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HOST FISHES FOR FOUR FEDERALLY ENDANGERED FRESHWATER MUSSELS (UNIONIDAE) IN THE APALACHICOLA-CHATTAHOOCHEE-FLINT BASIN

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ABSTRACT

We determined host use and glochidial metamorphosis success of four federally endangered mussel species from the Apalachicola-Chattahoochee-Flint River Basin. Fishes of 19-27 species in a total of 14 families were tested as potential hosts for each mussel species. Metamorphosis of *Pleurobema pyriforme* was observed only on six minnow species (Cyprinidae): *Cyprinella venusta, Nocomis leptocephalus, Notropis amplamala, N. lutipinnis, Pimephales promelas* and *Semotilus atromaculatus*, and metamorphosis success was >27% for all six species. Metamorphosis of *Medionidus penicillatus* was observed only on four darter species (Percidae): *Etheostoma inscriptum, E. swaini, Percina crypta,* and *P. nigrofasciata*, but metamorphosis success varied among species and was highest on *E. inscriptum* (40%) and *P. nigrofasciata* (39%). Metamorphosis of *Hamiota subangulata* was observed only on three species of black basses (Centrarchidae): *Micropterus cataractae, M. coosae,* and *M. salmoides*, and metamorphosis success was >78% on all three species. Metamorphosis of *Amblema neislerii* was observed on 23 species in seven families, indicating that this species is a host generalist, but metamorphosis success varied widely among species. These data augment existing host information for these species and provide a clearer picture of host breadth and the relative suitability of host species.

KEY WORDS *Amblema neislerii, Pleurobema pyriforme, Hamiota subangulata, Medionidus penicillatus,* life history, glochidia

INTRODUCTION

The Apalachicola-Chattahoochee-Flint Basin (ACF) in eastern Alabama, northwestern Florida, and western Georgia has a diverse mussel fauna of 32 species, including eight endemic species (Brim Box & Williams, 2000; Williams et al., 2008). Dams, water pollution, and more recently, heavy and contentious water withdrawal (Pierce et al., 1984; Ruhl, 2005) all have contributed to declines in the mussel fauna, and six species in the basin are listed as federally endangered or threatened. Life history information is needed for the conservation of these species.

Metamorphosis of mussel larvae (glochidia) to juvenile mussels usually requires parasitism on fishes, but host use varies from generalists that can use multiple fish species, to specialists that can metamorphose on only one or a few species (Kat, 1984; Barnhart et al., 2008; Haag, 2012). Despite the recent proliferation of host studies, host information remains lacking for many species and much available information is incomplete or potentially inaccurate (Haag & Warren, 2003). Accurate and comprehensive knowledge of host fishes is necessary for mussel recovery because suitable hosts must be available in sufficient numbers and at the appropriate time, and captive propagation programs also depend on this information (NNMCC, 1998; Haag & Williams, 2014). Host information exists for five of the federally listed ACF mussel species (O'Brien & Brim Box, 1999; O'Brien & Williams, 2002; Fritts et al., 2012a), but for most, only a limited number of potential hosts were tested and quantitative assessment of host suitability was not conducted. The objective of this study was to provide more comprehensive host information and measures of metamorphosis success on fishes for *Hamiota subangulata, Medionidus penicillatus, Pleurobema pyriforme,* and *Amblema neislerii.*

METHODS

Adult female mussels were collected from the ACF Basin from October 2010 to May 2012 (Tables 1-4). We inspected the gills of each mussel by slightly opening the valves with either an oyster knife for *A. neislerii* or with our thumbnail for *H. subangulata, P. pyriforme,* and *M. penicillatus*. Gravid females were identified by the presence of swollen gills. We collected five gravid mussels of a particular species on each sampling date and transported them in river water in aerated coolers to the Aquatic Science Laboratory at the University of Georgia (UGA) where they were held in dechlorinated municipal water at 17-20º C. Female *P. pyriforme* and *A. neislerii* released glochidia spontaneously in the laboratory within two days, and we extracted glochidia from *H. subangulata* and *M. penicillatus* within seven days of the original collection by flushing the gills with water through a syringe. We returned all females to their collection site within 28 days after collection.

Host suitability trials were conducted following Neves et al. (1985) and Fritts et al. (2012a). For each mussel species we tested the suitability of 19-27 fish species representing a total of 14 families (Tables 1-4). Most fishes were collected from rivers and ponds throughout Georgia using a nylon seine and backpack electrofisher, but some species were obtained from federal, state, or private hatcheries. Collections of wild fish focused on locations where mussels were not present to avoid potential acquired immunity from previous glochidial exposures (Dodd et al., 2006). Fish were transported to UGA in aerated coolers or hauling tanks and held in dechlorinated tap water until the experiments began. Fish nomenclature follows Page et al. (2013).

Glochidia viability for each female was quantified prior to host trials by adding a saturated sodium chloride (NaCl) solution to a subsample of glochidia and counting the number of glochidia that closed their shells in response (Lefevre & Curtis, 1912; Fritts et al., 2014). If viability was less than 90%, glochidia from that female were not used in the experiment. Glochidial viability was >90% for all five individuals of *H. subangulata, M. penicillatus,* and *P. pyriforme* but for only three individuals of *A. neislerii*. For each species, glochidia were pooled from all females with >90% viability then enumerated by placing them in a known volume of water, counting the number of viable glochidia in ten 200-µl subsamples, and extrapolating the total number of viable glochidia from the mean subsample count. A standardized inoculation suspension was then made by diluting to a concentration of 4000 viable glochidia L-1.

Potential hosts were exposed to glochidia by allowing the fish to swim for 15 min in the inoculation suspension. Glochidia were kept in suspension with vigorous aeration and a large-bulb pipette. Following exposure, fish were removed from the inoculation suspension, rinsed with fresh water to remove unattached or loosely attached glochidia that might influence estimates of metamorphosis success (see subsequent), and placed in holding chambers. All fishes were held in individual tanks (one fish per tank) in a modified recirculating aquaculture system (AHAB®; Aquatic Habitats Inc., Apopka, Florida, USA), except for Threadfin Shad (*Dorosoma petenense*). Threadfin Shad are difficult to hold in captivity, and all individuals of this species were held in a single 800-L round, communal tank to increase survival. Static water changes (50% renewal) were conducted daily after siphoning the bottom of the tanks (see subsequent).

