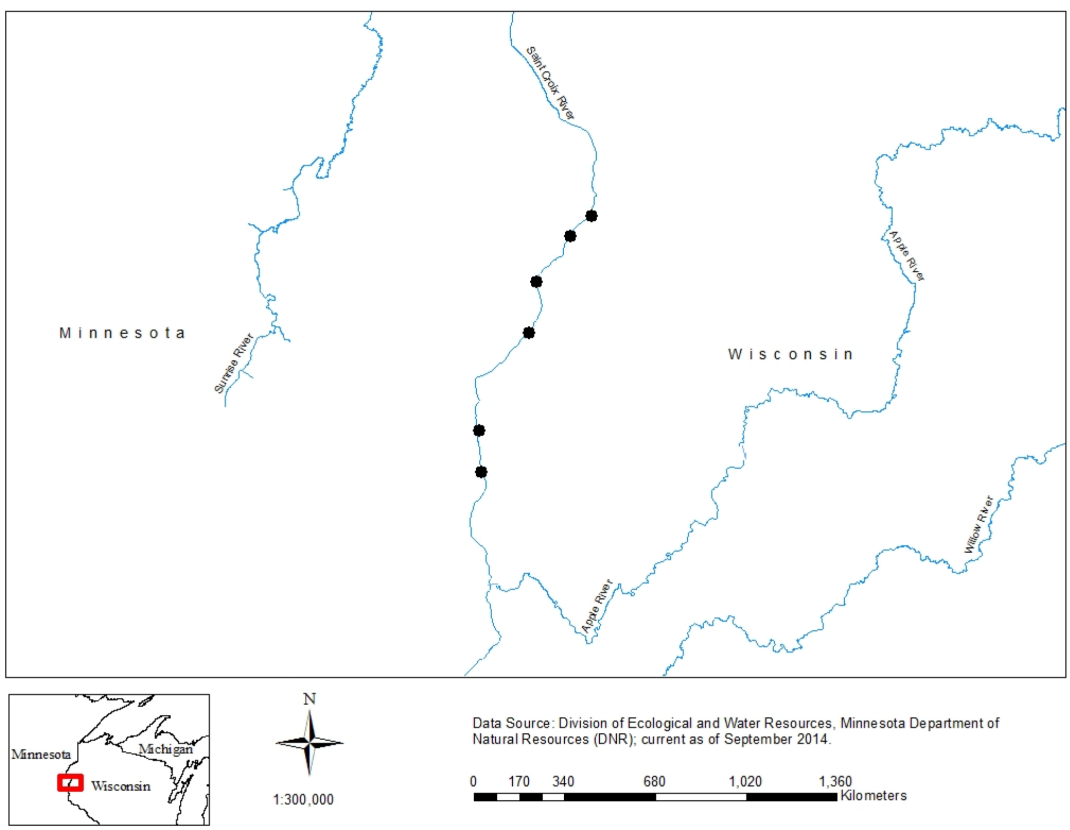

Figure 3. Approximate current distribution of *Q. fragosa* in St. Croix River, Minnesota/Wisconsin.

Table 1. Summary of sampling conducted since 1996 at Interstate study area on St. Croix River, Minnesota/Wisconsin. Each year, all mussels were counted within one square meter quadrats randomly placed within the study area. Differences in mean density are not statistically significant.

Year of Sampling	No. Quadrats	No. Live *Q. fragosa* found	Estimated Density of *Q. fragosa* (no./m^2)	Source
1996	140	4	0.03	(Doolittle and Heath 1997)
2000	150	3	0.02	(Heath et al. 2001)
2004	164	0	0	(Wisconsin Department of Natural Resources et al. 2004)
2009	163	8	0.05	(Mussel Coordination Team 2010)
2014	148	10	0.07	D. Kelner, U.S. Army Corps of Engineers, *in litt.*

Hornbach and Hove (2008, p. 13) used an alternative technique – adaptive cluster sampling – to estimate the population size in the St. Croix River at about 13,000 "between Interstate State Park and Franconia, Minnesota", maintaining that their estimate was more accurate than previous attempts. Although approximately three times more efficient than simple random sampling for monitoring *Q. fragosa* (Hornbach et al. 2010, p. 257), adaptive cluster sampling may not be the most efficient method for estimating the abundance of *Q. fragosa* in the St. Croix River. Hornbach and Hove (2008, p. 14) recommended that "future population estimates be made with stratified random sampling, and that the number of randomly selected quadrats collected at high density sites be increased."

Despite the limited ability to draw inferences regarding population trends of *Q. fragosa*, total mussel density at the 'Interstate' site was significantly lower in 2009 than it was in 1988 (Mussel Coordination Team 2010, p. 3). This was largely due to the decline in density of fawnsfoot (*Truncilla donaciformis*), which was the only species with a statistically significant declining trend between 1988 and 2009 (Mussel Coordination Team 2010, p. 19). No trend analyses have incorporated the 2014 data yet, but estimated density of all live mussels at the Interstate mussel bed in 2014 was 12/m^2 compared to 11/m^2 in 2009 (D. Kelner, U.S. Army Corps of Engineers, *in litt.* 2014).

The *Q. fragosa* population appears to be recruiting individuals through reproduction in the St. Croix River. As part of assessing viability of *Q. fragosa* populations, recovery task 5A1 instructs us to, "Conduct surveys until data demonstrate recruitment to the population in 8 of the 11 age classes aged 2 to 12 years" (U.S. Fish and Wildlife Service 1997). Counts of external annuli, although only able to provide approximate ages for mussels (Anthony et al. 2001, p. 1349-1350), indicate steady recruitment for *Q. fragosa* in the St. Croix River (Fig. 3). Moreover, the length-frequency distribution does not indicate any significant gaps in any size classes of *Q. fragosa* in the St. Croix River (Fig. 4). *Quadrula fragosa* younger than 4 years old can be difficult to identify and may be under sampled by the opportunistic methods employed (U.S. Fish and Wildlife Service 1997, p. 12).

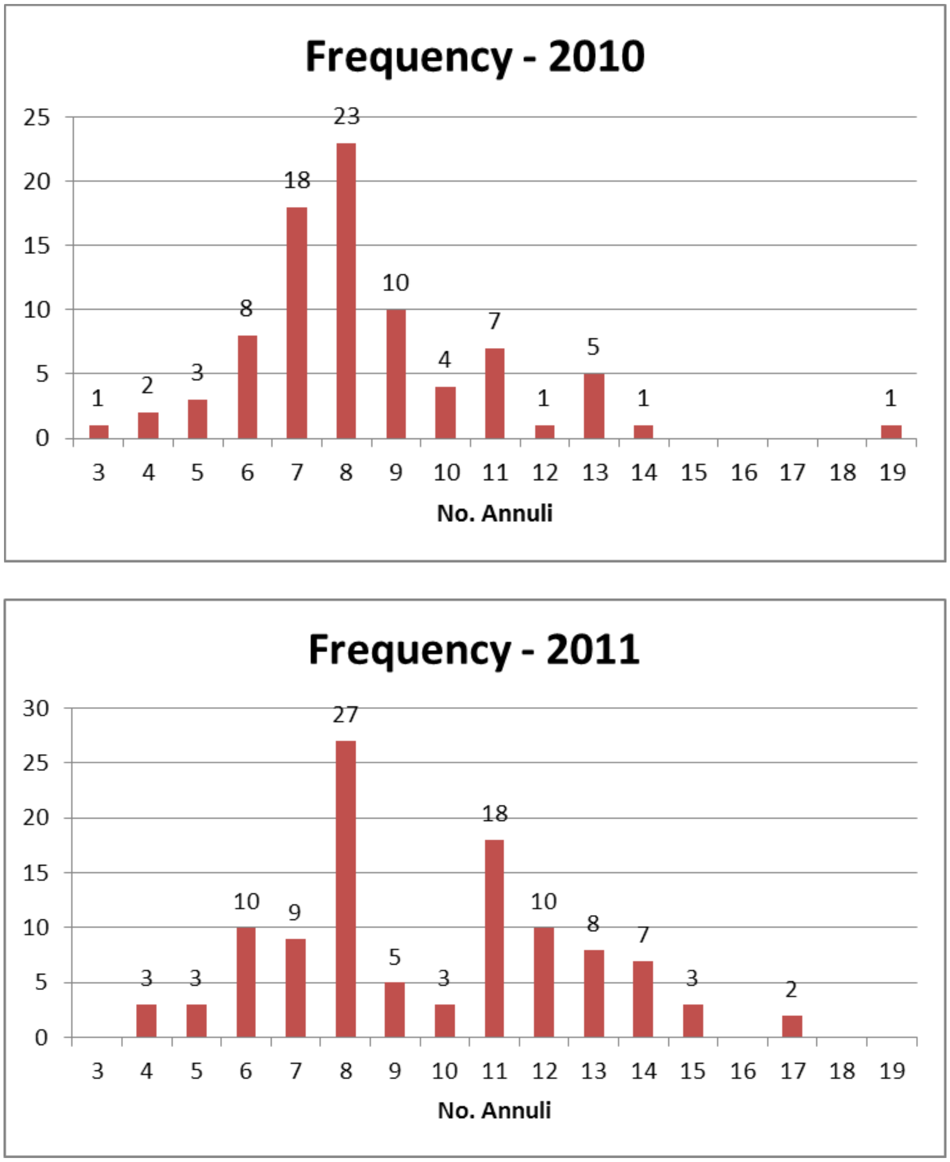

Figure 4. Frequency distribution of approximate ages (number annuli) of live *Q. fragosa* captured during targeted searches in July 2010 and 2011 in the St. Croix River.

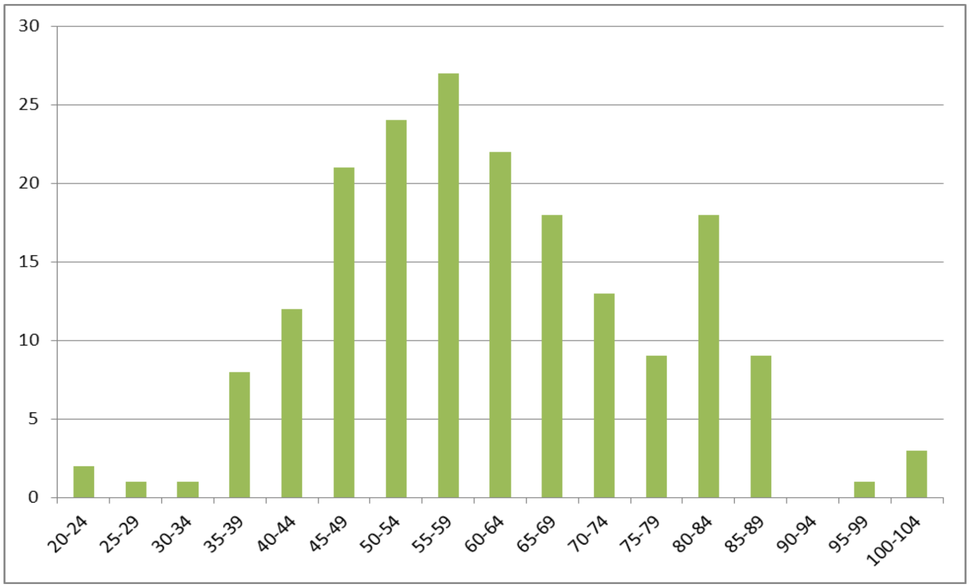

Figure 5. Length frequency distribution of *Q. fragosa* (n=189) found during targeted searches for *Q. fragosa* in the St. Croix River in 2010 and 2011. Only the 2011 length measurement was included for the three *Q. fragosa* that were collected in both years.

Although the primary population of *Q. fragosa* in the St. Croix River is in the Interstate area described above, about 400 individuals also occur in the river near Hudson, Wisconsin as a result of propagation efforts. These mussels were first discovered during sampling of the Hudson Higgins Eye Essential Habitat Area (U.S. Fish and Wildlife Service 2004, p. 89) in July 2014. An interagency team of biologists concluded that these mussels likely spilled from a propagation cage in the fall of 2005 when they were very approximately 2-3 mm in width (G. Wege, *in litt.* 2014). The mussels descended from one or two female *Q. fragosa* that were collected from the Interstate population in the fall of 2004 and taken to Genoa National Fish Hatchery where they were used to infest 100 channel catfish.

Bourbeuse River – Missouri

Since 2001, at least five live unique *Q. fragosa* have been recorded at two sites on the "middle reach" of the Bourbeuse River (Fig. 6; A. Roberts, U.S. Fish and Wildlife Service, pers. comm. 17 September 2008). The first live specimen was found in 1997 by Andy Roberts and Mike Davis discovered the second site – about five miles upstream – in 2005 (S. McMurray, Missouri Department of Conservation, pers. comm. 19 September 2008). During return visits to the upstream site, biologists relocated the same individual several times and found an additional three *Q. fragosa* in 2009 (S. McMurray, pers. comm. 19 September 2008; A. Roberts, U.S. Fish and Wildlife Service, Columbia, MO, pers. comm. 17 August 2009).

Currently available data on *Q. fragosa* in the Bourbeuse River do not allow us to make any assessments of the species' viability in the river. Extensive and intensive survey efforts would be needed to estimate population density, distribution, recruitment, and age structure.

