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A comparison of genetic diversity between sympatric populations of the endangered Winged-Mapleleaf (*Quadrula fragosa*) and the Pimpleback (*Amphinaias pustulosa*) in the St. Croix River, U.S.A.

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Abstract: Assessing genetic variation in species of conservation concern is critical for developing sound recovery strategies. In this study, we compared sympatric populations of two related species, the endangered *Quadrula fragosa* (Conrad, 1836) and its common relative *Amphinaias pustulosa* (Lea, 1831), using standard genetic parameters such as allelic richness, heterozygosity, and effective population size. Our primary aim was to determine if a small population size and isolation from conspecifics had negatively affected the genetic diversity of this population of *Q. fragosa*. By comparing the endangered species to a related and sympatric, common species we can assess the rare species for genetic effects associated with reduced population size, and in addition, develop management targets for what a recovered *Q. fragosa* population looks like genetically. Examination of eight microsatellite loci indicated that *Quadrula fragosa* exhibited reduced genetic variation when compared to *A. pustulosa* at all measures, however, no evidence of a genetic bottleneck or inbreeding was discovered for either species. A comparison of known fish hosts and reproductive period for these two species point to competition for fish hosts as one possible explanation for the smaller population size of *Q. fragosa*. We discuss the implications of our findings for the conservation and management of freshwater mussels.

Key words: Unionidae, river, rare species, invertebrates, impoundment

The spatial distribution of populations can be an important factor affecting the genetic characteristics of a species. Rare or endemic species with limited geographic distributions tend to have lower levels of genetic variation than species with a more widespread distribution. These differences can arise due to the effects of natural selection, inbreeding, or drift, and the impact of these processes is greater on the small population sizes that are typical of most endangered species (Wright 1945, Lande and Barrowclough 1987). Additionally, rare species often consist of several small, isolated populations, which can also reduce levels of genetic diversity within populations and enhance differences among them due to restricted gene flow (Delaney *et al.* 2010). Comparisons between rare and widespread species (*e.g.*, Avise and Hamrick 1996, Frankham 1996, Cole 2003, Furches *et al.* 2013) can provide valuable information for conservation managers developing recovery plans for species of conservation concern. For example, in the absence of a meaningful comparison with a related species, interpreting the significance of measures of genetic diversity for rare species that have complex or poorly understood life histories can be problematic. In such situations distinguishing between low genetic variation due to life history attributes and low variation due to anthropogenic impacts such as habitat fragmentation may be critical for making effective management decisions (*e.g.*, Hoehn *et al.* 2007). A comparison of related rare and common species can factor out shared traits such as breeding system or life history that affect population genetic variation (Karron *et al.* 1988, Cole 2003), and, thereby, clarify interpretation of the results.

The Unionoida or freshwater mussels are among the most endangered faunas in North America (Haag 2012). Of the ~300 recognized species in North America, nearly 48% are considered to be of conservation concern (Ricciardi and Rasmussen 1999). The winged mapleleaf mussel, *Quadrula fragosa* (Conrad, 1836), is a federally endangered species that historically occurred in at least 34 rivers in the Mississippi, Tennessee, Ohio, and Cumberland River drainages of North America, but has suffered severe range reductions (USFWS 1991, Hove *et al.* 2012). The species is currently known from a total of five populations in Arkansas (Ouachita River and Saline River), Missouri (Bourbeuse River), Oklahoma (Little River), and Minnesota/Wisconsin (St. Croix River) (Hemmingsen 2008, Hove *et al.* 2012). Of the five known remnant populations, the most extensively studied occurs in a 20-kilometer stretch of the lower St. Croix River (Fig. 1). The St. Croix River is in a moderately to minimally disturbed watershed with high water quality, and is protected as a National Wild and Scenic River since 1968. Surveys of the lower stretch of the river between 1988 and 1992 for *Q. fragosa* located only 77 individuals. In recent years, recruitment to this population has been low; there has not been a large cohort recruited to the population since 1984 (USFWS 1997, Hove *et al.* 2012).