The outflow from each AHAB tank was equipped with a 100-µm mesh filter cup to recover sloughed glochidia or metamorphosed juveniles released from the fish. Contents of the filter cups were examined one day after inoculation and every second day thereafter. Immediately prior to examining filter cups, water velocity in the tanks was increased for 15 min to flush glochidia and juveniles from the bottom of the tanks into the cups. The bottom of the large communal Threadfin Shad tank was siphoned daily through a large filter (20-cm diameter) equipped with 100-µm mesh to recover sloughed glochidia or metamorphosed juveniles. Contents of the filters were then rinsed into Bogorov trays and glochidia and juveniles were counted under a stereomicroscope. An individual fish was removed from a trial if three consecutive observations revealed no glochidia or juveniles, but prior to removal, the individual's gills were examined to insure that no encysted glochidia remained. The number of glochidia that attached to each fish during inoculation was estimated as the sum of sloughed glochidia and metamorphosed juveniles recovered throughout the duration of each trial. Percent metamorphosis (%M) for each individual fish was calculated by dividing the number of juveniles by the sum of glochidia and juveniles recovered from that fish.

Water temperature, dissolved oxygen (DO), and pH in the holding systems were measured daily with a Hydrolab Quanta (Hach Hydromet, Loveland, Colorado). Ammonia concentrations were monitored weekly using a LaMotte colorimeter (LaMotte Co., Chestertown, Maryland). Water chemistry parameters were maintained within suitable levels for aquatic organisms throughout all trials (DO=7.6-8.4 mg L^{-1}, pH=6.8-7.8, total ammonia= <0.1 mg L^{-1}). All fishes survived the host trials for all four mussel species.

RESULTS

Nineteen fish species in five families were tested as potential hosts for *P. pyriforme*. Metamorphosis was observed on six minnow species (Cyprinidae): *Cyprinella venusta, Nocomis leptocephalus, Notropis amplamala, N. lutipinnis, Pimephales promelas* and *Semotilus atromaculatus* (Table 1). *Cyprinella venusta* and *Semotilus atromaculatus* had the highest metamorphosis success at 58.3% and 52.7%, respectively, but metamorphosis was relatively high on all suitable hosts and confidence intervals overlapped broadly among species. The only minnow species that produced no juvenile metamorphosis was *Notropis texanus*. The glochidia of *P. pyriforme* were released as fragile pink conglutinates, similar to those described for other *Pleurobema* (e.g., Hove & Neves, 1994; Haag & Warren, 1997).

Twenty-four fish species in seven families were tested as potential hosts for *M. penicillatus*. Metamorphosis was observed on all four darter species (Perci-

TABLE 1

Fish species tested as potential hosts for *Pleurobema pyriforme*. HR denotes hatchery reared species; all other species were field collected. %M is the mean percent metamorphosis across all individual fishes of a species (N). Female mussels were collected from Sawhatchee Creek, Early County, Georgia, May 2011. Water temperature ranged from 22-23° C during the trial. The dashed line (—) indicates a non-host species (i.e., metamorphosis = 0%).

Fish species	N	Days to metamorphosis (hosts) or rejection (non-hosts)	%M ± 95% CI
Cyprinidae			
Cyprinella venusta	5	11-21	58.3 ± 13.2
Nocomis leptocephalus	5	11-21	38.0 ± 11.7
Notropis amplamala	4	13-24	38.5 ± 22.1
Notropis lutipinnis	5	11-21	27.7 ± 21.6
Notropis texanus	1	5	—
Pimephales promelas (HR)	2	11-21	38.5 ± 7.8
Semotilus atromaculatus	1	13-21	52.7
Catostomidae			
Hypentelium nigricans	1	3	—
Ictaluridae			
Ameiurus brunneus	1	3	—
Ameiurus natalis	1	3	—
Ictalurus punctatus	4	3	—
Centrarchidae			
Lepomis auritus	1	3	—
Lepomis cyanellus	4	3	—
Lepomis macrochirus	4	3	—
Lepomis punctatus	2	3	—
Micropterus salmoides (HR)	5	3	—
Percidae			
Etheostoma inscriptum	6	3	—
Percina crypta	2	3	—
Percina nigrofasciata	3	3	—

dae) tested: *Etheostoma inscriptum, E. swaini, Percina crypta,* and *P. nigrofasciata* (Table 2) Metamorphosis success was highly variable among darter species and between the two trials. Mean metamorphosis success on *Percina nigrofasciata* was only 19.7% in trial A (female mussels collected in May 2011) but 58.5% in trial B (female mussels collected in January 2012). Overall, metamorphosis success was highest on *Percina nigrofasciata* (39.1%; mean of both trials) and *Etheostoma inscriptum* (39.9%), and it was low on the other two darter species (mean metamorphosis: 2.5-7.9%). Two *Ichthyomyzon gagei* carried glochidia for 12 days after inoculation but no juveniles were recovered.

TABLE 2

Fish species tested as potential hosts for *Medionidus penicillatus*. HR denotes hatchery reared species; all other species were field collected. %M is the mean percent metamorphosis across all individual fishes of a species (N). Female mussels were collected from Sawhatchee Creek, Early County, Georgia, May 2011 (Trial A), and January 2012 (Trial B). Water temperature ranged from 19-20° C during Trial A, and 22-23° C during Trial B.

	N		Days to metamorphosis (hosts) or rejection (non-hosts)		%M ± 95% CI	
Fish species	A	B	A	B	A	B
Petromyzontidae						
Ichthyomyzon gagei	–	2	–	12		
Anguillidae						
Anguilla rostrata	–	1	–	1		
Cyprinidae						
Cyprinella trichroistia	–	4	–	9		
Cyprinella venusta	4	–	3	–		
Nocomis leptocephalus	4	–	3	–		
Notropis amplamala	–	4	–	5		
Notropis chalybaeus	–	4	–	3		
Notropis lutipinnis	4	–	3	–		
Pimephales promelas (HR)	2	–	3	–		
Semotilus atromaculatus	2	–	3	–		
Ictaluridae						
Ameiurus brunneus	1	–	3	–		
Ameiurus natalis	2	–	3	–		
Ictalurus punctatus	4	–	3	–		
Noturus leptacanthus	–	2	–	3		
Aphredoderidae						
Aphredoderus sayanus	–	3	–	6		
Centrarchidae						
Lepomis auritus	3	–	5	–		
Lepomis cyanellus	4	–	3	–		
Lepomis macrochirus	4	–	5	–		
Lepomis punctatus	2	–	3	–		
Micropterus salmoides (HR)	4	–	5	–		
Percidae						
Etheostoma inscriptum	4	–	20-30	–	39.9 ± 5.7	–
Etheostoma swaini	–	3	–	15-24	–	2.5 ± 4.9
Percina crypta	5	–	23-30	–	7.9 ± 12.8	–
Percina nigrofasciata	3	5	20-28	13-29	19.7 ± 16.4	58.5 ± 7.8

Twenty-six fish species in eight families were tested as potential hosts for *H. subangulata*. Metamorphosis was observed on all three species of black bass (Centrachidae) tested: *Micropterus cataractae, M. coosae,* and *M. salmoides* (Table 3). Metamorphosis success was consistently high on all three species (78-88%). *Lepomis cyanellus* and *L. gulosus* carried glochidia for 12 days after inoculation but no juveniles were recovered from either species.