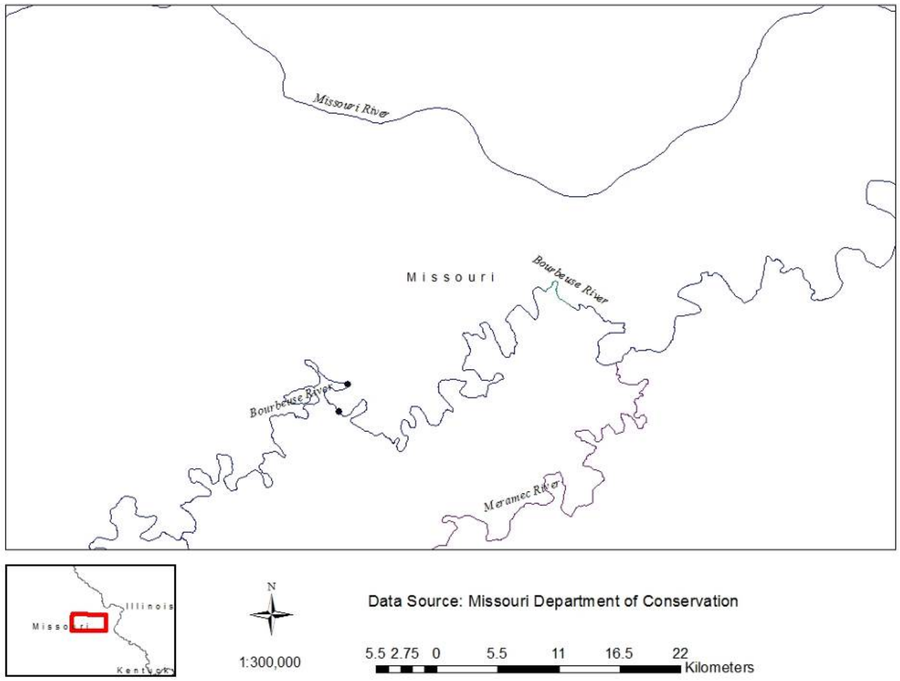

Figure 6. Locations of recent records of live *Quadrula fragosa* in Bourbeuse River, Missouri.

Arkansas

Although the two rivers inhabited by *Q. fragosa* in Arkansas are connected – Saline River is a tributary to the Ouachita River – the two populations are discrete per the definition in the recovery plan – "sufficiently geographically isolated from each other so both are unlikely to be affected by a single stochastic event, such as a toxic spill or a disease outbreak" (Fig. 7; U.S. Fish and Wildlife Service 1997, p. 19). In each river *Q. fragosa* typically occurs downstream of long and deep pools that provide habitat for their reproductive hosts, blue catfish and channel catfish (Harris 2006, p. 10, C. Davidson, U.S. Fish and Wildlife Service, Conway, AR, pers. comm. 2012). Biologists have sampled numerous beds inhabited by *Q. fragosa* in each river, but additional surveys are needed to estimate the species' total abundance and distribution (Harris 2006).

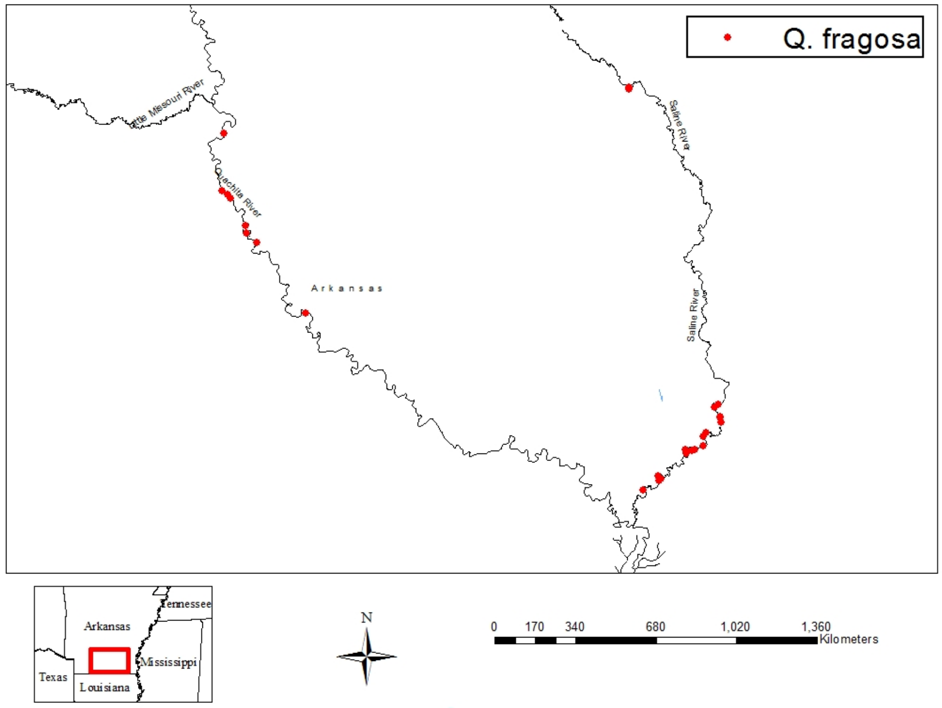

Figure 7. Recent records of live *Quadrula fragosa* in the Ouachita River and Saline River, Arkansas.

Ouachita River

In the Ouachita River, *Q. fragosa* has been found in a few mussel beds distributed along an approximately 72-km (45 river-mile) reach (Harris 2006, p. 12). The population also extends into the lower 0.4 km of Little Missouri River (Davidson 1997, p. 47). In four beds sampled near Camden, Arkansas, population estimates for *Q. fragosa* ranged from 217 to 1770 (Harris 2006, p. 10). The species was absent from the furthest upstream sites in a high gradient (steeply sloped) reach with fast flows (Harris 2006, p. 10). Length frequency data for *Q. fragosa* from the Ouachita River (Harris 2006) are not as broadly distributed as found in the St. Croix River (Fig. 4), but are available for only 20 individuals (Fig. 8).

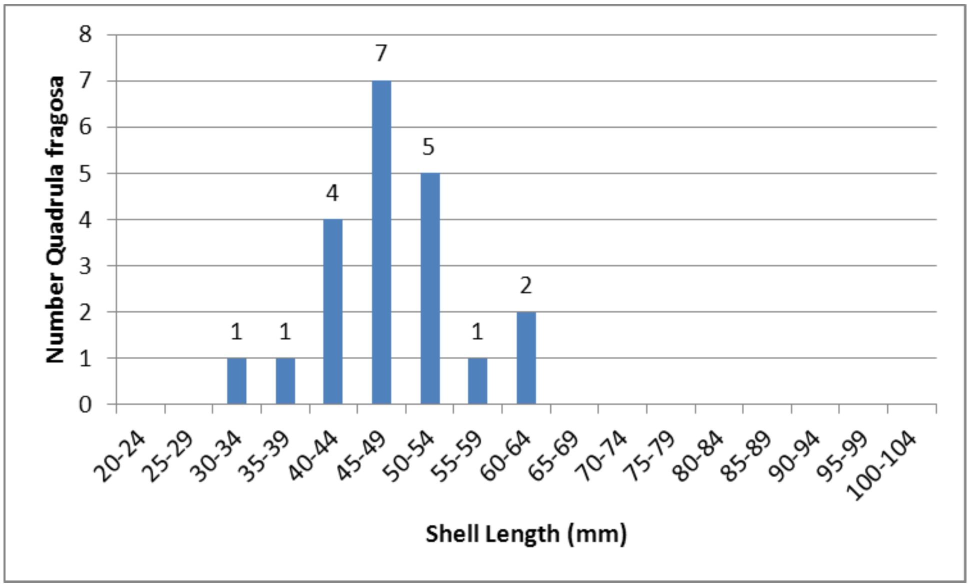

Figure 8. Length frequency distribution of *Q. fragosa* (n=20) from Ouachita River (Harris 2006).

Saline River - Arkansas

Q. fragosa was first found in the Saline River in 2001 when Davidson and Clem (2002, p. 12) recorded a single specimen. In 2003 and 2004 they found eight additional live specimens at five additional sites (Davidson and Clem 2004, p. 13).

Q. fragosa is patchily distributed and not common in the Saline River, but the species' total abundance may be substantial. During an extensive five-year sampling effort workers confirmed *Q. fragosa* at only six of the 169 mussel beds or "concentrations" surveyed – the species comprised only nine of the 21,316 live mussels recorded (Davidson and Clem 2002, p. 41-48; 2004, p, 7-13; Harris 2006). *Q. fragosa* typically occurs downstream of long and deep pools that provide good habitat for blue catfish and channel catfish in the Saline River, but the species is absent from some of the river's largest and most diverse mussel beds (C. Davidson, U.S. Fish and Wildlife Service, Conway, AR, pers. comms. 2009 and 2012). Despite its rarity relative, however, population estimates for the fourteen beds in which it has been recorded range from 125 to 11,281 (Table 2) and some beds remain unsampled.

Table 2. Summary of quantitative sampling data for Saline River, Arkansas, 2005 – 2012. Saline River Basin (SRB-) mussel bed numbers or Site Names are taken directly from reports (Harris 2006 and C. Davidson, U.S. Fish and Wildlife Service, Conway, AR, pers. comms. 2012 and 2013; Davidson 2015).

Bed (SRB-) or Site Name	Sampling Year	*Q. fragosa* Population Estimate +/- 95% Confidence Interval	Bed Area (m^2)
6M	2014	No estimate available	2,700
8M	2014	100 +/- 176	1,900
9M	2014	No estimate available	600
10M	2014	1188 +/- 1153	9,900
70	2008	202 +/- 360	2,625
70	2011	396 +/- 324	2,625
71	2008	356 +/- 341	2,895
71	2011	198 +/- 198	2,475
141	2005	9217 +/- 4114	9,275
141	2010	1615 +/- 6933	1,400
143	2012	737 +/- 714	1,290
145	2005	510 +/- 253	850
145	2011	850 +/- 731	850
145	2011	11,281 +/- 59,153	16,700
146	2005	540 +/-415	1,020
146	2011	491 +/- 244	540
147	2011	811 +/- 858[3]	2,850
152	2005	1214 +/- 742	2,760
152	2011	2981 +/- 1653	8,280
153	2012	125 +/- 219	3,120
155	2012	1280 +/- 1586	8,000
157	2012	1512 +/- 1026	5,400
158	2012	480 +/- 583	6,000
159A	2011	2235 +/- 1136	4,860
159B	2011	4199 +/- 2893	17,650

Some beds originally sampled in 2005 were resurveyed in 2011 after severe flooding occurred in both 2008 and 2009. Population estimates at the two furthest upstream sites near Mt. Elba [Saline River Beds (SRB) 70 and 71] were not obviously different after the floods, but the species' mean density had declined by about 82 percent at SRB 141 (Table, 2; C. Davidson, 2012 pers. comm.). Before the floods, SRB 141 may have contained more *Q. fragosa* than any other bed range wide. Among the other sites resampled, *Q. fragosa* numbers were similar at one site, increased by about 125 percent at another, and increased sharply at SRB 152, due to the discovery of a section of the bed not detected during sampling in 2005 (Table 2).

Surveys conducted in the Saline River continue to reveal now locations for the species, suggesting that the species' complete distribution in the river is not yet settled. In 2011, *Q. fragosa* was found at two additional Saline River sites – SRB 159A and 159B. The discovery of these beds extended the length of river known

[3] SRB 147 redefined from Davidson and Clem (2004).

to be inhabited by *Q. fragosa* by about 8 river miles (Table 2). *Q. fragosa* was found in five additional beds in 2012 (Table 2) and in 2014 a survey that repeated the Davidson (1997) survey extended the species known distribution by an additional 11 river miles – in 1997 no *Q. fragosa* had been encountered in the studied reach, but in 2014 the species was found at several localities (C. Davidson, pers. comm. 2014).

External annuli counts of live *Q. fragosa* are not available for Saline River, but the age structure of the species may be approximated by the length frequency distribution of 81 and 23 *Q. fragosa* found in quadrat samples in 2011 and 2012, respectively (Fig. 9).

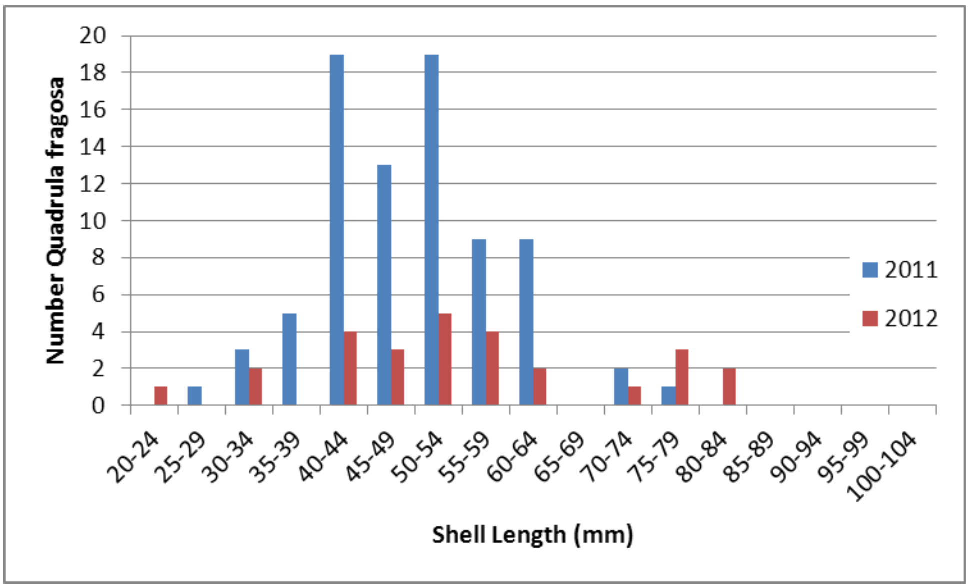

Figure 9. Length frequency data for *Q. fragosa* found in quadrats in Saline River, Arkansas, in 2011 and 2012 (n=81 and 23, respectively). (C. Davidson, USFWS, Conway, AR, unpubl. data).