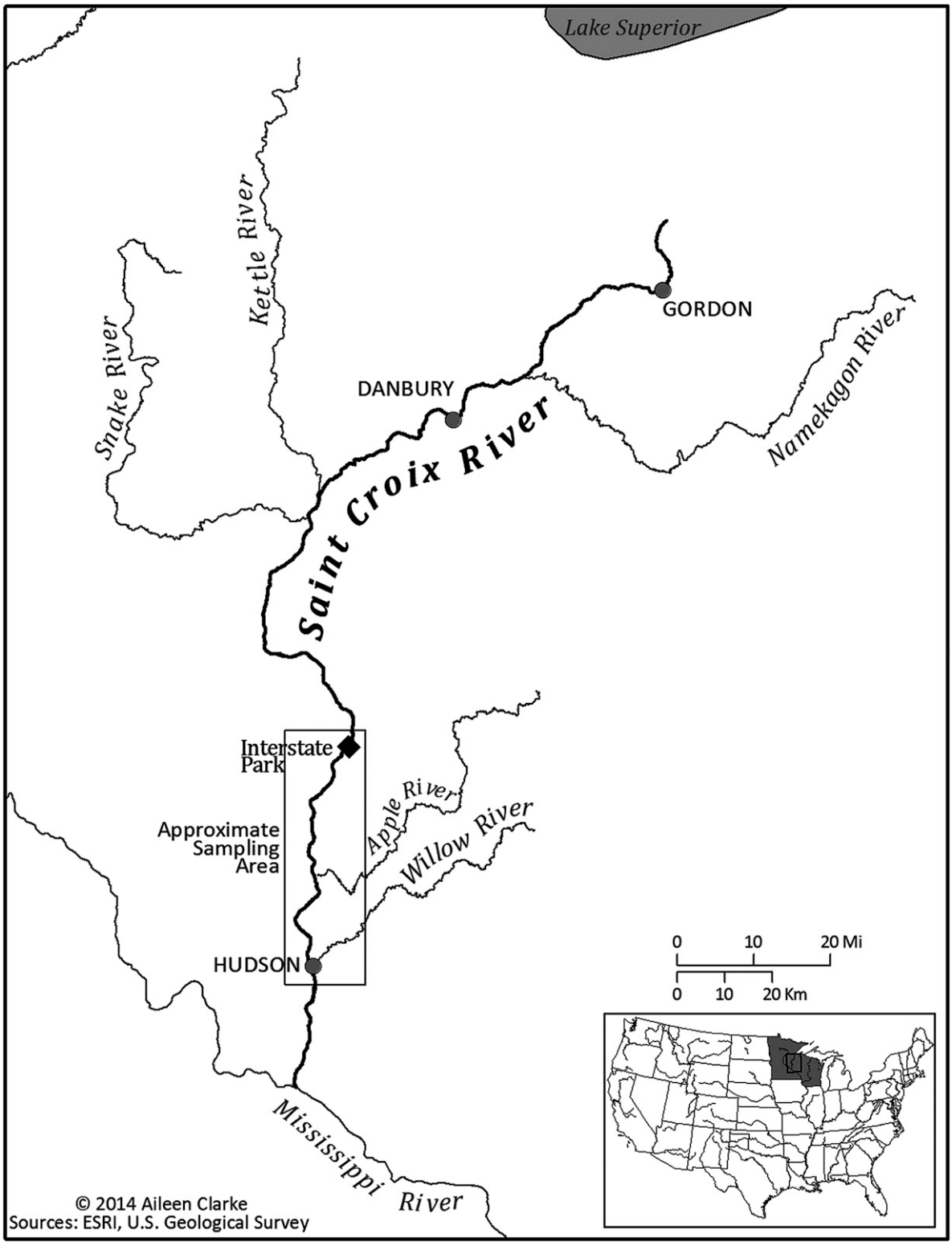

Figure 1. Map of St. Croix River including the approximate location of the section sampled for this study. Inset map indicates the location of the St. Croix River in the U.S.A.

In this study we compared the St. Croix River population of *Quadrula fragosa* and a sympatric population of a related, widely distributed common species, *Amphinaias pustulosa* (Lea, 1831) using microsatellite markers and standard measures of genetic diversity. These two species were formerly grouped together in the genus *Quadrula* Rafinesque, 1820, but phylogenetic analyses by Serb *et al.* (2003) indicated this large, monphyletic group of mussels included several lineages that corresponded to formerly recognized genera and have since been recognized as such (*e.g.*, Graf and Cummings 2007). Population surveys for mussels in the St. Croix River estimate the density of *Q. fragosa* individuals to be between 0.008–0.022/m^2 or ~13,000 individuals (Hornbach *et al.* 2010). Estimates for the density of *A. pustulosa* in the same reach were considerably higher, and were between 1.14–1.58/m^2 (D. Heath and D. Hornbach, pers. com.). Recovery plans for imperiled species often include goals for maintaining a particular number of populations, but do not include goals for the genetic characteristics of these populations. By comparing endangered species (*Q. fragosa*) to a closely related, sympatric common species (*A. pustulosa*) we may be able to develop management targets for what the genetic diversity of a recovered *Q. fragosa* population might look like. Due to its scarcity, we predicted that *Q. fragosa* would exhibit values for measures of genetic diversity that were typical of a smaller, less genetically diverse population than the non-imperiled *A. pustulosa*.