Twenty-seven fish species in nine families were tested as potential hosts for *A. neislerii*. Metamorphosis

TABLE 3

Fish species tested as potential hosts for *Hamiota subangulata*. HR denotes hatchery reared species; all other species were field collected. %M is the mean percent metamorphosis across all individual fishes of a species (N). Female mussels were collected from Spring Creek, Miller County, Georgia, October 2010 (Trial A), and May 2011 (Trial B). Water temperature ranged from 19-21° C for both trials.

	N		Days to metamorphosis (hosts) or rejection (non-hosts)		%M ± 95% CI	
Fish species	A	B	A	B	A	B
Acipenseridae						
Acipenser brevirostrum (HR)	2	–	3	–		
Acipenser fulvescens (HR)	2	–	3	–		
Acipenser oxyrinchus	2	–	3	–		
Esocidae						
Esox niger	–	1	–	3		
Cyprinidae						
Cyprinella venusta	4	–	3	–		
Hybopsis rubrifrons	2	–	1	–		
Nocomis leptocephalus	4	–	1	–		
Notropis lutipinnis	4	–	3	–		
Pimephales promelas (HR)	4	–	3	–		
Semotilus atromaculatus	4	–	5	–		
Catostomidae						
Hypentelium nigricans	2	–	3	–		
Minytrema melanops	–	1	–	3		
Ictaluridae						
Ameiurus brunneus	2	–	3	–		
Ameiurus natalis	2	–	3	–		
Ictalurus punctatus	6	–	3	–		
Noturus leptacanthus	2	–	1	–		
Aphredoderus sayanus	–	3	–	3		
Centrarchidae						
Lepomis auritus	9	–	5	–		
Lepomis cyanellus	6	–	12	–		
Lepomis gulosus	–	5	–	12		
Lepomis macrochirus	7	–	8	–		
Micropterus cataractae	–	2	–	17-35	–	82.7 ± 3.3
Micropterus coosae	–	4	–	12-21	–	87.7 ± 3.2
Micropterus salmoides (HR)	9	1	16-30	19-35	78.2 ± 4.6	85.6
Percidae						
Etheostoma inscriptum	4	–	3	–		
Percina nigrofasciata	2	–	3	–		

was observed on 23 species in seven families (Table 4). Metamorphosis success was variable both among and within families. Metamorphosis success was consistently high only on darters (*Etheostoma fusiforme, E.*

TABLE 4

Fish species tested as potential hosts for *Amblema neislerii*. HR denotes hatchery reared species; all other species were field collected. %M is the mean percent metamorphosis across all individual fishes of a species (N). Females were collected from the Apalachicola River, Gulf County, Florida, May 2012. Water temperature ranged from 22-23° C during the trial. The dashed line (—) indicates a non-host species (i.e., metamorphosis = 0%). The asterisk (*) indicates that fish were held in a communal tank rather than individually, which precludes estimation of individual variability in metamorphosis.

Fish species	N	Days to metamorphosis (hosts) or rejection (non-hosts)	%M ± 95% CI
Clupeidae			
Dorosoma petenense	12	13-18	6.6*
Cyprinidae			
Nocomis leptocephalus	7	10-15	6.7 ± 3.7
Notropis amplamala	4	10-15	2.6 ± 0.7
Notropis lutipinnis	2	10-18	25.3 ± 46.7
Notropis texanus	3	10	—
Pimephales promelas (HR)	8	10-15	12.6 ± 12.3
Pteronotropis grandipinnis	3	10-18	46.2 ± 15.2
Ictaluridae			
Ameiurus brunneus	4	10-13	1.3 ± 2.3
Ameiurus melas	1	10-13	1.9
Ameiurus natalis	2	10-13	1.9 ± 1.2
Ictalurus punctatus	1	13-18	0.2
Noturus leptacanthus	1	3	—
Aphredoderidae			
Aphredoderus sayanus	1	5	—
Poeciliidae			
Gambusia holbrooki	1	10-13	8.3
Moronidae			
Morone saxatilis	9	10-18	28.0 ± 7.6
Centrarchidae			
Lepomis auritus	3	10-15	6.2 ± 3.5
Lepomis cyanellus	6	10-18	58.0 ± 7.4
Lepomis gulosus	6	10-18	12.0 ± 7.4
Lepomis macrochirus	2	10-15	9.3 ± 1.9
Lepomis marginatus	1	10-13	7.3
Lepomis megalotis	3	10-18	18.2 ± 23.8
Lepomis punctatus	1	10-13	3.4
Micropterus salmoides (HR)	6	10-15	8.1 ± 3.0
Percidae			
Etheostoma fusiforme	2	10-15	42.6 ± 9.1
Etheostoma inscriptum	5	10-18	56.5 ± 9.5
Etheostoma olmstedi	3	10-18	56.1 ± 4.1
Elassomatidae			
Elassoma zonatum	1	5	—

inscriptum, E. olmstedi; 43-57%). Similarly high metamorphosis was observed on a minnow (*Pteronotropis grandipinnis*, 46%) and a sunfish (*Lepomis cyanellus*, 58%), but metamorphosis success varied widely on other species in these families. Metamorphosis was consistently weak on catfishes (Ictaluridae, 0.2-1.9%), and no juveniles were recovered from a madtom (*Noturus leptacanthus*). Only four out of 27 fish species produced no juveniles. The mature glochidia of *A. neislerii* were released in a loose mucous matrix similar to that reported by O'Brien and Williams (2002).