Little River – Arkansas and Oklahoma

Since 2005, *Q. fragosa* has been found in twelve sites in the Little River, Oklahoma (Fig. 10) and in 2013 it was found for the first time in the Arkansas section of the river (Davidson et al. 2014, p. 26). Galbraith et al. (2008, p. 16) found *Q. fragosa* at four sites in 2005; Allen and Vaughn (Fig. 6, Allen and Vaughn 2008, p. 17) found it at seven additional sites in 2006; and, Davidson (pers. comm. 2009) found it at a single site in 2009. Davidson's 2009 site was 0.5 mile upstream of the mouth of the Mountain Fork River, but *Q. fragosa* likely does not occur in Little River for some distance downstream of this confluence due to cold water releases from the dam that forms Broken Bow Lake (Fig. 7, Allen and Vaughn 2008, p. 16). The effects of cold water from Mountain Fork River on native mussels in the Little River are not apparent after the river passes into Arkansas (C. Davidson, pers. comm. 2013); therefore, the effects to mussel communities may dissipate at some point before the river reaches the Arkansas border (Vaughn and Taylor 1999, p. 915).

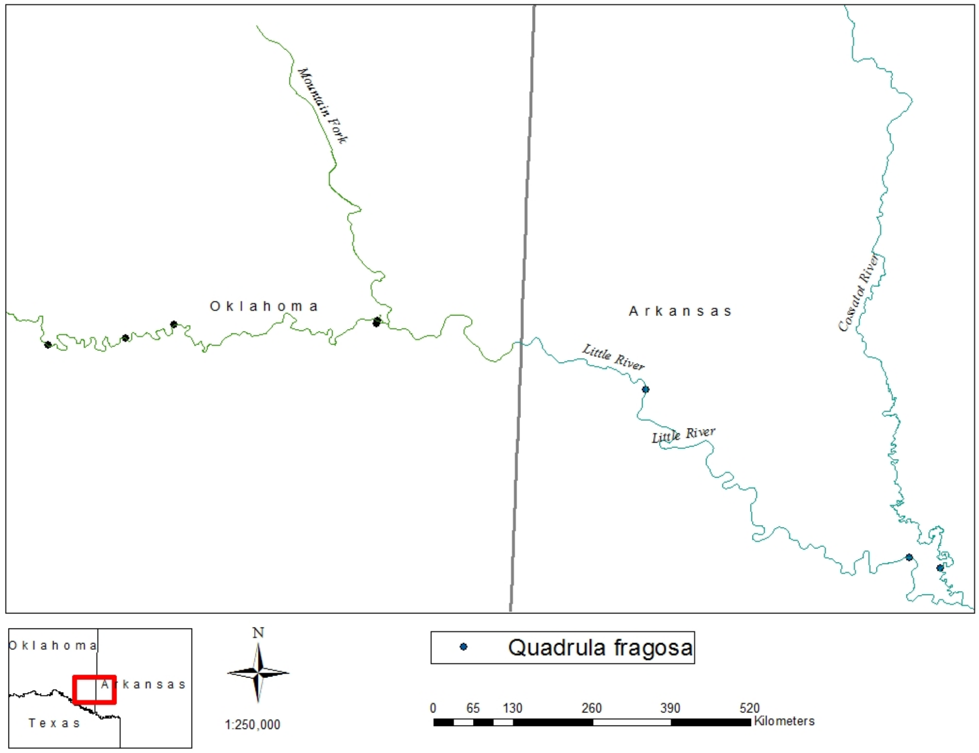

Figure 10. Locations of recent records of live *Q. fragosa* from the Little River in Oklahoma and Arkansas and of one dead specimen found in 2013 in the Cossatot River in Arkansas (Allen and Vaughn 2008; Galbraith et al. 2008, Oklahoma Biological Survey, unpubl. data, C. Davidson, U.S. Fish and Wildlife Service, unpubl. data; J. Harris, pers. comm. 2013).

Allen and Vaughn (2008, p. 36-40) quantitatively sampled six mussel beds in the Little River in 2006. At each bed they placed six transects across the river and counted all mussels present within four quadrats spaced at regular intervals along the transect – a total of 24 quadrats per bed. They detected *Q. fragosa* in four of the six beds and at three additional sites where only timed searches were conducted (Allen and Vaughn 2008, p. 36-40). At the four sites where *Q. fragosa* was detected in quadrats its estimated density ranged from 0.2 (three sites) to 0.5 (one site) (D. Allen and C. Vaughn, unpubl. data, University of Oklahoma 2012). Lengths are available for 13 *Q. fragosa* found during the 2006 surveys (Fig. 11; D. Allen and C. Vaughn, unpubl. data, University of Oklahoma 2012).

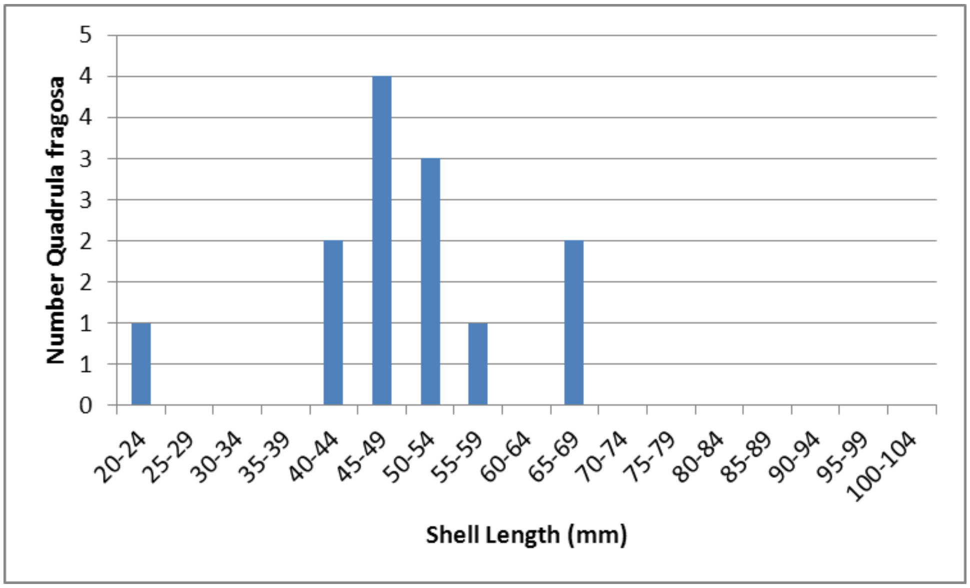

Figure 11. Length frequency distribution for *Q. fragosa* (n=13) detected during quantitative and qualitative sampling on Little River, Oklahoma, 2006 (D. Allen and C. Vaughn, unpubl. data, University of Oklahoma 2012).

In 2011, Vaughn and Atkinson (2011) documented extensive mussel mortality on the Little River during a severe drought. At one site they "recorded 19 species of freshly dead mussels in habitat that would normally be submerged", although no dead *Q. fragosa* were recorded (Vaughn and Atkinson 2011). In late July 2012, drought conditions again prevailed and flows in the Little River reach inhabited by *Q. fragosa* were at about 15% of median levels. The Oklahoma Climatological Survey (U.S. Fish and Wildlife Service 1997, p. 3) expects the state to experience an increasing frequency and severity and droughts, which could pose a significant threat to *Q. fragosa* in Little River.

Cossatot River – Arkansas

Based on the condition of a specimen collected in 2013 it appears likely that *Q. fragosa* is also extant, albeit rare, in the Cossatot River in Arkansas. One fresh dead *Q. fragosa* was among 5463 live mussels collected during the recent and comprehensive survey of the Cossatot River (Fig. 12), which is a tributary of the Little River (Fig. 10).

Figure 12. Photos of *Q. fragosa* specimen found in Cossatot River, Arkansas (Fig. 10) in 2013 (J. Harris, pers. comm. 2013).

2.3.1.3 Genetics, genetic variation, or trends in genetic variation (e.g., loss of genetic variation, genetic drift, inbreeding, etc.):

Genetic Distinctiveness of Q. fragosa

In the 1991 listing rule the Service referred to "a disagreement" about whether *Q. fragosa*, is a distinct species or a subspecies of *Quadrula quadrula* (U.S. Fish and Wildlife Service 1991, p. 28346). Since then, significant new information has been developed to establish *Q. fragosa* as a distinct taxon. When the species was listed in 1991, there were no genetic data available to support the contention that *Q. fragosa* and *Q. quadrula* were separate species (Hornbach et al. 1998, p. 6). Research conducted by Hornbach et al. (2003, p. 8), Serb and Harris (2008, p. 23), and Hemmingsen (2003, p. 8) confirmed that *Q. fragosa* is distinct from *Q. quadrula*. In addition, Serb and Harris (2008, p. 24), Hemmingsen et al. (2009), and Hemmingsen (2008, p. 58) developed a microsatellite library that allowed them to confirm that *Q. fragosa* in the northern part of the species' range (St. Croix River, Minnesota and Wisconsin) and in the southern part of its range (Arkansas, Missouri, and Oklahoma) descended from a common ancestor and constitute a coherent group that is distinct from other *Quadrula*. Genetic differences have developed, however, among the five remaining populations that are roughly proportional to their geographic separation (2010, p. 4).

Genetic Diversity of St. Croix River Population

A recent study conducted in the St. Croix River suggests that *Q. fragosa* there may be threatened by reduced genetic diversity that may have resulted from inbreeding. Roe (2010, p. 3-4) analyzed the genetic diversity of *Q. fragosa* in the St. Croix River based on 52 unique samples. He found "significant excess homozygosity" at three of the 20 loci he examined and estimated the effective population size at 149 (Frankham et al. 2009, p. 261).

Inbreeding – "the production of offspring from mating of individuals related by ancestry"(Frankham et al. 2009, p. 262-265) – is one cause of excessive homozygosity. In outbreeding species, inbreeding "results in the decline of reproductive fitness" and "exposes deleterious recessive alleles" (2010, p. 5). Therefore, Roe (2010, p. 5) recommended monitoring the St. Croix River population for signs of low recruitment that may indicate a reduction in reproductive fitness. Roe pointed out that decreased heterozygosity could also be caused by self-fertilization if *Q. fragosa* is among the mussel species that exhibit this trait and predominantly self-fertilize. Self-fertilizing species may be less sensitive to inbreeding than outbreeding species, but they may require "a greater emphasis on conservation of multiple populations" and the maintenance of larger population sizes" to guard against the threat of "mutational accumulation" (Roe 2010, p. 5).

Measures of an individual species' genetic diversity may be of greater use when compared to closely related sympatric species. Roe (Fig. 4, U.S. Fish and Wildlife Service 1997, p. 45-46) suggested comparing *Q. fragosa* to "a related sympatric species (*Q. pustulosa*), which is not endangered, to "reveal whether *Q. fragosa* is exhibiting low or normal genetic diversity." Comparison of the St. Croix River population to other *Q. fragosa* populations may also be useful.

2.3.1.4 Taxonomic classification or changes in nomenclature:

No new information has arisen since completion of the recovery plan that would suggest any changes in taxonomy or nomenclature, but see the genetics section immediately above.

2.3.1.5 Spatial distribution, trends in spatial distribution (e.g. increasingly fragmented, increased numbers of corridors, etc.), or historic range (e.g. corrections to the historical range, change in distribution of the species' within its historic range, etc.):

Extant populations – including those discovered after approval of the species' recovery plan in 1997 – are described in section 2.3.1.2.

New Information on Historical Distribution of Q. fragosa

Based on review of published accounts and review of museum records, the historical distribution of *Q. fragosa* was almost completely described in the recovery plan (U.S. Fish and Wildlife Service 1997, p. 10). Since then, however, historical occurrences have been documented in the Big Sioux River and James River in South Dakota (Skadsen 2000, p. 12; Perkins and Backlund 2003, p. 8) and in the Marais des Cygnes River in Kansas (Fig. 13; D. Stansberry, *in litt.* 22

August 2002). In 2005 and 2006 biologists resurveyed the One Hundred and Two River in northwest Missouri and several rivers in northeast Missouri tributary to the Missouri River, where *Q. fragosa* was recorded by Utterback in the early 1900s, but found no *Q. fragosa* (S. McMurray, pers. comm. 19 September 2008).

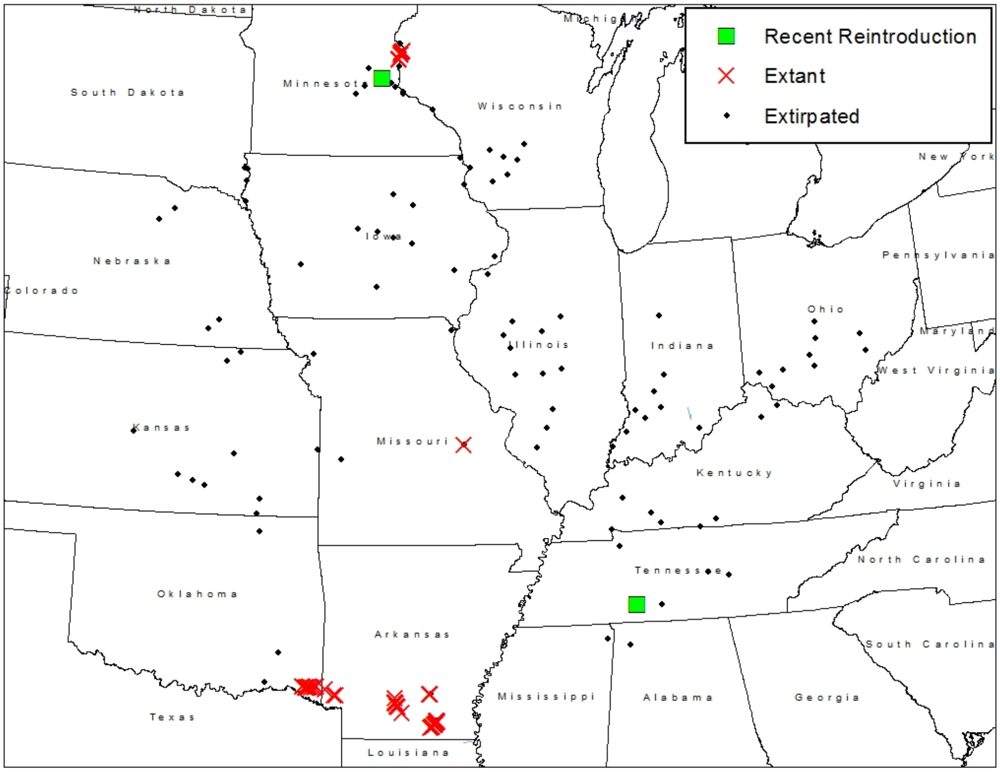

Figure 13. Approximate locations of historical (extirpated) and current (extant) records of *Q. fragosa*. Current locations include St. Croix River, Minnesota/Wisconsin; Bourbeuse River, Missouri; Ouachita River, Saline River, Little River, and Cossatot River, Arkansas; and, Little River, Oklahoma. Also shown are two sites where the species has been reintroduced - Mississippi River, Minnesota and Duck River, Tennessee – in 2012 and 2014, respectively.