MATERIALS AND METHODS

Specimens of *Quadrula fragosa* were collected from the only portion of the St. Croix River known to harbor this species. Forty individual *Q. fragosa* were collected from the St. Croix River within ~3 km distance downstream from Interstate Park with the assistance of the USFWS. Samples for DNA extraction were collected using a non-destructive method (Henley *et al.* 2006), and genomic DNA was extracted in laboratory using the Gentra Puregene Tissue Kit (Qiagen) following manufacturers instructions for buccal cells. When combined with 13 samples previously analyzed by Hemmingsen *et al.* (2009), the total number of *Q. fragosa* individuals included in this study was 53. In addition, *Amphinaias pustulosa* samples were collected from the St. Croix River between Interstate Park and the town of Hudson, WI as part of a different study (Szumowski *et al.* 2012), and genomic DNA from 39 individuals included in that study were used for comparison to the *Q. fragosa* samples. Voucher and locality information for *A. pustulosa* samples are detailed in Szumowski *et al.* (2012).

In addition to the microsatellite markers developed for *Quadrula fragosa* previously used by Hemmingsen *et al.* (2009), additional polymorphic markers that were developed for *Q. fragosa* by Genetic Identification Services (GIS), Chatsworth, CA, were used in this study (Table 1). Polymerase chain reaction (PCR) amplification of microsatellite loci was carried out in 10 µl reactions using the BIOLASE™ PCR kit (Bioline, Boston, MA) and approximately 2 ng of genomic DNA. Reactions were performed in Eppendorf Master Cycler thermal cyclers under the following conditions: 94 °C/3min; [94 °C/40 sec.; 49–58 °C/40 sec.; 72 °C/30 sec.] × 35 cycles; 72 °C/4 min. PCR products were visualized on 1% agarose gels, and the size of the alleles were then determined via capillary electrophoresis at the Iowa State University DNA Facility using an Applied Biosystems 3730 DNA Analyzer.

Table 1. Microsatellite loci, primers, annealing temperature, repeat motif, number of alleles, observed heterozygosity (H_o) and expected heterozygosity (H_e) for *Quadrula fragosa* in this study.

Locus	Primers	Temp. °C	Motif	Size range (bp)	# alleles	H_o/H_e
Qf A130	Hemmingsen *et al.* 2009	58	TG	298–320	12	0.792/0.877
Qf C109	Hemmingsen *et al.* 2009	55	TATG	185–225	9	0.698/0.739
Pc C6	F 5′-gcagtgtatggcaatgaaca-3′ R 5′-gcgtaataacctgtgacctcc-3′	55	TACA	213–265	13	0.788/0.822
Pc C105	F 5′-ttgcatgtgtcacttcatactg-3′ R 5′-gcacctacctacctatctctcg-3′	49	TACA	172–214	10	0.604/0.570
Qf C6	F 5′-ccacattacacacatacacg-3′ R 5′-cgcctcaagacctctgac-3′	58	TACA	220–277	14	0.865/0.819
Qf C12	F 5′-gacggacagatgaaatagatgc-3′ R 5′-cttttgttgcgatgttagtcg-3′	58	TACA	248–320	15	0.904/0.865
Qf D5	F 5′-cgcattatcagacacagtgg-3′ R 5′-caaccatagtcacacttgagca-3′	56	TAGA	230–254	7	0.604/0.773
Qf D110	F 5′-tctgctggaactagacaggtg-3′ R 5′-cggataagaaagaagggacac-3′	58	TAGA	176–220	11	0.849/0.853

Raw data output was visualized and alleles called using GeneMarker® software (Softgenetics, State College, PA). Microsatellite genotypes were examined for the presence of null alleles using the software MICRO-CHECKER (van Oosterhout *et al.* 2004). Because null alleles can bias population genetic analyses, any loci that were identified as possibly including null alleles were excluded from later analyses.