DISCUSSION

Three of the mussel species in our study appear to be host specialists, and patterns of host use in these species were in close agreement with previous information about these species or related species. A single minnow species (*Pteronotropis hypselopterus*) was previously identified as a suitable host for *P. pyriforme*, but four additional minnow species were unsuitable (O'Brien and Williams, 2002). All other species of *Pleurobema* for which host use is known are specialists on minnows to varying extents (e.g., Yokley, 1972; Weaver et al., 1991; Hove & Neves, 1994; Hove et al., 1997; Haag & Warren, 1997, 2003; Layzer et al., 2003; White et al., 2008; Culp et al., 2009). However, specialists on minnows may use either a broad array of minnow species (e.g., *Fusconaia cerina, Pleurobema collina, P. oviforme, Theliderma metanevra*; Weaver et al., 1991; Hove & Neves, 1994; Haag & Warren, 2003; Fritts et al., 2012b) or only one or a few closely related species (e.g., *F. burkei, P. decisum, P. strodeanum, T. intermedia*; Yeager & Saylor, 1995; Haag & Warren, 2003; White et al., 2008). *Pleurobema pyriforme* clearly is a member of the former group based on its ability to metamorphose robustly on an array of minnow species. Another feature shared by many minnow specialists, regardless of the breadth of host use, is robust metamorphosis on *Cyprinella* (Haag & Warren, 1997, 2003; White et al., 2008; Fritts et al. 2012b), and this feature is shared by *P. pyriforme*. Although other minnow species also facilitated robust metamorphosis, *Cyprinella venusta* or *Semotilus atromaculatus* are good candidates for use in captive propagation because they are abundant, widely distributed, and easily procured.

Percina nigrofasciata and another darter species, *Etheostoma edwini*, were previously identified as hosts for *M. penicillatus* (O'Brien & Williams, 2002), and together with our results this shows that this species is a specialist on a broad array of darter species similar to other *Medionidus* (Zale & Neves, 1982; Haag & Warren, 1997, 2003). *Percina nigrofasciata* is a good candidate for use in propagation because it is widely distributed and abundant throughout the Gulf Coastal Plain. Our finding of specialization on black basses by *H. subangulata* also is concordant with previous information about this species and other members of *Hamiota* (Haag & Warren, 1997; Haag et al., 1999; O'Brien & Brim Box, 1999). *Hamiota altilis* was also reported to use *Lepomis cyanellus* as a host, but this species was considered only marginally suitable because metamorphosis was low and variable among trials (Haag et al., 1999). We did not recover any juvenile *H. subangulata* from *L. cyanellus*, but that species and *L. gulosus* held glochidia for 12 days after inoculation, suggesting that metamorphosis may be possible on this species. *Micropterus salmoides* is a good candidate for use in propagation of *H. subangulata* because this species is readily available from hatcheries, and we found no difference in metamorphosis success on hatchery raised *M. salmoides* and wild individuals of other *Micropterus* species.

We confirmed that *Amblema neislerii* is a host generalist. A previous study reported metamorphosis of this species on five fish species in three families (O'Brien & Williams, 2002), but our results provide a clearer picture of the wide host breadth of this species. In addition, we observed metamorphosis on one species (*Gambusia holbrooki*) not considered a host by O'Brien and Williams (2002), but that study observed metamorphosis on *Notropis texanus*, which did not produce juveniles in our study. These results show that *A. neislerii* is capable of metamorphosing on many fishes, but considerable variation in host suitability exists among fish species. Metamorphosis success was consistently high only on darters, and these fishes are good candidates for use in propagation of *A. neislerii*. Because *A. neislerii* releases glochidia in mucus threads that apparently entangle fishes by chance, the benthic habits of darters may expose them to glochidia more frequently, which in turn may have resulted in the close relationship between these species. However, another group of benthic fishes, catfishes, were consistently poor hosts for *A. neislerii*.

Apart from the tribe Anodontini, generalist host use is poorly documented and appears to be rare in North American mussel species (Haag, 2012), and confirmation of this host strategy has several important implications. Because it can metamorphose on many fish species, *A. neislerii* may be limited by host abundance to a lesser extent than specialists. On the other hand, its broad host use means that it could compete for hosts with many other mussel species. Finally, the ability of *A. neislerii* to metamorphose robustly on the migratory *Morone saxatilis* suggests that population structure may be influenced by long distance dispersal to a greater extent than mussel species that are specialists on more sedentary fish species. Studies of the suitability of other migratory species such as sturgeons, Skipjack Herring (*Alosa chrysochloris*), and Alabama Shad (*Alosa alabamae*)

also would be desirable. *Amblema plicata* also has been reported to parasitize fishes from 10 different families (Howard & Anson, 1922; Coker et al., 1921; Weiss & Layzer, 1995), but most of these potential hosts are unconfirmed, and remarkably, a comprehensive, quantitative host suitability study has never been conducted for this species. *Amblema plicata* is one of the most abundant and widespread species in North America, and better information is needed to more fully evaluate the evolutionary and conservation significance of host use in this genus.

The unique life cycle of freshwater mussels complicates the protection of these species because managers must consider the status not only of mussels but of host fish populations (McCargo & Peterson, 2010). All four species in this study use common fishes as hosts and therefore may have been affected by changes in fish populations to a lesser extent than mussel species that are specialists on imperiled fishes (e.g., Fritts et al., 2012a). Nevertheless, comprehensive host information is necessary for development of holistic management strategies, and these studies remain an important research need for mussel conservation (Haag & Williams, 2014).

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MITOCHONDRIAL DNA VARIATION IN THE EASTERN PONDMUSSEL, *LIGUMIA NASUTA* (BIVALVIA: UNIONOIDA), IN THE GREAT LAKES REGION

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ABSTRACT

Most freshwater mussel species in the Great Lakes colonized the region from the Mississippi River basin and few appear to have colonized from Atlantic coast rivers. The Eastern Pondmussel, *Ligumia nasuta*, is widespread along the Atlantic coast but occurs elsewhere only in the Great Lakes, suggesting that it is one of the few Great Lakes species of Atlantic origin. Great Lakes populations are now imperiled following invasion of the lakes by dreissenid mussels. We examined patterns of diversity in the mitochondrial CO1 and ND1 genes in *L. nasuta* populations in the Great Lakes and in Atlantic coast rivers. Genetic diversity was low in Great Lakes populations and included only one CO1 and two ND1 haplotypes, all of which were also found in Atlantic coast populations. Genetic diversity was higher in Atlantic coast populations and included four CO1 and six ND1 haplotypes. Pairwise Φ_{ST} revealed significant genetic differentiation for both genes between Atlantic coast and Great Lakes populations but not within Great Lakes populations. These results suggest that all populations of *L. nasuta* in the Great Lakes are derived from a single, small founder group that colonized from an Atlantic coast river. As such, Great Lakes populations may be considered a single management unit and conservation efforts based on propagation or translocation should be limited to use of Great Lakes source stock to prevent introduction of non-native haplotypes.