2.3.1.6 Habitat or ecosystem conditions (e.g., amount, distribution, and suitability of the habitat or ecosystem):

Q. fragosa habitat varies in some basic physical characteristics, such as substrate size, but the species seems to consistently inhabit relatively dense and diverse mussel beds. This was recognized in the recovery plan based on work conducted in the St. Croix River by Dan Hornbach, was later found to be true in the Little River (Allen and Vaughn 2008, p. 5), and was reconfirmed in the St Croix River (Hornbach and Hove 2008, p. 1).

Hornbach and Hove (2008, p. 14-15) and Hornbach et al. (2010) only found *Q. fragosa* in areas with high mussel density in the St. Croix River where hydraulic measurements were indicative of low sediment deposition rates. *Q. fragosa* habitats also had coarser and more compacted sediments then areas where mussels densities were low (Hornbach et al. 2010, p. 258). They did not find *Q. fragosa* in all areas where mussel densities were high and there were no apparent differences in habitat characteristics between high density mussel areas with and without *Q. fragosa* (2008, p. 5).

It is unclear whether high mussel densities facilitate *Q. fragosa* population growth or if other factors are responsible. Allen and Vaughn (2008, p. 15) suggested that a high density of common mussels increases substrate stability and reduces the likelihood that mussels of rare species are displaced by high flows – "mussels themselves, by the presence of their shells and through their burrowing activities, stabilize streambed sediments, decrease shear stress, and thus create more appropriate microhabitat for other mussel species."

2.3.1.7 Other:

Propagation of Q. fragosa

Propagation of *Q. fragosa* to reestablish populations in historically occupied habitats or to augment existing populations seemed an obvious high priority when we were aware of only one extant population in the 1990s. Despite the discovery of four additional populations, it is still a high priority to offset significant levels of threats (U.S. Army Corps of Engineers 2013). Therefore, continued efforts to propagate the species are warranted to restore the species to additional rivers, to augment existing populations, or both.

Since efforts to develop propagation methods for *Q. fragosa* were initiated, the Service and its partners have focused mainly on developing and refining techniques. Production of *Q. fragosa* for release has been minimal compared to results achieved with another endangered mussel, Higgins eye (*Lampsilis higginsii*), which is a spring/summer long-term brooder (Wege et al. 2007, p. 22-24). The short-term fall brooding habits of *Q. fragosa* present considerable challenges for propagation.

Two parallel efforts to propagate *Q. fragosa* are ongoing – one in the northern part of the species' range that uses St. Croix River mussels for broodstock and one in the south that is based on broodstock from the Saline River. The two efforts have used somewhat different approaches, which are summarized below. Each relies on obtaining larval *Q. fragosa* from wild broodstock to infest host fish.

St. Croix River (Northern) Effort

Wege et al. (2007) and Delphey et al. (2008, p. 1; 2011, p. 4) provide a detailed summary of the activities associated with the St. Croix River propagation effort. Each year, a group of 8-10 biologists typically spend two days searching for *Q.*

fragosa in the species' core habitat near St. Croix Falls, WI (e.g., Hove et al. 2012, p. 50). *Q. fragosa* found during searches are placed into one of eight aggregations established within the general search area – an approximately 2.4 km (1.5 mile) reach of the St. Croix River. Biologists return to the aggregations in late August or early September to begin searches for brooding *Q. fragosa*, which are continued once every 2-3 days until brooding has apparently ended for the year.[4]

Brooding *Q. fragosa* found during searches conducted between late August and early October are taken to Genoa National Fish Hatchery near Genoa, Wisconsin. At the hatchery they are allowed to release glochidia and conglutinates to infest host fish (Eckert and Baran 2011). Infected host fish are overwintered at the hatchery. Biologists use the model derived by Steingraeber et al. (2007, p. 308) to track development of encapsulated *Q. fragosa* glochidia and in the spring place the infected fish into cages in the St. Croix River until any juvenile *Q. fragosa* have likely detached (Wege et al. 2007, p. 24). Biologists attempt to stock fish in the cages as near as practicable to the anticipated date of detachment to reduce the likelihood that fish will die in the cages before the glochidia detach (Wege et al. 2007, p. 23). Juvenile mussels are then allowed to develop on natural substrate inside the cage, which are checked periodically.

The St. Croix River effort first produced a single year-class of mussels in 2006 (Wege et al. 2007, p. 23). Eleven of the 24 juveniles found in cages (Barnhart et al. 2007, p. 71) in September 2006 remained alive in September 2011 and nine were still alive in the summer of 2012 when they were released into the Mississippi River in St. Paul, MN. In 2014 approximately 400 *Q. fragosa* from the same cohort were found near the cage site used in 2006. These mussels were found during mussel sampling that was unrelated to the propagation effort. The *Q. fragosa* were left in the approximate location where they were found – in the St. Croix River near Hudson, Wisconsin. Buccal swab samples were collected from 30 individuals in August 2014 and will be analyzed to confirm to evaluate genetic diversity and to help determine whether we will leave them in their current location or move them to another site for reintroduction.

In addition to the Mississippi River site where the nine *Q. fragosa* were released in 2012, three additional reintroduction areas are currently under consideration for the northern effort – Chippewa River and Wisconsin River (Wisconsin); and, St. Croix River, upstream of St. Croix Falls, Wisconsin (Minnesota/Wisconsin border) (U.S. Army Corps of Engineers 2013).

In 2008 and 2009 the U.S. Army Corps of Engineers (Corps) used a set of biological and physical factors to rank the current suitability of 31 rivers and river

[4] *Q. fragosa* were not aggregated in 2012 and a high number of brooding *Q. fragosa* were discovered, suggesting that annual aggregation is not necessary. In future years, we may change to alternate year aggregation and we will aggregate *Q. fragosa* as early in the year as is feasible to minimize the likelihood of adversely affecting reproduction.

reaches historically inhabited by *Q. fragosa*. The streams assessed were all in the Upper Mississippi River drainage. The biological factors included current mussel density and diversity, the presence of three mussel species typically associated with *Q. fragosa*, especially snuffbox (*Epioblasma triquetra*), and the presence/absence of zebra mussels. Physical factors included water quality (contaminants, dissolved oxygen, and sedimentation) and stream order (size). The following rivers and river reaches historically occupied by *Q. fragosa* had the highest suitability ranks:

- St. Croix River (Minnesota/Wisconsin) above the St. Croix Falls dam;
- Iowa River (Iowa) from the English River downstream to the Cedar River; Wisconsin River (Wisconsin) from the Prairie du Sac dam to the Mississippi River;
- Iowa River from the Coralville Reservoir dam downstream to the English River; and,
- Mississippi River (Minnesota) from St. Anthony Falls downstream to the Minnesota River (site of the 2012 reintroduction).

Although not included in the Corps' screening of potential reintroduction areas, the Chippewa River in Wisconsin is now included as one of the top three sites for reintroduction of the species. No specimens of *Q. fragosa* have been found in Chippewa River, but it likely occurred there historically. It flows into a reach of the Mississippi River that contains numerous historical records and its "hydrology and erosive nature (shifting sand, tanic acid)" likely have eroded many relic shells (Heath et al. 2004, p. 23). Heath et al. (2004, p. 23-24) listed reasons why reintroduction of Higgins eye (*Lampsilis higginsii*) should be pursued in the Chippewa River, which we think also hold true for *Q. fragosa*: the river's size, current mussel fauna, proximity to historic populations, lack of zebra mussels, and presence of mussel aggregations."

The Corps followed up its screening of historic habitats with mussel surveys in highly ranked rivers and river reaches to more precisely determine whether any might be suitable for *Q. fragosa* reintroduction. Surveys conducted in the Wisconsin River near Portage, WI in 2008 found three areas that seemed at least marginally suitable for *Q. fragosa* based on substrate composition and mussel diversity and abundance (Dunn and Badgett 2009, p. 12). The sporadic occurrence of zebra mussels in the Wisconsin River study area is a concern, but they appear to not currently be affecting native mussel populations there (2009, p. 6).

Hove et al. (Hove et al. 2009, p. 21) surveyed mussel communities in four locales in the St. Croix River upstream of the St. Croix Falls Dam and found that two may be suitable for reintroduction of *Q. fragosa*. In each area they found 17 species of live mussels, including, substantial numbers of two species that use channel catfish as a reproductive host – *Quadrula pustulosa* and *Cyclonaias tuberculata*. Total mussel density at the two sites was not significantly different than that found in the area currently occupied by *Q. fragosa*, downstream of the St. Croix Falls Dam (Barnhart 2006).

The lower St. Croix River was also not included among the sites evaluated by the Corps for reintroduction of *Q. fragosa*, presumably due to the presence of zebra mussels in the area. After finding about 400 *Q. fragosa* that likely were lost from propagation cages in 2006, however, the interagency Mussel Coordination Team may focus on this site for additional releases. The area is an Essential Habitat Area for Higgins eye, another federally endangered species.

Saline River/Duck River (Southern Effort)

The Duck River reintroduction effort, which relies on the Saline River (Arkansas) population for broodstock – has been in the planning phase for several years and led to the first release of propagated *Q. fragosa* into the middle portion of the river in 2013. The Service and Tennessee Wildlife Resources Agency have identified suitable reaches and sites within the lower Duck River for eventual reintroduction.

In Arkansas, the Service, Arkansas Game & Fish Commission, and Missouri State University have been working collaboratively since 2005 to test and refine propagation techniques. Juvenile mussels are collected from host fish in recirculating propagation systems and reared in a compact recirculating mussel rearing system designed for captive grow-out of early juvenile mussels (Barnhart et al. 2007). Once juveniles grow large enough – to "taggable size"– they can be transferred to the release site (Barnhart 2009, p. 10).

The Service and its partners released 103 subadult *Q. fragosa* into the Duck River in September 2013 (C. Davidson, pers. comm. 2014). Recently the Service has also begun working with Natchitoches National Fish Hatchery in Louisiana to broaden the number of facilities involved in propagation activities and the types of techniques investigated.

2.3.2 Five-Factor Analysis (threats, conservation measures, and regulatory mechanisms)

In the recovery plan, the Service reiterated four reasons for the original determination that *Q. fragosa* warranted listing as endangered in 1991 (U.S. Fish and Wildlife Service 1991, p. 28347-28348). We summarize and provide an updated perspective on each below:

Reason 1: Q. fragosa has been eliminated from nearly all of its original 11-state range and is now known from a single extant population along one 20-kilometer reach of the St. Croix River.

Q. fragosa is now known to occur in four of the 15 states in which it is known to have occurred historically. Although a marked change from its presumed distribution in 1997, it is still absent from a substantial proportion of its historical range (Table 3, Fig. 13).

Table 3. Rivers from which *Q. fragosa* has been extirpated (Angelo et al. 2009; U.S. Fish and Wildlife Service 1997, p. 45; J. Harris, pers. comm. 2013). Based on information obtained from Nebraska Game and Parks Commission (S. Schainost, pers. comm. 2013), Blue River and Bow Creek, Nebraska, were removed from the table that described historical distribution of *Q. fragosa* in the recovery plan.

Waterbody	State(s)
Cedar River	IA
Des Moines River	IA
Iowa River	IA
Raccoon River	IA
Illinois River	IL
Kaskaskia River	IL
Sangamon River	IL
Spoon River	IL
Ohio River	IN, OH
Wabash River	IN
West White River	IN
Blue River	KS
Fall River	KS
Marais des Cygnes River	KS
Neosho River	KS
Soldier Creek	KS
Spring River	KS
Verdigris River	KS
Walnut Creek	KS
Cumberland River	KY, TN
Licking River	KY
Minnesota River	MN
Mississippi River[5]	IL, IA, MN, WI
Vermillion River	MN
Fox River	MO
One Hundred and Two River	MO
Osage River	MO
Raccoon Creek	OH
Scioto River	OH
Boggy Creek	OK
Kiamichi River	OK
Little River	OK
Big Sioux River	SD
James River	SD[6]
Duck River	TN
Harpeth River	TN
Tennessee River	GA, TN
Baraboo River	WI
Wisconsin River	WI

[5] *Q. fragosa* was reintroduced into Mississippi River at one location in Minnesota in 2012 (Delphey et al. 2013, p. 3).
[6] Perkins and Backlund (2003)

Reason 2: The remnant population is thought to be small and therefore vulnerable to stochastic disturbances, such as toxic substance spills or low water levels.

It is still true that the St. Croix River population may be "vulnerable to stochastic disturbances" because it still only inhabits an approximately 9-km long reach of the river. The population has sustained three distinct low-flow events – sustained periods with below-median discharges – with no clear effects to population viability. The population remains vulnerable to spills, although a spill contingency plan has been developed to reduce this threat.

In the 1991 listing rule, the Service rather extensively described the threat to the species posed by the operation of the dam on the St. Croix River at St. Croix Falls, Wisconsin (U.S. Fish and Wildlife Service 1991, p. 28347). Since then, Xcel Energy signed a memorandum of understanding with Wisconsin Department of Natural Resources to operate the dam in a run-of-river mode and to avoid causing low flows and substantial changes in flow levels that harmed *Q. fragosa* and other aquatic fauna.