Linkage disequilibrium and deviation from Hardy-Weinberg equilibrium were estimated using the program GENEPOP (Raymond and Rousset 1995). Genetic diversity of each population sample was summarized in several ways: allelic richness (*A*), or the number of alleles per locus, both uncorrected and rarefacted for sample size as calculated by HP-RARE (Kalinowski 2005); the unbiased expected heterozygosity (UH_e); the inbreeding coefficient (*F*); the effective number of alleles per locus (A_e); and Shannon's Information Index were calculated in GenAlEx 6.5 (Peakall and Smouse 2006). The linearized version of the Shannon's Information Index (as a measure of the effective number of alleles) was calculated following Jost (2006). The number of alleles per locus (uncorrected and rarefacted) for each population was compared using the Wilcoxan Signed Rank Test, and the Shannon's Information Index values for each population were compared using the *t*-test (Hutcheson 1970). Two different contemporary estimates of the effective population size (N_e) were obtained from the microsatellite genotypes generated in this project for each species using approaches that are based on a single temporal population sample. One method is based on genetic disequilibrium, and is implemented in the program LDN_E (Waples and Do 2008), whereas the other uses an approximate Bayesian computation to estimate N_e and is implemented in ONeSAMP (Tallmon *et al.* 2008).

The range of *Quadrula fragosa* has declined dramatically in the last several decades and surviving remnant populations may have experienced reductions in size as well (USFWS 1997). Populations that experience a sharp reduction in size will often display a reduction in both allele numbers and heterozygosity. The program BOTTLENECK 1.201 (Cornuet and Luikart 1996) was employed to detect the genetic signature of population decline that would indicate a recent bottleneck. The microsatellite genotypes for both species were tested for evidence of excess heterozygosity using the Wilcoxon sign-rank test as implemented in BOTTLENECK under both the infinite alleles (IAM) and the step-wise mutation (SMM) models. In addition, the software calculated the distribution of allele frequencies, as bottlenecked populations often exhibit a rightward shift in the mode of allele frequencies (Luikart *et al.* 1998).

RESULTS

Of the 20 loci that were identified as polymorphic for *Quadrula fragosa*, 14 were also polymorphic for *Amphinaias pustulosa*. Of these 14 loci, six were removed from further analysis due to evidence of null alleles in either species resulting in a total of eight loci for cross-species comparison (Table 1). *Amphinaias pustulosa* exhibited a higher number alleles (uncorrected and rarefacted) than *Q. fragosa* at six of the eight loci examined (Table 2). The difference in the number of alleles was significant based on the Wilcoxon's Signed Rank Test ($p < 0.05$). At all other measures of allelic diversity, *A. pustulosa* exhibited higher values than *Q. fragosa* (Table 3). Although *Q. fragosa* exhibits lower allelic diversity, it is generally 70–75% of the diversity seen in *A. pustulosa*. The inbreeding coefficient indicated little reduction in heterozygosity for either species relative to that of a randomly mating population.

The estimates of N_e for each population are presented in Table 4. Both approaches used indicate that *Amphinaias pustulosa* has a larger effective population size (N_e = 919–2303) that is up to 25 times as large as *Quadrula fragosa* (N_e = 57–94).

Table 2. Uncorrected and corrected (rarefacted) number of alleles per locus at eight microsatellite loci for *Quadrula fragosa* and *Amphinais pustulosa* (Lea, 1831). Wilcoxon's signed-rank test indicates *A. pustulosa* has more alleles per locus ($P < 0.05$).

	Uncorrected Number of alleles			Corrected number of alleles		
Locus	***Q. fragosa***	***A. pustulosa***	**Sign**	***Q. fragosa***	***A. pustulosa***	**Sign**
QfA130	12	23	+	11.4	23	+
QfC109	9	6	-	8.5	6	-
PcC6	13	18	+	12.2	18	+
PcC105	10	13	+	9.9	13	+
QfC6	14	22	+	13.6	22	+
QfC12	15	12	-	14.5	12	-
QfD5	7	9	+	6.9	9	+
QfD110	11	21	+	10.9	21	+

Table 3. Measure of allelic diversity. Shannon Diversity compared using the *t*-test (Hutcheson 1970). * indicates significantly higher Shannon diversity ($P < 0.001$).