KEY WORDS Endangered mussels, genetic variation, Laurentian Great Lakes, phylogeography, glaciation, Atlantic coast

INTRODUCTION

The diverse mussel fauna of the Laurentian Great Lakes upstream of Niagara Falls (referred to here as the upper Great Lakes) is a result of dispersal into the region following the end of the Wisconsin glaciation about 11,000 years ago. Most species (about 40) colonized the region from the Mississippi River basin (van der Schalie, 1963; Graf, 2002) and genetic evidence suggests that there were multiple colonization routes (Elderkin et al., 2007, 2008). Only two species are thought to have colonized the region from Atlantic coast river systems: the Eastern Pondmussel, *Ligumia nasuta* (Say, 1817) and Eastern Elliptio, *Elliptio complanata* (Lightfoot, 1786). The dearth of Atlantic coast species is a result of the long-standing barrier of Niagara Falls and the limited number of post-glacial colonization routes between Atlantic coast rivers and the upper Great Lakes (Mandrak & Crossman, 1992; Strayer & Jirka, 1997; Larson & Schaetzl, 2001; Lewis et al., 2012). In contrast, Lake Ontario and the St. Lawrence River system downstream of Niagara Falls have a higher proportion of Atlantic coast mussel species, suggesting that this region has had more exchange with other Atlantic coast rivers (Haag, 2012).

Ligumia nasuta is widely distributed in Atlantic coast rivers from South Carolina to Maine (Nedeau et al., 2000; Price, 2005). In the upper Great Lakes, *L. nasuta* was locally common but restricted mainly to the Lake Erie and Lake St. Clair watersheds and a small portion of the Lake Huron and Lake Michigan watersheds, and it was widely distributed downstream of Niagara Falls (COSEWIC, 2007; Watters et al., 2009). The distribution and abundance of *L. nasuta* in the Great Lakes

was greatly reduced after introduction of invasive dreissenid mussels (*Dreissena* spp.) (Nalepa et al., 1991; Schloesser et al., 1996; Zanatta et al., in press), and the species is in danger of extirpation from the region. Because it remains widely distributed in Atlantic coast rivers, *L. nasuta* is considered "apparently secure" globally (NatureServe, 2013). However, the genetic relationship of surviving Great Lakes populations to those on the Atlantic coast is unknown.

We examined DNA sequence variation in the mitochondrial COI and ND1 genes in populations of *L. nasuta* in the Great Lakes and Atlantic coast rivers. We use these data to 1) examine the colonization history of the species in the Great Lakes, and 2) provide information necessary for management and conservation of the species.

METHODS

A total of 64 individuals were collected in 2011 and 2012 from 17 sites within five major watersheds or geographical regions: northern Michigan (Lake Michigan and Huron drainages), Lake St. Clair, Lake Erie, Lake Ontario (including the St. Lawrence River system), and Atlantic coast rivers (Fig. 1; Table 1). Mussels were col-