On Little River, Ouachita River, and Saline River *Q. fragosa* is more broadly distributed than on the St. Croix River and may be less vulnerable to stochastic events; the size and distribution of the population on the Bourbeuse River is poorly understood, but the species is only known to inhabit a two-mile reach of the river. The potential resiliency of the Saline River population may have been demonstrated by the outcome of severe flooding in 2008 and 2009. Sediment deposition and channel movements caused by the floods reduced the extent of one major bed by about 80%, but surveys in 2011 indicated that at least three beds still support over one thousand *Q. fragosa* and another supports more than 5000 (Table 2).

In the Saline River and Ouachita River, respectively, *Q. fragosa* occur within patches (beds) along 123-km and 72-km reaches – about fourteen and eight times longer, respectively, than the reach inhabited by the species on the St. Croix River. Although the size and distribution of mussel populations may, in general, be directly related to their resiliency to stochastic events, many other factors must be considered to assess and mitigate the level of threat. These factors may include proximity to roads, traffic volume on those roads, and current and future land use within the watershed. Therefore, any spill contingency plans completed for river reaches inhabited by *Q. fragosa* should be reviewed to better understand the threat of spills and other sources of stochastic threats must also be documented and addressed.

2.3.2.1 Present or threatened destruction, modification or curtailment of its habitat or range:

Drought and Water Withdrawals in Oklahoma – Little River

Allen and Vaughn report that plans have been proposed to sell water from the Little River (Peterson et al. 2011, p. 120-121). Water withdrawals are likely to negatively affect populations of freshwater mussels unless adequate minimum flows are maintained (Vaughn and Atkinson 2011). *Q. fragosa* in the Little River may already be threatened by severe droughts, which are projected to increase in frequency and severity throughout the 21st century in Oklahoma (Oklahoma Climatological Survey 2011, p. 2).

Sedimentation in St. Croix River

Increasing levels of fine sediments and increasing flow velocities may be developing threats to *Q. fragosa* in the St. Croix River (U.S. Army Corps of Engineers 2013, p. 41). *Q. fragosa* appears to rely on factors that also are necessary for high mussel density and diversity (Allen and Vaughn 2008, p. 5; Hornbach and Hove 2008, p. 1). Therefore, reductions in mussel density and diversity where *Q. fragosa* occurs may also be indicative of a developing threat to the species. Hornbach and Hove (2008) found a decrease in the density of juvenile mussels, increases in proportions of fine sediments, and signs of increasing flow velocities in the reach of the St. Croix River inhabited by *Q. fragosa*.

2.3.2.2 Overutilization for commercial, recreational, scientific, or educational purposes:

No new information.

2.3.2.3 Disease or predation:

No new information.

2.3.2.4 Inadequacy of existing regulatory mechanisms:

Q. fragosa is not listed as endangered or threatened under Oklahoma's endangered species statute. The species is listed as "extirpated" in Missouri, but we expect that status will be changed to endangered. In Wisconsin and Minnesota the species is listed as endangered under the respective state statutes.

2.3.2.5 Other natural or manmade factors affecting its continued existence:

A recent genetic analysis of the *Q. fragosa* population in the St. Croix River indicates that the population may be at "heightened risk for extinction due to erosion of genetic diversity via genetic drift and inbreeding" (Roe 2010, p. 4). Although other factors may be responsible for indications of inbreeding, this population should be monitored for any signs that inbreeding depression.

2.4 Synthesis

The recovery criteria for *Q. fragosa* focus on ensuring that a minimum number of viable populations will persist in protected habitats in at least two or three separate tributaries to the Mississippi River for downlisting and delisting, respectively (U.S. Fish and Wildlife Service 1997, p. 19). These criteria were approved based on the presumption that there was only one extant population of *Q. fragosa* – in the St. Croix River on the border between Minnesota and Wisconsin.

For this synthesis, we will assess the status of *Q. fragosa* in light of the recovery criteria (U.S. Fish and Wildlife Service 1997, p. 19).

Number and Viability of Discrete Populations
Based on the recovery criteria, at least three viable populations of *Q. fragosa* are needed to change the status of the species from endangered to threatened and at least five are needed to delist the species (U.S. Fish and Wildlife Service 1997, p. 29). To assess viability of each population based on the recovery plan we need to review data on recruitment and age structure, population size relative to the size of the minimum viable population, and "genetic structure" (Table 4, U.S. Fish and Wildlife Service 1997, p. 29).

Population viability data are most comprehensive for St. Croix River (Table 2). Although not strictly in agreement with the recovery plan's standard for recruitment – i.e., "demonstrate recruitment to the population in 8 of the 11 age classes aged 2 to 12 years (U.S. Fish and Wildlife Service 1997, p. 29) – data from targeted searches in 2010 and 2011 are indicative of consistent recruitment with possible indications of variation in the strength of year classes (Fig. 3). There is an estimate of population size in the St. Croix River – 13,000 (Hornbach and Hove 2008, p. 1) – but we have not determined a minimum viable population size.

Recruitment/Age Structure
Age of individual mussels may be approximated by counts of external annuli (growth cessation lines) on shells. The distribution of external annuli counts, and perhaps also of shell lengths, may allow us to approximate the recruitment and age structure of *Q. fragosa* populations. Based on external annuli counts and length measurements for 189 unique *Q. fragosa* from 2010 and 2011, it appears as if *Q. fragosa* has been consistently recruiting individuals into the population for approximately the past fifteen years (Fig. 3). Length-frequency distributions for Ouachita River and Saline River *Q. fragosa* are also fairly broad (Figs. 5-6), but there is relatively little data because lengths are only available for *Q. fragosa* found in quadrat samples and annuli counts are not available. Data are insufficient to estimate age structure of *Q. fragosa* in the Bourbeuse River (Table 3).

Genetic Structure

Population genetics data are only available for St. Croix River. We have not described a genetic structure for populations that would be "consistent with the minimum viable population determination", as is suggested in the recovery plan (U.S. Fish and Wildlife Service 1997, p. 27). Roe (2010, p. 4) found potential evidence of inbreeding based on an analysis of genetic data collected from 52 St. Croix River *Q. fragosa*.

Population Persistence

The criteria relative to persistence of populations state that each population must have been extant for 24 years following colonization or establishment. This criterion may have been intended to ensure that any reintroduced population had become firmly established and self-perpetuating, but we include known persistence times for each population in Table 4. These obviously represent only minimum times of existence for these populations. This criterion also calls for "Three consecutive surveys taken at approximately 5-year intervals" that "demonstrate population levels to exceed the MVP determination made in Task 2D1" (U.S. Fish and Wildlife Service 1997, p. 29).

Long-term Habitat Protection

This criterion calls for the drafting and approval of a "watershed management plan" by the Service that "demonstrates all potential threats to the population have been identified and either eliminated, mitigated, or otherwise provided for." Although the Service and its conservation partners generally monitor and evaluate known and potential threats to each population, this has not been carried out in the manner specifically called for in the recovery plan. Due to the discovery of four new populations since approval of the recovery plan and the changes that may have affected the St. Croix River population (U.S. Army Corps of Engineers 2013), an updated analysis of threats for each population is warranted.

Table 4. Summary of data that may be used to assess viability of *Q. fragosa* populations.

Population	Population Size	Population Size Comments	Recruitment/Age Structure	Genetic Structure	Receiving Tributary to Mississippi River	Years Persisted (through 2012)	Persistence
St. Croix River	13,000	Population estimate (Hornbach and Hove 2008, p. 1)	Evidence for recruitment for year classes, 3-14 (U.S. Fish and Wildlife Service, unpubl. data 2010-2011)	Possible evidence of inbreeding (Roe 2010)	Direct tributary to Mississippi River	≥43	Recent evidence of presence since 1969 (U.S. Fish and Wildlife Service 1997, p. 69)
Bourbeuse River	Unknown	Only five unique individuals have been recorded	Insufficient data	Population genetics study has not been conducted	Meramec River	≥13	First discovered in 2001
Ouachita River	At least approximately 3388	Sum of mean population estimates for four beds (U.S. Fish and Wildlife Service 1997, p. 29); only a minimum population estimate may be derived – some beds remain unsampled	No external annuli counts available; length frequency distribution available based on 20 individuals (Fig. 5).	Population genetics study has not been conducted	Red River	≥18	First discovered in 1996
Saline River	At least approximately 24,207	Sum of most recent mean population estimates for each of nine beds (Table 1)	Length frequency data available from Harris (unpubl. data) and Davidson (Fig. 6);	Population genetics study has not been conducted	Red River	≥13	First discovered in 2001
Little River	Unknown	Density estimates available for four of the eleven sites inhabited by *Q. fragosa* on Little River, but bed sizes are unknown (D. Allen and C. Vaughn, unpubl. data, University of Oklahoma 2012).	Length frequency distribution (Fig. 7) based on only 13 specimens; additional data needed to assess recruitment and age structure	Population genetics study has not been conducted	Red River	≥9	First discovered in 2005

3.0 RESULTS

3.1 Recommended Classification:

____ Downlist to Threatened
____ Uplist to Endangered
____ Delist (*Indicate reasons for delisting per 50 CFR 424.11):*
____ *Extinction*
____ *Recovery*
____ *Original data for classification in error*
_X__ No change is needed

3.2 Recovery Priority Number: 2C

No change in priority number.

Brief Rationale:

Three of the five remaining populations of *Q. fragosa* are subject to high degrees of threats due in part to their restricted distributions and isolation from other populations. Low flows associated with drought pose a high degree of threat to the Little River population. The species' recovery potential is high due to the existence of at least two populations that appear to be large and somewhat resilient to stochastic disturbances, such as major floods. In addition, progress has been made to propagate the species. Although large numbers of the species have not yet been produced by propagation efforts, it has resulted in at least nominal reintroductions of the species into three different locations and robust interagency efforts are ongoing to explore and refine techniques.

4.0 RECOMMENDATIONS FOR FUTURE ACTIONS

1. Revise the recovery criteria and tasks to reflect the change in the species' status due to the discovery of four additional populations and other changes since 1997.

2. Describe the magnitude and immediacy of threats to the four newly discovered *Q. fragosa* populations and update that information for the St. Croix River population. Rank threats in priority – high, medium, and low – and describe actions that should be taken to address each threat, beginning with the high priority threats and proceeding to low priority threats.

3. If spills of toxic materials are a threat to one or more populations, develop spill contingency plans to minimize the likelihood of significant adverse effects to the potentially affected populations.

4. Complete sampling needed to describe the abundance and distribution of *Q. fragosa* in the Bourbeuse River, Little River, Ouachita River, and Saline River.

5. Determine whether additional populations of *Q. fragosa* should be established to consider the species for reclassification or delisting. That is, are three and five populations, respectively, sufficient to consider the species for reclassification or delisting? Important considerations include: conserving the species' genetic diversity and ensuring that the species is able to withstand demographic and environmental variation.

6. Revise the recovery criteria to include practicable metrics for determining population viability and persistence. These metrics must be feasible to implement in light of likely funding and logistical constraints and should minimize disturbance of benthic organisms and their habitats. The Service should then develop methods to assess the status of *Q. fragosa* populations in light of the metrics.

7. Convene the recovery team to identify any research questions that, if answered, would be likely to have a significant benefit to our ability to propagate *Q. fragosa*.

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PLAN FOR CONTROLLED PROPAGATION, AUGMENTATION, AND REINTRODUCTION

Winged Mapleleaf
(*Quadrula fragosa*)

A Cooperative Interagency Plan


March 2013

Prepared by:

USFWS Winged Mapleleaf Propagation Committee

Phil Delphey, Tony Brady, Stephanie Chance, Chris Davidson, Nathan Eckert, Andy Roberts, and Nick Utrup

Personnel from the following agencies or organizations reviewed at least one version of this plan and provided helpful comments:

Arkansas Game and Fish Commission

Illinois Department of Natural Resources

Iowa Department of Natural Resources

Macalester College

Minnesota Department of Natural Resources

Missouri Department of Conservation

National Park Service

Southwest Missouri State University

U.S. Army Corps of Engineers

U.S. Fish and Wildlife Service

U.S. Geological Survey

University of Minnesota

Wisconsin Department of Natural Resources

INTRODUCTION

Winged mapleleaf (*Quadrula fragosa*) is a freshwater mussel that occurred historically in at least 41 rivers in 16 Midwestern states (Figure 1). U.S. Fish and Wildlife Service (Service) listed it as endangered under the Endangered Species Act in 1991 (56 Federal Register 28345-28349). The primary causes for the decline of winged mapleleaf are uncertain, but several potential factors were identified: changes in water and sediment quality, habitat loss resulting from impoundments, chemical contaminants, stochastic events (e.g., drought), and invasive species.

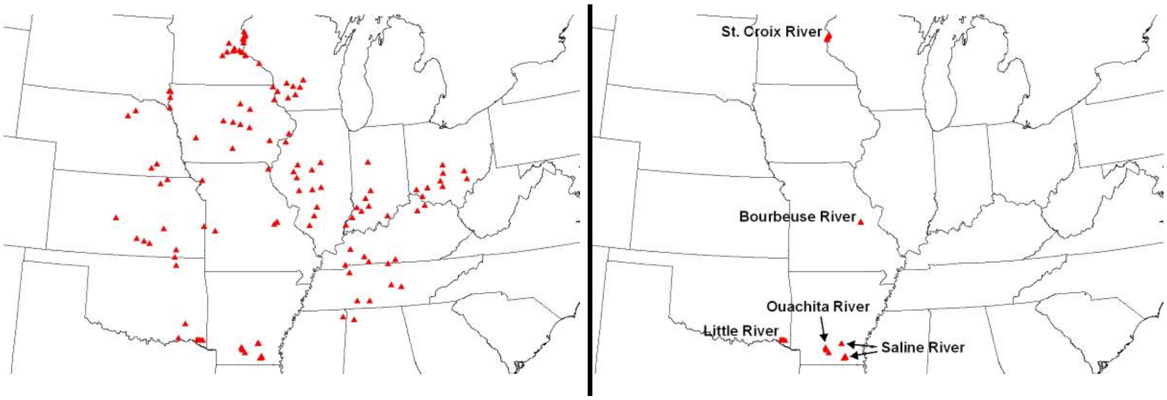

Figure 1. Historical records (left) and current locations (right) of winged mapleleaf (*Quadrula fragosa*) populations.