	Quadrula fragosa	*Amphinais pustulosa*
Avg. # Alleles	11.4 (0.94)	15.5 (2.3)
Expected heterozygosity [(2N / (2N-1)) * H_e]	0.797 (0.036)	0.848 (0.036)
Inbreeding Coefficient [(H_e - H_o) / H_e = 1 - (H_o / H_e)]	0.032 (0.033)	0.072 (0.028)
Effective # Alleles [1/(Σ pi^2)]	5.501 (0.49)	7.679 (1.38)
Shannon Information Index [-1 x Σ (pi x Ln (pi))]	1.922 (0.12)	2.22 (0.18) *
Effective # Alleles (linearized Shannon Index) exp [-1 x Σ (pi x Ln (pi))]	6.83	9.21

Neither *Q. fragosa* nor *A. pustulosa* displayed a significant excess of heterozygosity under either the IAM or the SMM models that would be indicative of a recent genetic bottleneck and neither species displayed a mode-shifted distribution of alleles that is typical of a recent bottleneck (Table 4).

DISCUSSION

Although molecular data have been generated for several unionid mussels that are endangered or of special concern to aid in specimen identification (*e.g.*, Roe *et al.* 2001, Boyer *et al.* 2011) or for the purpose of species delimitation (*e.g.*, Roe and Lydeard 1998, Inoue *et al.* 2014), our study is the first to provide a direct comparison of genetic diversity between closely-related, sympatric mussel species where one species is endangered and the other is common or widespread. In fact, as far as we were able to determine, this is the first study of its kind in animals, despite the fact that many similar comparative studies have been performed in plants (*e.g.*, Viana e Sousa and Lovato 2010, Takahashi *et al.* 2011, Chung *et al.* 2012).

Amphinaias pustulosa has a higher genetic diversity than the sympatric *Quadrula fragosa* population for all comparisons made. This is not surprising based on the greater population densities documented for *A. pustulosa* in the St. Croix River (D. Heath and D. Hornbach, pers. com.), as the correlation between population size and genetic diversity of a population is well established (Frankham 1996, Montgomery *et al.* 2000). Reductions in the range size of *Quadrula fragosa* were noted as early as 125 years ago and throughout the early 20th century (USFWS 1997). The possibility that the lower observed genetic variation of *Q. fragosa* might have been due to a dramatic reduction in population size was investigated, but the genetic signature of a population bottleneck was not found. Bottlenecks are transient events and are thought to be detectable less than 4.0N_e generations into the past (Luikart and Cornuet 1998) after which time a new mutation-drift equilibrium will be established in the population. Based on estimates of time to sexual maturity of other species of *Quadrula* Rafinesque, 1820 (3 to 9 years of age; Haag and Staton 2003) a mean generation time of six years indicates that if the early 20th century reductions were severe enough to result in a bottleneck of *Q. fragosa*, the signature of such an event should still be detectable.

As no evidence of a bottleneck was detected in *Quadrula fragosa*, other factors must, therefore, explain the lower census size, genetic diversity and comparatively small N_e observed for this species. One alternative is that a small census population size and low N_e is "normal" for *Q. fragosa;* that is, although contraction of the species range has certainly occurred (Hove *et al.* 2012), perhaps local population sizes have been

Table 4. Sample size, estimated effective population size calculated using LDN_e and ONeSAMP and 95% confidence intervals and credible limit respectively. Results of BOTTLENECK analysis: Wilcoxon sign-rank test for evidence of heterozygote excess and allele frequency distribution.

	Quadrula fragosa	*Amphinais pustulosa*
Sample size	53	39
LDN_e		
Est. N_e	93.8	2302.6
95% CI (parametric)	66.2–150.3	275.9–∞
95% CI (jackknife)	62.4–168.8	175.6–∞
ONeSAMP		
Est. N_e	57.0	919.0
95% CL	51.9–62.6	365.6–3998.1
BOTTLENECK		
Wilcoxon sign-rank *P*-values (IAM, SMM)	0.098, 0.098	0.156, 0.994
Allele Distribution	normal	normal

small historically. Factors such as fluctuations in population size, mating system dynamics (*e.g.*, inbreeding, low host availability), or metapopulation structure involving local extinctions and recolonizations (Pimm *et al.* 1989, Hedrick 1996) could result in such small populations. These factors have not been extensively examined for freshwater mussels in general, but the available information on *Q. fragosa* can be used to attempt to eliminate individual factors.