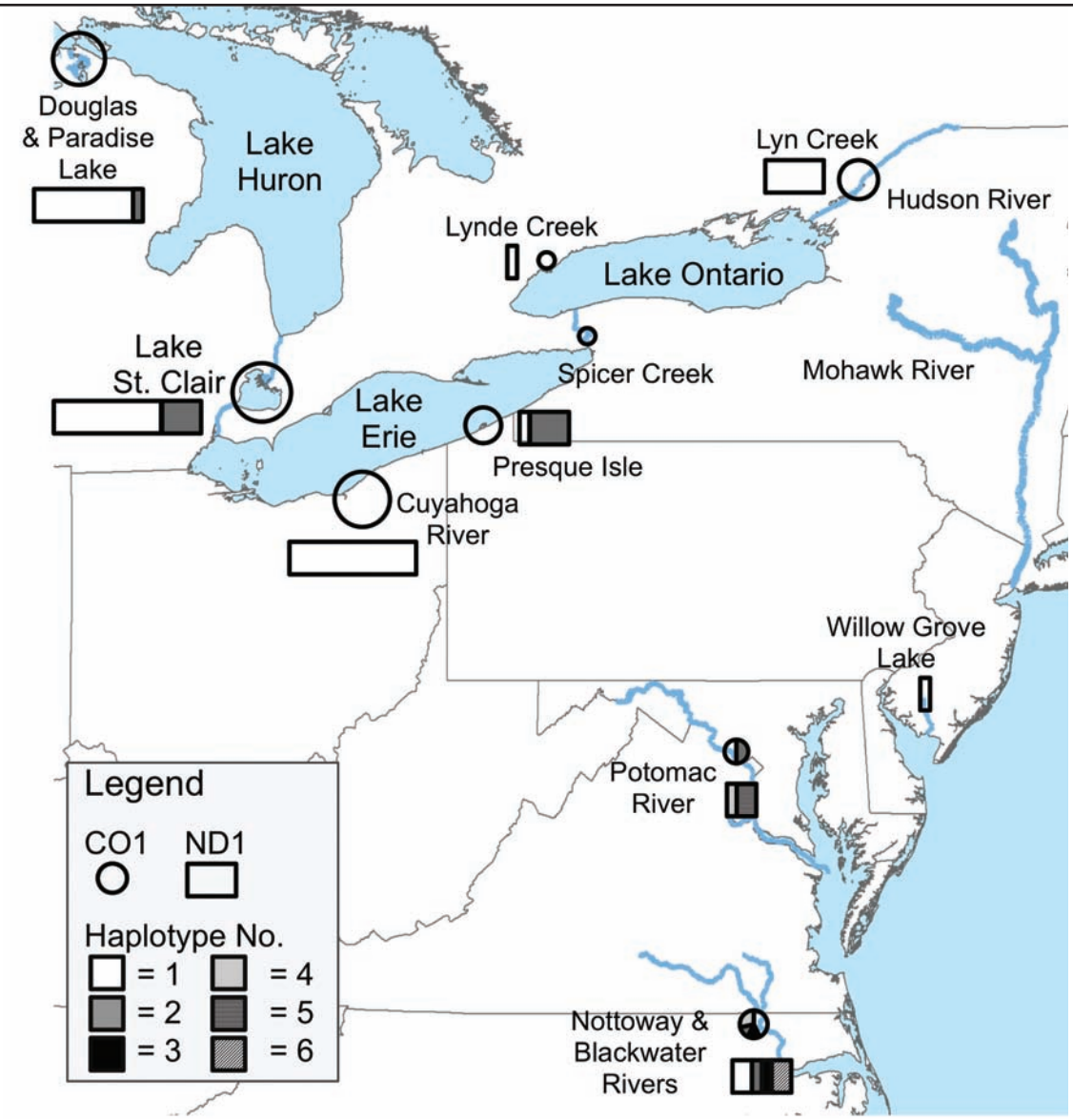

FIGURE 1

Sampling sites and haplotype frequencies for *L. nasuta*. The size of circles (CO1) and rectangles (ND1) indicates the relative sample sizes (number of individuals) for each gene. CO1 haplotype circles are centered over the sampling location area they represent. Note that CO1 and ND1 mtDNA sequences were not resolved at some sites and these sites lack the corresponding symbol. Some closely adjacent sample sites are represented by a single symbol representing pooled results for those sites (e.g., Presque Isle); Table 1 provides a complete list of sample sites

TABLE 1

Sampling sites for *Ligumia nasuta*. Sites were pooled by region for statistical analysis (see text). Sites were pooled by population for depiction of haplotype frequencies on Fig. 1.

Region	Population	Site
Northern Michigan	Douglas Lake and Paradise Lake	Douglas Lake, Cheboygan Co., Michigan Paradise Lake, Emmet and Cheboygan Co., Michigan
Lake St. Clair	Lake St. Clair	Big Muscamoot Bay, St. Clair Co., Michigan Goose Bay, St. Clair Co., Michigan Little Muscamoot Bay, St. Clair Co., Michigan Bass Bay, Walpole Island First Nation, Ontario, Canada
Lake Erie	Cuyahoga River	Cuyahoga River, Geauga Co., Portage Co., Ohio
	Presque Isle	Thompson Bay, Erie Co., Pennsylvania Presque Isle Bay, Erie Co., Pennsylvania Duck Pond, Erie Co., Pennsylvania
	Spicer Creek (Niagara River)	Spicer Creek, Grand Island, Erie Co., New York
Lake Ontario	Lynde Creek (Lake Ontario)	Lynde Creek, Durham Region, Ontario, Canada
	Lyn Creek (St. Lawrence River)	Lyn Creek, Leeds and Grenville Co., Ontario, Canada
Atlantic coast	Willow Grove Lake (Maurice River)	Willow Grove Lake, Salem Co., New Jersey
	Potomac River	Potomac River, Montgomery Co., Maryland
	Nottaway and Blackwater rivers	Nottaway River, Southampton Co., Virginia Blackwater River, Franklin, Virginia

lected with clam rakes or by hand with SCUBA and snorkeling. Two methods were used to collect DNA: a swab of mucus from the foot, which was stored in sterile lysis buffer (Henley et al., 2006); or a clip of mantle tissue stored in 95% ethanol (Berg et al., 1995). The collection method depended on permit restrictions for rare species in each state or province. Each mussel was gently opened along the ventral margin <1 cm to obtain the sample, after which the mussel was returned to the substrate. All samples were stored at -20°C in the laboratory. Only female lineage mtDNA was sampled because methods required to obtain gonadal tissue for male lineage mtDNA are typically lethal.

DNA was extracted from samples using an overnight digestion with proteinase K. The alcohol extraction method of Sambrook et al. (1989) was used for mucus samples and Qiagen DNeasy extraction kits were used for mantle clips. Genomic DNA was stained with SYBR Green (or Ethidium Bromide) and electrophoresed in a 1.5% agarose gel to confirm presence. Two mtDNA regions were amplified, the mitochondrial cytochrome c oxidase subunit 1 (CO1) and the NADH dehydrogenase subunit 1 (ND1) using primers described in Campbell et al. (2005). Samples from the Cuyahoga River were run at Cleveland State University in 25 µL volumes consisting of 10 µL of deionized water, 5.5 µL of a 5X buffer, 2.75 µL of 2.5 mM dNTPs, 2.75 µL of each primer at 2.5 mM, 2.75 µL of 0.25 mM $MgCl_2$, and 0.15 µL Taq polymerase. All other samples were run at Central Michigan University in 10 µL volumes, made of the mixture of 1 µL 10X Buffer, 1 µL bovine serum albumin, 0.3 µL of forward primer, 0.3 of reverse primer, 0.2 µL of dNTP, 5.15 µL of deionized water, and 0.05 µL Taq polymerase per sample. To each assay 1 µL extracted DNA was added. If the initial PCR reaction did not work, an additional 0.2 µL of $MgCl_2$ replaced an equal amount of water. The thermocycler amplification conditions for both mtDNA regions were as follows: denaturation at 92-94°C for 2 minutes; five cycles of 92-94°C for 40 seconds; 40°C for 40 seconds; 72°C for 90 seconds; 25 cycles of 92°C for 40 seconds; 50°C for 40 seconds (or 49°C for all cycles), and 72°C for 90 seconds. Completed reactions were held at 4°C and then placed in the freezer. Primers were removed from amplified samples using a QIAquick© PCR Purification Kit or an Exonuclease I (Amersham Biosciences cat# E70073X, 10 U.ml) and shrimp alkaline phosphatase (SAP) (Amersham Biosciences cat# E70092X 1U.ml) (78 ml ddH_2O, 2 ml ExoI, 20 ml SAP) reaction to denature enzymes, and incubation at 37°C for 40 min followed by 80°C for 20 min. Amplified samples were sequenced on an ABI 3730 (Applied Biosystems).

The sequences of the two mtDNA regions were aligned and edited using BIOEDIT (Hall, 1999) and MACCLADE (Maddison and Maddison, 1997) software. Haplotypes were identified using COLLAPSE v.1.2 software (Posada 2011). A haplotype network for both mtDNA regions was constructed using TCS v.1.21 software (Clement et al., 2000). Due to limited sample sizes at many sites, we pooled sites within the five watersheds or geographical regions described previously (see Table 1) to examine large-scale patterns of genetic diversity. Differences among these regions in haplotype differentiation (Φ_{ST}), gene diversity, nucleotide diversity, and the number of haplotypes per group were examined using analysis of molecular variance (AMOVA) implemented in ARLEQUIN (Schneider et al., 2000). CO1 and ND1 mtDNA sections were analyzed separately because sequencing was not successful for both genes in all individuals.