When the Service completed the recovery plan for winged mapleleaf in 1997 (U.S. Fish and Wildlife Service 1997) the species was known to occur only in the St. Croix River, on the boundary between Minnesota and Wisconsin (Fig. 1). Since then four other populations have been confirmed – in the Ouachita River (Posey et al. 1996, p. 97) and Saline River (Davidson and Clem 2002, 2004), Arkansas; in the Bourbeuse River, Missouri (A. Roberts, U.S. Fish and Wildlife Service, pers. comm. 17 September 2008; S. McMurray, Missouri Department of Conservation, pers. comm. 19 September 2008); and, in the Little River, Oklahoma (Allen and Vaughn 2008, p. 3).

The discovery of four populations outside of the St. Croix River has changed the context for the recovery of winged mapleleaf, but the species' propagation is still warranted. The species only inhabits a small portion of its historical range. In addition, extant populations face significant threats and are largely isolated – only the Arkansas populations may be secure and interconnected. Therefore, attempts to propagate the species are warranted to facilitate augmentation of populations or reintroduction of the species into rehabilitated habitats. In addition, captive propagation could produce individuals for scientific studies that could be important for the recovery of the species in the wild.

St. Croix River – Minnesota and Wisconsin
Nearly all of the winged mapleleaf in the St. Croix River occur in a 9-km reach that represents only about 9% of the species' original distribution in the river (Wisconsin Department Natural Resources 2002, p. 2, Hornbach and Hove 2008, p. 13). Hornbach and Hove (2008, p. 13) used adaptive cluster sampling to estimate the population size in the St. Croix River "between Interstate State Park and Franconia, Minnesota" at about 13,000. Threats to the St. Croix River population include human-mediated introduction of zebra mussels (*Dreissena polymorpha*) and increasing levels of fine sediments (Hornbach and Hove 2008, p. 15). The population's isolation and concentration also render it vulnerable to stochastic events, such as toxic spills.

Ouachita River and Saline River - Arkansas
Arkansas contains two populations of winged mapleleaf – in the Ouachita River and Saline River. Saline River is a tributary to Ouachita River, so these populations are presumed to be interconnected. In each river winged mapleleaf occurs downstream of long and deep pools that provide habitat for blue catfish (*Ictalurus furcatus*) and channel catfish (*I. punctatus*), the species' reproductive hosts (C. Davidson. U.S. Fish and Wildlife Service, Conway, AR, pers. comm., 2012).

In the Ouachita River, winged mapleleaf occurs within an approximately 72-km long reach (45 river miles, RM, Harris 2006, p. 12). Among the four beds where it was detected by Harris (2006, p. 8) the species' estimated abundance ranged from 217 to 1770 – it was not detected at two other beds that were in a reach with high gradients and fast flows and that were the furthest upstream of the six beds sampled. Winged mapleleaf also occurs in the lowermost reach of the Little Missouri River, but these mussels are considered to be an extension of the Ouachita River population (Davidson 1997, p. 47).

In the Saline River, winged mapleleaf occurs in at least 14 mussel beds between Mt. Elba and Stillion Accesses (Fig. 2, Table 1, Davidson and Clem 2002, Davidson and Clem 2004). In 2010 and 2011, the Service and Arkansas Game and Fish Commission (AGFC) resampled beds that Harris (2006) had surveyed in 2005. The largest bed had declined in extent by 82 percent due to channel changes caused by flooding in 2008 and 2009. This was a significant impact to the species – the bed may have contained more winged mapleleaf than any other bed range wide. At another of the four sites, however, the estimated abundance of winged mapleleaf increased from about 510 to about 11,821 due to a previously undocumented segment of the bed (C. Davidson, pers. comm. 2012). At the two remaining of Harris' (2006) study sites, winged mapleleaf abundance was similar at one site compared to 2005 and increased at the other by about 125 percent.

Table 1. Summary of quantitative sampling data for Saline River, Arkansas, 2005 – 2012 (C. Davidson, U.S. Fish and Wildlife Service, Conway, AR, pers. comm. and unpubl. data 2012).

Bed (SRB-)	Sampling Year	*Q. fragosa* Population Estimate +/- 95% Confidence Interval	Bed Area (m^2)
70	2008	202 +/- 360	2,625
70	2011	396 +/- 324	2,625
71	2008	356 +/- 341	2,895
71	2011	198 +/- 198	2,475
141	2005	9217 +/- 4114	9,275
141	2010	1615 +/- 693[1]	1,400
143	2012	737 +/- 714	1,290
145	2005	510 +/- 253	850
145	2011	850 +/- 731	850
145	2011	11,281 +/- 5915[2]	16,700
146	2005	540 +/-415	1,020
146	2011	491 +/- 244	540
147	2011	811 +/- 858[3]	2,850
152	2005	1214 +/- 742	2,760
152	2011	2981 +/- 1653	8,280
153	2012	125 +/- 219	3,120
155	2012	1280 +/- 1586	8,000
157	2012	1512 +/- 1026	5,400
158	2012	480 +/- 583	6,000
159A	2011	2235 +/- 1136	4,860
159B	2011	4199 +/- 2893	17,650

Bourbeuse River – Missouri

Winged mapleleaf was first documented in Missouri's Bourbeuse River in 2001, but the species' distribution and abundance in the river is still not well understood. The first live specimen was found at river mile 53.9 in 1997 by Dr. Chris Barnhart (Missouri State University) and four more unique individuals have been documented since then, including at river mile 56 (A. Roberts, U.S. Fish and Wildlife Service, pers. comm. 17 September 2008; S. McMurray, Missouri Department of Conservation, pers. comm. 19 September 2008). During return visits to the upstream site, biologists have relocated the same individual several times and in 2009 they found an additional three winged mapleleaf (S. McMurray, pers. comm. 19 September 2008; A. Roberts, U.S. Fish and Wildlife Service, Columbia, MO, pers. comm. 17 August 2009).

Little River - Oklahoma

Beginning in 2005, winged mapleleaf has been found in eleven sites in the Little River, Oklahoma. Galbraith et al. (2008, p. 16) found winged mapleleaf at four sites in 2005 and Allen and Vaughn (Fig. 6, Allen and Vaughn 2008, p. 17) found it at seven additional sites in 2006. The downstream distribution of the species may not extend past its confluence with Mountain

[1] Floods in 2008 and 2009 floods reduced extent of SRB 141.
[2] Previously undiscovered portion of SRB 145 sampled in 2011.
[3] SRB 147 redefined from Davidson and Clem (2004).

Fork River due to cold water releases from the dam that forms Broken Bow Lake (Fig. 7, Allen and Vaughn 2008, p. 16). Exhaustive surveys in the Arkansas portion of Little River have not resulted in any additional winged mapleleaf records (C. Davidson, U.S. Fish and Wildlife Service, pers. comm., 30 March 2009); if they do occur in the Little River in Arkansas, they may be "very rare" (C. Davidson, pers. comm. 7 January 2010).

Allen and Vaughn (2008, p. 36-40) quantitatively sampled six mussel beds in the Little River in 2006 by placing six transects across the river and counting mussels within four regularly spaced quadrats along each transect – a total of 24 quadrats per bed. They detected winged mapleleaf at four of the six sites and at three additional sites where only timed searches were conducted (Allen and Vaughn 2008, p. 36-40). At the four sites where winged mapleleaf was detected in quadrats its estimated density ranged from 0.2 (three sites) to 0.5 (one site) (D. Allen and C. Vaughn, unpubl. data, University of Oklahoma 2012).

In 2011, Vaughn and Atkinson (2011) documented extensive mussel mortality on the Little River during a severe drought. At one site they "recorded 19 species of freshly dead mussels in habitat that would normally be submerged", although no dead winged mapleleaf were recorded (Vaughn and Atkinson 2011). In late July 2012, drought conditions again prevailed and flows in the Little River reach inhabited by winged mapleleaf were at about 15% of median levels. The Oklahoma Climatological Survey (U.S. Fish and Wildlife Service 1997, p. 3) expects the state to experience an increasing frequency and severity and droughts, which could pose a significant threat to winged mapleleaf in Little River.

JUSTIFICATION FOR CONTROLLED PROPAGATION, AUGMENTATION, AND REINTRODUCTION

Historical winged mapleleaf population declines and current threats to extant populations warrant the use of captive propagation to ensure the species' recovery. The primary criterion of the winged mapleleaf recovery plan is to protect five discrete viable populations in at least three tributaries of the Mississippi River (U.S. Fish and Wildlife Service 1997). The populations in Arkansas' Ouachita River and Saline River may be viable (C. Davidson, pers. comm. 2012), but the populations in the three other rivers face significant threats or are of uncertain abundance and distribution (e.g., Bourbeuse River).

The Service and its partners have tried to propagate winged mapleleaf for several years with only moderate success. Since 2005 captive propagation efforts have been pursued at Genoa National Fish Hatchery (St. Croix River broodstock), Missouri State University, Natchitoches National Fish Hatchery, and Kansas City Zoo (Saline River broodstock).

Recovery Plan Tasks Related to Propagation

The recovery plan called for several tasks to facilitate the reintroduction and conservation of the species.

Task 2Cl, Reproductive phenology: Determine the phenology of reproduction.

Status: The reproductive phenology of the winged mapleleaf in the St. Croix River is now well understood. We now know that the brooding period for winged mapleleaf in the St. Croix River (Minnesota and Wisconsin) runs from early/mid-September to early/mid-October (Heath et al. 1999, p. 1-3), although its precise timing varies from year to year. In the Saline River, winged mapleleaf brooding occurs typically during mid-October (C. Davidson 2012, pers. comm.).

Task 2C2, Glochidial host: Identify the glochidial host(s)

Status: Steingraeber et al. (2005, 2007) confirmed both blue catfish and channel catfish as reproductive hosts for winged mapleleaf.

Task 4: Reestablish Q. fragosa populations in historical range.

Task 4A, Translocation:

Task 4Al, Translocation protocol: Evaluate translocation techniques and establish a translocation protocol.

Status: No translocation protocol has been developed for winged mapleleaf. This reintroduction plan focuses on release of propagated subadults into rehabilitated historical habitats. It does not include translocation of wild winged mapleleaf to reestablish populations, although we may later evaluate that as an option if propagation is not successful.

Protocols for propagating and reintroducing winged mapleleaf are being developed. In the northern part of the species range, broodstock are typically collected in from the St. Croix River and transported to Genoa National Fish Hatchery where they are used to infest channel catfish. The infested fish are overwintered at the hatchery before being placed in cages in the spring before glochidia have detached. Cages are then checked periodically for successfully transformed juvenile winged mapleleaf. Some juveniles have been further grown-out in alternative cage designs (silos) before out-planting at reintroduction sites. As of late 2012, nine winged mapleleaf subadults have been released into the Mississippi River in St. Paul, Minnesota.

In Arkansas, Service biologists and their partners have tried a variety of approaches. An attempt to rear winged mapleleaf juveniles in silos in the Saline River failed when the silos were buried under bed load. Progeny of Saline River broodstock are being grown out partially in the lab of Dr. Chris Barnhart, Missouri State University, and in floating upweller systems (FLUPSY) at the Kansas City Zoo. In Arkansas, Chris Davidson, Conway, Arkansas Field Office, is also experimenting with the cage technique used in Minnesota/Wisconsin in collaboration with Natchitoches National Fish Hatchery. This includes a comparison of growth and survival between cages held in raceways at the hatchery and cages placed into a river.

Agencies collaborating on this effort are considering various alternatives to the above basic protocols, including releasing host fish into rivers soon after infestation.

> *Task 4A2, Suitable habitat: Identify rivers within the historical distribution of Q. fragosa which have suitable physical, chemical, and biological habitat for reintroduction of Q. fragosa. Give priority to the following factors when selecting translocation sites:*
>
> > *a) Rivers close to the St. Croix so environmental and climatic factors will be similar to those to which the St. Croix River population is adapted and so new populations might function as a metapopulation.*
> >
> > *b) Rivers having sufficient long-term protection (such as mussel sanctuaries, state or National parks) so they will qualify under the guidelines for population habitat protection in Task 5C.*
> >
> > *c) Rivers at low risk from colonization by Dreissena spp.*

Status: The Service's Twin Cities Field Office began to identify rivers and streams in the historical range of winged mapleleaf whose habitat and water quality are likely conducive to supporting a reintroduced population of this species. The St. Paul District of the Corps picked up that effort in 2008 as part of its implementation of reasonable and prudent measures contained in a biological opinion completed by the Service in 2000. The Corps used a set of biological and physical factors to rank the current suitability of 31 rivers and river reaches historically inhabited by winged mapleleaf in the Upper Mississippi River basin. The biological factors included current mussel density and diversity, the presence of three mussel species typically associated with winged mapleleaf in the St. Croix River, especially snuffbox (*Epioblasma triquetra*), and the presence of zebra mussels. Physical factors included water quality (contaminants, dissolved oxygen, and sedimentation), and stream order (size). The following rivers ranked highest in suitability: St. Croix River (Minnesota/Wisconsin) above the St. Croix Falls dam; the Iowa River (Iowa) from the English River downstream to the Cedar River; the lower Wisconsin River

(Wisconsin) from the Prairie du Sac dam to the Mississippi River; Iowa River from the Coralville Reservoir dam downstream to the English River; and, the Mississippi River (Minnesota) from St. Anthony Falls downstream to the Minnesota River. A fourth site, Chippewa River, Wisconsin, is now also under consideration. Chippewa River contains high quality mussel habitat and is likely part of winged mapleleaf's historical range despite the lack of records.