Past surveys indicate that *Quadrula fragosa* has been uncommon or rare for approximately the last century (USFWS 1997). *Amphinaias pustulosa* has generally been considered to be one of the most common species of *Quadrula* over the same period (i.e., Oesch 1984), and no information indicating dramatic fluctuations of these populations was found. Current *Quadrula fragosa* populations are separated by great distances, the nearest known population is in the Bourbeuse River in Missouri > 1000 river km from the St. Croix population. The large distances between populations imply that the extant populations are genetically isolated, and, thus, no metapopulation structure exists. However, other species of *Quadrula* have shown low genetic differentiation across equally great distances (Berg *et al.* 1998) and further investigation is warranted, as a range-wide study of the genetic structure of remaining *Q. fragosa* populations has yet to be conducted. The small census and effective population size estimates suggest the possibility of inbreeding depression, yet this conclusion is not supported by the genetic data as the inbreeding coefficient which indicates only a 3% reduction in heterozygosity relative to a randomly mating population. Because of the high probability that sibling larval mussels attach to, and likely excyst from, the same host fish at the same time and location, inbreeding is likely to be common among freshwater mussels due to the close spatial arrangement of related individuals in a mussel bed. This feature of their natural history is common to both *Q. fragosa* and *A. pustulosa* however, and, therefore, is unlikely to explain the differences observed between these two species. Available information indicates little or no recent recruitment of individuals for the St. Croix *Q. fragosa* population (USFWS 1997, Hove *et al.* 2012).

Like all unionid mussels, both *Quadrula fragosa* and *Amphinaias pustulosa* are dioecious and have a life history that includes a specialized larva known as a glochidium that must attach to a fish host in order to complete metamorphosis to the juvenile stage. The available information on substrate composition and water depth and velocity preferences for *Q. fragosa* indicates its habitat requirements are similar to the larger mussel community in the St. Croix River (including *A. pustulosa*) (Hornbach *et al.* 1996, USFWS 1997). Although the life histories of these two species are very similar, there are differences in reproductive ecology between *Q. fragosa* and *A. pustulosa.* One notable difference between *Q. fragosa* and *A. pustulosa* is the time of year that female mussels are gravid and release their larvae. Mussels in the genus *Quadrula* are short-term brooders (Haag 2012); typically, eggs are fertilized in the spring or summer and brooded in the gills of the female until mature ~2–6 weeks, and once mature, the larvae are soon released (Yokely 1972). Most of the species formerly placed in the genus *Quadrula,* brood glochidia during the 16-week period between May and August (Hove *et al.* 2012). In the St Croix River, *Amphinaias pustulosa* and *Theliderma metanevra* (Rafinesque, 1820) both display this typical May to August brooding pattern. Gravid *Q. fragosa* however, have only been observed brooding between 8 September and 8 October in the St. Croix River, with the percentage of gravid females peaking in late September (1997–2010). *Quadrula fragosa* is currently the only Mississippi River Basin mussel species of any genus that has been reported as having such a truncated fall brooding period (Hove *et al.* 2012), releasing glochidia 2-3 months later than its relatives (Heath *et al.* 1998, USFWS 2000)

Another discernable difference between *Quadrula fragosa* and *Amphinaias pustulosa* is the number and species of host-fish utilized by their larvae. Hove *et al.* (2012) conducted host suitability trials on 67 potential hosts of *Q. fragosa*, representing 72% of fish species and 79% of fish families native to mid- and lower reaches of the St. Croix. In laboratory trials, the only fish species on which *Quadrula fragosa* larvae have been shown to successfully transform to juveniles are blue catfish, *Ictalurus furcatus* (Valenciennes, 1840) and channel catfish, *Ictalurus punctatus* (Rafinesque, 1818) (USFWS 2000, Hove *et al.* 2002, Steingraeber *et al.* 2004, Hove *et al.* 2012). Furthermore, blue catfish are not native to the St. Croix River, and their one-time presence was the result of an introduction (Fago and Hatch 1993); they have not been reported from the St. Croix River since they were stocked in 1977 (Phillips *et al.* 1982). In contrast, although no systematic study of the hosts of *A. pustulosa* has been conducted (M. Hove, pers. comm.), *A. pustulosa* larvae have been documented using flathead catfish, *Pylodictis olivaris* (Rafinesque, 1818), black bullhead, *Ameiurus melas* (Rafinesque, 1820), and channel catfish (*I. punctatus*) as hosts (Howard 1913, Coker *et al.* 1921), with brown bullhead, *Ameiurus nebulosus* (Lesueur, 1819), white crappie, *Pomoxis annularis* (Rafinesque, 1818), and shovelnose sturgeon, *Scaphirhynchus platorynchus* (Rafinesque, 1820) as additional potential hosts (Surber 1913, Coker *et al.* 1921). It seems clear, based on only a few studies that *A. pustulosa* uses a larger number of hosts than *Q. fragosa*. It has been shown that host number and utilization of different hosts can affect the population structure and genetic diversity of parasites (Blouin *et al.* 1995, Ericson *et al.* 1999). To date, no studies have examined the effect of host number or host utilization on the genetic diversity and connectivity of freshwater mussel populations.