RESULTS

The CO1 sequencing provided a 453 bp fragment from 64 individuals and the ND1 sequencing gave a 511 bp fragment from 61 individuals (Genbank Accession numbers KM656075-KM656083). Both mtDNA gene segments exhibited little variation within the Great Lakes including Lake Ontario and the St. Lawrence River. Only one CO1 haplotype and two ND1 haplotypes were found in these populations (Fig. 1; Table 2). In contrast, four CO1 and six ND1 haplotypes were recovered in Atlantic coast populations. All Great Lakes haplotypes were present in and among the most common haplotypes in Atlantic coast populations even though sample numbers were generally low across all of the Atlantic coast populations sampled. All haplotypes in all regions differed by just one or two point mutations from the most common type (Fig. 2). Gene diversity and nucleotide diversity for both genes also were low in Great Lakes populations; CO1 was invariant and ND1 showed very low diversity except in Lake Ontario where it was invariant (Table 2). Gene diversity and nucleotide diversity for both genes were substantially higher in Atlantic coast populations than in the Great Lakes (Table 2).

The percentage of variation explained by partitioning among the five regions was 38% ($P < 0.0001$) for CO1 and 10% for ND1 ($P = 0.0128$), and more variation was present within Atlantic coast populations than within all of the Great Lakes samples combined (Table 2). Pairwise Φ_{ST} revealed significant genetic differentiation for both genes only between the Atlantic coast populations and each of the four Great Lakes regions and there were no differences within the Great Lakes (Table 3).

DISCUSSION

Genetic variation in *Ligumia nasuta* was low in all Great Lakes populations compared to Atlantic coast

TABLE 2

Variation in the mitochondrial CO1 and ND1 genes of *Ligumia nasuta* among five regions. N is the number of individuals sampled.

Region	CO1			
	N	Gene Diversity	Nucleotide Diversity	No. of Haplotypes
Northern Michigan	12	0.0000	0.0000	1
Lake St. Clair	14	0.0000	0.0000	1
Lake Erie	21	0.0000	0.0000	1
Lake Ontario	7	0.0000	0.0000	1
Atlantic coast	9	0.7778	0.0030	4
	ND1			
Northern Michigan	12	0.1667	0.0003	2
Lake St. Clair	15	0.4190	0.0008	2
Lake Erie	17	0.3824	0.0007	2
Lake Ontario	7	0.0000	0.0000	1
Atlantic coast	10	0.8889	0.0023	6

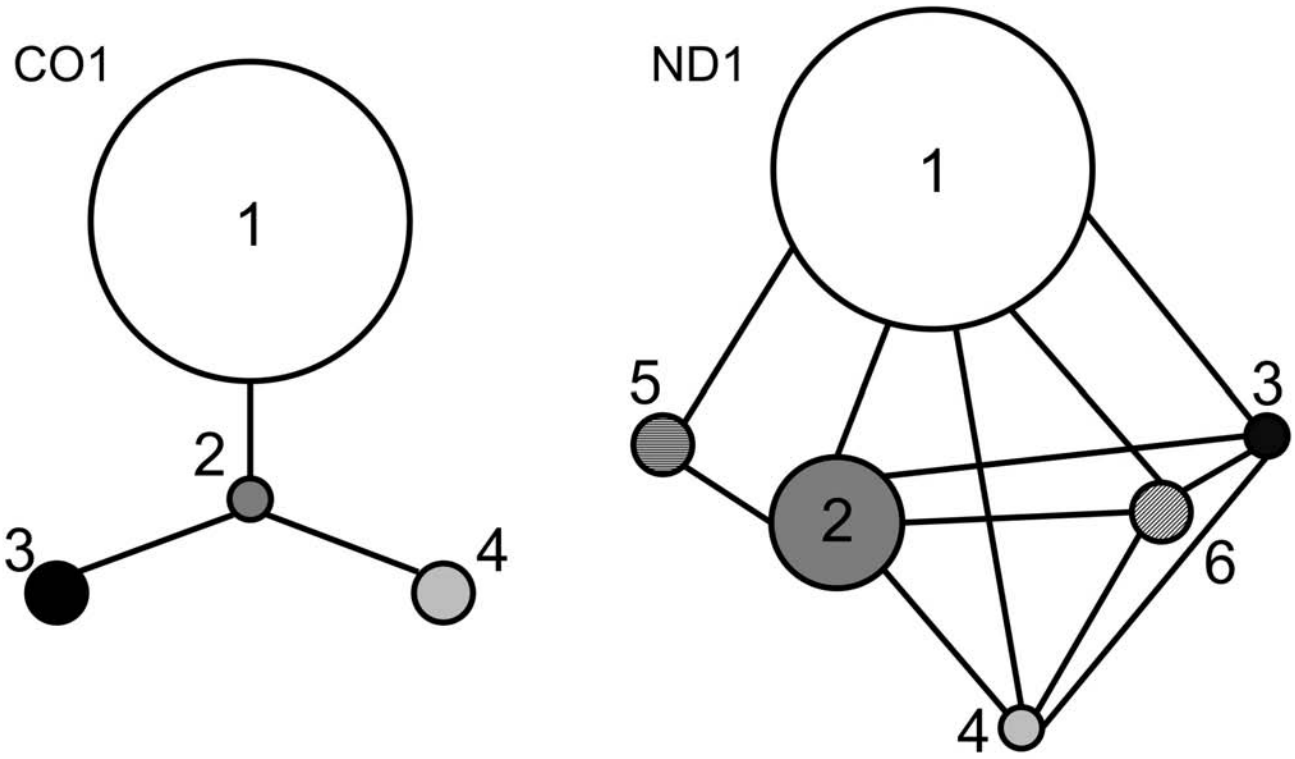

FIGURE 2

Spanning network of mtDNA haplotypes at the CO1 and ND1 loci for *L. nasuta*. The connecting lines represent a single base pair difference between adjoined haplotypes. The relative size of the circles represents the frequency of the haplotypes in all samples. Haplotype numbers are referenced on Fig. 1.

TABLE 3

Pairwise Φ_{ST} values for *Ligumia nasuta* mitochondrial ND1 (above diagonal) and CO1 (below diagonal) genes among five regions. Values with asterisk are statistically significant (α= 0.05).