The Service's Arkansas and Tennessee Field Offices and the Tennessee Wildlife Resources Agency have identified the lower Duck River as a reintroduction site within the southern range of the species. Improvements in water and habitat quality that support a diverse mussel fauna and confirmation in 2009-2010 that the lower Duck River has the best habitat and host fish availability based on conditions observed in the Little, Ouachita, and Saline River have helped to confirm the Duck River as a suitable reintroduction area (C. Davidson, pers. comm. 2012).

Task 4B, Mussel culture and propagation:

Task 4Bl, in situ vs. ex situ: Evaluate in situ vs. ex situ approaches to recovery and develop methods consistent with the findings.

Status: The Service and its state and federal partners have been working since about 2003 to conduct studies and to try to develop and apply techniques to propagate winged mapleleaf in the northern part of the species' range. The studies confirmed the suitable hosts (Thiel and Newton 2003, Steingraeber et al. 2005, Steingraeber et al. 2007). The collaborating agencies have also developed and refined techniques to facilitate collection of brooding females, maximize the likelihood of successful transformation of juveniles, appropriately time the placement of infested catfish into cages *in situ*, and support *in situ* growth and development of subadult winged mapleleaf for release (Thiel and Newton 2003, Steingraeber et al. 2005, Brady and Aloisi 2007, Steingraeber et al. 2007, Wege et al. 2007, Brady and Aloisi 2008, Delphey et al. 2008, Brady 2009, Brady and Aloisi 2009, Delphey et al. 2009, Eckert and Aloisi 2010, Eckert and Baran 2011, Delphey et al. 2012).

Techniques are now developed and mostly standardized, but success has been limited thus far with only nine subadult winged mapleleaf released thus far. We will not consider the current methods to be final until we are able to demonstrate significant production of subadult winged mapleleaf at the scale that would support successful reintroductions. The discussion of protocols above summarizes the methods attempted thus far and the variety of techniques being evaluated.

PROPAGATION AND REINTRODUCTION OF WINGED MAPLELEAF – 2005 THROUGH 2011

Propagation and reintroduction of freshwater mussels may be described as a four-step process, with at least some of the steps overlapping: 1) develop techniques to propagate a species in numbers sufficient to reestablish genetically diverse populations; 2) select reintroduction areas; 3) carry out reintroductions; and, 4) monitor reintroduced populations.

Two parallel efforts to propagate winged mapleleaf are ongoing – one in the northern part of the species' range that uses mussels from the St. Croix River and one in the south based on the Saline River population. Thus far the two efforts have used somewhat different approaches.

St. Croix River (Northern) Effort

Wege et al. (2007, p. 22-24) provided a detailed summary of the activities carried out by biologists involved with the St. Croix River propagation effort. Each year, a group of 8-10 biologists spend two days searching for winged mapleleaf in the species' core habitat near St. Croix Falls, WI (Delphey et al. 2008, p. 1, Delphey et al. 2011, p. 4). Winged mapleleaf found during searches are placed into one of eight aggregations established within the general search area – an approximately 2.4 km (1.5 mile) reach of the St. Croix River. Biologists return to the aggregations in August and September to search for brooding winged mapleleaf, which are taken to Genoa National Fish Hatchery near Genoa, Wisconsin. At the hatchery, brooding winged mapleleaf are allowed to release conglutinates containing glochidia (larvae). Propagation biologists at the hatchery then expose channel catfish to the glochidia to allow for attachment.

Host fish are overwintered at the hatchery and in the spring placed into cages in the St. Croix River until any juvenile winged mapleleaf have likely detached and fallen to the bottom of the cage (Eckert and Baran 2011). Biologists use the model derived by Steingraeber et al. (2007, p. 308) to track development of encapsulated winged mapleleaf glochidia. Shortly before glochidia are ready to detach from the host fish, biologists transport the infested fish to the river and place them into closed-bottom cages; the fish are subsequently released into the river once glochidia have detached. Biologists attempt to place fish into cages in the river as near as practicable to the anticipated date of detachment to reduce the likelihood that fish will die in the cages before the glochidia detach (Wege et al. 2007, p. 24). Juvenile mussels are then allowed to develop on natural substrate inside the cage.

The St. Croix River effort first produced a single year-class of mussels in 2006 (2005/2006 year class - Wege et al. 2007, p. 23). Eleven of the 24 juveniles found in cages in September 2006 (Wege et al. 2007, p. 23) remained alive in September 2011. Five were in "silos" (Barnhart et al. 2007, p. 71) in the Mississippi River in St. Paul, Minnesota and six were in a cage (Wege et al. 2007, p. 127) in the St. Croix River near Houlton, WI. Between 2011 and 2012, two of the winged mapleleaf in the silos died; the remaining nine subadult winged mapleleaf were released into the Mississippi River in St. Paul, MN in August 2012.

Saline River/Duck River (Southern Effort)

The Duck River reintroduction effort – relying on the Saline River in Arkansas population for broodstock – has been in the planning phase for several years. The Service and Tennessee Wildlife Resources Agency have identified suitable reaches and sites within the lower Duck River for eventual reintroduction. In Arkansas, the Service, Arkansas Game & Fish Commission, and Missouri State University have been working collaboratively since 2005 to develop propagation techniques for the southern population. The methods used may be summarized as follows: juvenile mussels are collected from host fish in recirculating propagation systems and reared in a compact recirculating mussel rearing system designed for captive grow-out of early juvenile mussels (Barnhart 2006). Once juveniles grow large enough, they are transferred to the release site where they are placed into silos (Barnhart et al. 2007) that are placed on the bottom of the river. Although silos have been used thus far, other techniques for release and grow-out of juvenile mussels may be used in the future.

The Service and its partners are planning to release subadult winged mapleleaf in 2013 or 2014, depending on success of searches for brooding females in Saline River and subsequent growth and survival of juveniles. The Service will develop a site plan (see below), in cooperation with its partners, before any release. Recently the Service has also begun working with Natchitoches National Fish Hatchery to broaden the number of facilities involved in propagation activities and the types of techniques investigated.

GOAL AND OBJECTIVES

The goal of this augmentation and reintroduction program is to restore viable populations of winged mapleleaf to appropriate portions of historical range through augmentation of existing populations or reintroduction. The objectives of this plan are to:

1) Establish basic protocols for propagating winged mapleleaf mussels.
2) Communicate and coordinate among partners before relocation of wild stock or the release of hatchery stock to the wild. No reintroductions would occur until site plans and appropriate coordination is complete.

The purposes of captive propagation, reintroduction, and augmentation are to:

1) Reduce the risk of the species' extinction by increasing the number and distribution of viable populations;
2) Restore populations to historical portions of the species' range into suitable habitat; and,
3) Provide for recovery and the potential for delisting.

PARTNERS

In addition to the Service's Ecological Services and Fisheries Divisions, the Corps' St. Paul and Rock Island Districts, U.S. Geological Survey, National Park Service – St. Croix National Scenic Riverway and Mississippi National River & Recreation Area , University of Minnesota, Macalester College, Southwest Missouri State University, and States of Minnesota, Wisconsin, Iowa, Illinois, Missouri and Arkansas Game and Fish Commission have been cooperating in studies of life history, genetics, and in developing holding and propagation technology for winged mapleleaf. The Kansas City Zoo is participating with the Service and Dr. Chris Barnhart, Missouri State University, on winged mapleleaf propagation, using floating upweller systems (FLUPSY) on ponds within their facility.

DEFINITIONS

Augmentation - Augmentation describes the increase in numbers of a snail or mussel species within a defined area of habitat through the transplantation of adults from other locations or through the release of propagated individuals. Augmentation is appropriate when the population size of a listed species is minimal within an occupied area; the population is experiencing recruitment failure, or both. It is also appropriate where the species may be absent within apparently suitable habitat that is contiguous with and accessible to occupied habitat.

Augmentation increases the likelihood of population success for spawning, fertilization, host fish infestation by mussels, and recruitment within sparsely occupied habitat. It may be used to expand the range of a species within habitats accessible to existing populations, reducing the likelihood of extirpation due to localized catastrophic events.

The potential for augmentation with endangered or threatened mussels from existing populations is limited and many species have low numbers of surviving individuals. In addition, augmentation with hatchery produced juveniles carries the potential of disease introduction and genetic swamping. The latter may be addressed by ensuring that a suitable portion of the population's genetic diversity is adequately represented among the released individuals.

Reintroduction - Reintroduction describes the establishment of adults or subadults into historically occupied river and stream reaches where the species no longer occurs and where we do not expect natural immigration from extant populations. Reintroductions may be accomplished by transplanting adults from extant populations or through the release of propagated individuals; the latter is the only technique that is being proposed under this plan. Adult translocation would likely only occur in the event of a severe threat that would likely result in significant adult mortality (e.g., imminent infestation by zebra mussels from a known upstream source) or if we determine that propagation may not be a feasible means of establishing reintroduced populations. The reintroduction of winged mapleleaf into areas of historical habitat will be considered when the Service and its partners have established that the conditions that led to the extirpation of the species have been eliminated or improved (e.g., water chemistry, flow, etc.) to the extent that populations are likely to grow and persist.

CONTROLLED PROPAGATION

Each action carried out under this plan, including initial trials and subsequent reintroduction attempts, will require detailed site-specific plans before implementation. In general, any party proposing to conduct controlled propagation of winged mapleleaf must abide by the following guidelines:

- complete a detailed plan outlining the expertise, facilities and methodology, source of stock (fish hosts and mussels), and disposition of progeny;
- provide justification for the work, including benefits to the species, likely impacts to source populations and to mussel communities and habitats at reintroduction sites;
- obtain all necessary state and federal permits;
- describe all necessary precautions to be taken to prohibit the potential introduction or spread of diseases and parasites into controlled environments or suitable habitat and to minimize the likelihood of killing or harming wild individuals during augmentation or reintroduction;
- conduct all activities in a manner that will prevent the escape or accidental introduction of individuals outside of their historical range;
- keep detailed notes and records of life history observations, fecundity, survival and mortality, water chemistry, seasonality, identity of wild individuals used for propagation,

and any other conditions/observations important to successful propagation of these species, and an explanation of fish management guidelines for any fish to be used for propagation and how those guidelines would comply with state regulations (e.g., use of disease-free fish) and allow for the conservation of native fish stocks.

POPULATION AUGMENTATION OR REINTRODUCTION

To protect genetic integrity, biological diversity, and to avoid conflicts, all reintroduction activities will be coordinated with all affected Recovery Partners (i.e., U.S. Fish and Wildlife Service field and regional offices, other affected federal or state agencies, riparian landowners, and other potentially interested and affected members of the public.

Partners wishing to plan, sponsor, or conduct specific reintroduction actions will produce a Site Reintroduction Plan (Site Plan) before conducting any activities. Site Plans will be developed and distributed to the appropriate FWS Field and Regional Office(s) as early as practicable before the subject activities are implemented. Collection of gravid females, successful production of progeny, number of progeny produced, etc. is difficult to predict; Site Plans, however, should include as much information as possible, including:

- the exact location where animals are to be introduced;
- status of the target species at the site and why reintroduction is necessary to augment or introduce the species;
- an Alternatives Analysis (see Alternatives, below);
- relationship of the reintroduction site to other populations of the target species;
- current habitat conditions at the reintroduction site;
- possible limiting factors at the reintroduction site, including invasive species (e.g., zebra mussels);
- source of the animals for reintroduction (adults, juveniles -- hatchery-produced or wild);
- source of the stock (location and drainage);
- monitoring plan and responsibilities;
- cooperating and responsible partners;
- identification of potentially interested and affected members of the public;
- a copy of all appropriate permits; and,
- any other pertinent information.

The Service will notify all Recovery Partners, and any other affected private or public entity identified by the Partners, of planned reintroduction activities and will provide them with the Site Plan at least 20 days before relocating or releasing animals in the wild. Sites Plans are subject to the final approval of the Field Supervisor in the Service's Twin Cities Field Office, which is the national lead office for the recovery of this species.

Site Selection

Sites for reintroduction activities should be selected based on criteria identified above, including historical and current distribution of the species, habitat conditions, and past, present or future threats. Selected sites should be used and monitored for a period of at least ten years or until there is evidence of success or failure. Concentrating efforts at a site will reduce monitoring costs. The species' recovery plan (U.S. Fish and Wildlife Service 1997) may suggest additional monitoring to assess the status of augmented or reintroduced populations relative to the recovery criteria.

Monitoring

Monitoring is critical to determine whether or not reintroduction activities are successful, whether future efforts should be carried out, and whether specific changes should be made to increase the likelihood of success or to improve efficiency. The facilities and/or Partner conducting the release are responsible for developing and implementing a monitoring plan, unless otherwise specified in the Site Plan. Regardless, the Site Plan should clearly state who is responsible for monitoring.

At a minimum, the monitoring plan shall include at least three years of annual monitoring, beginning the year after reintroduction or augmentation is carried out, with at least one additional year after year ten.

Monitoring reports will be prepared and distributed to all affected partners – at least those partners and potentially affected parties identified in the Site Plan.

Stock

The use of adults for propagation shall only be done after an assessment of their potential affects to the source populations. The section below contains a brief assessment of the potential for affecting the two populations that we are currently using as sources – St. Croix River and Saline River. Where feasible, removal of mussels for translocations should affect less than 5% of the donor population. Hatchery progeny used for reintroduction should come from parental stock in the drainage nearest to the reintroduction site, whenever possible, but the best available genetic information should also be assessed. The Service and its *partners* may also consider factors other than inter-population distance (e.g., similarity of habitat, host fish communities, etc.) when deciding which population would be most appropriate as a source for a reintroduction site.

St. Croix River

Hornbach et al. (2010) estimated the population of winged mapleleaf at 13,000. We typically collect less than ten brooding females each year for use in propagation, but may collect and use 30 individuals per year. If we assume an equal sex ratio of 1:1 and that one-quarter to one-half of all females are mature[4] then 30 brooding females may only include about 1-2% of the mature females in the St. Croix River each year. It seems unlikely that this would have a significant impact on the population.