Since 1974, there has been a loss of fish diversity in the St. Croix River (Fago and Hatch 1993). These species include

the shovelnose sturgeon, goldeneye, *Hiodon alosoides* (Rafinesque, 1819), skipjack herring, *Alosa chrysochloris* (Rafinesque, 1820), pallid shiner, *Notropis amnis* (C. L. Hubbs, 1951), river shiner, *Notropis blennius* (Girard, 1856), weed shiner, *Notropis texanus* (Girard, 1856), and the mud darter, *Etheostoma asprigene* (S. A. Forbes, 1878 in Jordan, 1878). Of the fish species no longer found in the St. Croix River, only one, the shovelnose sturgeon is known to be a host for species of the genus *Quadrula* (Surber 1913, Coker *et al.* 1921), although other minnow species and at least one other darter species are known as hosts or are potential hosts for other species of *Quadrula* in different drainages (i.e., Yeager and Neves 1986). The loss of the shovelnose sturgeon, a likely host for *Amphinaias pustulosa,* from the St. Croix River, could have increased competition between *A. pustulosa* and *Q. fragosa* for channel catfish hosts. The partitioning of host resources by different species has not been well studied in freshwater mussels (but see Rashleigh and DeAngelis 2007, Haag 2012). Niche theory predicts that two species cannot use the same resource in the same way and as a result the inferior competitor will be denied access to that resource. The truncated, later brooding period of *Q. fragosa* relative to other *Quadrula* species could be evidence of an inferior competitors' response to competition for the same host resources. This shift in the reproductive period of *Q. fragosa* to later in the year could cause the observed lower recruitment in *Q. fragosa*. Low or reduced recruitment would be a result of less frequent encounters with primary host fishes, utilization of secondary (inferior) hosts, or environmental conditions less conducive to larval development on host fish or successful settlement post excystment. Determining which, if any of these putative causes are responsible for the low abundance of *Q. fragosa* in the St. Croix will require additional research on host preferences of *A. pustulosa*, and host preferences and brooding period of *Q. fragosa* in other extant populations.

Conservation implications

Quadrula fragosa exhibits both lower genetic diversity and smaller population size than the more common *Amphinaias pustulosa.* However, the magnitude of the difference between the two species is much greater for metrics of population size than it is for measures of genetic diversity. *Quadrula fragosa* exhibits 73% of the allelic diversity and 94% of the expected heterozygosity of the more common *A. pustulosa;* in contrast, the census population size of *Q. fragosa* is only 2% and the effective population size estimates only 4–6% of *A. pustulosa. Quadrula fragosa* exhibits low levels of recruitment, which is a concern for the long-term survival of the St. Croix River population, one of only five known populations in the U.S. Because small populations are more vulnerable to loss of genetic diversity due to drift over time, we recommend that the St. Croix River *Q. fragosa* population should be monitored for evidence of genetic drift as well as signs of recruitment. One possible solution to the small population size would be supplementation of the St. Croix population with propagated individuals, although this would only represent a short-term solution if the lack of recruitment persisted. Prior to the initiation of the propagation of *Q. fragosa*, a genetic management plan should be developed for the breeding program to prevent loss of existing genetic diversity and increase the population size to reduce the effects of genetic drift (i.e., Princée 2001, Wang 2004). A range-wide evaluation of genetic diversity for *Q. fragosa* should also be conducted to confirm conspecificity and to estimate levels of genetic diversity and historical connections between the remaining populations.

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