	Northern Michigan	Lake St. Clair	Lake Erie	Lake Ontario	Atlantic coast
Northern Michigan	-	0.033	0.007	-0.051	0.155*
Lake St. Clair	0.000	-	-0.062	0.121	0.165*
Lake Erie	0.000	0.000	-	0.089	0.172*
Lake Ontario	0.000	0.000	0.000	-	0.114*
Atlantic coast	0.386*	0.415*	0.498*	0.290*	-

populations where limited sampling revealed numerous haplotypes and much higher overall genetic diversity. Together with the common occurrence of all Great Lakes haplotypes in Atlantic coast populations, these results suggest that Great Lakes populations were established by a single, small founder group from an Atlantic coast river system or a larger group from a single source population with low genetic variation. Either scenario is consistent with the hypotheses that 1) *L. nasuta* is one of the few upper Great Lakes species to have colonized the region from Atlantic coast rivers, and 2) there were few opportunities for such exchanges. An unexpected result was the low genetic diversity of Great Lake populations downstream of Niagara Falls. Our sample sizes were lowest in this region, but these results suggest that there also have been few opportunities for faunal exchange between the St. Lawrence River system and other Atlantic coast river systems.

The low genetic diversity of Great Lakes populations of *L. nasuta* is in contrast to other species that colonized the region from the Mississippi River basin. In the Lake Erie watershed alone, *Amblema plicata* (Say, 1817) had at least six CO1 haplotypes out of 36 known haplotypes across its range (Elderkin et al., 2007), and *Pyganodon grandis* (Say, 1829) had 34 CO1 haplotypes out of 45 haplotypes across the northern portion of its range (Krebs et al., in press). Across the Great Lakes region, *Elliptio dilatata* (Rafinesque, 1820) had four to seven haplotypes per site out of 38 haplotypes across its range, and *Actinonaias ligamentina* (Lamarck, 1819) had six to eleven haplotypes per site out of 73 haplotypes across its range (Elderkin et al., 2008). These results are consistent with the idea that some Mississippi River basin species reached the Great Lakes via multiple routes.

Other Mississippian species in the Great Lakes have lower genetic diversity comparable to that seen in *L. nasuta*. *Fusconaia flava* (Rafinesque, 1820), had only three CO1 haplotypes in the Lake Erie watershed compared to 13 found across its range (Burdick & White, 2007), and *Epioblasma torulosa rangiana* (Lea, 1839) in the Sydenham River (Lake St. Clair watershed) had two CO1 haplotypes out of 10 haplotypes found across its range (Zanatta & Murphy, 2007). *Venustaconcha ellipsiformis* (Conrad, 1836) in the Lake Huron and Lake Michigan watersheds had three CO1 haplotypes and one ND1 haplotype out of 13 haplotypes found in the Mississippi River basin (Zanatta & Harris, 2013). These mixed results highlight the diverse and complex history of post-glacial dispersal into the Great Lakes from the Mississippi River basin (see Graf, 2002) as opposed to the apparently more limited dispersal from Atlantic coast rivers.

The genetic similarity among *L. nasuta* populations throughout the Great Lakes suggests that they can be treated as a single management unit. However, the Great Lakes management unit clearly is genetically distinctive from the Atlantic coast populations we sampled. Until more information becomes available, recovery efforts in the Great Lakes based on captive propagation or translocation should be limited to use of Great Lakes source stock to avoid introduction of non-native haplotypes. Sampling from populations in additional Atlantic coast rivers, particularly those in previously glaciated regions (e.g., Hudson and Mohawk rivers), may reveal other suitable source populations for conservation efforts and may refine our understanding of the evolutionary history of Great Lakes populations of *L. nasuta*.

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Gastropod Distribution and Status

Genetics

Guidelines and Techniques

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Mussel Distribution and Status

Outreach

Propagation and Restoration

WALKERANA The Journal of the Freshwater Mollusk Conservation Society

OUR PURPOSE

The Freshwater Mollusk Conservation Society (FMCS) is dedicated to the conservation of and advocacy of freshwater mollusks, North America's most imperiled animals. Membership in the society is open to anyone interested infreshwater mollusks who supports the stated purposes of the Society which are as follows:

1) Advocate conservation of freshwater molluscan resources;

2) Serve as a conduit for information about freshwater mollusks;

3) Promote science-based management of freshwater mollusks;

4) Promote and facilitate education and awareness about freshwater mollusks and their function in freshwater ecosystems;

5) Assist with the facilitation of the National Strategy for the Conservation of Native Freshwater Mussels (Journal of Shellfish Research, 1999, Volume 17, Number 5), and a similar strategy under development for freshwater gastropods.

OUR HISTORY

The FMCS traces it's origins to 1992 when a symposium sponsored by the Upper Mississippi River Conservation Committee, USFWS, Mussel Mitigation Trust, and Tennessee Shell Company brought concerned people to St. Louis, Missouri to discuss the status, conservation, and management of freshwater mussels. This meeting resulted in the formation of a working group to develop the National Strategy for the Conservation of Native Freshwater Mussels and set the ground work for another freshwater mussel symposium. In 1995, the next symposium was also held in St. Louis, and both the 1992 and 1995 symposia had published proceedings. Then in March 1996, the Mississippi Interstate Cooperative Research Association (MICRA) formed a mussel committee. It was this committee (National Native Mussel Conservation Committee) whose function it was to implement the National Strategy for the Conservation of Native Freshwater Mussels by organizing a group of state, federal, and academic biologists, along with individuals from the commercial mussel industry. In March 1998, the NNMCC and attendees of the Conservation, Captive Care and Propagation of Freshwater Mussels Symposium held in Columbus, OH, voted to form the Freshwater Mollusk Conservation Society. In November 1998, the executive board drafted a society constitution and voted to incorporate the FMCS as a not-for-profit society. In March 1999, the FMCS held it's first symposium "Musseling in on Biodiversity" in Chattanooga, Tennessee. The symposium attracted 280 attendees; proceedings from that meeting are available for purchase. The second symposium was held in March 2001 in Pittsburgh, Pennsylvania, the third in March 2003 in Raleigh, North Carolina, the fourth in St. Paul, Minnesota in May 2005, the fifth in Little Rock, Arkansas in March 2007, the sixth in Baltimore, Maryland in April 2009, the seventh in Louisville, Kentucky in 2011, and the eighth in Guntersville, Alabama in 2013. The society also holds workshops on alternating years, and produces a newsletter four times a year.

TO JOIN FMCS OR SUBMIT A PAPER

Please visit our website for more information at http://www.molluskconservation.org

Or contact any of our board members or editors of WALKERANA to talk to someone of your needs. You'll find contact information on the inside back cover of this publication.