Saline River

The Service and its partners have not completed population estimates on all the mussel beds inhabited by winged mapleleaf in the Saline River. Therefore, it is not possible to accurately estimate the overall population size. However, based on population estimates from beds that are inhabited by winged mapleleaf, it is reasonable to assume that the population exceeds 25,000 (Davidson 2012, pers. comm.). We typically collect six to 12 brooding females for use in propagation, but this number may increase to 30 individuals per year as the Duck River reintroduction effort increases. If we assume an equal sex ratio of 1:1 and that one-quarter to one-half of all females are mature[5], 30 brooding females would include one percent or less of the mature females in the Saline River each year. It seems unlikely that this would have a significant effect on the population.

REPORTING

Recovery Partners conducting propagation trials, reintroduction releases, or monitoring studies will provide an annual report of activities to the FWS[6] and other involved partners, including:

- a brief description of the activities, including objectives and status;
- list of cooperators, if any;
- activities conducted or obstacles to achieving monitoring, propagation, or reintroduction efforts; and,
- a brief description of the status of targeted A/R populations, if known.

GENETIC CONSIDERATIONS

Genetic analyses have confirmed that the individuals that comprise the five populations of winged mapleleaf belong to the same species (Hornbach et al. 1998, p. 6, Serb and Harris 2003, p. 8, Hemmingsen 2008, p. 23), but pronounced genetic distances have developed among the

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[4] The minimum shell length and number of annuli found for brooding females by Heath et al. (2000) was 65 mm and eight, respectively. Of the 109 winged mapleleaf collected during searches by hand in the St., Croix River in 2011, 48% exceeded these measurements.

[5] The minimum shell length and number of annuli found for brooding females by Heath et al. (2000) was 65 mm and eight, respectively. Of the 109 winged mapleleaf collected during searches by hand in the St., Croix River in 2011, 48% exceeded these measurements.

[6] Annual reports will be submitted, at a minimum, to the endangered and threatened species chief in U.S. Fish and Wildlife Service (Service) Region 3 and to Ecological Services field offices, other regional offices, and to partners, as appropriate. Copies of annual reports will be kept on file at the Service's Twin Cities (Minnesota) Ecological Services Field Office in Bloomington, MN. For addresses of USFWS field offices, see Appendix I.

populations in approximate proportion to their geographic separation (Hemmingsen 2008, p. 58). We assume that these distinctions are representative of local adaptations. Therefore, with the exception of progeny originating from the Saline River and Ouachita River we intend to keep offspring from different source populations separate. Given the connectivity of the Saline River to the Ouachita River, we will consider individuals in these two rivers to be genetically similar and will treat them as belonging to a single lineage unless future analyses support keeping these two populations separate. Genetic data is unavailable for extirpated populations, so individuals from the closest geographical region will be used for brood stock in reintroduction efforts.

One of the purposes of the propagation program is to reduce or alleviate the loss of genetic diversity within drainages. Therefore, all possible measures to maintain or increase genetic diversity will be implemented based on the genetic analyses cited above. Past and current measures include: (1) collecting brood stock from multiple locations within each river, if feasible; (2) genetic sequencing of brood stock; and, (3) controlled release of captive-reared young at multiple locations in reintroduction rivers. Future measures that will be implemented as needed include ensuring that individual brood stock are not used during multiple propagation efforts.

Numbers of Broodstock

Adequate numbers of broodstock must be used to ensure that reintroduced populations reflect the genetic diversity of their source population (Roe 2010). The progeny of ten and thirty contributing founders, for example, would be expected to retain 95% and 98%, respectively, of the source population's heterozygosity (Frankham et al. 2009). In addition to heterozygosity, allelic diversity – including the frequency of rare alleles – may also be an important consideration. Marshall and Brown (1975 - cited in Frankham et al. 2009) "recommended that the number of founders be sufficient to obtain, with a 95% certainty, all the alleles with a frequency greater than 0.05" – at least 30 founders would be needed to meet this recommendation – more founders would be needed to ensure that alleles present at even lower frequencies are captured (Frankham et al. 2009).

To ensure that at least 30 females contribute relatively equal numbers of subadults to each reintroduction effort, it may be necessary to use more than that to infest host fish. We will eventually assess the genetics of winged mapleleaf found in reintroduction areas, but several years will elapse before we can determine the genetic composition of reintroduced populations. In addition, typical propagation methods do not facilitate determining the number of progeny produced by each individual female. It may take several years to find sufficient numbers of broodstock. For example, in recent years, an average of 4-5 brooding females have been found and used for infesting host fish from the St. Croix River – ten in good years; ten have typically been used in the Southern effort, but unexpected events have precluded any propagation in some years.

Basic Protocols

Each site plan will describe basic protocols to ensure that the above genetic considerations are addressed and will also include the following, as appropriate:

- To allow us to track and evaluate genetic information (e.g., relatedness) of females used as broodstock, we will take genetic (e.g., buccal swab) samples from each female used to infest host fish. These samples will be analyzed, based on available funding. Results of genetic analyses will be kept on file at the Twin Cities Field Office, U.S. Fish and Wildlife Service, 4101 American Blvd. E., Bloomington, MN 55425 and at other locations, as appropriate.

- To avoid using any individual winged mapleleaf for propagation more than once, gravid mussels used to produce juveniles will be uniquely marked and returned to the approximate point of capture.

- All actions will be carefully documented in annual reports. These reports will contain the identifying numbers of any mussels used for propagation or that are released or translocated. Reports will include any information or observations related to the success or failure of propagation activities, with information as specific as possible regarding each individual female used for broodstock. This information may help us to evaluate the relative contribution of each female to the number of progeny produced for release.

RISKS

The USFWS controlled propagation, augmentation, and reintroduction policy (56 Federal Register 56916-56922) recommends assessing the following ecological and genetic risks associated with captive propagation, augmentation, or reintroductions.

1. *Removal of natural parental stock that may result in an increased risk of extinction by reducing the abundance of wild individuals and reducing genetic variability within naturally occurring populations* - The number of propagated individuals that will eventually be released back into the wild will far outweigh the number of individuals removed from the wild for brood stock. When gravid females are collected from the wild, fish hosts can be infested with glochidia and juveniles can be reared at significantly higher survivorship rates than those estimated in the wild. When individuals are reared to larger sizes for release, substantially more winged mapleleaf can survive to sexual maturity and contribute to population growth. In addition, individuals used for broodstock are typically returned to the habitats in which they were found within several days of their removal, as recommended by Hoftyzer et al. (2008, p. 1221), and rarely show signs of ill effects. Measures to avoid reducing genetic variability are addressed in the ***Genetic Considerations*** section.

2. *Equipment failures, human error, and other potential catastrophic events that may cause the loss of some or all of the population being held or maintained in captivity or cultivation* - The maintenance of winged mapleleaf at more than one facility reduces the potential extent of adverse effects that could occur as a result of a catastrophic event at any single facility. Limiting the number of facilities reduces the potential for human error and other issues which may result from the involvement of unqualified personnel. Natchitoches National Fish Hatchery (NNFH) and Genoa National Fish Hatchery (GNFH), for example, have staff trained and experienced in the care and handling of winged mapleleaf. GNFH has an alarm system, two emergency life support systems and a back-up generator on the mussel building to abate the risk of power failure. Protocols are in place with the staff to deal with emergency situations in which these safeguards fail. GNFH maintains separate filtration, fish culture equipment, temperature control and aeration for tanks to prevent the spread of disease and to abate power outage risks. Unauthorized disturbance of animals at these facilities is prevented by restricted access and secured housing. Measures are in place to prevent individuals from escaping confinement of aquariums and raceways. These measures include: maintaining sufficient freeboard (i.e., minimum of 18 inches above waterline) in raceways, securing aquarium lids, and securing covers on outdoor raceways.

   At Missouri State University, winged mapleleaf are cultured for the first few months in laboratory recirculating upweller systems. Older (>5 mm) animals are moved to larger scale upwellers, either floating or in raceways, at the Kansas City Zoo. Access to the culture facilities is restricted. The systems are checked at least once daily, including weekends (C. Barnhart, Missouri State University, pers. comm. 4 November 2012).

   Each facility will also strive to minimize the density of mussels in any propagation unit to further ensure that any event will not lead to catastrophic loss of mussels.

3. *The potential for an increased level of inbreeding or other adverse genetic effects within the populations that may result from the enhancement of only a portion of the gene pool*- Measures to address this risk are identified in the ***Genetic Considerations*** section.

4. *Exposure to novel selection regimes in controlled environments that may diminish a species' natural capacity to survive and reproduce in the wild* – This risk is diminished by the use of wild broodstock. Changes that may occur over multiple generations of captive breeding would not be an issue as long as we continue to use only unique wild broodstock. Development of a captive broodstock is not planned. There is some potential for selection to occur during the "artificial" infestation of host fish (Hoftyzer et al. 2008, p. 1221).

5. *Genetic introgression, which may diminish local adaptations of the naturally occurring populations* - This risk is eliminated by housing Ozark Hellbenders based on river drainages (as outlined in the ***Genetic Considerations*** section).

6. *Increased predation, competition for food, space, mates, or other factors that may displace naturally occurring individuals, or interfere with foraging, migratory, reproductive, or other essential behaviors* – Stocking rates of propagated individuals will be based on historical density data (if available), the availability of habitat, and estimated carrying capacities. Therefore, increased predation risk and competition for resources should be minimized.

7. *Disease transmission* – Extensive effort has been undertaken at Genoa National Fish Hatchery and Natchitoches National Fish Hatchery to prevent and control disease transmission, and risks have been addressed to the greatest extent possible. At GNFH, for example, each lot of fish is tested twice annually to assure that stock for propagation remains disease free. Any lot of fish that tests positive for a bacterial, viral or parasitic agent is destroyed and a new lot is certified clean before it can be brought on station. Tanks are thoroughly disinfected after each batch of fish is removed. Fish hauling trucks are disinfected after returning from stocking trips and all dive gear is also disinfected between field trips.

ALTERNATIVES ANALYSIS

Controlled Propagation

Controlled propagation requires the temporary removal of reproductively mature individuals from the wild for propagation in captivity. Risks include mortality during collection and in the hatchery, failure to spawn, mortality of larvae and young in the hatchery, mortality of reintroduced juveniles, and lack of knowledge of environmental requirements of the species in the hatchery and at reintroduction sites. Controlled propagation may become an important tool to save winged mapleleaf from eventual extinction due to acute and chronic threats and it is the desired of the options under consideration in this plan.

Direct Translocation

Collection and translocation of adult mussels into suitable habitat, or to augment a declining population, requires less intervention than controlled propagation. Risks involve potential mortality during collection and relocation, reduction in size of parental population, and lack of knowledge of environmental requirements of the species in the hatchery and at reintroduction sites. This plan would not authorize translocation of adult winged mapleleaf to reestablish populations, although we may later evaluate that as an option if propagation is not successful.

'Do Nothing'

Foregoing attempts to propagate and reintroduce winged mapleleaf could hinder our ability to recover the species. Winged mapleleaf formerly occurred in 41 rivers and 16 states, but is now reduced to five populations if four states. Two of the five extant populations may be viable, but the other three either face clear and imminent threats or are of unknown viability. If we waive attempts to reintroduce the species into historically occupied habitats we would be limited to efforts to protect and increase the viability of surviving populations. Protection and restoration activities (e.g., reducing sedimentation, preventing zebra mussel infestation, etc.) are critical to the recovery of winged mapleleaf, but we should further reduce the likelihood of the species' extinction by increasing the number and distribution of populations while minimizing risks to existing populations.

CONTROLLED PROPAGATION PLAN REVIEW

This Plan is a working document that is subject to modification based on results of current and future research and recovery activities involving mollusk propagation, augmentation, or reintroduction. Recovery Partners are encouraged to provide comments and suggestions to Mr. Phil Delphey, U.S. Fish and Wildlife Service, Bloomington, MN 55425 (see Appendix I for contact information). FWS will conduct an annual review of the Plan and will incorporate new information, protocols, etc. as they become available.

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APPENDIX I: USFWS ECOLOGICAL SERVICE FIELD OFFICE CONTACTS

Arkansas
Chris Davidson
U.S. Fish and Wildlife Service
110 South Amity Road, Ste. 300
Conway, AR 72032
chris_davidson@fws.gov

Minnesota
Phil Delphey
U.S. Fish and Wildlife Service
4101 American Blvd. E.
Bloomington, MN 55425
phil_delphey@fws.gov

Missouri
Andy Roberts
U.S. Fish and Wildlife Service
101 Park DeVille Drive
Columbia, Missouri 65203-0057
andy_roberts@fws.gov

Tennessee
Stephanie Chance
U.S. Fish and Wildlife Service
446 Neal Street
Cookeville, TN 38501
stephanie_chance@fws.gov

Wisconsin
Nick Utrup
U.S. Fish and Wildlife Service
2661 Scott Tower Drive
New Franken, WI 54229
nick_utrup@fws.gov

U.S. FISH AND WILDLIFE SERVICE
5-YEAR REVIEW OF WINGED MAPLELEAF

Current Classification: ___Endangered___

Recommendation resulting from the 5-Year Review
____ **Downlist to Threatened**
____ **Uplist to Endangered**
____ **Delist**
_X__ **No change is needed**

Appropriate Recovery Priority Number: _2C__

Review Conducted By: Phil Delphey

FIELD OFFICE APPROVAL:
Lead ~~Field Supervisor~~, U.S. Fish and Wildlife Service

Approve________________ Date 21 April 2015

REGIONAL OFFICE APPROVAL:
for **Assistant Regional Director, Ecological Services, U.S. Fish and Wildlife Service, Midwest Region**

Approve________________ Date 5/15/15

for **Cooperating Assistant Regional Director, U.S. Fish and Wildlife Service, Southeast Region**

Signature________________ Date 5/6/2015

for **Cooperating Assistant Regional Director, U.S. Fish and Wildlife Service, Southwest Region**

Signature________________ Date 5/12/15